5th Annual Research Day

10th October 2014, Friday

Scudder Auditorium

Christian Medical College
Vellore
PHOTOGRAPHS OF POSTER PRESENTATIONS
Aberrant Niche signaling and its role in the etio-pathogenesis of Ulcerative Colitis

INTRODUCTION
- Colonic stem cell self-renew, differentiate and migrate from the cues given by the niche.
- Increasing evidences suggest an epithelial abnormality is central to the development of Ulcerative colitis.
- However the role of the niche in the etio-pathogenesis of UC is poorly understood.

AIM
To explore the role of the niche in the etio-pathogenesis of Ulcerative colitis in an animal model of and in patient mucosal biopsies.

MATERIALS AND METHODS

RESULTS
- Unaltered gross changes prior to inflammation
- Mucous depletion in the lower crypt prior to inflammation

CONCLUSIONS
Impaired stem cell function may play a role in the etio-pathogenesis of Ulcerative colitis.

ACKNOWLEDGEMENTS
Funding By: Centre for Stem Cell Research (CSCR)
CSCR Animal Facility, Histopathology Laboratory, Core Facility
Background

Extrypoiesis involves a multi-step differentiation process. The molecular processes that are involved in each stage of extrypoiesis that drive the cells from hematopoietic stem cell stage to terminally differentiated red cells have yet not been fully understood.

Ex-vivo differentiation of hematopoietic stem cells and progenitors to erythroid cells is an useful tool to conduct studies to understand transcriptional and epigenetic mechanisms of human extrypoiesis.

Recent studies revealed the potential role of micro RNAs (miRNAs) during hematopoietic stem cell differentiation and a few miRNAs have been identified to be critical for extrypoiesis and globin gene regulation.

We performed ex-vivo erythroid differentiation of CD34+ hematopoietic stem cells and progenitors (HSPCs) and the RNA obtained from the cultured erythroid cells at different stages of erythroid differentiation were used for small RNA sequencing.

We identified several miRNAs which were differentially expressed in HSPCs and erythroid cells and there were several other miRNAs that were transcriptionally co-regulated in these cells. ChIP-sequencing analysis of GATA1 and H3K4Me3 showed that these miRNAs have erythroid specific expression.

This study helped us to identify the miRNAs that are required for HSC maintenance and extrypoiesis.

Results and discussion

![Graph A](image1.png)
![Graph B](image2.png)

Fig 1: Ex-vivo erythroid culture system: A) Flow cytometry analysis of surface markers B) Giemsa staining C) Globin gene expression pattern D) Haemosiderin expression by nIHC.

- We achieved a robust proliferation of erythroid cells from CD34+ cells and they showed characteristic morphology, surface marker expression and globin gene expression during ex-vivo extrypoiesis

- We observed 10 micro RNA clusters containing co-regulated miRNA in extrypoiesis. The miRNAs in the clusters were either upregulated or downregulated.

- We observed a strong binding of GATA1 and active epigenetic marker H3K4Me3 near the up-regulated miRNAs but not near the down-regulated miRNA.

- We identified an additional miRNA, miR-4732, in the miRNA-144-451 cluster which has been shown to have important roles in extrypoiesis.

Table 1: List of 10 miRNA clusters that are up or downregulated in human extrypoiesis.

<table>
<thead>
<tr>
<th>miRNA Cluster</th>
<th>Expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR-182, miR-193 &amp; miR-36</td>
<td>Up</td>
</tr>
<tr>
<td>miR-461, miR-444 &amp; miR-4732</td>
<td>Up</td>
</tr>
<tr>
<td>miR-192, miR-6749, miR-6750 &amp; miR-20a</td>
<td>Down</td>
</tr>
<tr>
<td>miR-145, miR-143 &amp; miR-191</td>
<td>Down</td>
</tr>
<tr>
<td>miR-22a, miR-17, miR-18a, miR-19a, miR-20a &amp; miR-191</td>
<td>Down</td>
</tr>
<tr>
<td>miR-431, miR-433, miR-37 &amp; miR-555</td>
<td>Down</td>
</tr>
<tr>
<td>miR-371b, miR-371a &amp; miR-373</td>
<td>Down</td>
</tr>
<tr>
<td>miR-9, miR-125a</td>
<td>Down</td>
</tr>
<tr>
<td>miR-10a, miR-18b, miR-20b, miR-190-2, miR-920-2 &amp; miR-363</td>
<td>Down</td>
</tr>
<tr>
<td>cluster of 41 miRNAs</td>
<td>Down</td>
</tr>
</tbody>
</table>

Materials and methods

- Ex-vivo erythroid culture: Mobilized peripheral blood CD34+ cells were isolated and cultured in modified 2 phase liquid culture condition
- Flow cytometric analysis: Expression of surface markers CD34, CD45, CD71 (early erythroid marker) and CD235a (late erythroid marker) were analyzed in different stages of extrypoiesis
- Giemsa Staining: Morphological changes during ex-vivo extrypoiesis were evaluated by Giemsa staining
- Globin gene expression analysis: Real time PCR analysis was carried out to estimate the miRNA levels of α and β globin genes in cultured erythroid cells
- Haemoglobin analysis: Levels of haemoglobin A, A2 and F were measured by Biocentric haemoglobin VARIANT analyzer
- Small RNA sequencing: RNA obtained from different stages of extrypoiesis were used for small RNA sequencing. Small RNA library was prepared and analyzed in Illumina Hiseq 1000 platform and the data was analyzed by using in-house Perl script
- ChIP-sequencing analysis: The GATA1 and H3K4Me3 enrichment were identified from publicly available data GSE63694 (ref).

CONCLUSION

1. We have systematically screened for miRNAs in ex-vivo extrypoiesis by small RNA sequencing.
2. We observed that the majority of miRNAs were down-regulated during extrypoiesis differentiation.
3. Most of the differentially expressed miRNAs are intronic in location.
4. Several miRNA clusters with co-expressed miRNAs were identified.
5. We found a new member, miR-4732, in the miRNA-144-451 cluster and all the three miRNAs in the cluster are up-regulated during extrypoiesis.
6. Erythroid specific expression of miRNAs is regulated by cell specific transcription factors.

References:
Demonstration of high endothelial venules in human postpartum Fallopian tube

Minu Rekha B.*, Santosh Joseph Benjamin**, Visalakshi Jayaseelan***, J. Suganthy*
*Department of Anatomy, **Department of Obstetrics and Gynaecology, ***Department of Biostatistics, Christian Medical College, Vellore

Introduction

Mucosa associated lymphoid tissue (MALT) is characterized by the presence of lymphoid aggregates and high endothelial venules (HEVs), the specialized postcapillary venules, that are concerned with lymphocyte trafficking in secondary lymphoid tissues. Presence of intraepithelial lymphocytes (IELs) and lymphoid aggregates have been demonstrated in the Fallopian tube suggesting that it belongs to MALT. However, HEVs are said to be absent. Yet, an incidental finding in our department showed the presence of HEVs in postpartum Fallopian tubes. In addition to lymphocytes, the HEVs act as a migratory route for procursors of dendritic cells. This study was done to confirm the presence of HEVs in the postpartum Fallopian tube.

Aim and Objectives

AIM:
To look for the presence of HEVs and lymphocyte trafficking through them in the mucosa of the human postpartum Fallopian tube

OBJECTIVES:
1. To quantify IELs per 100 mm length of epithelium under light microscopy.
2. To quantify the mucosal HEVs per mm² under light microscopy.
3. To look for correlation between numbers of IELs and mucosal HEVs.
4. To look for high endothelial venules, lymphocyte trafficking through them and their association with dendritic cells under electron microscopy.

Materials and Methods

Approval was obtained from the Institutional Review Board, Christian Medical College, Vellore.

Sample collection and processing:
Ampullary part of Fallopian tubes were collected from ten women who underwent lower segment caesarean section with sterilization and processed for electron microscopy.

Light microscopy:
The serial sections stained with 1% toluidine blue were used to study the HEVs and IELs. Measurements were done using cells/n image analysing software.

1. Number of HEVs were counted per 4 mm² area of the mucosa in each sample.
2. Number of IELs were counted per 100 mm length of the epithelium in each sample.

Statistical analysis:
The data were statistically analysed using the SPSS version 17.0:
1. Descriptive statistics
2. Spearman’s rho correlation and simple linear regression between the number of mucosal HEVs and IELs were done.

Transmission electron microscopy (TEM):
1. HEVs and lymphocyte trafficking through them were looked for.
2. Association of HEVs with dendritic cells were looked for.

Results

Intraepithelial lymphocytes:
• Seen both singly or in rows as "string of pearls".
• Seen at different levels within the epithelium.

Figure 1: Light microscopic pictures of IELs in the mucosa of postpartum Fallopian tube. A) IELs arranged in rows (arrow), B) IELs arranged at different levels of the epithelium (arrowheads).

High endothelial venules:
• Lined by cuboidal endothelium
• Predominantly subepithelial in location
• IELs were constantly located in the epithelium overlying HEVs
• In addition to lymphocytes, neutrophils were present in the lumen
• Association of dendritic cells with HEVs was noted

Figure 2: HEVs (arrow heads) in the lamina propria of postpartum Fallopian tube seen under light microscope. A) HEV lined by cuboidal endothelium. B) Neutrophils (+) in the lumen of HEV.

Figure 3: TEM pictures of HEVs. A) Endothelium (E) is cuboidal in nature. Note an IEL overlying the HEV (arrow). B) Platelet. C) Lymphocytes (L) within the lumen of HEV. D) Red blood cell. E) Lymphocyte within the endothelium of HEV suggesting transendothelial migration; arrow head–globular mitochondria, "+-" vesicles. D) Neutrophil (N) within the lumen of HEV. Red arrow head–membrane contact of neutrophil with HEV. P–pericyte, black arrow heads–basal lamina, E) A dendritic cell (DC) seen in association with a HEV.

Quantitative analysis:
• The mean number of IELs per 100 mm length of the basement membrane of the epithelium was 771.35 ± 300.65.
• The mean number of the mucosal HEVs per square mm² area was 19.23 ± 7.05.

Table 1: Frequency of HEVs and IELs in the human postpartum Fallopian tube (n = 10).

<table>
<thead>
<tr>
<th>Group</th>
<th>Variable Studied</th>
<th>Mean</th>
<th>Standard Dev.</th>
<th>Range</th>
<th>Min</th>
<th>Max</th>
<th>IQR</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>HEVs/mm²</td>
<td>19.23</td>
<td>7.05</td>
<td>6.00</td>
<td>11.50</td>
<td>28.00</td>
<td>(12.26–24.31)</td>
</tr>
<tr>
<td>2</td>
<td>IELs/mm²</td>
<td>771.35</td>
<td>300.65</td>
<td>400.00</td>
<td>1200.00</td>
<td>1100.00</td>
<td>(524.80–857.75)</td>
</tr>
</tbody>
</table>

IQR = Interquartile range (25 percentile and 75 percentile).

Discussion

High endothelial venules:
• HEVs act as a migratory route for the circulating immune cells.
• Contrary to earlier studies, the present study showed many HEVs in the mucosa of postpartum Fallopian tube.
• Transendothelial migration of lymphocytes was seen.
• Earlier reports state that HEVs are channels that are specific for lymphocytes alone and neutrophils are not associated with HEVs. However, they were seen in association with HEVs in the present study.

Intraepithelial lymphocytes:
• The presence of IELs in the mucosa of the Fallopian tube was considered.
• To be physiological and not due to sapling/cysts.
• They might act as a first line of defence against infections and thereby prevent infertility.
• They play an important role in immune tolerance, facilitating the transport of sperm and blastocyst without triggering a local immune response.

Dendritic cells in association with HEVs:
• The presence of dendritic cells seen in association with HEVs shows that the lymphocytes migrating through the HEVs might possibly interact with these dendritic cells to set an immune response.

Conclusion

The presence of IELs, HEVs and the significant correlation between them confirms that Fallopian tube is a member of MALT and that HEVs are the migratory route of lymphocytes in the postpartum Fallopian tube.

Limitations

There was no baseline data on human non-pregnant Fallopian tube available for the variables studied. So the study results could not be compared.

References

**BACKGROUND**

Cervical cancer is the 4th most common cancer in the world with a very high incidence rate reported in India. Cervical cancer evolves from various grades of dysplastic changes that can be detected with Pap smears. Interpretation of histopathological and cytological specimens is subject to interobserver variation. Thus it was essential to develop an immunohistochemical marker for objective assessment.

**AIMS & OBJECTIVES**

- To evaluate the diagnostic utility of p16 to differentiate between reactive, premalignant and malignant squamous and glandular lesions of cervix.
- To assess the effectiveness of Pap smear as screening tool.

**MATERIALS AND METHODS**

- 120 cervical biopsies (15 cases each of CIN 1, 2, 3 and squamous cell carcinoma, 22 cases of adenocarcinoma and 38 reactive cases) were studied and p16 immunohistochemistry was performed. Score out of 8 was given based on intensity and proportion of p16 expression. Corresponding cytological diagnoses were also evaluated.

**RESULTS**

- Sensitivity of p16 in CIN 1, 2, 3, squamous cell carcinoma, adenocarcinoma were found to be 73.7%, 93.3%, 93.3%, 100% and 95.4%.
- Specificity of p16 in cervical squamous and glandular lesions was 85.7% and 80%.

| CIN 1 | 11/15 (73.3%) | 4/11 (27.8%) | 3/11 (27.8%) | 4/11 (27.8%) |
| CIN 2 | 14/15 (93.3%) | 9/14 (64.3%) | 5/14 (36%) | 4/14 (28.6%) |
| CIN 3 | 15/15 (100%) | 4/15 (26.7%) | 1/15 (6.7%) | 5/15 (33.3%) |
| SCC | 15/15 (100%) | 15/15 (100%) | 1/15 (6.7%) | 6/15 (40%) |
| AdC | 21/22 (95.5%) | 18/21 (86%) | 3/21 (14.3%) | 0/21 (0%) |
| Reactive | 6/38 (16%) | 6/38 (16%) | 0/38 (0%) | 0/38 (0%) |

- p16 over expression also correlated with stage of tumour, lymph node metastasis, local and distant metastasis. It did not correlate with grade of the tumour.
- Intensity of expression correlated better with lesion severity than proportion.
- 15.7% of reactive epithelium showed patchy weak cytoplasmic positivity for p16.
- The time taken for a dysplastic lesion to progress into a higher grade lesion was 16 months.
- Among the morphological parameters used to diagnose cervical dysplasia, mitotic count was found to be the only parameter which showed statistically significant difference among the subtypes.
- Koilocytosis was significantly associated with CIN 1 and CIN 2 but not with CIN 3. All the cases of koilocytosis were negative for p16.
- The most common presenting symptom was vaginal discharge in CINs and cervical growth in carcinoma.
- 15.8% of women with CIN 1 were referred for biopsy due to abnormality in Pap smear.
- Mean age of diagnosis of CIN 1, 2, 3, SCC and AdC were 43.3 years, 41.4 years, 48.6 years, 52 years and 50 years.
- 47.1% of women presented with advanced stages of carcinoma (Stage III/IV).
- Cytology had sensitivity of 35.7%, 69.2% and 50% for low grade lesions, high grade lesions and glandular lesions respectively.

**CONCLUSIONS**

- p16 is a reliable marker to detect cervical dysplastic lesions and can differentiate benign mimics from dysplastic lesions.
- Over expression of p16 is associated with higher stage of tumour, nodal and distant metastasis.
- Pap smear test alone has a low sensitivity for low grade lesions. However, it is still considered an effective screening tool due to its high sensitivity for high grade lesions and low cost.
- A diagnosis of cervical dysplasia should be made in conjunction with clinical and immunohistochemical examination.
- Further studies are required to unfold the molecular mechanisms of cervical carcinogenesis.

**REFERENCES**

Outcomes of Cochrane Systematic Review Protocol Development Workshops in India

Mathew R J*, Bhaumik S**, Barnabas J P*, Parathasarathy V*
* South Asian Cochrane Network and Centre, Vellore, India
** Bio Medical Genomics Centre, Kolkata, India

Introduction:
One of the primary activities of the 14 independent centres of the Cochrane Collaboration is to provide training about methods to conduct systematic reviews. It is imperative to analyse the outcomes of the training activities provided.

Objectives:
To analyse the outcomes of the Systematic Review Protocol Development workshops (PDW) held at the South Asian Cochrane Network and Centre (SASIANCC).

Methods:
Data regarding participants for the PDW conducted at the SASIANCC between June 2007 and February 2013 was collected and any additional data was retrieved from Archie.

Results:
205 participants have taken part in the 8 PDWs run by SASIANCC since 2007. About 37% of workshop participants were females and the regional distribution of the participants are shown in Figure 1. The job profile of the participants has been described in Table 1.

Only 70 (34.1%) participants had Archie accounts, 67 of whom are registered as authors (including 1 inactive) or possible contributors and remaining were either referees or consumers. Of the 66 active authors who registered titles, 34 (51.5%) had registered prior to attending the PDW and 32 (48.5%) after. The average time taken to register a title after attending a PDW was approximately 10 months (range 1 month-4 years). Most PDW participant authors were from the Neonatal group (5), Oral health group (5) and the Infectious disease group (5).

Conclusion:
PDW participants need to be followed up and facilitated so that they can overcome the barriers that prevent them from registering as Cochrane authors. These barriers need to be identified by further studies and strategies to address these barriers need to be implemented. There is also a need for diversification to ensure a more uniform regional spread.

Figure 1. State-wise distribution of PDW

Table 1. Profile of PDW participants- according to Job title

<table>
<thead>
<tr>
<th>Sl. No</th>
<th>Job Title</th>
<th>Number</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Deputy Directors &amp; Deputy Generals</td>
<td>14</td>
<td>6.8</td>
</tr>
<tr>
<td>2</td>
<td>Students (under graduate &amp; post graduate)</td>
<td>25</td>
<td>12.5</td>
</tr>
<tr>
<td>3</td>
<td>Junior Residents</td>
<td>30</td>
<td>14.6</td>
</tr>
<tr>
<td>4</td>
<td>Additional &amp; Assistant Professors</td>
<td>48</td>
<td>23.4</td>
</tr>
<tr>
<td>5</td>
<td>Associate Professors</td>
<td>11</td>
<td>5.4</td>
</tr>
<tr>
<td>6</td>
<td>Professors</td>
<td>22</td>
<td>10.7</td>
</tr>
<tr>
<td>7</td>
<td>Project Managers</td>
<td>5</td>
<td>2.4</td>
</tr>
<tr>
<td>8</td>
<td>Research Scientists/Officers</td>
<td>21</td>
<td>10.2</td>
</tr>
<tr>
<td>9</td>
<td>Others (including Physiotherapists, Public health professionals, Librarians)</td>
<td>26</td>
<td>12.7</td>
</tr>
</tbody>
</table>

* For 3 participants the job titles was unknown
CARDIOVASCULAR RISK FACTORS, LIFE STYLE AND SOCIODEMOGRAPHIC DETERMINANTS: A CROSS SECTIONAL COMPARATIVE STUDY AMONG ADULT URBAN POPULATION IN SOUTH INDIA

Annie K.1, Amudha Poobalan 2, Sushil John 1, Geraldine McNeil 4, Rita Isaac5
1, 3 RUHSA Department, Christian Medical College, 2 LCUC, Christian Medical College, 4 University of Aberdeen

Background:
Rapid urbanization and industrialization with associated changes in life-style particularly in relation to diet and physical activity have led to a rise in cardiovascular diseases in India. Levels of obesity are increasing in low and middle income countries as a result of new dietary habits and sedentary ways of life (Cecchinoli 2010, Lancet). Countries such as India, undergoing rapid economic and nutrition transitions with improved living standards, are facing public health challenges of a shift from under to over nutrition problems (Wang Y, 2009). In the past two decades, several studies have reported an increase in overweight adults in the urban Indian population (Gopinath N 1994, Misra 2001) with a prevalence of 9.3% overweight/obesity in men and 12.6% in women. According to WHO estimates, by 2020, chronic non communicable diseases, mostly associated with diet will take over two third of the global burden of disease in terms of mortality and morbidity.

Aim:
To measure the prevalence of risk factors for cardiovascular diseases in the urban population of Vellore, Southern India.

Methodology
Study Design
A community based cross sectional survey of urban poor comparing with urban affluent population in South India.

Sample size: Sample size was calculated based on the minimum feasibility. 95% confidence intervals of the likely proportions of higher BMI (ranging from 10% to as high as 40%) were estimated. Proposed to study 100 subjects each in different strata.

Sample Selection: A random sampling of consenting eligible participants from the urban slum of Vellore town and eligible consenting participants from Rotary, Innerwheel and Ladies Recreational clubs of Vellore town.

Study instrument: An interviewer/self administered structured questionnaire consisting of socio demographic characteristics, socio economic factors, physical activity, dietary intake and dietary diversity was used. Anthropometric measurements like weight, height and BMI was measured along with cardiovascular risk factors such as diabetes mellitus, hypertension and dyslipidemia were assessed.

Study Period: January to May 2014

Results:
• The mean age of the participants was 39.29 years (SD 11.2years)
• 82.6% were currently married, 17.4% were either single, widowed or separated
• 34.4% of the participants had graduated or had a professional degree or a Diploma, while 15.7% were illiterate.

One third (33.7%) were daily wage earners and one fourth (25.3%) were house wives.

Prevalance of underweight, overweight and obesity:
• According to the WHO classification of BMI, two third (67.4%) of the study population had one or other form of malnutrition.

• Urban affluent population had significant higher prevalence of obesity 25.6% Vs 11.7% (p = 0.030)

Conclusion:
The study found that about quarter of the affluent population is obese, nearly 50% of the people in slums are diabetic while about half the population in both areas have high LDL levels, although more than 90% of both groups practiced vigorous physical activity.

Acknowledgements:
Participants, Rotary Clubs, Innerwheel and Ladies Recreational Clubs of Vellore; Prof. Jacob John; and UGC UKIERI (UK-India Education and Research Initiative) for funding the study.

References
Comparison of G1P[8] gene sequences of Rotarix vaccine and wild-type strains in India

Robinson Peter, Gagandeep Kang
The Wellcome Trust Research Laboratory, Division of Gastrointestinal Sciences, Christian Medical College, Vellore

Introduction

- In India rotavirus accounts for 39% of all diarrheal diseases in children ≤5 years of age which requires hospitalization.
- Globally, G1P[8], G2P[4], G3P[8], G4P[8] and G9P[8] genotype and recently G12, are most frequent isolates from humans.
- Two rotavirus vaccines, Rotarix, a human live attenuated monovalent G1P[8] and RotaTeq, a human-bovine reassortant vaccine containing human genotype (G1-G4 and P[8]) with bovine WC3 (G6P[5]) backbone are currently licensed in many countries including India.
- After introduction of these vaccines there is a notable decrease in the rotavirus infection in developed countries but in developing countries like India, vaccines are used mainly in the private sector.
- The current study was designed to report on G1P[8] wild type strains prior to widespread vaccine introduction and compare them with strains from children given Rotarix vaccine.

Materials and methods

- Genotyping was performed on ELISA (Premier™ Rotaclone ELISA, Meridian Biosciences; Cincinnati, Ohio) positive samples by hemi-nested multiplex PCR for VP7 and VP4 genes.
- A total of (n=44) stool samples positive for G1P[8] rotavirus strains (children given Rotarix vaccine n=18, children enrolled in a national surveillance study (NRS) n=26).
- Samples were further characterized by sequencing VP7 and VP4 genes using Sanger’s automated cycle sequencing method.
- Analysis: Sequences obtained were aligned with the respective reference strain sequences using BioEdit Sequence Alignment Editor and phylogenetic analysis was conducted using MEGA version 6 software. Nucleotide sequence comparison was carried out using BioEdit software.

Results

- Of the 44 samples, sequences were obtained for both G- and P-types. NRS study (26/26) and in children who received vaccine (12/18). In children who received vaccine only six samples had first round PCR product for VP7 gene.
- Phylogenetic analysis showed that 26/26 sequences from NRS study were wild type, and in children who received vaccine 9/18 were similar to the Rotarix vaccine and 9 samples were wild type.
- Further analysis of sample sequences with reference sequences (wild type and Rotarix vaccine type) from genbank showed significant changes in the nucleotide position.

The following changes were observed in the nucleotide positions of G- and P-type sequences

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Nucleotide position</th>
<th>Wild type strain/samples</th>
<th>Rotarix vaccine strain/ samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>G 1</td>
<td>473</td>
<td>T</td>
<td>C</td>
</tr>
<tr>
<td></td>
<td>693</td>
<td>C</td>
<td>T</td>
</tr>
<tr>
<td></td>
<td>604</td>
<td>A</td>
<td>G</td>
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<tr>
<td></td>
<td>704</td>
<td>T</td>
<td>C</td>
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<td></td>
<td>723</td>
<td>A</td>
<td>G</td>
</tr>
<tr>
<td></td>
<td>726</td>
<td>G</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>765</td>
<td>A</td>
<td>G</td>
</tr>
</tbody>
</table>

- Fig. 1 Phylogenetic dendrogram of the VP7 gene G1 of wild type and rotarix vaccine strain.
- Phylogenetic analysis was performed using MEGA 6.0 and genetic distance is calculated using Tamura 3- parameter model. phylogenetic tree was constructed using neighbor joining methods with 1000 bootstrap replicates.
- S = rotavirus isolates from surveillance study, Vaccinate = children who received vaccine.

- Fig. 2 Phylogenetic dendrogram of the VP4 gene P8 of wild type and rotarix vaccine strain.
- Phylogenetic analysis was performed using MEGA 6.0 and genetic distance is calculated using Tamura 3- parameter model. phylogenetic tree was constructed using neighbor joining methods with 1000 bootstrap replicates.
  - S = rotavirus isolates from surveillance study, Vaccinate = children who received vaccine.

Conclusion

- In the present study, both G1P[8] wild type and Rotarix vaccine strains were identified in children. It was surprising to find that several Rotarix recipients were carrying wild-type G1P[8] strains. This finding needs to be correlated with time of vaccination and follow up.
- Therefore the sequences of G1P[8] strains identified in children need further analysis, particularly after vaccine introduction,
  - To determine whether or not vaccine virus is being shed.
  - Whether wild type strains are continuing to infect children and to track evolution of these viruses.
Rotavirus infections in a community based cohort in Vellore, India

Robin P. Lazarus*, Anu Paul, Beryl P. Gladstone, Indrani Mukhopadhyya, Gagandeep Kang

The Wellcome Trust Research Laboratory, Division of Gastrointestinal Sciences, Christian Medical College, Vellore, India

1. Introduction

- The burden of infection in communities determines the spread of rotavirus infection and disease in susceptible populations.
- Group A rotaviruses causes severe, dehydrating diarrhea mainly in young children <2 years of age.
- Transmission
  - Feco-oral
  - Airborne
  - Person to person contact
- Rotavirus gastroenteritis is usually accompanied by vomiting and fever.
- Rotavirus is excreted in large numbers during diarrhea.

2. Methods

- Study population
  - 3 contiguous slums in Vellore, India
  - 452 children enrolled at birth followed till 3 years of age.
  - Bimonthly surveillance and diarrheal stool samples collected.
  - 373 children completed 3 years follow up.
- Screening and Molecular characterization
  - ELISA (Dako Rota IDEIA, Ely, UK)
  - RT-PCR to genotype VP7 and VP4 genes.

- Statistical Analysis
  - Incidence rates – Poisson regression equations.
  - Diarrheal severity – Vesikari scores
  - Risk factors associated with symptomatic and asymptomatic were compared using multiple logistic regression.

3. Results

- Burden of disease
  - 94.4% children in the cohort were infected and had a total of 1149 episodes of rotavirus infections.
  - Incidence of rotavirus infection was 1.04 (0.97-1.1) per child per year
    - Asymptomatic – 0.75
    - Symptomatic – 0.29

4. Conclusion

- Rotavirus is the most common cause of gastroenteritis in the community and causes the majority of severe disease.
- Interventions like health education, sanitation, appropriate treatment and vaccines are needed to decrease rotavirus disease in communities.
Epidemiology of Rotavirus Infection in Children < 5 Years Hospitalized with Acute Gastroenteritis in India

Rajesh Arumugam¹, Sudhir Babji¹, Anna Simon² and Gagandeep Kang¹.
¹Department of Gastrointestinal Sciences, Christian Medical College, Vellore, Tamil Nadu.
²Department of Child Health, Christian Medical College, Vellore, Tamil Nadu.

Background

- Recognized as the leading cause of infantile viral gastroenteritis worldwide in the absence of vaccine use.
- Responsible for 78,500 diarrheal death each year in India.
- Non-enveloped, double-stranded RNA viruses; Family - Reoviridae.
- Code for 6 structural (VP1, 2, 3, 4, 5, 6) & 6 non-structural proteins (NSP1–NSP6).
- Classified as 7 distinct groups A-G with groups A, B, C rotavirus found in both humans and animals.
- Group A rotaviruses further classified into G and P types.
- Currently, 27 G and 35 P types have been identified.
- Considerable research carried out on rotavirus disease in India in different settings.
- Collation of data frequently not possible due to the differences in study design, populations examined and testing methodologies.
- Multi-centric surveillance system in India was established in 2005, jointly under the supervision of the Indian Council for Medical Research (ICMR) and the Centers for Disease Control and Prevention (CDC), Atlanta, USA.
- There were 5 centres in India. Standardized protocols for enrollment and diagnostic evaluation of children hospitalized with diarrhea for rotavirus data were used. Strains of rotavirus circulating in the study population were characterized by standardized molecular methods.
- Rotavirus detected in 40% of all diarrheal admissions (Dec 2005–June 2009).
- To build on the success of this network, it was proposed to extend the surveillance activities at sites located across the country in different geographical zones from.

Aim & Objectives of the Study

- Establish a multicentric surveillance system to estimate the proportion of diarrhoea hospitalizations attributable to rotavirus among children <5 years of age.
- To characterize (G and P) types prevalent strains of rotavirus in the population under surveillance.
- To monitor trends in incidence of hospitalizations.
- To perform extensive evaluation of strains not identified/typed by standard techniques including sequencing the genome of these strains.

Criteria

Enrollment criteria:
- All children under 5 years of age presenting to the hospital with acute gastroenteritis and requiring hospitalization for rehydration for at least 6 hours were enrolled in the study.

Exclusion criteria:
- Any child >60 months with diarrhea or not requiring supervised oral or intravenous rehydration was excluded.

Study Sites

- This is a multi-centre surveillance project conducted across the country in 4 Zones involving 4 Referral and 7 Regional Centres.
  - North zone: AIIMS, New Delhi.
  - East zone: NICE, Kolkata.
  - West zone: NIV, Pune.
  - South zone: CMC, Vellore.
- The study is coordinated by the ICMR and the NIE.
- CMC – Responsible for the QC/QA during the surveillance.
- Responsible for the characterization of untypeable samples received from referral and regional centres.

Study Design

- Admission of Child (<5 yrs) with diarrhea
- Collection of clinical data and Blood sample
- Transport of sample to laboratory
- Sample receipt in the laboratory
- Testing of rotavirus by EIA (for all samples)
- PCR (for EIA positive samples)
- Exclusion of other viruses by MPx
- Check for true positive using MPx PCR
- Sequence of 3^rd round products or specific primers with alternate primers

Results

Diarrhoeal admissions and rotavirus positivity (Sep 2012 to Jun-2014)

<table>
<thead>
<tr>
<th>Clinical Site</th>
<th>No. of Children Admitted with Diarrhoea</th>
<th>No. of Children Enrolled in Study</th>
<th>No. of stool samples collected</th>
<th>No. of stools tested for Rotavirus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vellore (S1)</td>
<td>866</td>
<td>740</td>
<td>864</td>
<td>203</td>
</tr>
<tr>
<td>Kollam (S2)</td>
<td>650</td>
<td>600</td>
<td>602</td>
<td>179</td>
</tr>
<tr>
<td>Thrissur (S3)</td>
<td>207</td>
<td>260</td>
<td>198</td>
<td>105</td>
</tr>
<tr>
<td>Lucknow (S4)</td>
<td>308</td>
<td>386</td>
<td>388</td>
<td>130</td>
</tr>
<tr>
<td>Hyderabad (S5)</td>
<td>650</td>
<td>327</td>
<td>317</td>
<td>86</td>
</tr>
<tr>
<td>New Delhi (S6)</td>
<td>667</td>
<td>666</td>
<td>612</td>
<td>268</td>
</tr>
<tr>
<td>Tiruchirappalli (S7)</td>
<td>348</td>
<td>342</td>
<td>342</td>
<td>91</td>
</tr>
<tr>
<td>Total</td>
<td>3788</td>
<td>3272</td>
<td>3123</td>
<td>1156</td>
</tr>
</tbody>
</table>

Distribution of Genotypes (3rd RV Positives)

<table>
<thead>
<tr>
<th>City or Town</th>
<th>Genotype</th>
<th>No. of Stools Positive</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vellore</td>
<td>G1P[8]</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>Thrissur</td>
<td>G1P[8]</td>
<td>1</td>
<td>5%</td>
</tr>
<tr>
<td>Lucknow</td>
<td>G1P[8]</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>Hyderabad</td>
<td>G1P[8]</td>
<td>1</td>
<td>25%</td>
</tr>
<tr>
<td>New Delhi</td>
<td>G1P[8]</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>Tiruchirappalli</td>
<td>G1P[8]</td>
<td>1</td>
<td>3%</td>
</tr>
<tr>
<td>Total</td>
<td>G1P[8]</td>
<td>2</td>
<td>6.1%</td>
</tr>
</tbody>
</table>

Discussion

- Rotavirus detected in 37% of all diarrheal admissions from September 2012 to June 2014.
- G1P[8] was the most common genotype followed by G2P[4].
- G12P[6] genotype was the next most common genotype seen in Delhi.
- Significant differences in prevalence rates of various strains in different geographic regions of the same country.
- This study highlights the high incidence of rotavirus gastroenteritis in India and emphasizes the importance of strain surveillance studies to keep up with the changing epidemiological profile of rotaviruses.
Household food insecurity in urban Vellore - determinants and trends
Nikitha Dharmaraju, Nirupama Arulappan, Sonam Shah, Beeson Thomas, Sam Marconi D, Venkata Raghava Mohan
Department of Community Health, Christian Medical College, Vellore, India

**BACKGROUND**
- The National Family Health Survey India 2005 says that about 1/3rd of the world's hungry live in India, where around 20 crore Indians who go to bed hungry every night.
- Around 212 million of our population are undernourished.
- It is estimated that over 7000 Indians die of hunger every day and 25, 00,000 every year in the post independence era.
- With nearly a fourth of its 1.1 billion population hungry, India is the World's hunger capital.
- Food insecurity is defined as - "Access by all people at all times to enough food for an active, healthy life:"
- Food security includes at a minimum - the ready availability of nutritionally adequate and safe foods, and - an assured ability to acquire acceptable foods in socially acceptable ways.
- Food insecurity is defined as "Limited or uncertain availability of nutritionally adequate and safe foods or limited or uncertain ability to acquire acceptable foods in socially acceptable ways:"

**AIM**
- To assess the level of food security in the urban slums of Vellore, its association with various demographic variables and to assess the change in trend of food security since 2009

**METHODOLOGY**
- Cross sectional study was conducted in five urban slums of Vellore using a two stage cluster sampling technique.
- The sample size was calculated using 4pa/d2. P=75% from the earlier study in Vellore, which refers to any form of food insecurity. And taking design effect to be 2, the sample size was calculated to be 150.
- 150 households were selected using systematic random sampling from the five clusters.
- After obtaining verbal consent, a semi structured questionnaire was administered to the respondent of the selected families.
- Household level food security was assessed using the US Department of Agriculture— "Household Food Security Scale".
- Different levels of food security were determined in terms of 'Food secure', 'Food insecure without hunger' and 'Food insecure with hunger' over last one month period based on the responses.
- Statistical associations between food security and demographic variables were tested and change in level of food security over the period of 5 years was studied.

**RESULTS**
- Ninety percent (135/150) of the respondents were housewives.
- Majority (64%) of the families were nuclear families.
- Mean family size was 4.6 & median was 5.
- Majority (73%) of the families belonged to the 'upper lower' socio-economic status.
- 81% of the households had access to public distribution system.
- Nearly 45% of the households were financially in debt.
- More than half (57.3%) of the households were food insecure.
- Out of which 26.7% were insecure without hunger; 27.3% were insecure with moderate hunger and 3.3% were insecure with severe hunger.
- The proportions of households with 'food insecurity and hunger' were more among the lower socio-economic classes. 67.7% of those with low SES are food insecure. 75% of families with debts were food insecure.
- Lower socio-economic status and indebtedness were significant determinants of household level food insecurity in urban slums of Vellore.
- The proportion of households which were food insecure has increased to 47.7% in 2014 as compared to 25.4% in 2009.
- The proportion of houses which were 'Food insecure with hunger' has reduced by half over the last five years (30.6% vs. 61.5%).

**DISCUSSION**
- According to the Tamil Nadu Civil Supplies Corporation 1.78,82,593 people are being benefited through the system and it is being implemented through 33,222 fair price shops functioning under various agencies in the state.

**CONCLUSION**
- Even though more than half of the families still face varying degree of food insecurity, severe forms of food insecurity have declined in urban slums of Vellore over the last 5 years.
- The probable reason for this decline in food insecurity being the universal establishment of PDS and its present system which became effective in 2011.

**REFERENCES**
- Prevalence of food insecurity among households with children in Coimbatore, India Nurses Institute, C. Impagam ON.
- College of Applied Science and Technology, Department of Family and Consumer Sciences, Illinois State University, Normal, IL 61790-4508, USA.
- Dipali Kondhikoppadhy et al Enduring starvation in silent population: a study on prevalence and factors contributing to household food insecurity in two rural populations in Bankura, West Bengal.
Knowledge, attitude and practice of pesticide usage and its adverse health effects among farmers in a rural village of South India.


Guide by: Dr. Reginald Alex, Professor, Emergency Medicine, CMC, Vellore – 632002.

Background

Farmers use pesticide to protect crop production and food materials from insects, fungi, rodents and other organisms by destroying them. However, studies have reported that unsafe usage and handling of pesticides has resulted in high exposure and adverse health effects to the farmers.

Aim

The aim of this study was to find the knowledge, attitude and practice of pesticides usage and its adverse health effects among farmers living in Kilirasampet village, South India.

Methods

The study involved 59 farmers, both men (83%) and women (17%) from Kilirasampet village of Kanyakumari Block, Vellore district. Data was collected using interviewer-administered, piloted semi-structured questionnaire designed to elicit socio-demographic information, knowledge, attitude and practices towards method of pesticide usage, storage, relevant knowledge on health effects and its prevention by a door to door survey method after standardization among interviewers.

Results

The mean age ± SD of the farmers was 50.08 ± 14.5 years.

Several pesticides were used by the farmers including banned pesticides like Endosulfan3 (6.8%) and Monocrotophos3 (1.7%). This can be attributed to the finding that local shopkeepers were the source of knowledge providers for twenty-two (37.3%) farmers regarding pesticides.

A significant number of farmers (54.2%) purchased pesticides and used it without storing indeed others stored it in their houses (13.6%) and farm shed (32.2%) for later use. The empty pesticide containers were reused by thirty-five (59.3%) farmers for household purposes such as dippers in bathroom.

Forty-eight (81%) farmers were directly involved in spraying pesticides on crops. After spraying the pesticides, most of the farmers had the habit of washing hands (84.7%) followed by shower (74.6%) and changing clothes (44.1%).

Poor awareness regarding pesticide poisoning was reported among one-fourth (23.3%) of the farmers. However, other farmers had knowledge about the routes of exposure such as inhalation (40.7%), oral (25.4%), dermal (7.7%) and ocular (2.5%). Symptoms reported by the farmers which occur immediately after pesticide spraying are fever, boils, body ache, headache, vomiting, breathing difficulties, skin irritation, skin allergy and eye irritation.

Thirty-one (52.5%) farmers didn’t use any personnel protective equipment (PPE) while spraying pesticides. The unavailability of PPE, its cost and discomfort during work were reported as the reasons for not using them. Few felt (6.8%) that there is no use of wearing it. Among those who used PPE (47.5%) poor practices were followed such as cotton cloth towels as mask, plastic covers as gloves. Only few used rubber gloves to protect their hands.

Conclusion

The results of the study have demonstrated that there is extremely low level of knowledge and awareness about pesticides usage (23.8%), PPE usage (59.3%), storage and disposal of pesticides container (59.3%). Based on the results of our survey, we recommend awareness education programs and behavior change strategies to improve the level of knowledge on use of pesticides, eliminate existing attitude and practice towards pesticides usage and its health effects. Alternate pest control strategies such as use of bio pesticides and integrated pest management (IPM) could also be recommended.

Reference:

Introduction
Drug Information Centre (DIC) is a service unit committed to providing unbiased drug information as it relates to therapies, pharmacoeconomics, education, and research programmes to healthcare professionals. In CMC, DIC was established in 1984 and is well equipped with qualified drug information pharmacists, primary, secondary and tertiary literatures, internet and intranet facilities, computers and telephone.

Objective
This study was conducted to assess the pattern of drug information queries received and answered by DIC over 15 months. The objectives were to acquire, analyze and interpret data on parameters such as professional status of requestor, mode of receipt and reply of query, type of query, purpose of query and references used.

Results and Discussion
- A total of 2010 queries were received and answered during the study period.
- Physicians formed the major group with 814 (41%) queries followed by nurses (n=595; 30%) and pharmacists (n=462; 23%).
- Most of the queries (92%) were received and answered via telephone while 6% of queries were received during ward rounds and 1% directly.

- Patient care related queries were the most common (87%).
- Internet/search engines served as the main source of reference (n=952; 47%) followed by textbooks, intranet, journals and others.

Conclusion
- The DIC in a teaching hospital handles a wide range of queries from various healthcare workers, meets specific healthcare needs and serves as a valuable source of drug information.
- Patient care is the purpose behind majority of the queries to DIC where unbiased and reliable drug information may be sought 2.
- The impact of this service on improving patient care and minimizing errors warrants further study.

Acknowledgements
The authors sincerely acknowledge all drug information pharmacists for proper documentation of queries.

References
RELATIONSHIP BETWEEN SLEEP QUALITY AND GLYCAEMIC CONTROL AMONG SUBJECTS WITH TYPE 2 DIABETES MELLITUS
Abijah Princy B*, Valliammal Babu**, Sheela Durai ***, Asha #, Antonymsamy ###
*Junior lecturer, College of Nursing, CMC, Vellore, **Professor, College of Nursing, CMC, Vellore, ***Associate professor, College of Nursing, CMC, Vellore, #Associate professor, Physician, Department of Diabetes & Endocrinology, CMC, Vellore, ###Head of the Department (Biostatistics), CMC, Vellore

BACK GROUND
- Sleep disturbance has evolved as an unrecognized health issue among the diabetic.
- Meticulous information on sleep quality in Type 2 Diabetes based on glycemic control is limited and rarely explored.
- Poor sleep quality (disturbed/decreased sleep) is associated with glucose intolerance, insulin resistance and reduced acute insulin response to glucose (Spiegel, 2009).
- Sleep quality is an important predictor of HbA1c (Knutson 2006).
- Poor sleep quality and less efficient sleep significantly correlated with worse glycemic control in patients with Type 2 Diabetes mellitus (Bar-YF, 2012).

AIM
A descriptive study to assess the relationship between sleep quality and glycemic control among subjects with Type 2 Diabetes Mellitus in Diabetes and Endocrinology Outpatient Department of Christian Medical College, Vellore.

OBJECTIVES
- To assess the sleep quality among subjects with Type 2 Diabetes Mellitus.
- To assess the glycemic control among subjects with Type 2 Diabetes Mellitus.
- To determine the relationship between sleep quality and glycemic control among subjects with Type 2 Diabetes Mellitus.
- To determine the association of sleep quality and glycemic control with selected demographic and clinical variables.

METHODOLOGY
A descriptive study was conducted in the Outpatient Department of Diabetes and Endocrinology of Christian Medical College, Vellore.

Sample size & sampling method
A total of 500 subjects with Type 2 Diabetes Mellitus were selected using total enumeration sampling method.

Criteria for sample selection
Subjects with Type 2 Diabetes Mellitus:
- For more than one year, on oral hypoglycaemic agents or insulin therapy, subjects understanding English, Hindi, Tamil or Bengali were included.
- Acute illness with impaired cognition, admitted in ward or with illnesses like Chronic Renal failure, Liver Failure, COPD, Asthma, Primary Obstructive Sleep Apnea or with Type 1 Diabetes, Pregnancy and Gestational Diabetes were excluded.

DATA COLLECTION
- Written informed consent was obtained.
- The Pittsburgh Sleep Quality Index scale self-rated questionnaire was provided.
- Demographic variables and the clinical variables (clinical diagnosis, age of onset of illness, duration of illness, mode of treatment, other illnesses, history of medication intake influencing sleep quality, day time sleepiness, tea/coffee intake, smoking & alcohol intake, height, weight, BMI, neck circumference & waist circumference) were obtained.
- HbA1c taken within the last 6 months of the visit was assessed.

RESULTS
Figure 1: Distribution of subjects with Type 2 Diabetes based on overall sleep quality (N=500)
- 63.6% Good quality sleep
- 36.4% Poor quality sleep

Table 1: Correlation of various components of sleep quality with glycemic control among subjects with Type 2 Diabetes (N=500)
<table>
<thead>
<tr>
<th>Variable</th>
<th>r Value</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjective sleep quality vs HbA1c</td>
<td>-0.085</td>
<td>0.058</td>
</tr>
<tr>
<td>Sleep latency vs HbA1c</td>
<td>0.078</td>
<td>0.082</td>
</tr>
<tr>
<td>Sleep duration vs HbA1c</td>
<td>0.092</td>
<td>0.040</td>
</tr>
<tr>
<td>Nocturnal sleep efficiency vs HbA1c</td>
<td>-0.039</td>
<td>0.387</td>
</tr>
<tr>
<td>Sleep disturbances vs HbA1c</td>
<td>0.081</td>
<td>0.094</td>
</tr>
<tr>
<td>Use of sleep medications vs HbA1c</td>
<td>-0.038</td>
<td>0.600</td>
</tr>
<tr>
<td>Daytime dysfunction vs HbA1c</td>
<td>0.092</td>
<td>0.039</td>
</tr>
</tbody>
</table>

Table 2: Median glycemic index according to the level of sleep quality among subjects with Type 2 Diabetes

<table>
<thead>
<tr>
<th>Quality of sleep</th>
<th>HbA1c (%)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Good sleep quality</td>
<td>7.80 (7.7-9.2)</td>
<td>0.01*</td>
</tr>
<tr>
<td>Poor sleep quality</td>
<td>8.25 (7.0-9.8)</td>
<td></td>
</tr>
</tbody>
</table>

- Low positive correlation of 0.09 (p=0.036) existed between sleep quality and glycemic control.
- Statistically significant relationship existed between glycemic control and sleep disturbance (p=0.018).
- Statistically significant association existed between: Sleep quality and the location of the house (p=0.001) glycemic control with gender and occupation (p=0.051) sleep quality with medication intake (p=0.005) and day time sleep (p=0.028).
- Glycemic control with duration of illness (p=0.003) mode of treatment (p=0.000) and tea/coffee intake. (p=0.040)

CONCLUSION
- Majority of Type 2 Diabetics have poor sleep quality and poor glycemic control.
- Progressive rising trend of HbA1c existed as the sleep quality worsened.
- The findings of the study highlight the need for optimal sleep quality in maintaining the glycemic levels in Type 2 Diabetes besides medications and other lifestyle modifications.
- Incorporation of sleep hygiene as a part of routine management of Type 2 Diabetes is essential with periodic sleep quality assessment and health education on sleep hygiene will enhance sleep quality and promote optimal glycemic control in subjects with Type 2 Diabetes.

REFERENCE
Effect of Complex Decongestive Physical Therapy in Subjects with Chronic Venous Insufficiency

S. Sandeep Kumar, Andrew Babu, E. Sandeep Kumar, Joanna M Popsi, Merlyn Tilak, Dr. Edwin Stephen, Department of Physical Medicine and Rehabilitation, Department of Vascular Surgery, Christian Medical College, Vellore.

Background

- Chronic Venous Insufficiency (CVI) is the 7th most common condition for medical referral.
- Complex decongestive physical therapy (CDP) is being widely used in the conservative management of this condition.
- Several studies have shown promising results, but patient compliance was not satisfactory in clinical practice due to the long duration of therapy.

Aim of the study

The purpose of this study was to find out the effect of short duration treatment (3 Days) of CDP in subjects with CVI.

Methodology

Design Overview:
Quasi experimental pre-test and post-test design

Setting and participants:
Physiotherapy Outpatient department. 45 subjects recruited after written consent.

Outcome Measures:
1. Limb girth measurement- tape
2. Pain- Visual Analogue Scale
4. Quality of life (QOL)-LymphedemaQOL

Procedure:
Subjects with CVI who fulfilled the inclusion criteria were assessed by a Physiotherapist (PT1) who was blinded to the treatment given. The baseline assessment included circumference of limb, pain, gait and quality of life (QOL).

CDP was given to the subjects for 3 days by another Physiotherapist (PT2) which included
- Manual lymphatic drainage (MLD)
- Skin care
- Compression bandaging
- Exercise
- Nutritional care

Compression garments.

Following 3 days of treatment, a final assessment was done by PT1.

Conclusion

Short period of CDP does improve outcomes and is a promising physiotherapy management which warrants further studies with control group, in Subjects with CVI.

Limitation

Lack of placebo or non-treatment group

Reference

2. The long-term effects of CDP on lower extremity secondary lymphedema and Quality of life. Sung Joong KIM, PT, PHD Department of Physiotherapy, Kangwon National University.

Acknowledgement

1. Institutional Review Board for approval.
2. Fluid Research Grant from Institution.

**The authors declared no conflicts of interest.**
SYMPTOM SEVERITY AND QUALITY OF LIFE AMONG PATIENTS WITH LOWER URINARY TRACT SYMPTOMS (LUTS) SUGGESTIVE OF BENIGN PROSTATIC HYPERPLASIA (BPH)

* MRS. ANGELINE JEEYA RANI. MSc (N), ** MRS. BEULAH PREMKUMAR. MSc, M.PHIL.,
** DR. CHANDRASINGH, M.S, M.CH., ** MRS. EMILY DANIEL, MSc (N), ** DR. SELVARAJ ., PH.D

Department of Urology, Christian Medical College, Vellore.

BACKGROUND:
As men age, they have a significant risk of having lower urinary tract symptoms associated with an enlarged prostate. The manifestation such as frequency, intermittency, nocturia, urgency, urge incontinence and dribbling as Lower Urinary Tract Symptoms (LUTS) can seriously affect the patients daily activities and self concept. The major concern is early identification and treating LUTS. A nurse can relatively do a proper assessment, diagnostic work up, stratify the patient who present with LUTS and recommend treatment consisting of watchful waiting and educate about lifestyle modification.

AIM:
This study was aimed to assess the severity of symptoms and quality of life among patients with Lower Urinary Tract Symptoms suggestive of Benign Prostatic Hyperplasia in Urology OPD and admitted in wards of Christian Medical College, Vellore.

METHODOLOGY:
A descriptive study design was used to assess the severity of symptoms and quality of life among subjects with LUTS for more than 6 months. A total of 150 subjects were selected using enumerative sampling technique. Data was collected through International Prostate Symptom Score(IPSS) questionnaire to assess severity of symptoms and King's Health Questionnaire(KHQ) to assess their quality of life. Descriptive statistics and Pearson correlation coefficient was used for statistical analysis.

RESULTS:

![Figure 1: International Prostate Symptom Score](image)

The overall perception of symptom severity using IPSS score revealed that majority 52% had moderate symptoms, about one third 36% had severe symptoms.

![Figure 2: Correlation between Symptom severity and Quality of Life among LUTS](image)

There is a strong correlation between symptom severity and Quality of Life. Severe the symptoms, worse the quality of life.

![Figure 3: Association between symptom severity and Quality of Life](image)

Figure depicts that the quality of life was affected in only 15.6% of the subjects with mild or moderate symptoms, the percentage of those whose quality was affected was significantly more (40.8%) in the subjects with severe symptoms. 84% of those with mild or moderate symptoms and 59% of those with severe symptoms had good quality of life.

CONCLUSION:
Men with lower urinary tract symptoms can be helped in by nursing care by identifying symptoms early by proper assessment, assisting in diagnostic procedures and educating them on preventive aspects such as lifestyle modification. Hence they can have a better quality of life in the society.

Acknowledgement
KHQ © Linda Cardozo and Con Kelleher, College of Nursing Research Committee.
PREVALENCE OF ILIOPSOAS TRIGGER POINTS IN DESKBOUND WORKERS
- A CROSS SECTIONAL STUDY

*Department of Physical Medicine and Rehabilitation, **Staff Student Health Services, ***Department of Statistics, Christian Medical College, Vellore, India.

Background

- Prolonged sitting, especially when combined with a slouched posture shortens the iliopectineus muscles.
- Low back pain (LBP) can develop as the tight iliopectineus generates tension in its tendinous attachment to the lumbar spine. 1
- A few studies exist which show that LBP has an iliopectineus trigger point (ITP) component. 2
- There is lack of data on its prevalence in deskbound workers.

Objective

To find out the prevalence of ITPs in Deskbound workers.

Subjects and Methods

Study Participants

- 15 deskbound workers between the age group of 20 - 60 years.

Study Setting

- This study was conducted in the Physiotherapy outpatient unit of Christian Medical College, Vellore.

Outcome measure

- The visual Analog Scale (VAS) was used to measure the back pain and palpation with a tenderness grading was used to detect the presence of trigger points.

Assessment

- Demographic data and a back pain assessment was carried out by a physiotherapist and maintained in a separate folder.
- The principal investigator (PI) blinded to the initial assessment assessed for ITPs using tenderness grading scale by palpation.

Table and Figures

Prevalence of iliopectineus trigger points

<table>
<thead>
<tr>
<th></th>
<th>POSITIVE</th>
<th>NEGATIVE</th>
</tr>
</thead>
<tbody>
<tr>
<td>FREQUENCY</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>PERCENTAGES</td>
<td>67</td>
<td>33</td>
</tr>
</tbody>
</table>

Low back pain and iliopectineus trigger points

<table>
<thead>
<tr>
<th></th>
<th>ITP POSITIVE</th>
<th>ITP NEGATIVE</th>
<th>P VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>YES</td>
<td>% 88.9</td>
<td>11.1</td>
<td>0.025</td>
</tr>
<tr>
<td>NO</td>
<td>% 33.3</td>
<td>66.7</td>
<td></td>
</tr>
</tbody>
</table>

Results

Among the deskbound workers 67% had ITPs and 60% had a history of LBP. Among the subjects with LBP, 88.9% had ITPs.

Another finding in this study was the presence of ITPs in subjects who did not have LBP (33.3%).

Conclusions

- 67% of deskbound workers had ITPs.
- 88.9% of those with history of LBP had ITPs.

(p value = 0.025).

However, presence of ITPs in subjects without LBP (33.3%) is probably an early finding, which could be treated for prevention of LBP. But further studies in this field are required for more conclusive evidence.

Limitations

Due to the nature of the assessment the PI could not be blinded to the initial assessment.

References


Acknowledgements:

- Institutional Review Board for approval.
- Fluid Research Grant from Institution.

The Authors declared no conflicts of interest.
“EFFECT OF WEIGHTED DIAPHRAGMATIC BREATHING EXERCISE FOLLOWING THORACOTOMY: A CASE SERIES”

SAMUEL JOHNSON Doss A, PIERRE EDWIN D, LENNY VASANTHAN T.
DEPARTMENT OF PHYSICAL MEDICINE AND REHABILITATION, CMC, VELLORE.

INTRODUCTION

Inadequate inspiratory effort occurs due to pain and inhibition of muscles following surgery to the chest wall. This leads to inadequate sputum expectoration, thereby causing alveolar collapse. As a result, there is an increase in the work of breathing leading to muscle fatigue. (1)

Preoperative inspiratory muscle training using incentive spirometer performed in several patient populations was found to increase inspiratory muscle strength. (2)

We propose a novel method of strengthening diaphragm using diaphragmatic weights.

OBJECTIVES

To check the effectiveness of preoperative weighted diaphragmatic breathing exercise on diaphragm muscle strength and chest expansion postoperatively.

MATERIALS & METHODS

Study Design: Case series (n=7).

Study setting: Kamala Nehru Ward, CMC, Vellore.

Inclusion criteria: Patients undergoing Thoracotomy surgery.

Exclusion criteria:
- Pregnancy
- Infants
- Arrhythmia
- Patients with pacemakers.

Tools and Materials:
- Epigastric board
- Weight discs
- Respiratory Pressure Monitor.

OUTCOME MEASURES

Outcome Measures
- Maximum Inspiratory Pressure and
- Chest expansion.

Assessment done:
1. On the day of referral
2. The day before surgery and
3. Postoperative day 3.

PROCEDURE

Patients were recruited based on the inclusion/exclusion criteria.

An initial weight of 2 kilograms was started and weight was increased based on the fatigability of the patient (use of accessory muscles of respiration).

Intervention was given twice a day for 1 week.

RESULTS

Weighted diaphragmatic breathing exercise done preoperatively has a positive effect on improving MIP before surgery, its role in preventing postpulmonary complication needs to be studied.

REFERENCE


DISCLOSURE

- Conflicts of interest: None known
- Funding: Fluid Research Grant, Silver IRB, CMC, Vellore

CONCLUSION

- Out of the 7 patients, 5 patients showed improvement of Maximum Inspiratory Pressure (MIP) after training.
- The median decrease in Maximum Inspiratory Pressure on POD 3 when compared to baseline was 40%.

Chart 1: Changes in Inspiratory pressure.

- Chest expansion in the apical segments was increased when compared to lower thoracic levels (Chart 2 & Chart 3) on POD 3 indicating diaphragm inhibition (Chart 4).

Charts 2-4: Chest expansion at various levels.

I sincerely thank Mr. John and Mr. John Samuel for their help and support towards this Poster.


**BACKGROUND**

- Very Low Birth weight infants are at risk of adverse neurodevelopmental outcomes.
- Early identification is important to offer intervention programs to minimize the functional consequences of cerebral palsy (CP).
- Assessment of general movements (GMA) appears to have the potential for predicting the development of CP with a high degree of certainty at an early stage.
- There are no studies which have looked at GMs in Indian infants.

**AIMS AND OBJECTIVES**

- To evaluate the applicability of GMA in Indian infants.
- To evaluate the association between GMA and motor function at one year.

**METHODOLOGY**

- **Design:** A prospective observational cohort study.
- **Setting:** Tertiary care neonatal unit in south India.
- **Participants and Methods:** Video recordings of a cohort of 327 VLBW babies born in Christian Medical College, Vellore was taken between 9-15 weeks post term age (The Fidgety movements period) and analyzed according to Assessment of Motor Repertoire (AMR) by two observers masked for the infants' medical history.
- Fidgety movements (FMs) were classified as abnormal if absent, sporadic or exaggerated and as normal if continuously or intermittently present. Movement character was classified as normal if smooth and fluent and abnormal if monotonous, stiff or jerky. Motor function at 1 year corrected age was assessed using the Peabody Developmental Motor Scales (PDMS-2).
- **Statistical Analysis:** The variables Gross Motor Quotient (GMQ), Fine Motor Quotient (FMQ) and Total Motor Quotient (TMQ) were transformed using logarithmic transformation in parallel with reversing the data due to zero or below zero values. Hence, to get mean values, statistics were performed using a General linear model and the GMQ, FMQ and TMQ reversed lg10 were used to get $p$ values.

**RESULTS**

- There was a significant correlation between normal and abnormal FMs and the Gross Motor Quotient of the PDMS-2 ($p=0.006$).
- Presence of FMs and presence of smooth and fluent movement character when compared to abnormal FMs and monotonous, stiff and jerky movement character was significantly associated with higher scores in the Fine Motor and Total Motor Quotient ($p=0.007$).
- When FMs were present, smooth and fluent movement character was significantly associated with a higher score on Gross Motor Quotient of the PDMS-2 ($p=0.005$).
- The sensitivity of the test was calculated to be 22.7% and the specificity is 88.2%. The positive predictive value is 16% and the negative predictive value is 92%.

**CONCLUSION**

- This study documents applicability of General Movement Assessment in a low-resource setting in south India and contributes to GMA validity.
- There is a significant association between general movements and motor function assessed by the PDMS-2 at one year.
- Longer follow-up studies are needed to evaluate the neurodevelopmental outcomes and relationship to GMA in this population.

**REFERENCES**

SYMPTOM EXPERIENCE AND QUALITY OF LIFE AMONG PATIENTS WITH BREAST CANCER FOLLOWING CANCER TREATMENT

Ms. Sumi Sara Eapen – MSc(N) Junior Lecturer, Department of Medical and Surgical Nursing, CMC, Vellore
Dr. Punitha Ezhilarasu MSc (N), PhD, Professor and Head, Department of Medical and Surgical Nursing, CMC, Vellore
Mrs. Josephine Sukumaran, RN, RM, MSc(N), Professor, Department of Medical and Surgical Nursing, CMC, Vellore
Dr. Deepak Abraham, MS, PhD, Professor, Department of Endocrine Surgery, CMC, Vellore
Ms. Tunny Sebastian Lecturer, Department of Biostatics, CMC, Vellore

Introduction
A diagnosis of breast cancer causes significant physical, emotional, social, and economic and vocational upheaval.

Background of the study
It is projected that the annual number of deaths due to cancer deaths will increase from 7.6 million to 12 million by 2030 (WHO, 2023).

Moreover, unlike survival for other cancers, which levels off after 5 years, 62% of women diagnosed with breast cancer survive 10 years and 77% survive 15 years (American Cancer Society, 2012).

Statistics show that more and more women with breast cancer cross the set 5-year survival period after breast cancer treatment.

In India, the 5-year survival rate is 74.8%. During this course of survival, these patients experience a myriad of symptoms which can considerably reduce their Quality of Life (QOL).

Methodology
RESEARCH APPROACH: A quantitative research approach

RESEARCH DESIGN: A descriptive correlational design

SETTING OF THE STUDY: The study was conducted in the outpatient departments of radiation therapy of the Christian Medical College, Vellore

POPULATION: Women who were diagnosed to have breast cancer and treated at CMC, Vellore

SAMPLE: It comprised of all those who attended the radiation therapy outpatient department of CMC, Vellore, provided they fulfilled the inclusion criteria.

Inclusion criteria
1. Women who could communicate in English, Hindi, Tamil, Malayalam and Bengali.
2. Women who have completed a period of at least 3 months after surgery and has completed any one or a combination of treatment modalities.

Exclusion criteria
1. Women who do not give consent for the study.
2. Women who have metastasis to the brain.
3. Women who are on psychotropic medication.

Sample size
A total of 143 study subjects were selected using the total enumerative sampling technique, based on the inclusion criteria.

Data collection instrument
Data were collected using standardized scales namely the Memorial Symptom Assessment Scale (MSAS), which assessed the frequency, severity and distress of 12 symptoms and the Functional Assessment of Cancer Therapy – Breast (FACT-B) which assessed the quality of life of these patients. These were self-administered questionnaires.

Ethical consideration
Written consent was obtained from the study subjects.

Data analysis
Analysis was done using SPSS version 17. Data were analysed in terms of mean, median, range and percentages, Pearson’s correlation coefficient, ANOVA and independent t test.

Results
Symptom Experience of the patients with breast cancer

Comparison of Symptom Experience among selected demographic and clinical variables

The difference in QOL between patients with breast cancer in relation to their marital status, religion, location of residence, educational and socioeconomic classes were statistically significant (p<0.05).

However, there were no significant differences in the symptom experience and quality of life scores according to selected clinical variables.

Conclusion
The findings suggest that symptom experience can considerably affect the quality of life of patients with breast cancer after cancer treatment. There is a need to educate breast cancer patients, their caregivers and nurses on management of these symptoms. A well managed symptom experience will improve the quality of life and thus make survivalship meaningful.
TITLE OF THE STUDY
A study to assess the effectiveness of structured induction training programme on the knowledge and competency of nursing practice for the newly recruited staff nurses.

BACKGROUND
- A consistent specially induction framework within the acute care hospital facility is essential to the development of both generic and specialty knowledge and skills that transfer to different domains.
- Providing a structured and progressive learning with accessible resources applicable to the context in which learning is applied supports nursing care demands in clinical practice today.
- A broad consultative process among hospitals at the facility is essential so that patient governance is monitored and evaluated.

This article describes the structure, content and delivery of a 2-week novice nurse structured induction programme using a newly implemented knowledge and skills framework. While the programme is conducted at a tertiary referral hospital in India.

OBJECTIVES
1. To assess the knowledge of nursing recruits on the selected nursing practice among the controlled group.
2. To assess the knowledge of nursing recruits on the selected nursing practice among the experimental group.
3. To assess the competency of nursing recruits on the selected nursing practice among the controlled group.
4. To assess the competency of nursing recruits on the selected nursing practice among the experimental group.
5. To identify the effectiveness of the structured induction programme by comparing the knowledge and competency of the nursing recruits on the selected nursing practice between the control and experimental group.
6. To find the association between the demographic features and the knowledge and competency of the selected nursing practice in both control and experimental group.

METHODS
- Quasi experimental design was used.
- A total of 106 registered nurses who were fresh graduates or with 1-2 years experience in a private hospital, recruited, were randomly included in the study.
- Test of randomization was used and the study was conducted.
- Informed consent was obtained from the subjects.
- Permission was also obtained from the Institutional ethical board as well as the head of the Nursing Department, to conduct the study.

The structured teaching module used in the induction program were prepared by experienced faculty members.

DATA ANALYSIS
- The data collected was arranged, analysed and tabulated. Descriptive statistics such as frequency, mean were used to describe the demographic details. Descriptive statistics such as frequency, mean, standard deviation and percentage were used to determine the level of knowledge and practice of the recruits.

RESULTS CONTD....
- Results analysis revealed that there was no Statistically significant improvement in the knowledge of novice staff nurses who underwent the conventional induction programme.

CONTROL GROUP-PRE TEST & POST TEST KNOWLEDGE

<table>
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<tr>
<th></th>
<th>Mean</th>
<th>Std. Dev.</th>
<th>Correlation</th>
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<tr>
<td>Pre-test</td>
<td>71.10</td>
<td>17.67</td>
<td>0.35</td>
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<tr>
<td>Post-test</td>
<td>75.28</td>
<td>15.87</td>
<td>0.75</td>
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RESULTS CONTINUED....
- There was very significant increase in the knowledge of novice nurses who underwent the structured induction programme.
- The study results revealed that in all 14 domains of competency of the novice nurses.
- There was a highly statistically significant improvement in skills (P < 0.001).

CONCLUSION
The study was intended to guide the development of ward/unit competency program, which is to enable nursing practice to be dynamic, relevant and responsive to the changing needs of patients as well as to enhance staff development.

Results reveal that the programme was very effective and there is a strong relationship in the development of novice nurses for quality nursing care.

REFERENCES
2. E. Jesse.ferguson@beds.ac.uk
3. 3.http://her.sagepub.com/content/20/12/201/abstract
# Incidence and Factors Influencing Post Mastectomy Upper Limb Lymphedema

**Dr. Geley, Dr. Soniya Gupta, Dr. Ashish Kumar Gupta, Dr. Paul M.J.**

**Christian Medical College, Vellore**

## Introduction
- Arm lymphedema is a complication following surgery for breast cancer.
- It varies from mild swelling to an incapacitating condition. It has a major effect on physical condition, quality of life, functional status, family and finances.
- The worldwide reported incidence of lymphedema varies from 10-60%.
- Hayes et al. reported an incidence of 33% between 6 to 18 months of surgery.

## Aim
- To study the incidence and factors influencing post mastectomy lymphedema.

## Methods
- Prospective cohort study involving 103 adult women who underwent surgery for carcinoma breast.
- Serial measurements of the affected side arm were taken preoperatively and postoperatively at 3, 6 and 12 months.
- Various Risk factors assessed.
- The statistical analysis was done using chi square test with SPSS version 16.

## Risk Factors

<table>
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<tr>
<th>VARIABLES</th>
<th>STUDY GROUP % (n)</th>
<th>LYMPHEDEMA GROUP % (n)</th>
<th>LYMPHEDEMA ABSENT % (n)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGE INTERVAL</td>
<td>STUDY GROUP %</td>
<td>LYMPHEDEMA GROUP %</td>
<td>LYMPHEDEMA ABSENT %</td>
<td>P value</td>
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<tr>
<td>&lt;40 years</td>
<td>17.5 (18)</td>
<td>27.7 (5)</td>
<td>72.3 (13)</td>
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<td>41-60 years</td>
<td>71.8 (74)</td>
<td>25.7 (19)</td>
<td>74.1 (55)</td>
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<td>&gt;65 years</td>
<td>10.7 (11)</td>
<td>18.2 (2)</td>
<td>81.8 (9)</td>
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## Discussion
- According to Deo et al., prevalence of lymphedema is 33.5%.
- Stage of disease, body surface area, loco regional radiotherapy, presence of co-morbid conditions and anthracycline based chemotherapy had emerged as significant risk factors.
- Guedes Neto the incidence of arm edema within one year of breast cancer treatment was 73%.
- Many of our patients present in stage III or IV requiring extensive node dissection and aggressive post surgical therapy, which are known risk factors of lymphedema.
- Norman et al. breast cancer is the disease of working women. Paradoxically, many of our patients were homemakers.

## Results and Conclusions
- Total number of cases - 103
- The incidence of lymphedema post mastectomy in our institute was 25.24%.
- BMI more than 30 was found to be a significant factor causing lymphedema.
- Stage of the disease did not have statistical significance as risk factor for lymphedema.
- The number of nodes removed and number of nodes involved with carcinoma infiltration did not have a statistical significance.
- Modified radical mastectomy could not be proven as a risk factor when compared to breast conservation surgery.
- Radiotherapy to axilla was a significant risk factor compared to radiotherapy sparing axilla.

## References
- Nele Devgoogh, Marie-Rose Chisimwana, Lude Geraerts et al., Effect of manual lymph drainage in addition to guidelines and exercise therapy on arm lymphedema related to breast cancer: randomised controlled trial. BMJ 2013; 346:f3526 doi: 10.1136/bmj.f3526
INTRODUCTION
At present, mammography & B-mode ultrasound are the modalities routinely used for evaluation of the breast. The current practice of using sonoarcographic BIRADS lexicon has resulted in standardization of breast reporting however overlapping features result in indeterminate lesions, thereby subjecting many of these lesions to an invasive biopsy.
A technique which would improve lesion characterization and improve the specificity of diagnosis is highly desirable to avoid unnecessary biopsies.

Acoustic Radiation Force Impulse (ARFI):
Elastography is a recently introduced technology which is based on the observation that malignant tissue is stiffer compared to normal tissue. In 2002, the concept of using short duration acoustic forces called push pulses to create local tissue displacements and detection of displacements by conventional ultrasound methods was introduced.

AIM & OBJECTIVES
AIM: To assess the role of Acoustic radiation force impulse (ARFI) elastography in differentiating benign & malignant lesions in patients with solid breast lesions, using histopathological diagnosis as reference standard.

OBJECTIVES:
1. To assess the role of virtual touch quantification (VTQ) and Virtual touch imaging (VTI) of ARFI using the following variables:
   a) Shear wave velocity (SWV) within the lesion and surrounding normal tissue using VTQ.
   b) SWV ratio (lesion SWV / surrounding tissue SWV) using VTQ.
   c) Area Ratio (AR) of lesion on VTI & B-mode using VTI.
   d) Elasticity scoring based on a visual 5 point system using VTI.
2. To compare performance of ARFI (VTI & VTI) with B-mode USG in the prediction of malignancy.
3. To compare performance of combined criteria (USG BIRADS + ARFI) to that of USG BIRADS alone in the prediction of malignancy.

METHODS
Prospective observational study approved by the Institutional review board.

Inclusion criteria:
1. Consecutive patients with solid breast masses - solid / mixed with solid component >1cm.
2. Size <1cm.
3. Histopathological diagnosis after ARFI.

Exclusion criteria:
1. Calcified lesions with non-calcified area <1cm
2. Prior biopsy/TAC/thermoablation on the same breast

Equipment
- Technique of examination:
  Step 1: B-mode USG image acquisition & BIRADS categorization
  Step 2: VTI mode
  Step 3: 3D mode
  SWV measured within and outside the lesion

- Histological scores:
  - Elasticity scores:
    - Elasticity score 1 ≤ B-mode USG Image showing a well-defined hypoechoic lesion. B-IOMI score showing a mosaic pattern similar to the surrounding breast parenchyma.
    - Elasticity score 2 ≤ B-mode USG Image showing a well-defined hypoechoic lesion. B-IOMI score showing a mosaic pattern with a mixture of dark and bright areas.
    - Elasticity score 3 ≤ B-mode USG Image showing a less-defined hypoechoic lesion. B-IOMI score showing a mosaic pattern with no increase in size.
  - Area ratio:
    - 1.0 ≤ Area ratio

- Statistical analysis:
  - Student t-test (independent samples) was used for comparing the variables. Receiver operating characteristic (ROC) curves were used to compare diagnostic performance of B-mode USG, VTI, VTQ and combined VTI + VTQ.
  - Correlation coefficient was calculated using bivariate analysis. P values < 0.05 was taken as statistically significant.

RESULTS
A total of 130 breast lesions were included in the final analysis. Patient demographics:
- Mean age was 55 years (range 14-66 years).
- 122 were female (98.4%) and 2 were male (1.6%).

- 1 lesion characteristics:
  - Histopathologic subtypes:
    - Fibroadenoma (n=81)
    - Adenosis (n=6)
    - Inflammatory (n=4)
    - Benign phylloides (n=3)
    - Malignant:
      - Invasive ductal carcinoma (n=13)
      - Invasive lobular carcinoma (n=1)
      - Medullary carcinoma (n=1)

- 2 global score:
  - VTI scores:
    - Elasticity score 1 ≤ B-mode USG Image showing a well-defined hypoechoic lesion. B-IOMI score showing a mosaic pattern similar to the surrounding breast parenchyma.
    - Elasticity score 2 ≤ B-mode USG Image showing a well-defined hypoechoic lesion. B-IOMI score showing a mosaic pattern with a mixture of dark and bright areas.
    - Elasticity score 3 ≤ B-mode USG Image showing a less-defined hypoechoic lesion. B-IOMI score showing a mosaic pattern with no increase in size.

- Area ratio:
  - Area ratio 1.0 ≤ Area ratio

- 4 shear wave velocity (SWV)

CONCLUSION
Our results show that ARFI elastography using VTI and VTQ can reliably differentiate between benign and malignant breast masses. - The combined BIRADS + ARFI VTI elastography showed better performance (AUC 0.952) as compared to BIRADS alone.

Using VTI to modify the sonoarcographic BIRADS category could potentially reduce the number of biopsies.

We conclude that ARFI may be used as an adjunct modality with the existing B-mode ultrasound and mammography, particularly in BIRADS 4 lesions.

REFERENCES:
INTRODUCTION

Neurological tumors of the larynx are extremely rare. It accounts for only less than 1.5% of all benign tumors of larynx*. They are of 2 types- schwannomas and neurofibromas, both are nerve sheath benign tumors, of which schwannomas are more frequent. We present these 2 unusual tumors of the larynx. They present in any age group, mostly seen in 4th – 5th decade. Schwannomas arises from perineural schwann cells and neurofibroma, from perineural fibrocytes. Obstructive symptoms such as dysphonia, stridor, dysphonia and dysphagia are the common presentations. Though slow growing, it can become fairly large before causing symptoms. Supraglottis (aryepiglottic fold) is the commonest site, seen on laryngoscopy as a smooth mucosal bulge. CT +/- MRI with contrast is helpful in revealing the extent of the disease. Definitive diagnosis is based on histology. Histological diagnosis for schwannoma (Enger and Weiss criteria)** is based on presence of capsule, identification of Antoni A and B areas, a positive reaction of tumor for S-100 protein. However, histologically neurofibroma is a non encapsulated tumor, schwann cells seen intertwined with axons and collagen fibres, involving the nerve fascicles. Surgical resection remains the mainstay of treatment.

1st Case – Laryngeal Schwannoma

- 24 year old gentleman with hoarseness of voice and difficulty in swallowing for 10 months
- Flexible Laryngoscopy: Smooth mass seen over the interarytenoid region
- CT - Well defined mass arising from posterior hypopharyngeal wall
- Surgical approach- Endoscopic transoral laser excision
- Microscopy-
  
  Schwann cells intertwined with fibrocytes
  S-100 positivity on IHE

Follow up- After 6 months, patient asymptomatic. No evidence of recurrence on repeat laryngoscopy.

2nd Case - Laryngeal Neurofibroma

- 18 years old boy presented with hoarseness of voice since childhood and difficulty in breathing since 1 month
- Flexible Laryngoscopy: Smooth bulge seen over the right aryepiglottic fold. Bilateral vocal cords not visualized.
- CT- Well defined poorly enhancing mass arising from Supraglottis abutting thyrohyoid membrane & splaying thyroid cartilage
- Surgical approach- Initially a trans oral endoscopic laser excision was attempted but the vascularity of the tumor along with difficulty in excising the lateral component of the tumor splaying the thyrohyoid membrane necessitated a conversion to an open lateral pharyngotomy approach for complete tumor excision.
- Microscopy-

“nucleus free zones” that lie between the regions of nuclear palisading termed ‘Verocay bodies’ (green arrow)

Follow up- Repeat laryngoscopy done 6 months later and showed no evidence of recurrence. Normal mobile vocal cords

REFERENCES


CONCLUSION

- Benign laryngeal neurological tumors are rare.
- Any age group can be involved.
- Hoarseness and dysphonia are the common presenting symptoms.
- Laryngoscopy shows a smooth mucosa covered bulge over aryepiglottic fold and false vocal cord obscuring the true vocal cord.
- CT and MRI scans are required for the extent and to plan the approach.
- Histology is the only clue to definitive diagnosis and to classify the tumors into types.
- Complete surgical resection is the mainstay of treatment and to prevent recurrence.
- In neurofibroma, patient should be evaluated for neurofibromatosis type 1 and 2.
- Long term follow up is needed.
DIAGNOSTIC ACCURACY OF FINE NEEDLE ASPIRATION CYTOLOGY & IMAGING IN PAROTID TUMORS: WHERE DO WE STAND?

Shruti Venkitachalam*, Pranay Gaikwad, A J Turkey, Rajinikanth J, J C Muthusami
Christian Medical College, Vellore

ABSTRACT

BACKGROUND: Parotid lesions account for 2-6.5% of head and neck neoplasms. They have varied aggressiveness and patterns of spread. Though being easily accessible by Fine Needle Aspiration Cytology (FNAC), the sensitivity and specificity is 84.9% and 80.9%, respectively. MRI is the method of choice in salivary gland masses to assess the extent of tumor, invasion of neighboring structures, perineural spread and lymph node staging. Differentiation of benign and malignant masses is often extremely difficult with imaging alone. The exact pre-operative evaluation of salivary gland tumours remains a major challenge and which investigation to employ remains debatable. The objective of this study is to evaluate the ability of FNAC and imaging to predict malignancy in parotid gland tumours.

METHODS: A retrospective study, between January 2010 and June 2014 done to include 199 consecutive patients who presented with parotid swellings. Fine needle aspiration cytology reports, imaging details and histopathology reports were analyzed.

RESULTS: 118 FNAC were suggestive of a benign pathology, while 25 were suggestive of malignancy. 23 were inconclusive, and were considered in the 'intention to treat' category. Amongst the imaging, 86 were suggestive of a benign pathology, while 27 were suggestive of malignancy. 154 histopathology reports were "benign" and 45 were "malignant". Based on the above clinical audit, the sensitivity, specificity, positive predictive value and negative predictive value of FNAC was found to be 63.85%, 80.77%, 84.26% and 44.43% respectively. The sensitivity, specificity, positive predictive value and negative predictive value of Imaging was found to be 42.86%, 84.04%, 44.43% and 84.04% respectively. The combined information gained from FNAC and imaging is superior to either modality alone and could be helpful in ruling out malignancy preoperatively, and hence helpful in tailoring the surgical treatment.

IMAGING

- Clinically & biologically divergent tumors can look similar under microscope
- Parotid gland: one of lowest FNAC accuracy rates amongst H&N sites
- Overall sensitivity & specificity of FNAC are 85.9% & 88-100% respectively
- FNAC diagnosis of malignant or suspicious lesion of the parotid region has positive and negative predictive values of 90% & 96.6% respectively

- Radiological assessment of lesion (benign/malignant): CT, MRI and US are comparable in terms of sensitivity, specificity and accuracy
- In v/o tumor localisation & margin characteristics, MRI has a 100% sensitivity and 84-100% specificity; and CT has a 100% sensitivity and 72-93.3% specificity
- In our review, the sensitivity of imaging was 42.86% and specificity was 84.04%
- New MR techniques such as DW-MRI and MR spectroscopy have shown promising results

CONCLUSION

- FNAC is safe and economical, yet, by itself, is often not able to exclude malignancy
- Preoperative evaluation extremely important in formulating treatment strategies
- For malignant tumors, with FNAC & Imaging combined: high specificity, low sensitivity
- In our experience, combined FNAC and Imaging is helpful in ruling out malignancy

RECOMMENDATIONS

- Design a prospective study to evaluate the combined efficacy of FNAC & Imaging in detecting malignancy in parotid gland, in view of limited evidence

REFERENCES

Lymphoma of the uterine cervix - A report of three cases

Anitha Thomas1, Thomas S Ram2, Anuradha Chandramohan3, Aby Abraham4, Ramani Manoj kumar, Rachel Chandy1, Abraham Peedicayil1
1Departments of Gynecologic Oncology, 2Radiation Oncology, 3Radiology, 4Hematology, 5Pathology
Christian Medical College, Vellore.

Introduction
- Lymphomas arise in the lymph nodes or other lymphatic tissues in two third of cases and extra nodal tissues in about a third of patients
- The commoner sites of occurrence being gastrointestinal tract and skin
- Genital tract lymphomas constitutes 1.5% of extra nodal lymphomas.
- Lymphoma of the cervix is a rare malignancy and contributes to about 1% of all cervical malignancies

Objective
- Primary cervical lymphoma is a rare clinical condition among the Non-Hodgkin’s lymphomas. Here we describe our experience with primary lymphoma of the cervix.

Methods
- Chart review of patients with lymphoma of the cervix during the period 2010-2012.

Results
- We had three cases of primary lymphoma of the cervix. The ages of these women ranged from 38 to 60 years.

Case 1
- 54 year old nulliparous, post menopausal obese lady with postmenopausal bleeding
- Pelvic examination revealed a large smooth cervical mass of 10x15cm which was displacing the body of the uterus upwards and effacing the external cervical os.
- Referred elsewhere as cervical fibroid
- MRI of her pelvis also revealed a large cervical fibroid pushing the uterus into the abdomen above the pubic symphysis.
- Biopsy taken from the mass was reported as B cell lymphoma.
- She was subsequently referred to the hematologist for further management.

Case 2
- 36 years old P212 presented with severe menometrorrhagia and anemia
- Examination revealed a smooth surface 12x12 large cervical mass which felt like a cervical fibroid going on to the upper part of the vagina. The mass was wedged within the pelvis.
- Patient had persistent heavy vaginal bleeding and was posted for emergency hysterectomy
- At surgery the uterus mobilization was extremely difficult with severe blood loss requiring transfusions.
- Post operatively biopsy was B cell lymphoma and she was referred for RCHOP chemotherapy.
- The tumor cells were CD20 positive, CD3 with background reactive lymphocytes, Cytokeratin, CD 10, H-caldesmon and SMA negative with MIB1 labeling index 60%.

Case 3
- 49 year lady presented post hysterectomy and bilateral salpingo-oophorectomy done elsewhere 3 months back for multiple fibroids, a possible cervical fibroid and benign cystadenoma of the left ovary.
- She had difficulty on voiding post operative period and needed to be on continuous bladder drainage since then.
- On clinical examination she had pallor and had a hard pelvic mass 20x20 cm which on bimanual examination felt posteriorly infiltrating the recto vaginal septum. She did not have any palpable lymphadenopathy.
- Computerized tomography scan of the abdomen revealed Post hysterectomy status and a large ill defined pelvic mass as in the images below
- Her outside slides were reexamined at our lab and were reported as B cell non Hodgkin’s lymphoma of the cervix
- FNAC of the mass was reported as B cell non Hodgkin’s lymphoma. (CD20+, CD3-, CD34-).

Outcomes
- Case 1: Treated successfully with 12 cycles of RCHOP, following which cranial radiation was given. She expired due to febrile neutropenia at her hometown.
- Case 2: She was treated with RCHOP with good response initially and now has metastatic disease on follow up at 9 months.
- Case 3: Once the diagnosis was proven to be lymphoma the patient was offered chemotherapy but she went to hometown for further treatment.

Discussion
- Lymphoma cervix though uncommon can mimic benign conditions like cervical fibroid
- Clinically distinguishing them from fibroids and other malignancies may be difficult.
- The common B symptoms of lymphomas are absent
- Common presenting symptoms would be irregular vaginal bleeding and discharge
- MRI imaging may be of great use in distinguishing the origin, type of these tumors as well as aid us in staging purposes
- Pap smears may not be very useful as they rarely cause surface abnormalities
- The role of punch biopsy also is controversial as these are deep seated lesions
- FNAC /FNAB biopsy may be useful in doubtful cases as the tissues obtained may then be subjected to various immuno histochemical stains

Treatment
- With the advent of newer agents like rituximab the success rates of chemotherapy regimens like CHOP(cyclophosphamide, Adriamycin, vincristine, and prednisolone) have gone up and resulted in better survival
- In some cases radiation is an option following chemotherapy especially if the lesion persists.
- Surgical excision post chemotherapy can be offered to a limited number of women.
- Role of primary surgical excision is not recommended as most of these tumours shrink with the administration of chemotherapy.

Conclusion
- Primary cervical lymphoma is a rare diagnosis often made after hysterectomy or LEEP. The mainstay of treatment is chemotherapy and surgery is usually reserved for a persistent cervical mass.
- Benign lesions like cervical fibroids as well as other malignancies may mimick lymphomas.
- Primary cervical lymphoma involving only the cervix are usually associated with good prognosis in spite of their large size unlike other cervical malignancies.

References
Introduction

The ability to completely resect a squamous cell carcinoma of the head and neck weighs heavily in the surgeon’s decision to use surgery in an attempt to eradicate the cancer. And the presence of disease (gross or microscopic) at the surgical margins represents a key prognostic factor for patient survival. Bakstas notes that 5% to 50% of carcinomas recur at the surgical margin of clear margins regardless of tumour stage. And especially in carcinoma of the tongue, the surgery is blinded when it comes to resection of deep soft tissue and this often leads to compromise of the surgical margins. (Bakstas J. Surgical margins in squamous cell carcinoma. Acta Otolaryngol 1986;99:233

Aims and Objectives

To review the surgical margins following glossectomy for Ca of the tongue and to look for:
1. Relative frequency of involved versus close and clear surgical margins.
2. The influence of tumour variables on the frequency of the above three categories
3. If margins are commonly involved/closed in a particular direction

Hypothesis

Wider marginal margins for oral tongue SCC may be justified in order to reduce the number of cases with close or involved surgical margins. The functional impact of this approach can be minimised if wider margins could be taken at only at certain areas, e.g. at the deep soft tissue margins.

Methods and Materials

- All biopsy proven and oral tongue SCC cases who underwent glossectomy between 2010-2014 were included in the study. Recurrent and Post RT cases were excluded from the analysis.
- Retrospective chart review was done for demographic, clinical and pathological data.
- The surgical margin nomenclature was standardised as anterior/ posterior/superior/inferior in a mucosal margin and deep soft tissue margin. The internal margin represents the floor of the mouth.
- Margins were labeled clear if clearance was more than 5 mm, case if 1 - 5 mm and involved if less than 2 mm.

Results

- There were 118 patients eligible for analysis. Overall, close margins occurred in 37 patients (31.3%), involved margins in 22 cases (18.6%) and clear margins in 59 cases (50.1%).
- Involved margins more commonly occurred at the mucosal aspect as compared to the depth.
- Involved or clear margin (floor of the mouth) was less frequently found to have involved margins.
- Patients with deeper tumours were significantly found to have mucosal margins involving tumour and close margins at the depth.
- Increasing T stage and N stage were significantly associated with involved mucosal margins.
- Preservation of lymph vascular invasion was associated with involved margins both at the mucosal aspect and at the depth.
- Patients who underwent primary closure of the defect had more close/involved margins at the mucosa as compared to the depth (not statistically significant).

Conclusion

- The inferior/lateral (FOM) and deep margins were frequently compromised, due to the anatomical difficulties visualizing and dissecting these margins.
- Alveolar gingival tissue needs to be taken along with the FOM tissue at the inferior/lateral aspect to improve the margin status.
- Adjunct techniques like intra-op use of Lugol's solution (*), auto-fluorescence visualization techniques (VEL scope) and per oral ultrasound to guide the resection at the depth (*), can be employed to reduce the frequency of close/involved margins.
- Local flaps/ free flaps can be employed more commonly to enable the surgeon to access the tumor adequately without the anxiety of closing the defect primarily.
- Wider mucosal margins for higher staged tumors/deeper tumors (similar to cutaneous melanoma) have to be evaluated in further prospective studies.

(*Completed studies at our Unit and yet to be published)
Non Medullary Thyroid malignancies in Primary hyperparathyroidism

Nitin Kapoor, Sahana Shetty, Nihal Thomas, Simon Rajaratnam, Deepak Thomas Abraham, M J Paul, Pooja Ramakanth, Regi Oommen, Nyla Shanthly, Marie Therese, Thomas V Paul

Departments of Endocrinology, Diabetes & Metabolism, Endocrine Surgery, Nuclear Medicine, Pathology
Christian Medical College, Vellore, Tamil Nadu, India.

Introduction
- Primary hyperparathyroidism (PHPT) and non-medullary thyroid cancer are the endocrine conditions seen in day to day clinical practice.
- Concomitant non-medullary thyroid cancer and PHPT is very rare.
- A coexistence of non-medullary thyroid cancer is found in 2.4% to 3.7% of the patients operated on for PHPT.

Material & Methods
- A retrospective study of histologically proven cases of primary hyperparathyroidism between the years 2007 - 2013.
- The diagnosis of PHPT was based on presence on hypercalcaemia (> 10.3mg/dl) with an non suppressed serum immunoreactive PTH level.

Patients with biopsy proven Primary hyperparathyroidism (PHPT) screened 2007-2013
165 total subjects screened
71 Men; 91 Women
Screened for the presence of documented thyroid malignancy
91/165(5%) patients found to have Non-Medullary thyroid malignancies

Results
- Mean age was much higher in patients with non-medullary thyroid malignancies (57 yrs. vs. 41 yrs.).
- All patients with thyroid malignancies were women.
- All but one patient were asymptomatic for their thyroid illness and were detected incidentally.
- None of these patients had family history of thyroid malignancies.
- The mean thyroid tumor size was 12 mm and none of the patients had any extra capsular invasion or lymph node metastasis.
- Three out of 5 patients had papillary thyroid carcinoma (PTC) and two had a follicular variant of papillary thyroid carcinoma (FVPTC).

Surgery
- Those with PTC underwent total thyroidectomy
- Subjects with FVPTC of thyroid -Hemi thyroidectomy initially followed by completion

Aims and Objectives
- To study the prevalence of non-medullary thyroid malignancies, in the presence of primary hyperparathyroidism.
- To look at the clinical profile of these subjects.

Conclusions
- The pathogenesis of this concurrence is not fully established.
- Thyroid carcinoma may coexist with parathyroid adenoma.
- Meticulous screening with an ultrasound examination of the neck may help in high risk subjects who are harboring both tumors.

References:
Doing the level best: a retrospective review of neck dissections in the treatment of oral squamous cell carcinoma
Lurstep Wanshnong, Pranay Gaikwad, Grace Rebecca
Christian Medical College, Vellore

Background

➢ The oral cavity is the most common site for squamous cancer of the upper aerodigestive tract.
➢ Lymph node metastasis indicate more aggressive/advanced cancer with a poor prognosis.
➢ With greater understanding of nodal spread, the MDT approach to manage neck nodes has changed over time.

Methods

➢ Retrospective review of medical records of 204 patients who underwent ipsilateral neck dissections for Oral Cancers between January 2009 and May 2014.
➢ Patients classified into two groups based on the types of neck dissection as:
➢ Selective neck dissection (SND-I-IV) and Radical/ Modified Radical Neck Dissection (RND/MRND).

Statistical Methods

➢ Frequency analysis was used in each group for the pathologically positive nodes at:
  ➢ All levels
  ➢ Each level

➢ Logistic regression for the association and predictability between:

  Positive nodal rates:
  ➢ Levels I-III with level IV
  ➢ Levels I-IV with level V
  ➢ Level II with level III
  ➢ With pT-stage, lymhovascular invasion, perineural invasion
  ➢ With depth of tumor in oral tongue and positive nodes.

Results

➢ Types of neck dissection (N = 204)

- SND I-IV
- RND/MRND

- Positive nodes:
- SND I-IV
- RND/MRND

- Percentage of positive nodes:
- Levels I-III
- Levels I-IV

Sub-site Distribution (N= 204)

- Tongue
- Floor of mouth
- Neck Masses

Primary Stage Profile

- pT stage
- T1
- T2
- T3
- T4

Pattern Of Nodal Spread-79/204

Types Of Neck Dissection

<table>
<thead>
<tr>
<th>Types of Neck Dissection</th>
<th>SND I-IV</th>
<th>RND/MRND</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alveolus</td>
<td>2 (7.1%)</td>
<td>26 (92.9%)</td>
<td>28</td>
</tr>
<tr>
<td>Buccal mucosa</td>
<td>5(6.9%)</td>
<td>67 (93.1%)</td>
<td>72</td>
</tr>
<tr>
<td>Floor of mouth</td>
<td></td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Tongue</td>
<td>38(38.0%)</td>
<td>62(62.0%)</td>
<td>100</td>
</tr>
<tr>
<td>TOTAL</td>
<td>45(38.0%)</td>
<td>159(62.0%)</td>
<td>204</td>
</tr>
</tbody>
</table>

Association of pT with pN+

Association of LVI with pN+

Conclusions

Positive node at:
- Any of the levels IIb, IIa,III
- Level IIa
- Level II
- Level Ib

Predicts level IV involvement p<0.002
Predicts IIb involvement p<0.001, Odds Ratio=20
Predicts IIa involvement p=0.004, Odds Ratio=6.5
Predicts IIa involvement p<0.001, Odds Ratio=7.2

➢ Involvement of level V is very rare even in N+
➢ Perineural invasion does not predict node involvement
➢ In tongue, >4mm depth of tumor may predict neck node spread (p=0.060)

Recommendations

➢ Based on our data, SND I-IV may be the minimum standard neck dissection as a prophylactic nC0 and therapeutic in n+ disease
➢ Level IIb nodes need to be included in all neck dissections
➢ Lymphovascular invasion is a better predictor of nodal metastases than perineural invasion.

References

Introduction:

- Cervical polyps are relatively common lesions and most commonly present in premenopausal and multiparous women between the ages of 30 and 50 years [1]. Incidence is 4-10% of all cervical lesions [2]. A cervical polyp is a growth protruding from the endocervix or endocervical canal. Polyps that arise from the endocervix are called endocervical polyps. The polyp usually develops as a result of chronic cervicitis or a response to a viral infection. They are soft, spherical, plastron-looking red masses and bleed easily when touched. They may be associated with pre-cancerous lesions secondary to the underlying endocervicitis.

- They are most commonly asymptomatic and are usually discovered on routine examination. They also present with intermittent pain or post-coital bleeding and vaginal discharge. Polyps are painless and, unless a patient has a bleeding abnormality necessitating her evaluation by a physician, she go undiagnosed.

- Histologically, polyps are composed of endocervical epithelium with a fibromuscular stalk. Differential diagnoses include papillary endocervical carcinoma or ciliated carcinoma protruding through the os, retained products of conception, granulomatous endometrial polyps that occasionally originate in the cervix, and proliferating submucous fibroids or endometrial polyps [3].

- Most cervical polyps can be grasped with a clamp and avulsed, and the base should be cauterized for hemostasis and to reduce the risk of recurrence. During pregnancy, the cervix is highly vascularized. If the polyp is stable and benign-looking, they should just be observed during the pregnancy and removed only if they are causing bleeding.

- Although they are mostly benign, carcinomatous change occurs in 1.7% of cervical polyps [2]. Some cervical polyps are associated with malignant or pre-malignant lesions of the cervix or endometrium.

- Most polyps are less than 3 cm in diameter. Giant cervical polyps with a size greater than 4 cm is rare and till now only 5 cases have been reported [4]. They occur in adult women, more rarely in children and are frequently interpreted as malignant neoplasms at the time of the presentation.

Case Report:

- A 15 year old girl presented with mass descending per vaginum and increasing white discharge since 4-5 months. She had regular cycles 3-6 days-30 but had menorrhagia and spastic dysmenorrhoea. She had no other complaints. On endocervical examination under anaesthesia, it showed a large 6x5 x5 cm polyp arising from the endocervix between 7 and 9 o'clock positions. Fig (1). It was extracted and was seen as an exophytic mass arising from upper vagina. The same was excised. The histopathology was reported as benign endocervical polyp. Gyno examination showed a 6x5 x5 cm mass arising from the endocervix, cut section it revealed cystic degeneration filled with mucinous material. Microscopy showed papillary endocervical epithelium with a large chronic inflammatory and blood vessels with thick fibromuscular stalks. She developed secondary haemorrhage seven days after surgery. Examination under anaesthesia showed the protrusion were controlled and vagina was packed. She was discharged in a stable condition.

Discussion:

- Only fifteen cases (Table 1) of giant cervical polyps have been reported so far [4]. In previous cases age of presentation ranged from 5 years to 61 years with mean age of 32 years [5]. Most of the patients presented with leukorrhea, menorrhagia and variable per-vaginal bleeding. In one pregnant patient, the polyp protruded from the external os mimicking early abortion [6]. There are only 3 young girls/less than (8 years) reported with giant cervical polyps and only one of them had an endocervical polyp. Our patient, a 15 years old girl presented with leukorrhea, menorrhagia and dyspareunia. She was diagnosed to have a giant endocervical polyp. The size of origin in ten out of fifteen giant cervical polyps was ecocervical (Table 2), while in four cases it was endocervical. In one case site of origin was not reported.

- In ten out of fifteen previous cases polypectomy was done while in one case no surgical procedure was done. In one case each radical hysterectomy and pan hysterectomy was done because of clinical suspicion of malignancy and endometrial hyperplasia was seen in one case. The youngest one (five years), was operated for submucous myoma.

- Exploratory laparotomy was done and polyp was excised through an incision in the lower uterine segment because the polyp extended into the endometrial cavity. In our case polypectomy was done.

Conclusion:

- To conclude giant endocervical polyps are rare. They are not common in young girls and polypectomy is sufficient to treat them. All reported cases of giant cervical polyps are benign and thought to be the result of reactive changes from long standing chronic inflammation. They can be misdiagnosed as malignancy and then hysterectomy is performed. Therefore proper knowledge of this entity and its clinical & USG presentation can save the patient from major surgery.

References:

Boerhaave’s syndrome: 10 years experience at a tertiary care hospital

Deshpande G., Samarasam I., Chandran B.S., Abraham V., George S.V., Mathew G.
Department of General Surgery, Christian Medical College and Hospital, Vellore, Tamil Nadu, India

BACKGROUND

• Spontaneous oesophageal rupture (Boerhaave’s syndrome) is an uncommon but serious condition associated with a diagnostic dilemma leading to delay in management.

METHODS

• A retrospective review of cases managed at Christian Medical College, Vellore, India
• Between 2004 and 2013

RESULTS

• Total no. of patients: 12
• Most common symptom at presentation was breathlessness.
• All the patients underwent chest X ray which showed pleural effusion.
• Six patients (50%) underwent chest tube insertion at other centre before referral.
• CT scan was confirmatory of the diagnosis
• Median diagnostic delay was 9 days (range 0 to 20 days)
• 75% of the patients underwent surgical intervention.
• The major factor influencing the plan for surgical intervention at presentation was the general condition of the patient.
• 4 patients underwent endoscopic stent placement.
• 30 day mortality was 8.3% (1 patient)
• Median ICU stay was 10.5 days (range 0–32 days)
• Median hospital stay was 25 days (range 12-60 days)

CONCLUSION

• The rarity of this condition and non specific symptoms at presentation often leads to diagnostic dilemma and delay in referral.
• Primary repair may be appropriate for ruptures diagnosed early.
• Management strategy consisted of adequate drainage of sepsis (surgical/radiological), repair/stenting of the oesophageal rent, appropriate antibiotics and adequate nutrition (feeding procedure).
• Non-operative management such as endoscopic stent placement and image guided drainage may help avoid the morbidity of surgery.

REFERENCES

**INTRODUCTION**

- Parapharyngeal space lesions constitute 0.5% of head and neck tumors.1
- Mostly benign lesions – commonest being salivary gland tumors.
- The parapharyngeal space can be divided into – pre-styloid and post-styloid compartments.
- Pre-styloid compartment usually give rise to salivary gland neoplasms.
- Post-styloid compartment (or carotid space)2 commonly give rise to tumors of neural origin.

**METHODS**

- Retrospective review of charts from June 2004 to June 2014.

**DEMOGRAPHICS**

- We had 24 patients.
- 11 females and 13 males.
- Mean age of 34.4 years (18-65 yrs)

**PRESENTATION**

- Symptoms:
  - Most common was swelling.
  - 2 had only intraoral swelling while the rest had external swelling also.
- Duration of symptoms at presentation:
  - Mean - 23.6 months (2.7-2 months)

**SURGERY**

- 26 surgeries in 24 patients.
  - 1 patient had a recurrent lesion within the study period.
  - 1 bilateral lesion – excised in separate sittings.
  - In 2 cases, the approach was unspecified.
  - All patients had an extraoral approach to excision of the tumors.

**DIAGNOSIS**

- n=24
  - Schwannoma
  - Sarcoma
  - Neurofibroma
  - Paranglioma
  - Pleomorphic adenoma
  - Adenoid cystic carcinoma
  - Meningioma

**FOLLOW UP**

- Follow up for 15 patients was available.
- Mean duration of follow-up:
  - 14.6 months (2-54 mths)

**DISCUSSION**

- Our series had a predominance of benign nerve sheath tumors.
- FNAC was mostly adequate when used, though in other studies intraoral FNAC has shown good results.4
- The cervical route without mandibulectomy was the preferred approach and was adequate even for large tumors.5
- The commonest postoperative complication was nerve palsies.

**CONCLUSION**

- Parapharyngeal tumors are rare tumors.
- Successful management requires cross-sectional imaging with angiography/embolization in appropriate cases.
- Preoperative counselling for postoperative neurological deficits is essential.
- Stroke is a rare but serious complication.
- Post-op rehab and long-term follow-up may improve QOL and detect significant residual disease or recurrence.

**REFERENCES**

Normal saline wash vs. no wash to reduce incisional surgical site infection in elective open colorectal resections – a randomized controlled trial

Augustin Abraham, Rohin Mittal, Mark Ranjan Jesudason, Benjamin Perakath
Surgery Unit 2 (Colorectal Surgery)
Christian Medical College, Vellore

BACKGROUND

- Surgical site infection (SSI) is one of the most common causes of morbidity and prolonged hospital stay among patients undergoing colorectal operations
- SSI rate is used as an indicator of quality of surgical care provided in an institution
- Various methods are used to prevent SSI
- Normal saline wound irrigation prior to skin closure has been considered as one of the prevention strategies to decrease SSI
- However, this has not been studied in colorectal resections in a human population

AIM

- To study the effect of normal saline wound irrigation in reducing the rate of abdominal incisional surgical site infection in open elective colorectal resections

METHODS

- Randomized controlled trial
- In a single colorectal unit of a tertiary care, teaching hospital
- Between December 2012 and August 2014
- IRB approved
- Registered with CTRI (Reg. No.)

Inclusion criteria

- All patients undergoing open elective colorectal resections

Exclusion criteria

- Laparoscopic surgery
- Emergency surgery
- Non consenting patients
- Intervention arm – Saline wash of the surgical wound after fascial closure
- Comparator arm – No saline wash
- Randomization with computer generated random numbers, with block randomization with variable block size
- Allocation concealment by consecutively numbered, sealed, opaque envelopes opened in the operating room after fascial closure
- Blinding – PI, patient and outcome assessor blinded
- Follow up till 4 weeks post operatively to look for SSI
- Data entered into predesigned proforma

RESULTS

- Sex distribution
- Age distribution
- Comparison between the 2 groups

CONCLUSION

- There is no significant benefit in irrigating the surgical wound with normal saline before skin closure to reduce incisional surgical site infection in patients undergoing elective colorectal resections

References

Submucous fibrosis in Oral Cancer – does it influence the prognosis?

Tirkey A J;* Rajinikanth J;* Thomas C T; Muthusami J C*; Gaikwad P.
*Department of General Surgery & Head and Neck Oncology
Christian Medical College, Vellore. Tamil Nadu, INDIA.

INTRODUCTION
- Submucosal fibrosis is a common pathology found in the patient using smokeless, chewable tobacco and areca nut seen in Indian and south East Asian region.
- Oral Sub mucosal fibrosis(OSMF) being one of the pre malignant condition causing oral squamous cell carcinoma. It has been hypothesized that patients having SCC on background of oral sub mucous fibrosis has better prognosis.
- It is attributed to blockage of the lymphatic's and reduced vascularity.

METHODS
- We have retrospectively analyzed the patients data who were operated in our department from the year January 2006 till November 2013 for oral malignancy.
- Patients with trismus, burning on taking spicy food, pale mucosa and presence of fibrous band in oral cavity were considered to have SMF.
- The patients with oral cancer were divided in two categories patient with oral canceroma (SCC without SMF) and patient having oral carcinoma on background of OSMF (SCC + SMF).
- We wanted to see, is there any difference in the spread of the disease in these two category of patients and does OSMF play a protective role.

RESULTS
DISTRIBUTION OF ORAL CANCER ACCORDING TO SUBSITES

<table>
<thead>
<tr>
<th>Subsites</th>
<th>Number of Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buccal mucosa</td>
<td>246 (41.5%)</td>
</tr>
<tr>
<td>Alveolus</td>
<td>137 (21.2%)</td>
</tr>
<tr>
<td>Gingiva</td>
<td>91 (15.3%)</td>
</tr>
<tr>
<td>Lip</td>
<td>31 (5.1%)</td>
</tr>
<tr>
<td>Tongue</td>
<td>13 (2.1%)</td>
</tr>
<tr>
<td>Others</td>
<td>38 (6.3%)</td>
</tr>
<tr>
<td>Total</td>
<td>600 patients</td>
</tr>
</tbody>
</table>

ASSOCIATION OF SMF IN ORAL CANCER

<table>
<thead>
<tr>
<th>Gender</th>
<th>Nodular positivity</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>46 (25.3%)</td>
<td>0.5</td>
</tr>
<tr>
<td>Female</td>
<td>330 (77.5%)</td>
<td>0.02</td>
</tr>
</tbody>
</table>

GENDER

426, 71%
174, 29%

INFLUENCE OF SMF ON NODAL SPREAD IN T1/T2 STAGE

<table>
<thead>
<tr>
<th>Type of Spread</th>
<th>Nodularism with SMF</th>
<th>Nodularism without SMF</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early T stage</td>
<td>24/69 (34.7%)</td>
<td>85/301 (28.2%)</td>
<td>0.3</td>
</tr>
<tr>
<td>Advanced T stage</td>
<td>40/71 (56.3%)</td>
<td>82/159 (51.5%)</td>
<td>0.5</td>
</tr>
</tbody>
</table>

INFLUENCE OF SMF ON NODAL SPREAD IN T3/T4 STAGE

<table>
<thead>
<tr>
<th>Type of Spread</th>
<th>Nodularism with SMF</th>
<th>Nodularism without SMF</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early T stage</td>
<td>17/29 (58.6%)</td>
<td>77/138 (56.1%)</td>
<td>0.5</td>
</tr>
<tr>
<td>Advanced T stage</td>
<td>14/26 (53.8%)</td>
<td>82/159 (51.5%)</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Analysis of Clinicopathologic findings between Oral cancer with SMF and without SMF

<table>
<thead>
<tr>
<th>Age</th>
<th>With SMF</th>
<th>Without SMF</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;50</td>
<td>162 (38.6%)</td>
<td>24 (42.1%)</td>
<td>0.9</td>
</tr>
<tr>
<td>&gt;50</td>
<td>137 (21.2%)</td>
<td>238 (41.5%)</td>
<td>0.02</td>
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</table>

<table>
<thead>
<tr>
<th>Gender</th>
<th>With SMF</th>
<th>Without SMF</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>46 (25.3%)</td>
<td>330 (77.5%)</td>
<td>0.5</td>
</tr>
<tr>
<td>Female</td>
<td>330 (77.5%)</td>
<td>130 (74.7%)</td>
<td></td>
</tr>
</tbody>
</table>

CONCLUSION
- There was no difference in age, gender or tumor differentiation between both groups of patients.
- Patients with SMF presented more often with advanced tumors.
- Nodal positivity in early or advanced stage disease is not altered by the presence of SMF.
- There was significantly increased presence of LVI in patients with SMF but no difference in PNI status.

REFERENCE
Efficacy of 1% Acetic Acid in Treatment of Chronic Wounds Infected with Pseudomonas Aeruginosa - Prospective RCTT

Dr. Amish Gohil, Dr. Pratik Barreto, Dr. Madhusudan, Dr. Kingsly Paul
Department of Plastic, Reconstructive and Microsurgery, Christian Medical College, Vellore

Introduction

- Chronic wounds are persistent wounds that don't respond to any sort of treatment.
- Such wounds are a major concern as they are not only a cause for morbidity but also require lengthy hospital stays, expensive antibiotics and specialized home care.
- The concept of using topical antibiotics on open wounds is to prevent and treat infections.
- Pseudomonas aeruginosa is a gram-negative opportunistic pathogen with innate resistance to many antibiotics.
- In economically backward places, patients cannot afford newer expensive drugs.
- Topically applied dilute acetic acid, cheap and easily available, has been found to be effective in such chronic wounds.

Aims & Objectives

- To evaluate the efficacy of acetic acid, in low concentration of 1%, as a local anti-septic agent, in chronic wounds infected with Pseudomonas aeruginosa.

Materials & Methods

- This was a PROSPECTIVE study conducted over a period of 6 months from October 1st, 2013 to March 31st, 2014 in the Department of Plastic Surgery, Christian Medical College, Vellore.
- Inclusion criteria: All patients with chronic wounds infected with P. aeruginosa.
- Exclusion criteria: Wounds resulting from massive burns, malignancy, immunosuppressed individuals, and individuals with sepsis.
- Thirty-two patients with chronic wounds infected with pseudomonas were enrolled in the study.
- Subjects were randomized (16 patients each) to the 1% acetic acid and saline dressing group. Method of randomization used was Permuted block randomization of size 2, 4, or 6 generated using SAS 9.1.3.
- All the patients under the study did not receive any systemic antibiotics during the study period and received twice daily dressings.
- Cultures were obtained by blinded independent wound evaluators.
- Test group patients were subjected to twice daily dressings with 1% acetic acid.
- Gauze soaked in 1% acetic acid solution were placed over the wound without squeezing them.
- Control group patients were dressed in a similar fashion but with normal saline gauze.
- On the 4th day and every third day after they underwent regular pus culture by Levine's method.
- The end point of the treatment was wound free of Pseudomonas aeruginosa.
- Data obtained included patient demographics, site of the wound, density of pseudomonas aeruginosa in the wound before starting the treatment and treatment time.
- Variables from both groups were analysed for P values less than 0.05.

Results and Analysis

<table>
<thead>
<tr>
<th>Variables</th>
<th>1% Acetic acid (n=16)</th>
<th>Saline (n=16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (in years)</td>
<td>41.2±13.5</td>
<td>42.4±16.7</td>
</tr>
<tr>
<td>Gender (% male)</td>
<td>68.8%</td>
<td>82.5%</td>
</tr>
<tr>
<td>Etiology</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Burns</td>
<td>5</td>
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</tr>
<tr>
<td>Trauma</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Cellulitis</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>Others</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Growth</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scanty</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>Moderate</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Heavy</td>
<td>14</td>
<td>10</td>
</tr>
</tbody>
</table>

The duration of treatment required to eliminate the pseudomonas from the wounds in the acetic acid group was on an average 7 days less than that required by the saline group. The difference was statistically significant.

<table>
<thead>
<tr>
<th>Duration</th>
<th>1% Acetic acid</th>
<th>Saline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Min.</td>
<td>3</td>
<td>8</td>
</tr>
<tr>
<td>Max.</td>
<td>11</td>
<td>17</td>
</tr>
<tr>
<td>Mean</td>
<td>5.5</td>
<td>12.25</td>
</tr>
</tbody>
</table>

Conclusion

- In the 1% Acetic acid group, irrespective of the antibiotic sensitivity, pseudomonas organisms were eliminated within the same time period i.e., 4.5 days.
- In the Saline group, susceptible organisms were eliminated on an average within 11.5 days and those organisms which were multidrug resistant were eliminated by 15.5 days.

Smartphone Photography And 3G Wireless Internet Technology- A Novel Method To Monitor Free Flaps

Dr. Akhila. S, Dr. Pranay Gaikwad, Dr. Cecil Thampachan Thomas, Dr. Rajinikanth J, Dr. Arif Ilhan Tirkey
Department of General Surgery, Unit I, Christian Medical College, Vellore

Introduction

✧ Microvascular reconstruction has become a cornerstone in the management of oral cancers after excision.
✧ Traditionally, free flaps have been monitored clinically by assessing color, turgor, temperature and capillary refill time.
✧ This study attempts to explore the role of Smartphone photography and transfer of images via 3G wireless internet technology as an adjunct to clinical monitoring.

Aims

✧ To study the role of Smartphone photography and 3G wireless technology for monitoring free flap.

Methods

✧ A prospective study was conducted from November 2012 to September 2014 to include all patients who underwent microvascular free flap reconstruction for post-excisional defects of oral cavity with a visible skin paddle.
✧ All flaps were clinically monitored for 120 hours postoperatively. During the same period, clinical photographs of free flaps using a Smartphone were taken with a standardized color card in the frame at 6 hourly intervals and transmitted to the Operating Surgeon(OPS) and another independent surgeon, Observing Surgeon1(ObS1).
✧ The clinical details at these points of time were revealed to OPS and another surgeon, Observing Surgeon 2(ObS2).
✧ The free flaps were assessed using a scoring system and findings were analyzed by the principal investigator (P.I).
✧ Inter-observer variability and accuracy rate of each observer in free flap assessment was analyzed.
✧ The decision on re-exploitation of free flap was made by OPS, although input from the other two surgeons (ObS1&ObS2) was provided when the free flap viability was questionable.

Results

✧ A total of 18 cases were analyzed with 100% free flap survival rate. Five patients were re-explored and salvaged completely.
✧ The indication for operation was neck haematoma in 4 patients and venous thrombosis in one patient.
✧ Venous thrombosis was identified by photograph and later on clinical grounds.
✧ The accuracy rate with the use of photographs was 100%.

Demographics

<table>
<thead>
<tr>
<th></th>
<th>N=18</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (mean, SD)</td>
<td>47.5 ±11</td>
</tr>
<tr>
<td>Male (%)</td>
<td>13 (72%)</td>
</tr>
<tr>
<td>Education-Graduates (%)</td>
<td>14 (78%)</td>
</tr>
<tr>
<td>Smoking (%)</td>
<td>7 (39%)</td>
</tr>
<tr>
<td>Tobacco (%)</td>
<td>9 (50%)</td>
</tr>
<tr>
<td>Co-morbidities (%)</td>
<td>7 (39%)</td>
</tr>
</tbody>
</table>

Conclusions

✧ Smartphone photography with 3G internet technology prove to be a useful adjunct to the clinical monitoring.
✧ The incorporation of this technique in the current protocols of free flap monitoring may help identify impending flap failures even before the arrival of the senior team member.

**INTRODUCTION**

- Near total amputation of the penis is a rare injury in younger men.
- An absent or inadequate penis is a devastating condition with significant psychological and physical impact on a victim's life.

**CASE REPORT**

- 20 year old male
- Rice mill worker
- Accidental entangling of his worn clothes into the milling machine conveyor belt
- Causing avulsion injury to his distal 3/4th penis.

- Following injury underwent debridement.
- His scrotum was intact with residual 4cms stump of penile shaft buried in the scrotal skin.
- Erectile function and ability to void urine were intact.
- Preoperatively supra pubic urinary diversion was done.

**OPERATIVE STEPS**

- Proximal based radial artery forearm flap of 10x15cms designed from the left forearm was used to reconstruct the phallus.
- Femoral artery, great saphenous vein and superficial inferior epigastric vein were dissected on the right groin.
- Anastomosis was achieved:
  - Radial artery → Femoral artery (end to side),
  - Venae committantes → Great saphenous vein (end to end),
  - Basilic vein → Superficial inferior epigastric vein (end to end).

- The creation of neourethra from 10x4cm ulnar skin by tube in tube method.

- The radial part of flap was then wrapped around to neourethra.
- Sutured ventrally to achieve the final tubular appearance of the phallus.

- The neourethra was then anastomosed to the residual urethral stump over 14F silicone urethral catheter.

**POST OPERATIVE RESULT**

- Patient had a healed neo-phallus with adequate length and good urinary stream.

**DISCUSSION**

- Borgora: Tubed abdominal flap
- Gilles: Staged abdominal pedicle
- Orticcocher: Gracilis myocutaneous flap
- Puckett and Montic: The groin flap was first used for penile reconstruction
- Descamps et al: Anterolateral thigh flap
- In 1980, the forearm flap first described by Chang.
- The radial forearm neurovascular fasciocutaneous free flap includes:
  - Volar forearm skin,
  - Underlying adipose tissue,
  - Sensory medial and lateral ante-brachial cutaneous nerves,
  - The basilic and cephalic veins and
  - Part of the deep fascia.

All these are connected to the radial artery and its venae comitantes by a thin intermuscular septum that contains cutaneous perforating vessels.

**CONCLUSION**

- Various tubed and distant flaps for reconstruction of penes were described, the free radial artery forearm flap allows reconstruction of neo-phallus is aesthetically and psychologically more acceptable.
- It enables the patients to void in standing position through distal meatus and phallus that has protective and erogenous sensation and of suitable size that permits successful coitus.

**REFERENCES**

4. Puckett CL, Monticelli R.: Construction of penile tubed flap using a tubed groin flap for the penis and a hydraulic inflation device. J Reconstr Microsurg. 4:1:52-50
ANATOMICAL VARIATION OF THE SAPHENOUS FASCIA IN THE INDIAN POPULATION

Department of Vascular Surgery, Christian Medical College, Vellore
Authors: Vimalin Samuel, Indrani Sen, Dheepak Selvaraj, Edwin Stephen, Sunil Agarwal

Introduction
- The GSV is the longest vein in the body originating from the medial saphenous to the level of groin skin crease.
- Enclosed within a deep plane of the hypodermis, over the muscular fascia.
- "Saphenous eye" sign - sine que non.
- Present understanding - complete from inguinal ligament upto ankle.

Uses
- Venous scan - A good understanding of the anatomy of the saphenous vein and fascia is important in doing a good venous scan.
- Endo Venous procedures - Injection of tumescence within the saphenous fascia is important in achieving a good heat sink effect, adequate anesthesia and good compression of the GSV.
- Therapeutic - Intermittent compression with pressures between 30 - 45 mm Hg has been described as the mainstay of treatment of chronic venous insufficiency. The principle of this treatment is compression of the GSV within the saphenous fascia.

In our experience with the Indian population, the saphenous compartment in not complete in a significant percentage of patients. We thus conducted this study to determine the anatomical variation of the saphenous fascia in the Indian population.

Methods
- Prospective study.
- 50 male patients, total of 100 limbs.
- We analysed the completeness of the saphenous fascia using ultrasonography done by a single member of the team.
- The completeness of the fascia was documented at the mid thigh, above knee, below knee, mid calf and above ankle in both legs.
- When the fascia was found to be incomplete, the distal most level at which the fascia was complete was documented.

Results

<table>
<thead>
<tr>
<th>Location</th>
<th>Mid thigh</th>
<th>Above knee</th>
<th>Below Knee</th>
<th>Mid calf</th>
<th>Upto ankle</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>10</td>
<td>3</td>
<td>13</td>
<td>2</td>
<td>72</td>
</tr>
</tbody>
</table>

Analysis
- 72% of our patients had complete saphenous fascia, extending up to just above the ankle, as described in literature.
- The most common variation of this anatomy was seen in 13% of patients, saphenous fascia ending above the knee.
- 10% of patients had the fascia ending in the mid thigh.
- 3% of patients had the fascia ending above the knee.
- 2% of patients had the fascia ending in the mid calf.

Conclusions
- The saphenous fascia as described in the western literature is complete in most of the population.
- However, there are variations in 28% of the study population.
- The vascular surgeon/sonologist must be aware of the variations in the GSV and saphenous fascia anatomy so as to perform a complete venous scan and endovenous ablation.
- Patients with chronic venous insufficiency requiring compression therapy may require higher pressures of compression if the GSV is not enclosed within the saphenous fascia.

Pitfalls in this study
- All the scans were done by a surgeon. Perhaps a radiologist would have been better experienced in doing the venous scans.
- An ultrasonogram is also known to be operator dependent.
- The role of stereo microscopy in studying the anatomy of the saphenous fascia has been documented. An ultrasound may be inadequate in some patients with subcutaneous edema or plenty of varices.
- A larger number of patients are required for validation of this study.
MALIGNANCY IN THYROID NODULES - A PROSPECTIVE STUDY OF CLINICAL PREDICTORS

Vimalin S, M.J Paul, Department of Endocrine Surgery, Christian Medical College, Vellore.

INTRODUCTION

- 5% - 15% malignancy rate in thyroid nodules.
- The challenge: Which nodule must we excise?
- Lacunae of data published from the Indian subcontinent.

STUDY DESIGN

- All adults detected with thyroid nodules that went on to have surgery.
- Clinical examination - Senior surgeon.
- Ultrasound thyroid - Radiologist.
- FNAC - Palpation guided, Bethesda criteria.
- Gold standard: Histopathology after surgery.
- Malignant - 102 Benign - 93.

RESULTS

- Studied 86 patients, p = 0.55.
- Older age was not a risk factor for malignancy.

USG

- Vascular: 37%, Cystic: 25%, Solid: 24%, Mixed: 15%
- Benign: 25, Malignant: 10%

SIZE

- Size: 0-3 cms: Benign 51, Malignant 13
- Size: 3-6 cms: Benign 29, Malignant 13
- Size > 6 cms: Benign 10, Malignant 12

GENDER

- Male: Benign 33, Malignant 69
- Female: Benign 29, Malignant 64

CONCLUSIONS

- Size, age > 50, male gender and compressive symptoms were not risk factors.
- FNACs done using the palpation technique results in poor NPV and is not of much use in predicting malignancy.
- Using ultrasound as an imaging tool requires experience and a combination of features to predict malignancy.

LIMITATIONS

- Study conducted in a single, tertiary care center with referral bias.
- Lack of a dedicated sonologist and cyto pathologist.
- We have now developed a team of pathologists and surgeons performing ultrasounds.
EFFECTIVENESS OF VIDEO ASSISTED TEACHING PROGRAMME ON THE KNOWLEDGE ATTITUDE AND PRACTICE OF MOTHERS OF LOW BIRTH WEIGHT BABIES REGARDING KANGAROO MOTHER CARE

JEBAKAMAL, EBENEZER ELLEN BENJAMIN, DOROTHY DEVAKIRUBAI
MATERITY NURSING DEPARTMENT, COLLEGE OF NURSING CMC VELLORE

BACKGROUND:
The neonatal period is one of the most vulnerable times in the human life, even though this forms a very small fraction of average life span - Yadav, 1998. Kangaroo Mother Care eases the baby through its new experience of being outside the mother's body for the very first time and helps the baby go through this period of transition with relatively higher degree of comfort and care.
Kangaroo Mother care is humanization of top-class technology and serves as a natural alternative to conventional care through incubators or warmers. It is important to educate mothers on the benefits and technique of Kangaroo Mother Care, as many research findings reveal, how mothers are ignorant of this cost-effective method which can save the lives of their Low birth weight babies.
Since educational video resources are a great way to add stimulus to teaching programme by raising the level of engagement and achievement during lectures, this study was performed to assess the effectiveness of Video assisted teaching programme on the knowledge, attitude & practice of mothers with Low Birth Weight babies regarding Kangaroo mother care.

OBJECTIVES OF THE STUDY:
To determine the effectiveness of the Video assisted teaching programme on the knowledge, attitude and practice of mothers regarding Kangaroo mother care.
To determine the association between the selected demographic and clinical variables on the knowledge, attitude and practice of mothers regarding Kangaroo mother care.

RESEARCH METHODOLOGY
Research Approach
Quantitative Approach
Research Design
Quasi-Experimental Research design
Setting of the Study
The study was performed in the Level I, Level II and Level III Neonatal care unit of CMC, Vellore
Population
All mothers of low birth weight babies with the birth weight between 1200 to 2000gms who fulfilled the inclusion criteria admitted into the neonatal care unit.
Sample Size
The sample size was 100, 50 in the control group and 50 in the experimental group.
Criteria for sample selection
Inclusion criteria
Low birth weight babies after 2 days of admission in the neonatal unit.
Low birth weight babies who are hemodynamically stable.
Mothers of low birth weight babies willing to participate in KMC.
Babies whose birth weight ranges above 1200gms to 2kg irrespective of their gestational age.
Mothers who can speak English, or Tamil.
Mothers who give consent to participate in the study
Exclusion criteria
Low birth weight babies who are hemodynamically unstable.
Low birth weight with major CNS anomalies.
Low birth weight babies whose mothers are not available.
Mothers with mental health problems.

INSTRUMENT:
Mothers were interviewed and data was collected using knowledge questionnaire, likert scale and Observational checklist developed by the researcher.

RESULTS:
In the present study, majority of the babies under both groups had a gestational age of 32 – 36 weeks with 60% in Control group and 60% in the experimental group. These findings are consistent with the study done by Nimalla, Routa and Washington (2006) which showed that NICU infants Mean gestational age was 33.88 weeks.
The study findings reveal that the post test knowledge, attitude and practice of mothers have improved considerably in the experimental group as compared to the control group which is similar to the study conducted by Singhvi, Subash and Kamala (2007).

CONCLUSION:
KMC has an advantage of being a stand-alone and inexpensive method that can be used by mothers in the community, as it is a cost effective way of improving the health outcomes of low birth weight babies. The study concludes that the Video assisted teaching programme is an effective method to improve the knowledge, attitude and practice of mothers regarding Kangaroo Mother Care.

REFERENCES:
INCIDENCE OF HYPERGLYCEMIA IN CHILDREN DURING INDUCTION PHASE OF CHEMOTHERAPY FOR ACUTE LYMPHOBLASTIC LEUKEMIA

INTRODUCTION: Acute lymphoblastic leukemia (ALL) is one of the commonest childhood cancers. Hyperglycemia is a known complication during various stages of chemotherapy with a reported overall incidence varying from 3.8–87%. Known risk factors for development of hyperglycemia during ALL chemotherapy include use of corticosteroids, L-Asparaginase, and hyperleukocytosis at diagnosis as well as stress of the disease process itself. In a pilot study done in our institution the incidence of symptomatic hyperglycemia in children with ALL was 4%, of which 67% presented in diabetic ketoacidosis. In the current study, children with ALL admitted in our unit between June 2013 and March 2014 were monitored during the induction phase of chemotherapy for development of hyperglycemia.

METHOD: Glycosylated haemoglobin (HbA1C) was done at admission. Random blood glucose was monitored once a day from days 1-35 of induction chemotherapy.

PILOT STUDY 2007-2013

<table>
<thead>
<tr>
<th>NUMBER OF CHILDREN TREATED FOR ALL BETWEEN 2007-2013</th>
<th>300</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHILDREN WITH SYMPTOMATIC HYPERGLYCEMIA</td>
<td>12 (6 MALES)</td>
</tr>
<tr>
<td>AGE AT DIAGNOSIS OF HYPERGLYCEMIA</td>
<td>4-14 years</td>
</tr>
<tr>
<td>TYPE OF ALL</td>
<td>PRE B ALL 9, T CELL ALL 3</td>
</tr>
<tr>
<td>ONSET OF HYPERGLYCEMIA IN RELATION TO THE PHASE OF CHEMOTHERAPY</td>
<td>INDUCTION 3, DELAYED INTENSIFICATION 8, RECONSIDERATION 1</td>
</tr>
<tr>
<td>PRESENTATION OF HYPERGLYCEMIA</td>
<td>DKA 8, ABDOMINAL PAIN 4, LETHARGY 3, POLYURIA, POLYDIPSIA 1</td>
</tr>
<tr>
<td>RBS AT DIAGNOSIS</td>
<td>240-1069 mg/dl</td>
</tr>
<tr>
<td>TREATMENT WITH INSULIN</td>
<td>12</td>
</tr>
<tr>
<td>RECURRENT/HYPERGLYCEMIA/INSULIN THERAPY &gt;7 DAYS</td>
<td>12</td>
</tr>
<tr>
<td>KNOWN RISK FACTORS FOR HYPERGLYCEMIA</td>
<td>STEROID USE 12, PANCREATITIS 1</td>
</tr>
</tbody>
</table>

RECRUITMENT OF SUBJECTS

7% RECRUITED
46% DECLINED ALL TKT
47% DECLINED PARTICIPATION

DURATION BETWEEN START OF TREATMENT AND DEVELOPMENT OF HYPERGLYCEMIA

<table>
<thead>
<tr>
<th>NUMBER OF DAYS STEROIDS GIVEN</th>
<th>NUMBER OF DAYS L- ASPARAGINASE GIVEN</th>
</tr>
</thead>
<tbody>
<tr>
<td>PATIENT 1</td>
<td>13</td>
</tr>
<tr>
<td>PATIENT 2</td>
<td>7</td>
</tr>
<tr>
<td>PATIENT 3</td>
<td>17</td>
</tr>
</tbody>
</table>

GLYCEMIC STATUS

- 75% EUGLYCEMIA
- 10.7% IMPAIRED GLUCOSE TOLERANCE
- 14.3% HYPERGLYCEMIA

COMPARISON BETWEEN HYPERGLYCEMIC AND EUGLYCEMIC CHILDREN

<table>
<thead>
<tr>
<th>HYPERGLYCEMIA</th>
<th>EUGLYCEMIA</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGE</td>
<td>12.33 (4-14)</td>
</tr>
<tr>
<td>SEX (M:F)</td>
<td>2:1</td>
</tr>
<tr>
<td>WBC COUNT</td>
<td>2,99,066 (15,000-7,57,400)</td>
</tr>
<tr>
<td>FAMILY HISTORY OF DIABETES MELLITUS</td>
<td>YES 33.3%</td>
</tr>
<tr>
<td>HbA1C</td>
<td>5.97% (5.50-6.40%)</td>
</tr>
<tr>
<td>AVERAGE NUMBER OF INFECTIONS</td>
<td>1 (0-2)</td>
</tr>
</tbody>
</table>

DEMOGRAPHY

- TOTAL NUMBER: 28
- MALE: FEMALE 18:10
- AGE DISTRIBUTION:
  - <12 months: 4 (14.3%)
  - 1 year - 10 years: 19 (63.3%)
  - > 10 years: 5 (17.5%)

SUMMARY

- Incidence of hyperglycemia was 10.71%.
- 14.3% of children had impaired glucose values.
- Only 1 of the 3 children with hyperglycemia was symptomatic with abdominal pain.
- One child needed insulin for 16 days. The other two children had transient hyperglycemia.
- Of the four children with initial WBC count >2 Lakh/cumm, one developed hyperglycemia while two children had impaired glucose values.

RELATIONSHIP BETWEEN HYPERLEUKOCYTOSIS AND HYPERGLYCEMIA

CONCLUSIONS AND RECOMMENDATIONS

- Children on ALL chemotherapy need regular monitoring of blood sugar level, particularly during the D1 phase, to prevent the dreaded complication of DKA.
- No children in this study developed hyperglycemia, as blood sugars were being monitored serially.
- Older age and higher WBC count at diagnosis are risk factors for developing hyperglycemia.
- HbA1C values at the start of therapy is a good monitoring tool.
- Children who develop hyperglycemia during induction therapy should be monitored during subsequent phases of therapy.
- Our results did not attain statistical significance due to the small sample size. Hence studies with larger numbers are needed to corroborate this data.
# Neurological manifestations of scrub typhus and determinants of outcome

**Swaratika Majumdar, George M. Varghese, Ravikar Ralph**

**Department of General Medicine, C.M.C. Vellore.**

## BACKGROUND
Scrub typhus accounts for 47.5% of patients with febrile illness requiring admission in our institute. It presents as a febrile illness with multisystem involvement and sepsis syndrome.

Meningitis and meningencephalitis, seen in nearly one-fifth of the cases, are the most well-known neurological manifestation of scrub typhus.

Focal neurological involvement like cranial nerve dysfunction, cerebellar, movement disorders and demyelinating disorders associated with scrub typhus, though reported, are rarely seen.

Though commonly attributed to vasculitis, pathogenic basis of nervous system involvement is largely unknown. Appearance of focal deficits typically in the second week of illness suggests an antibody-mediated phenomenon.

A comprehensive clinical description of neurological involvement in scrub typhus is lacking and warranted a detailed study.

## AIM and OBJECTIVES

**AIM:**
Estimate the incidence and describe the pattern of neurologic involvement among patients with scrub typhus admitted to Christian Medical College, Vellore.

**OBJECTIVES:**
1. To determine the cumulative incidence of neurological manifestations in scrub typhus
2. To describe the spectrum, temporal profile and sequence of neurological manifestation
3. Analyse factors predicting neurological manifestations

## METHODOLOGY

Single centre observational prospective cohort study

Included patients above 18 years with scrub typhus diagnosed either by presence of eschar or scrub IgM positivity or both.

### Methodology: Sequence of events

1. Patients fulfilling inclusion criteria for scrub typhus
2. Physical examination within 24 hours
3. Laboratory data collected within 24 hours
4. CSF analysis done at physician discretion
5. MRI Brain if focal deficits are present
6. Brief neurological examination every 48 hours till discharge/death
7. Follow-up at 2-4 weeks after discharge

## RESULTS

198 patients fulfilled inclusion criteria. Of these, 54 patients had new neurological deficits attributed to scrub typhus.

Cumulative incidence of neurological manifestations in scrub typhus was 27.27%.

Median time to development of manifestations from the onset of illness was 8 days (Range 1-28 days).

Time to resolution of neurological manifestation ranged from one to thirty three days with median duration of 4 days. 2 patients had persistent neurological sequelae at 6 months.

### Neurological manifestations in patients with scrub typhus

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Number of patients with neurological manifestation (n/N)</th>
<th>Time to development of manifestations from the onset of illness (median [Range])</th>
<th>Time to resolution of neurological manifestations (Days [Range])</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seizure</td>
<td>13 (6.56)</td>
<td>7</td>
<td>-</td>
</tr>
<tr>
<td>Altered sensorium</td>
<td>40 (20.5)</td>
<td>8</td>
<td>4 (1-25)</td>
</tr>
<tr>
<td>Aseptic meningitis</td>
<td>21 (10.60)</td>
<td>10</td>
<td>4.5 (1-20)</td>
</tr>
<tr>
<td>Meningoaensephalitis</td>
<td>17 (8.59)</td>
<td>7</td>
<td>1 (1-3)</td>
</tr>
<tr>
<td>Ophthalmos myelonic</td>
<td>2 (1.01)</td>
<td>5</td>
<td>14 (9-24)</td>
</tr>
<tr>
<td>New onset tremors</td>
<td>12 (6.06)</td>
<td>8.5</td>
<td>8.0 (2-24)</td>
</tr>
<tr>
<td>Rigidity</td>
<td>11 (5.56)</td>
<td>8</td>
<td>6.5 (2-31)</td>
</tr>
<tr>
<td>Ataxia</td>
<td>6 (3.05)</td>
<td>9</td>
<td>6.5 (1-24)</td>
</tr>
<tr>
<td>Cerebellitis</td>
<td>7 (3.54)</td>
<td>7</td>
<td>11.5 (2-24)</td>
</tr>
<tr>
<td>Cranial nerve palsy</td>
<td>5 (2.53)</td>
<td>9</td>
<td>2 (1-15)</td>
</tr>
<tr>
<td>Polymyopathy</td>
<td>3 (1.53)</td>
<td>17</td>
<td>19 (19-188)</td>
</tr>
<tr>
<td>ADEM</td>
<td>1 (0.51)</td>
<td>28</td>
<td>&gt;180</td>
</tr>
<tr>
<td>CVA</td>
<td>2 (1.01)</td>
<td>8</td>
<td>&lt;1</td>
</tr>
</tbody>
</table>

### Description of rare neurological manifestations in with scrub typhus

<table>
<thead>
<tr>
<th>Manifestation</th>
<th>Hemiparesis, aphasia</th>
<th>Right hemiparesis</th>
<th>Asymptomatic sensorimotor (\uparrow)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration of fever (days)</td>
<td>8</td>
<td>7</td>
<td>10</td>
</tr>
<tr>
<td>Onset of illness</td>
<td>28&lt;sup&gt;th&lt;/sup&gt; day</td>
<td>10&lt;sup&gt;th&lt;/sup&gt; day</td>
<td>17&lt;sup&gt;th&lt;/sup&gt; day</td>
</tr>
<tr>
<td>Time to resolution</td>
<td>Persistent deficits</td>
<td>&lt;6 hours</td>
<td>Persisting, non-progressive</td>
</tr>
<tr>
<td>CSF-TC/×1000</td>
<td>15/99</td>
<td>-</td>
<td>12/98</td>
</tr>
<tr>
<td>CSF Prot</td>
<td>53</td>
<td>-</td>
<td>12</td>
</tr>
<tr>
<td>Imaging</td>
<td>MRI: 1/6 ADEM</td>
<td>MR-(\uparrow)</td>
<td>MR-(\downarrow) with thickening of (\uparrow)</td>
</tr>
<tr>
<td>MR-(\uparrow)</td>
<td>-</td>
<td>-</td>
<td>MR-(\uparrow)</td>
</tr>
</tbody>
</table>

### Predictors of neurological outcome:

- Univariate analysis showed male gender, headache, lymphadenopathy, elevated creatinine and jaundice to be significant predictors of neurological outcome
- Multivariate analysis done for age, gender and occupation adjusted model showed only headache and elevated creatinine to be independent predictors of neurological outcome

### CONCLUSION

We found 27.27% of patients with scrub typhus to have neurological manifestations.

Cranial nerve dysfunction and cerebellitis were the most common focal deficits observed.

We found cases of ADEM, cerebral infarction and ophthalmos-myelonic which have been described in isolated case reports.

Persistent encephalopathy and polyneuropathy were noted for the first time in our study.

Manifestations commonly occurred in the second week of illness. All but 2 cases had complete resolution. Deficits due to ADEM and neuropathy persisted beyond six months.

Male gender, elevated creatinine and jaundice were predictors of neurological outcome.

Onset of focal syndromes typically in the late second week of illness suggests presence of antibodies against neuronal antigens which require further research.

### REFERENCES

Introduction:
- Stereotactic radiosurgery (SRS) is a non-invasive technique which aims to precisely deliver a high dose to the target volume in a single fraction and spare surrounding healthy tissue.
- SRS treatment is most frequently carried out using x-knife, gamma knife or cyberknife.
- The linac based SRS technique uses multiple non-coplanar arcs of circular beams or dynamically shaped beams or multiple static beams converging on to the machine isocenter, which is stereotactically placed at the center of the target volume.

Aim:
- To evaluate and compare the dosimetric performance of 3mm micro MLC (BrainLAB) with the 5mm MLC (Varian Millennium) in the stereotactic radiosurgery treatment of intracranial lesions.
- To analyze the treatment plans generated by these two MLCs to provide insight into the possible advantages of one compared with the other and the possible clinical implications.

What is Conformity Index?
- Conformity indices are used to compare competing plans, evaluate treatment technique, and assess clinical complications by quantifying the dose conformity to a target volume.
- SRS treatment plan require a high degree of conformity to minimize damage to the tissues that surround the target area.
- Clinically, this conformity is evaluated using a combination of visual inspection of the plan and evaluation of the dose-volume histograms.

Methods & Materials:
- Seventeen patients previously treated for acoustic schwannoma in our institution were retrospectively planned using Eclipse planning system with 3mm mMLC and 5mm MLC systems to evaluate the conformity indices, target volume dose coverage, organs at risk dose and the dose-volume histogram.

Results and Discussion:
- In this work, target volumes ranging from 0.6cm³ to 7.6 cm³ were analyzed. The conformity index was found to be from 1.12 to 2.17 (median 1.56) for the 3mm mMLC and 0.97 to 1.65 (median 1.3) for the 5mm MLC.
- An index situated between 1 and 2 except one index (2.17) which is considered to be a minor violation. In 5mm MLC plan, for two cases the indices were 0.97 and 0.99 which indicated the quality of conformation of the plan.
- The standard deviations of CI are 0.28, 0.92 and 0.18 for 3mm MLC, 2mm MLC and the difference between two MLCs respectively.
- The doses of Organ At Risk (OAR) such as eye, optic nerve, chiasm and brainstem in the plans were analyzed and 80% & 50% of dose coverage of target volume were measured.
- The measured target and OAR doses from the plans generated using the two MLCs were found to be similar and within the tolerance limits.

Conclusion: The target conformity of 5-mm MLC (Varian) and 3-mm micro-MLC (BrainLAB) were compared and was found to be approximately same. From this result we conclude that we can use 5mm MLC for SRS treatment when the linear accelerator with 3mm mMLC is unavailable for clinical use.

References:

Acknowledgment: This study was supported by the Department of Radiotherapy. Special thanks to my Guide Dr. B. Paul Ravindran who motivated and helped me in this study.
Introduction:

- Acute Myeloid Leukemia (AML) is the commonest adult leukemia with very poor Event Fress Survival/Overall Survival.
- Daunorubicin (Dnr) has been the choice of anthracycline in the induction chemotherapy of AML along with Cytastrabin for the past 4 decades. However, Drug resistance and relapse of the disease has been the major concern in the success of AML therapy.

- Elevated expression of ABC transporters (ABCR1 and ABC2) and metabolizing genes (CBR1 and CBR3) have been shown to influence treatment outcome in AML.
- Several factors, including pharmacokinetics (PK) of Dnr could have impact on treatment outcome as pharmacokinetic effects might limit the amount of drug that reaches the tumor.
- In this study, we analyzed the factors influencing plasma PK of Daunorubicin and its major metabolite Daunorubicinol (DOL) in AML patients receiving induction chemotherapy and compared PK parameters with clinical outcome (relapse).

Patients and Methods

Sixty four adult De-novo AML patients were included in the study after informed consent.

- Pharmacokinetic sampling:
  - Dnr was given as 1 hour bolus infusion at a dose of 60mg/m2/day for the first 3 days of induction chemotherapy. Peripheral blood were collected at different time points on the first day of induction chemotherapy.
  - Population Pharmacokinetic analysis:
    - Plasma levels of Dnr and DOL were measured using high performance liquid chromatography coupled with fluorescence detector. Population PK was analyzed by nonlinear mixed effects modeling using Monolix version 4.3.2.
    - Screening of polymorphisms in Dnr transporters and metabolizing genes
    - Common single nucleotide polymorphisms in ABCR1, ABC2, CBR1 and CBR3 genes were screened by PCR followed by either sequencing or restriction digestion.
    - In vitro cytotoxicity assay.
      - In vitro cytotoxicity of the primary AML cells to Dnr was assessed by MTT cell viability assay and IC50 was calculated using ADAPTS software.

Results

Sixty four AML patients receiving induction therapy with Ara-C Dnr were included in the study (36 male and 28 female).

Pharmacokinetic parameters of Dnr and DOL showed wide inter-individual variation among AML patients and listed in Table 1.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Dnr PK parameters</th>
<th>DOL PK parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC (ng.h/ml)</td>
<td>0.049-2.165 (0.227)</td>
<td>1.311-47.678 (6.812)</td>
</tr>
<tr>
<td>Clearance (L/hr)</td>
<td>41.561-2014.6 (404.86)</td>
<td>2.30-57.202 (14.99)</td>
</tr>
<tr>
<td>Volume of distribution (L)</td>
<td>1.6228-266.72 (26.9285)</td>
<td>5.91-286.05 (32.4395)</td>
</tr>
<tr>
<td>KL (1/hr)</td>
<td>2.546-31.937 (14.667)</td>
<td>5.82-13.621 (8.416)</td>
</tr>
<tr>
<td>Cmax (ng/ml)</td>
<td>0.09-2.766 (0.588)</td>
<td>0.16-0.96 (0.406)</td>
</tr>
</tbody>
</table>

Patients with variants of SNPs rs20572 (exon3) and rs9024 (3'UTR in CBR1) which were in complete linkage disequilibrium had a trend towards significantly higher Dnr AUC [median:0.2711; (0.04903-2.165) vs. median 0.2117; (0.0546-0.9036); p=0.06] than patients with wild-type (Figure 1).

Plasma pharmacokinetics of Dnr and DOL correlates with clinical outcome:

Among the 64 patients, complete remission (CR) was documented in 42 (65%) of which, 31% relapsed eventually [median time to relapse 328 (104-864) days].

Exposure and Clearance of Dnr were significantly lower and higher respectively in patients who relapsed (Table 2).

Also, IC50 of Dnr was higher in patients who relapsed (median:0.7 (0.02-1.46) vs. median: 0.23 (0.007-1.11); p=0.056) than in patients who did not relapse.

Table 2

<table>
<thead>
<tr>
<th>Pharmacokinetic parameters</th>
<th>Relapse</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No (n=29)</td>
<td>Yes (n=13)</td>
</tr>
<tr>
<td>Dnr AUC (ng.h/ml)</td>
<td>0.31 (0.1-1.74)</td>
<td>0.17 (0.049-2.165)</td>
</tr>
<tr>
<td>DOL AUC (ng.h/ml)</td>
<td>4.99 (1.3-16.5)</td>
<td>8.67 (1.6-20.1)</td>
</tr>
<tr>
<td>Dnr CL (L/hr)</td>
<td>329.31 (429.9-445.5)</td>
<td>546.54 (415.5-1632)</td>
</tr>
<tr>
<td>DOL CL (L/hr)</td>
<td>19.7 (6.1-57)</td>
<td>9.8035 (4.9-56)</td>
</tr>
<tr>
<td>Cmax (ng/ml)</td>
<td>237 (32-1692)</td>
<td>101 (31-415)</td>
</tr>
</tbody>
</table>

Conclusion:

- This is the first population PK report in AML which is designed to evaluate various factors which might influence Dnr and DOL PK in AML and our results suggest that plasma PK of Dnr influences clinical outcome in AML.
- Lower systemic exposure of Dnr in relapsed patients suggests that amount of drug required to completely eradicate the leukemic cells is not achieved and escalating dose might be an option to decrease relapse rates.
- Cellular pharmacokinetics of Dnr in these patients is ongoing in the lab, which will be more informative and help in prognosticating and dose adjustment for better treatment outcome.


The authors have no relevant conflicts of interest to disclose.
Evaluation of Line probe assay for the diagnosis of drug resistant tuberculosis

Joy S Michael, Marilyn Ninah, Priscilla Rupali, DJ Christopher
Christian Medical College, Vellore

Introduction

Tuberculosis has become a global crisis and though it has affected all countries, the greatest burden of the disease is borne by low and middle-income countries like India. Multi drug resistant tuberculosis (MDR-TB) which is defined as the resistance of Mycobacterium tuberculosis to the two main first line drugs in therapy, Rifampicin and isoniazid, has been a menace in the treatment of tuberculosis for over two decades.

The World Health Organisations (WHO) global tuberculosis report for 2013, estimates that India accounts for 64,000 cases of MDR-TB among notified patients with pulmonary tuberculosis.

Owing to the burden of multidrug resistant tuberculosis, molecular techniques have been approved by the WHO for the rapid diagnosis of the same.

Line probe assay was recommended in 2008 for diagnosis of smear positive pulmonary tuberculosis and Xpert MTB/RIF Assay was recommended in 2011 in for diagnosis of pulmonary TB and in addition for diagnosis of extrapulmonary and childhood TB in 2013.

However, few techniques have been used successfully on clinical samples, more especially smear negative samples. We aimed to evaluate a line probe assay for suspects of pulmonary tuberculosis regardless of their smear status.

Objectives

The objectives of the study were to compare the performance of line probe assay (GenoType MTBDRplus v2) with solid/liquid culture method, as well as the Xpert MTB/RIF assay for an early diagnosis of multidrug resistant tuberculosis (MDR-TB) in sputum samples.

Material and Methods

This was a prospective, diagnostic study conducted at Christian Medical College, Vellore, a tertiary care centre in South India.

The Mycobacteriology laboratory is an RNTCP accredited laboratory to perform culture and susceptibility testing.

Consecutive suspects of multidrug resistant pulmonary tuberculosis patients from January 2013 to August 2013 were enrolled in this study.

The line probe assay (LPA) was performed on all samples that fit the inclusion criteria and for whom a conventional solid (Li) and/or liquid culture (MGIT960) drug susceptibility testing (DST), and an Xpert MTB/RIF assay had been performed on.

Inclusion criteria :
1. Age >18 years
2. Signs and symptoms > 2 weeks of pulmonary tuberculosis
3. Previously treated patients (both new and follow up) or other reasons for suspecting MDR-TB
4. Culture and sensitivity testing should have been ordered
5. All new cases of tuberculosis not responding to first line ATT
6. All failures of new tuberculosis cases
7. Smear positive, at the 4th month of treatment
8. All contacts of MDR-TB cases with symptoms of pulmonary TB

Exclusion criteria :
1. Children less than 18 years of age
2. Patients with extra pulmonary TB

Results & Discussion

Of the 87 patients that were recruited, 71% were male and 29% were female in the third decade of life.

1/3rd of our patient population in our study were from Tamil Nadu and 1/4th were from West Bengal.

LPA was positive on 4 additional samples that were negative on culture. All 4 were clinical diagnosed as PTB and on treatment. 2 out of these 4 were also smear positive. All 4 samples were also positive by the Xpert(MTB/RIF) assay which indicates they were probably true positives.

Nine samples were LPA negative but culture positive. Five out of the 9 were also smear positive. All 9 were positive by Xpert(MTB/RIF) assay.

Thirteen samples were not detected by the LPA, whereas they were detected as M tuberculosis, by the Xpert(MTB/RIF) assay. Of these 13, 9 of the samples were smear negative or smear scanty samples. This was probably due to the lower detection limit of Xpert(MTB/RIF) assay.

One sample was Rifampicin resistant by the LPA, but was susceptible by conventional DST and Xpert(MTB/RIF) assay.

3 samples were detected by conventional DST as Isoniazid (INH) resistance (one of whom had monoresistance) but not by LPA. Literature shows that resistance for INH coded for by several mutations. Some of which such as oxyR-epsC and kasa gene and a mutation at codon 463 are not picked up by LPA.

Thirteen smear positive samples were not detected by LPA, 9 of these patients had scanty AFB in their smear. Eight of these grew M tuberculosis on culture and 10 were detected by the Xpert(MTB/RIF) assay.

The turnaround time was 6 hours for LPA, as compared to conventional culture and DST (8-12 weeks), though it is longer than for the Xpert(MTB/RIF) assay. However, it has the added advantage of detecting Isoniazid resistance.

Conclusion

Though LPA is rapid nucleic acid amplification test for diagnosis of drug resistant tuberculosis from smear positive tuberculosis, in a clinical diagnostic setting it has a moderate sensitivity of 80.3% versus microscopy.

It is however highly specific for diagnosing tuberculosis as well as detecting INH and Rif resistance.

Further studies on LPA with 2nd line drugs should be evaluated which would be useful for diagnosis of extensively drug resistant tuberculosis

References

1. WHO policy statement: molecular line probe assays for rapid screening of patients at risk of multidrug-resistant tuberculosis: http://www.who.int/tb/laboratory/line_probe_assays/en/

Incidence of necrotizing enterocolitis (NEC) among preterm babies born after preterm premature rupture of membranes (PPROM) in mother
Rajeev Reddy, Sridhar S, Vijay Gupta, Kallol George, Anil Kuvempilla, Anil Kumar Jana
Department of Neonatology, Christian Medical College, Vellore

INTRODUCTION
> Prematurity is a risk factor for development of NEC(1)
> NEC occurs in 1 to 3 per 1000 live births and 1.0 to 7.7 percent of admissions to neonatal intensive care units (NICUs) (2).
> Early recognition and aggressive treatment of NEC has improved clinical outcomes.

OBJECTIVES AND STUDY DESIGN
Objective: 1) To estimate the incidence of NEC among children born after preterm premature rupture of membranes (PPROM) in mother.

2) To compare the risk (relative risk) of developing NEC after preterm premature rupture of membranes with other causes of preterm births.

>. Design: Prospective cohort study of all preterm born over 11 months.
Nestecase-control study with babies with NEC as “cases” and those without NEC as “controls” in a 1:2 ratio.

> Subjects: All preterm babies born at a gestational age less than 37 weeks at Christian Medical College, Vellore.

RESULTS
• Between December 2012 and December 2013, the number of preterm deliveries in our hospital was 1028.
• Among them, the number of neonates with PPROM was 236(23%) and the rest were identified as non-PPROM group.
• The incidence of NEC in PPROM was 4.7% as compared to 2.3% in preterm without PPROM (p= 0.413)

Mortality in babies with PPROM and those without PPROM:

<table>
<thead>
<tr>
<th>Mortality</th>
<th>PPROM (n=236)</th>
<th>Non PPROM (n=792)</th>
<th>RR</th>
<th>95% CI</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>NEC</td>
<td>4.7%</td>
<td>2.3%</td>
<td>0.857</td>
<td>0.593-1.240</td>
<td>0.413</td>
</tr>
<tr>
<td>HMD</td>
<td>3.8%</td>
<td>3.6%</td>
<td>1.065</td>
<td>0.804-1.404</td>
<td>0.655</td>
</tr>
<tr>
<td>EOS</td>
<td>1.7%</td>
<td>1.5%</td>
<td>1.002</td>
<td>0.866-1.159</td>
<td>0.981</td>
</tr>
<tr>
<td>LOS</td>
<td>1.7%</td>
<td>1.9%</td>
<td>0.810</td>
<td>0.608-1.052</td>
<td>0.387</td>
</tr>
<tr>
<td>Survival</td>
<td>6.4%</td>
<td>4.7%</td>
<td>0.940</td>
<td>0.711-1.242</td>
<td>0.662</td>
</tr>
</tbody>
</table>

In the nested case control study, PPROM had an OR of 1.11 as risk factor for NEC (not significant).
• There was also trend towards higher incidence of early and late onset sepsis with NEC but there was no change in survival.

Nested case control study: Risk factors for NEC:

<table>
<thead>
<tr>
<th>Morbidity</th>
<th>NEC (n=29)</th>
<th>No NEC (n=58)</th>
<th>OR</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPROM</td>
<td>37.9%</td>
<td>27.6%</td>
<td>1.113</td>
<td>0.70-1.75</td>
<td>0.643</td>
</tr>
<tr>
<td>EOS</td>
<td>13.8%</td>
<td>0%</td>
<td>2.011</td>
<td>0.737-5.486</td>
<td>0.172</td>
</tr>
<tr>
<td>LOS</td>
<td>20.7%</td>
<td>1.7%</td>
<td>0.867</td>
<td>0.107-7.046</td>
<td>0.894</td>
</tr>
<tr>
<td>Survival</td>
<td>82.8%</td>
<td>91.4%</td>
<td>1.207</td>
<td>0.625-2.333</td>
<td>0.575</td>
</tr>
</tbody>
</table>

CONCLUSIONS
There was a trend towards increased incidence of necrotizing enterocolitis among children born after PPROM.

Similarly, an increased trend towards causation of NEC was observed in babies born to mothers with PPROM (P=0.413).

Early and better better antibiotic coverage of mothers with PPROM and More conservative feed management in babies after PPROM may help prevent NEC.

REFERENCES
**Therapeutic Drug Monitoring of Levetiracetam and Lamotrigine: Is there a Need?**

Girish S Naik¹, Rohit Kodagali², Binit S Mathew⁴, Denise H Fleming¹, Maya Thomas³, Vivek Mathew², Ratna Prabha Gupta¹

¹Department of Pharmacology and Clinical Pharmacology, CMC Vellore, TN, India
²Department of Neurology, CMC Vellore, TN, India

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**Introduction**

Newer anticonvulsants are often used as adjunctive treatment when patients are refractory to treatment with the older anticonvulsants and also used as monotherapy. Lamotrigine has high inter-individual pharmacokinetic variability which is significant when other anticonvulsant inducer or inhibitor medications are co-prescribed. (1) We focused on determining the proportion of patients on lamotrigine and levetiracetam who achieved concentrations within the reference range and the effect of anticonvulsant co-medication and age on the clearance of lamotrigine and levetiracetam.

**Objectives**

This study was an assessment of the therapeutic drug monitoring (TDM) data collected for levetiracetam and lamotrigine from a clinical setting. The proportion of patients in relation to the reference ranges for serum concentrations of lamotrigine and levetiracetam were estimated and the influence of age and anticonvulsant co-medication on their clearances were studied.

**Methods**

Information on levetiracetam (2011-2013) and lamotrigine (2008-2013) dose, trough concentration, age, sex, body weight and anticonvulsant co-medications prescribed was obtained from the TDM register and archived medical records. Patients were categorized into 4 groups based on anticonvulsant co-medication and further divided into 3 sub-groups based on age. Apparent clearance (CL/F) was compared across subgroups.

In each sub-group, the proportion of patients who achieved trough concentrations in the reference range for levetiracetam and lamotrigine was computed. Apparent clearance (CL/F) was compared across subgroups by one way ANOVA and factors which significantly predicted CL/F were identified by stepwise multiple linear regression.

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**Results**

Overall, 348 (330 patients) and 706 (493 patients) requests for levetiracetam and lamotrigine were included in the analysis. The figures (Figure 1 and 2) depict the proportion of requests in the reference range including the repeat requests for lamotrigine and levetiracetam respectively, while Table below shows the group-wise split of the requests falling within, below and above the therapeutic range for lamotrigine and levetiracetam.

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**Discussion**

The impact of age and co-medication on apparent clearance of levetiracetam was similar to an earlier reported study with limited number of patients. (2) For Lamotrigine, our findings were similar to those reported in earlier studies. (1) In our study, ~72% TDM requests for lamotrigine had trough concentrations within compared to a previous study in India in which only 43% within range. (3)

**Conclusion**

These findings emphasize the need to monitor lamotrigine and levetiracetam especially in children, as well as anticonvulsant co-medication prescribed in the treatment regimen.

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**References**

2. Mathew BS et al, Neuro India. 2012;60(2):146-149

**Acknowledgements**

The authors would like to thank the staff of Medical Records Department (MRD) for providing timely access to patient charts.
A Randomized Controlled Trial Comparing the Effect of Fortification of Human Milk with an Infant Formula Powder on the Growth of Very Low Birth Weight Babies

Vijay Gupta, Grace Rebekah, Yesudoss Sudhakar, Atanu Kumar Jana, Manish Kumar, Sridhar Santhanam, Anil Kuruvilla, Nirajan Thomas

Departments of Neonatology, Bio-statistics and Clinical Biochemistry, Christian Medical College Vellore

CTR/2013/11/004149

BACKGROUND

- Fortification of human milk has been recommended for Preterm infant nutrition for adequate catch up growth.
- In India only one HMF is available; LACTODEX HMF.
- Limitations:
  - Costly (75 – 80 Rs/day Rs 11.5 per 2 gm sachet)
  - Higher Vitamin A content
  - No Iron
  - Unavailability in smaller cities and towns

HYPOTHESIS

- Can commercially available infant formula which are far cheaper and widely available be used with equal benefit for human milk fortification?

AIM

- To study the effects of human milk fortification with an infant formula on the growth and biochemical parameters of preterm VLBW babies

MATERIAL AND METHODS

<table>
<thead>
<tr>
<th>Variable</th>
<th>Standard arm</th>
<th>Fortification arm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth weight, g</td>
<td>32 (33.2±3.3)</td>
<td>33 (33.2±3.4)</td>
</tr>
<tr>
<td>Gestational age, wk</td>
<td>29 ± 1.9</td>
<td>29 ± 1.9</td>
</tr>
<tr>
<td>Male</td>
<td>26</td>
<td>26</td>
</tr>
<tr>
<td>Female</td>
<td>26</td>
<td>26</td>
</tr>
<tr>
<td>Length at birth, cm</td>
<td>50 (50.2±2.4)</td>
<td>50 (50.2±2.4)</td>
</tr>
<tr>
<td>Head circumference at birth, cm</td>
<td>20 (20.1±2.2)</td>
<td>20 (20.1±2.2)</td>
</tr>
<tr>
<td>Birth as preterm</td>
<td>27 (27.4±2.4)</td>
<td>27 (27.4±2.4)</td>
</tr>
<tr>
<td>Neonatal illness</td>
<td>51 (51.6±0.9)</td>
<td>51 (51.6±0.9)</td>
</tr>
<tr>
<td>Maternal risk factors</td>
<td>38 (38.2±3.2)</td>
<td>38 (38.2±3.2)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>39 (39.5±3.2)</td>
<td>39 (39.5±3.2)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>38 (38.2±3.2)</td>
<td>38 (38.2±3.2)</td>
</tr>
<tr>
<td>Antenatal admission</td>
<td>38 (38.2±3.2)</td>
<td>38 (38.2±3.2)</td>
</tr>
</tbody>
</table>

Sample size calculation (Based on Indian study from Chandigarh):
For a study Power of 80% with Alpha error of 5% for a 2 tailed test, target sample size of 52 was calculated in each arm. Total 58 babies were recruited in each arm (to account for 10% loss to follow up)

Student's t-test for continuous variables and Pearson's chi-square and Fisher's exact test for categorical variables were used and P value of <0.05 was considered significant. Analysis was on an Intention to treat basis.

The primary outcome measure: weight gain in g/kg/day from the date of randomization until the baby reached 1800 g.

The secondary outcome measure:
- Linear growth (length gain in cm/week)
- Head circumference increase (Head circumference gain in cm/week)
- Duration of hospital stay
- Morbidity: feed intolerance, sepsis and necrotising enterocolitis

RESULTS

- The Institution review board and Ethics committee approved the study.
- Baseline demographic variables were comparable in both the arms (Table 1)
- Total 104 (89.8%) babies (52 in each arm) completed the study

PRIMARY OUTCOME: Significance weight gain in the fortification group as compared to the standard group (16.4±3.0 g/kg/day vs 15.8±3.1 g/kg/day, p<0.001). Fig 2 and Table 2. Secondary outcome: Linear growth, Head circumference gain in cm/week and duration of hospital stay though improved but not statistically significant.

CONCLUSION/KEY MESSAGE

- Fortification with infant milk powder can be a useful alternative for human milk fortification for feeding preterm VLBW babies in low resource settings.
Development of a Limited Sampling Strategy for Mycophenolate Mofetil in Adult Patients with Lupus Nephritis

Rohit Kodagali1, Binu S Mathew1, Denise H Fleming1, Gopal Basu2, V Tamilarasi2, Ratna Prabha Gupla2, Kalpana Ernest1

1Department of Pharmacology and Clinical Pharmacology, CMC Vellore, TN, India
2Department of Nephrology, CMC Vellore, TN, India

Introduction
Mycophenolate Mofetil (MMF), the immunosuppressive agent most commonly used to treat renal transplant patients and patients with certain autoimmune diseases such as Systemic Lupus Erythematosus (SLE). Due to the high inter-dose variability in drug exposure, therapeutic drug monitoring (TDM) has proved extremely beneficial in individualizing the MMF therapy.(1,2)

Aim
To develop a robust limited sampling strategy (LSS) to measure MPA AUC12-14h (Area under the concentration-time curve) in patients with lupus nephritis.

Methods
MPA plasma specimens from 30 patients were prospectively collected at time points trough (pre-dose) and then at 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, and 12 hours after patients had taken the Mycrease® brand (Panaceo Biotech, New Delhi) of mycophenolate mofetil (MMF) for lupus nephritis. The MPA measurement was done using HPLC with UV detection (3). Limited sampling strategies with a acceptable correlation coefficients (R²), bias and precision were developed by stepwise multiple regression analysis. The predictive performance of the LSS was validated using bootstrap validation. The LSS predicted and the observed MPA AUC12-14h were compared using Intra-class Correlation (ICC) and Bland Altman plot. All statistical analyses were done using R (version 3.1.5).

Results
Table 1: Baseline characteristics

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total patients</td>
<td>30</td>
</tr>
<tr>
<td>Age</td>
<td>29.33 (18-83)</td>
</tr>
<tr>
<td>Sex (MF)</td>
<td>4:25</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>55.53 (38-73)</td>
</tr>
<tr>
<td>Dose per kg body weight (mg/kg/day)</td>
<td>26.47 (8.62-63.83)</td>
</tr>
<tr>
<td>Serum creatinine (mg/dl)</td>
<td>1.02 (0.46-3.15)</td>
</tr>
<tr>
<td>Albunin (g/l)</td>
<td>4.2 (2.8-4.5)</td>
</tr>
</tbody>
</table>

* All values in Mean (Min-Max) format. 6 Median values.

Table 2: Basic pharamcokinetic parameters of the collected data

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC0-12h (mg/L)</td>
<td>45.12 (19.52-67.66)</td>
</tr>
<tr>
<td>Peak - Cmax (mg/ml)</td>
<td>16.70 (5.24-30.86)</td>
</tr>
<tr>
<td>Time to peak - tmax (h)</td>
<td>1.08 (0.5-2.5)</td>
</tr>
<tr>
<td>Trough - Cmin (mg/ml)</td>
<td>1.74 (0.17-5.1)</td>
</tr>
<tr>
<td>AUC/dose</td>
<td>1.83 (0.97-3.81)</td>
</tr>
<tr>
<td>Cj/dose</td>
<td>0.069 (0.006-0.153)</td>
</tr>
</tbody>
</table>

* All values in Mean (Min-Max) format.

The inter-patient variability calculated as coefficient of variation for the dose normalized AUC and Cj were 34.04 % and 61.81 % respectively. The spearman correlation was found to be highest between Cj and MPA AUC12-14h (r=0.781, p<0.000). The spearman correlation between the trough and MPA AUC12-14h, was found to be 0.63.

Discussion
This is the first report of the development of a LSS for MMF in Indian patients with lupus nephritis. Rahman et al reported that when the 3 point LSS for MPA was derived using multiple regression analysis, the mean bias and imprecision were 0.8 and 22.6 % respectively, (4) The advantage of our 4 point LSS is:

- It does not compromise on accuracy and precision of MPA AUC
- The patient only has to remain in the Unit for 4 hours for collection of lesser samples.
- Clinically also, there was no difference between the observed and LSS predicted MPA AUC12-14h
- The cost of the analysis is reduced from 3505 INR to 1340 INR on utilizing this LSS to predict MPA AUC12-14h.

Conclusion
This study has developed a reliable, clinically viable limited sampling strategy for MPA AUC12-14h in patients taking MMF for lupus nephritis using four time points and completed within four hours post dose.

References

Acknowledgements
The study was funded by CMC FLUID research grant. The authors would like to thank Dr. Visalakshi Jayaseelan for her help with statistics and Ms. Lily, Ms. Aash and Ms. Lavaneya for their help with sample collection and processing.
A STUDY OF CLINICAL PROFILE OF PSOAS ABSCESS IN CMC Vellore

Dr Joane R, Dr Ramya I, Dr Saumya S, Dr Jeethu, Dr Krishna, Dr Punitha
Department of Medicine
Christian Medical College, Vellore

INTRODUCTION

- Iliopsoas abscess is the collection of pus in the iliopsoas compartment.
- Classical presentation of psoas abscess is a triad of fever, pain and functional impairment. Most common presentation is back, hip or thigh pain. About 50% present with fever but usually nonspecific.
- Primary is caused by unknown cause or from distant site, while secondary is from a contiguous site like vertebra, GI tract etc.
- Risk factors include diabetes, intravenous drug use, HIV, renal failure, etc.
- Most common cause was MSSA or tuberculosis based on the local epidemiology.
- Mortality was higher in diabetics.
- Ultrasound is the ideal investigation with simultaneous advantage of percutaneous drainage (PCD). CT is the most useful and preferred investigation. MRI was preferred in secondary cases to look for the source.
- Percutaneous drainage has better success rate, lower hospital stay and lower mortality rate.

AIMS

- To study the clinical profile, etiology and outcomes of medical versus surgical management.

MATERIALS METHODS

- Retrospective observational study with review of medical records of patients admitted in medical wards from January 2009 till December 2013 who were diagnosed with psoas abscess based on imaging studies.

RESULTS

- Most patients fell in age group of 41 to 50 years.
- Fever was the most common presenting complaint, the classical triad was found only in 46.5% of the patients.
- Tuberculosis was the most common etiology found in our study.
- Most of the abscesses were secondary to spondylitis.
- In 53.5% patients a causative organism was isolated, of which tuberculosis was the most common.
- 46.5% patients were treated with percutaneous drainage.
- Average duration of stay was of 12 days.
- Complete resolution of symptoms was seen in 62.5% at the end of hospital stay.

DISCUSSION

INTRODUCTION
- Fibrucalculus Pancreatic Diabetes (FCPD) is an unusual disease, affecting patients like us.
- Insulin sensitivity is not well characterized in FCPD.
- Hypoglycemic Euglycemic Hyperinsulinemic Clamp: gold standard to determine insulin sensitivity.

AIMS AND OBJECTIVES
- To characterize insulin secretion and insulin sensitivity at the hepatic and peripheral tissues.
- To characterize the body composition, hepatic and skeletal muscle lipid content.
- To compare time with Type 1 Diabetes, Type 2 Diabetes and non-diabetic control subjects.

MATERIALS & METHODS
STUDY DESIGN
- Cross-sectional study
- Participating centers:
  - Group 1: 10 subjects with FCPD
  - Group 2: 15 subjects with T1DM
  - Group 3: 5 subjects with T2DM

PARAMETERS STUDIED
Detailed history, clinical examination
- Anthropometric measures:
  - Height, weight, BMI
  - Waist circumference and hip circumference
  - Skin-fold thickness:
    - Biceps, triceps, subscapular, abdomen, suprailiac area
  - Truncal and body fat by DEXA Scan (Hologic®)

NMR Spectroscopy: hepatic and muscle lipid estimation
- Principle: An unique non-invasive method to assess intra-muscular fat (IMCL) and extramuscular fat (EMCL). The use of high field strength magnets such as 3 Telsa instead of 1.5 Tesla gives a twofold greater frequency of separation.

SUMMARY AND CONCLUSIONS
- Insulin secretion rate by deconvolution method was markedly lower in FCPD (7.9±1.8 pmol/kg/min) relative to Non-diabetic (34.8±2.6 pmol/kg/min; p<0.0002) and T1DM subjects (40.8±10.8 pmol/kg/min; p<0.005).
- Insulin secretion in FCPD was similar to T1DM subjects (12.9±4.8 pmol/kg/min; p=0.1).
- Hepatic insulin sensitivity was significantly impaired in the FCPD (4.1±0.2 mg/kg/min) compared to Non-diabetic subjects (7.2±0.8 mg/kg/min; p<0.002).
- Peripheral insulin sensitivity was also significantly impaired in FCPD (9.6±0.4 mg/kg/min) relative to Non-diabetic subjects (13.5±1.4 mg/kg/min; p<0.001).
- DEXA and NMR spectroscopy showed significant decrease in % truncal fat, % total fat and % hepatic fat in comparison to T1DM and FCPD subjects.

NOVEL ASPECTS
- Pancreatic Hyperinsulinemic Euglycemic clamp to study insulin sensitivity in FCPD has not been reported before in literature.
- Use of C-peptide deconvolution techniques: unique model to assess insulin secretion rates in FCPD.
- Comparison with other types of diabetes mellitus in a single study.
- Use of DEXA and NMR spectroscopy to determine body fat content and hepatic and muscle lipid content.
- Concomitant fat biopsies, indirect calorimetry and next generation sequencing are being undertaken to understand the genotype-phenotype relationship and role of energy expenditure.
Absence of F343V, R357W and E360G mutation in carnitine palmitoyltransferase 1 and K329E mutation in medium-chain acyl-CoA dehydrogenase in four Indian patients with pregnancy-related liver disease

Raghupathy V1, Goel A2, Thangaraj KR1, Kupen CB1, Bhaskaran Sundar KAS1, Raj AK1, Jose RJ1, Benjamin SJ1, Ramachandran A1
1Department of Hepatology, 2Department of Gastroenterology, Christian Medical College, Vellore.

Background
- Acute fatty liver of pregnancy (AFLP) is a rare pregnancy related liver disorder manifesting in late pregnancy with acute liver failure.
- It is a mitochondrial hepatopathy attributable to defects in beta oxidation of fatty acids, and the role of placenta has been shown to be crucial in pathogenesis.
- The mitochondrial β oxidation of a fatty acids is a multi-step catabolic process carried out by at least 10 different enzymes.
- Carnitine palmitoyltransferase 1 (CPT1) controls entry of fatty acids into the mitochondrial matrix for oxidation after esterification with acyl CoA, while medium chain acyl-CoA (MCAD) functions in beta oxidation of fatty acids with chain lengths between C8 and C12.
- Mutations which induce functional alterations have been identified for both MCAD and CPT1 in the West. Three point mutations (F343V, R357W and E360G) have been identified in the gene for CPT1, which alter enzyme activity. Among these three mutations, F343V and E360G decrease enzyme activity while the other mutation resulted in decreased enzyme stability.
- The commonest nonsense mutation in MCAD is K329E, which resulted in decreased MCAD activity in caucasian patients.

Aim
To evaluate the presence of F343V, R357W and E360G mutations in the CPT1 gene and the K329E mutation in the MCAD gene in patients with AFLP.

Materials and methods
Clinical details of AFLP Patients:
3 patients with AFLP and 1 patient with HELLP syndrome (co-existent pre-eclampsia) were included for the study. All patients had an unyielding workup for the aetiology of acute liver disease, including serology tests for acute viral hepatitis A, B and E (Table 1)

<table>
<thead>
<tr>
<th>Baseline laboratory parameters in four patients with pregnancy-related liver disorder presenting in the third trimester</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum bilirubin (mg/dL)</td>
</tr>
<tr>
<td>-------------------------</td>
</tr>
<tr>
<td>Patient 1</td>
</tr>
<tr>
<td>Patient 2</td>
</tr>
<tr>
<td>Patient 3</td>
</tr>
<tr>
<td>Patient 4</td>
</tr>
</tbody>
</table>

DNA sequencing:
DNA was isolated from placenta and 100 ng placental DNA was used for PCR reaction using primers specific for exon 10 and 13 of CPT1 and exon 11 of MCAD. Purified PCR products were sequenced both in the sense and anti-sense orientation using the BigDye Terminator V3.1

Results
PCR and sequence analysis for the F343V, R357W and E360G mutation in exon 10 and Y498C mutation in exon 13 of CPT1

PCR and sequence analysis for the K329E mutation in exon 11 of MCAD

<table>
<thead>
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</tr>
<tr>
<td>Patient 3</td>
</tr>
<tr>
<td>Patient 4</td>
</tr>
</tbody>
</table>

Conclusion
None of the mutations identified in the West were seen in the 3 AFLP and 1 HELLP patient tested. Since the sample size is small, no definitive conclusions can be made regarding prevalence of these mutations in the Indian population. However, it is likely that molecular mechanisms involved in the pathogenesis of pregnancy related liver disorders in India may be different from those in the West.

Acknowledgements
The authors are grateful for funds received from Fluid Research Fund at Christian Medical College (Vellore, India) for this study.
A NOVEL TREATABLE CHILDHOOD MOVEMENT DISORDER: A CASE REPORT
Sangeetha Yoganathan*, Karthik Muthusamy*, Sniya Valsa Sudhakar**, Maya Mary Thomas*, Mathew Alexander*
*Department of Neurological Sciences**Department of Radiology

Introduction

Role of Manganese

- Trace metal essential for normal cell function and metabolism
- Manganese toxicity
- Impair dopaminergic, glutamatergic and GABAergic transmission
- Mitochondrial dysfunction
- Oxidative stress
- Neuroinflammation

Manganese toxicity

- Originally described as "manganism" in miners during the nineteenth century
- Manganese toxicity
- Environmental exposure
- Inherited disorder of manganese metabolism
- We describe a novel manganese transport defect identified in a child which is potentially treatable
- First case identified from Indian subcontinent

Case history

- Five year old girl from Tamil Nadu born second in birth order to second degree consanguineous parents
- No adverse perinatal events
- Attained age appropriate milestones
- Progressive tremor and dullness while drawing and writing noticed from three years of age
- Two years later she developed toe walking and abnormal intermittent twisting posture at ankles on right side
- Symptoms evolved over next few days and progressed to involve left side
- No cranial nerve involvement
- No history of hearing disturbances, cognitive decline, speech disturbances
- No history of exposure to manganese such as welding, battery factories, battery exposures, paints, homoeopathic medications or over the counter abuse ofephedrine containing drugs
- Family history not contributory

Examination

- Anthropometry: normal
- Bradynimia, bradykinesia
- Speech and memory: normal
- Spasticity involving both lower limbs
- Muscle stretch reflexes exaggerated
- Bilateral ankle clonus
- Dystonia involving right lower limb more than left

Neuroimaging

![CT images](image)

1.T2 axial image (A) shows bilateral symmetrical hyperintensity of the globus pallidus. Corresponding hypointensity is seen on T2 images (yellow arrow in B). Note the absence of blooming on SWI (yellow arrow in C).

2.T1 axial images showing hyperintensity of the striatonigral pathway and substantia nigra (yellow arrow in D).

3.Hypointensity is also seen along components of the Guillian-Mollaret triangle involving the dentate nucleus (white arrow in F), superior cerebellar peduncles (white arrow in E) and central tegmental tracts (yellow arrows in E and F).

Investigations

- Complete blood count: normal
- Mild elevation of liver enzymes
- Serum copper, ceruloplasmin, 24 hours urine copper: normal
- Blood manganese: 52.9 mg/dl (5-15 mg/dl)
- 24 hours urine manganese: 5.7 mg (0-2 mg/24 hour)
- Basic metabolic screening: normal
- Classical SLC30A10 mutation related to manganese deposition in brain-negative
- Novel manganese transporter gene mutation identified

Treatment

- Monthly intravenous sodium calcium EDTA 1000 mg/m2 in 2 divided doses for 5 days
- Vitamin C and iron supplementation
- Low manganese diet
- Tetrabenazine and Baclofen
- Occupational & physiotherapy

Follow up

Six months follow up

- Placed in school with good scholastic performance
- Improvement in writing and drawing skills
- Able to walk with AFO
- Significant reduction in spasticity and dystonia

Conclusion

- Inherited brain manganese accumulation is a novel and potentially treatable disorder
- Characteristic neuroimaging findings, clinical phenotype, blood manganese studies and genetic studies help in confirming the diagnosis
- Chelation therapy is effective in management
Clinical Profile and Outcome of Autoimmune Encephalitis in Children: Experience from a Tertiary Care Hospital

Sangeetha Yoganathan, Maya Mary Thomas, Sahil Kohli, Karthik Muthusamy, Mathew Alexander
Department of Neurological Sciences

Introduction

Complex category with diversity in
- Immunological associations
- Clinical manifestations
- Therapeutic outcomes

- Surveillance study in U.K.: 42% of patients with encephalitis had an identifiable infectious cause
- Prompt diagnosis & treatment with immunosuppression: improve or reverse symptoms
- If untreated: irreversible cognitive deficits, ongoing seizures & death

Anti-NMDAR Encephalitis

- Encephalitis of unknown origin: NMDAR antibodies in 1% of patients (aged between 18 and 35 years)
  [Ros et al. Neurology 2010]
- Multicentre, population-based prospective study of encephalitis in UK: 4% of patients had anti-NMDAR encephalitis
- Second most common immune-mediated cause, after ADEM and before all antibody-associated encephalitis
- Anti-NMDAR encephalitis rivals viral etiologies as a cause of encephalitis

- Autoimmune encephalitis with commonly described antibodies
  - Anti-N-Methyl D Aspartate receptor encephalitis
  - Anti-Basal Ganglia antibody associated encephalitis
  - Anti-Voltage-Gated Potassium Channel antibodies mediated encephalitis
  - Hashimoto encephalopathy

Objective

- To describe the clinical profile and outcome of children with autoimmune encephalitis

Study Methods

- Retrospective chart review
- Inclusion criteria: Children less than 15 years with acute or subacute encephalopathy
  - Clinical phenotype suggestive of autoimmune encephalitis with response to immunotherapy
  - Serological evidence of autoimmunity detected in blood or CSF
- Exclusion criteria: Children with proven metabolic disease or proven viral encephalitis
- Study period: December 2013 - July 2014

Results

- Children with serological evidence for autoimmune encephalitis or movement disorder: 9
  - Antibodies against NR2B subunit of NMDA receptor: 5
  - Antibodies against basal ganglia receptor: 3
  - Antibodies against both NMDA and a basal ganglia receptor: 1
- Children with seronegative autoimmune encephalopathy: 2
- Mean age at presentation:
  - Children with anti-NMDAR encephalitis: 58 months (range: 12-144 months)
  - Children with anti-basal ganglia antibody associated movement disorder: 71.3 months (range: 21-95 months)
- Mean time from onset of symptoms to admission:
  - Children with anti-NMDAR encephalitis: 70.6 days
  - Children with anti-basal ganglia associated encephalitis or movement disorder: 100 days
- Sex ratio in this study group: 5 girls and 4 boys

Table 1: Clinical presentation in our cohort

<table>
<thead>
<tr>
<th>Clinical symptom</th>
<th>Frequency (n=11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seizures</td>
<td>10</td>
</tr>
<tr>
<td>Super-refractory seizures</td>
<td>3</td>
</tr>
<tr>
<td>Speech disturbances</td>
<td>10</td>
</tr>
<tr>
<td>Excessive irritability</td>
<td>5</td>
</tr>
<tr>
<td>Personality changes or behavioural symptoms</td>
<td>5</td>
</tr>
<tr>
<td>Loss of milestones</td>
<td>10</td>
</tr>
<tr>
<td>Movement disorder</td>
<td>11</td>
</tr>
</tbody>
</table>

Table 2: Clinical spectrum in our cohort

<table>
<thead>
<tr>
<th>Clinical spectrum in our cohort</th>
</tr>
</thead>
<tbody>
<tr>
<td>Movement disorder</td>
</tr>
<tr>
<td>Personality changes or behavioural symptoms</td>
</tr>
<tr>
<td>Speech disturbances</td>
</tr>
</tbody>
</table>

- Somnolent or hypoactivity
- Motor weakness:
  - R stiff
  - Hand clapping
- Visual disturbance
- Ocular rotation
- Chorea/athetoid movements
- Confusional state and visual loss
- Imbalancy
- Slur
- Obsessive compulsive behaviors
- Aggression
- Social disorientation
- Reduced or absent
- Poor comprehension
- Inability
- Possible

Conclusion

- Autoimmune encephalitis in children is a potentially treatable condition
- High index of suspicion and early treatment would lead to better outcome in these children

Outcome in our cohort

- Mortality: none
- Morbidity: Follow up 3-6 months
  - Minimally conscious state: 1
  - Epilepsy: 5
  - Significant improvement: 9
  - Persistence of behavioural symptoms: 2

Algorithm

- Enccephalitis presentation: History & clinical examination
- Treatment: Start and adjust EEG-PEDS
- Inclusion criteria: Identified in neurology

Recurrent symptoms

Clinical presentation: Ataxia, Dizziness, Memory, Drowsiness

ALGORITHM

- All are treated for autoimmune encephalitis
- All are treated for ADEM/MS/AIDS
- All are treated for neuronal encephalitis
Study of the frequency and distribution of IL28B polymorphisms in hepatitis C virus infected patients and their association with virological markers and treatment response

P Ranjan1, Manikandan R1, GJ Fletcher1, J Sivakumar1, Grace R2, A Goel1, U Zachariah2, CE Eapen2, P Abraham1

1 Department of Clinical Virology, 2 Department of Hepatology, 3 Department of Biostatistics, Christian Medical College, Vellore

Background
- Hepatitis C virus (HCV) infection is a global health problem with a worldwide prevalence of around 2-3%, infecting more than 185 million people.
- India has over 10 million sero-positive individuals.
- Standard of care therapy: PEGylated Interferon plus Ribavirin.
- However, treatment is expensive and is associated with significant adverse effects, making serial monitoring of treatment response necessary.
- Rapid viral response (RVR) at 4 wk.
- End of treatment (ETR) response at 24/48 wk.
- Early viral response (EVR) at 12 wk.
- Sustained viral response (SVR) 24 wk after ETR.
- Factors associated with a favourable treatment response:
  - Viral factors:
    - Genotype 2/3
    - Low baseline viral load
    - Favourable viral kinetics
  - Host factors:
    - Younger age
    - Lower BMI
    - Female gender
    - Non-diabetic
    - East Asian ethnicity
    - Low baseline ALT
    - IL28B polymorphisms

- Single nucleotide polymorphisms near the IL28B gene (rs12979860 and rs8099917) have been found to be strong genetic predictors of treatment-induced viral clearance.

Objectives
1. To study frequency and distribution of IL28B polymorphisms in HCV infected patients.
2. To study and compare SVR rates in HCV genotype 1 and 3 infections.
3. To study the association of IL28B polymorphisms with SVR in HCV genotype 1 and 3 infected patients.
4. To study the association of other factors like age, gender, BMI, diabetes, pre-treatment viral loads, baseline ALT levels and treatment modality with SVR.
5. To study the association of IL28B polymorphisms with virological response during the course of treatment.

Funding
Institutional Review Board (IRB Min No: 8202 / 13.02.13) & Dept of Clinical Virology

Methodology
- Observational study conducted in Clinical Virology and Liver Clinic, CMC Vellore from April 2013 to August 2014.
- 57 hepatitis C virus genotype 1, 3 and 4 infected patients on therapy with Interferon (standard or pegylated) and Ribavirin recruited after informed consent.
- Patients with immunosuppression, HBV/HIV co-infection, on dialysis excluded.

Patient referred to Virology for HCV PCR/ genotyping

- BMI blood in EDTA tube
- Plasma used for viral load testing/ genotyping; DNA extracted from buffy coat
- PCR-RFLP for rs12979860 and rs8099917
- PCR- Sequencing on a subset of samples (to verify RFLP results)
- Detection of IL28B polymorphisms

Results
- Chromatogram showing sequence of rs12979860 (C/T)
- Chromatogram showing sequence of rs8099917 (T/G)
- The distribution of rs12979860 CC/ TT/CT genotypes was 60%, 33%, and 7% respectively. For rs8099917 genotype, the TT/CT/GG distribution was 72%, 23%, and 5% respectively.
- Of the 57 patients recruited, 34 completed follow-up during the course of the study.
- SVR was seen in 56% of cases (57% in genotype 1 and 54% in genotype 3).

- Responders
  - p = 0.012
- Non-responders
  - p = 0.068

- Responders
  - p = 0.001
- Non-responders
  - p = 0.077

Distribution of rs12979860 CC and non-CC genotypes

Distribution of rs8099917 TT and non-TT genotypes

- Variable: Genotype 1 vs. 3
  - P value: 1.0
- Age: 40 yrs vs. >40
  - P value: 0.426
- Gender: female vs. male
  - P value: 0.129
- BMI: ≤ 25 vs. >25
  - P value: 0.449
- RVR: achieved vs. not achieved
  - P value: 0.005
- Pre-treatment viral load: ≤600,000 vs. >600,000 IU/ml
  - P value: 0.710
- Treatment modality: PegIFN vs. Std IFN
  - P value: 0.217
- Diabetes
  - P value: 0.462
- Baseline ALT levels ≤105 vs. >105 IU/ml
  - P value: 0.08

Association of various factors with treatment response
- RVR predictive of SVR
- No association seen with other factors
- Larger studies needed to confirm the findings.

Conclusions
- Genotypes CC and TT commonest
- CC at rs12979860 associated with SVR and RVR
- SVR in genotype 1(57%) = SVR in 3 infections (54%)
- RVR predictive of SVR
- No association seen with other factors
- Larger studies needed to confirm the findings

References:
PNEUMOCOCCAL COLONISATION AND STREPTOCOCCUS SALIVARIUS AS A PROBIOTIC THERAPY

Mary John, Eileen M Dunne, Paul M Uddiehi, Catherine Salzke, Odilla Wijburg, Stephen O Lamuye
Department of Otolaryngology, Christain Medical College, Kerala, Tamil Nadu, India, Melbourne, Vic, Australia, Department of Otolaryngology, The University of Melbourne

ABSTRACT

Introduction
- Early nasopharyngeal colonization with high loads of pathogenic bacteria (including the pneumococcus) is associated with a high incidence of otitis media (OM).
- Pneumococcal vaccines have limited efficacy against OM.
- New strategies to protect against pneumococcal disease in early life are needed.

Methods: S. salivarius was added at varying doses added before, with, or after pneumococcal administration to human epithelial (CCL-23) cells in vitro and pneumococcal adherence was determined. Repeated nasogastric administration of S. salivarius was tested as an intervention in an infant mouse model of pneumococcal colonisation and otitis media. Healthy adult were given probiotic lozenges for one week and S. salivarius colonization of upper respiratory tract was examined.

RESULTS

Hypothesis
- The probiotic S. salivarius can inhibit the pneumococcal adherence to human epithelial cells in vitro.
- S. salivarius can inhibit pneumococcal colonization in mice and prevent OM.
- Oral intake of S. salivarius can colonize the upper respiratory tract.

METHODS AND MATERIALS

in vitro adherence assay

Figure 1: S. pneumoconiae was added to CCL-23 (HEPi-8) human to beathal cell line monolayers and incubated 5x3 h at 37°C. After washing to remove non-adherent bacteria, S. pneumoconiae were quantified by qPCR. The effect of S. salivarius on pneumococcal adherence was estimated at various doses and times of administration.

in vivo animal experiments

Repeated nasogastric administration of S. salivarius (10^9 CFU dose) was used as an intervention in an established model of otitis media in mice.

HUMAN STUDIES

Healthy adult volunteers (n=24) ingested S. salivarius lozenges 2x daily for one week and colonization was estimated by collecting nasopharyngeal and throat swabs at days 0, 7, 14, and 21.

CONCLUSIONS

In vitro assays
- S. salivarius inhibited pneumococcal adherence to epithelial cells in a time- and dose-dependent manner.

In vivo animal studies
- S. salivarius does not efficiently colonize infant mice.
- S. salivarius did not alter the pneumococcal numbers in ear in OM model.

HUMAN STUDIES

- S. salivarius naturally colonizes the human oral cavity.
- After oral intake, S. salivarius colonization increased in the oral cavity, but not in the nasopharynx of humans for up to 2 weeks.

Future directions
- Randomized double blind study using pernasal administration of S. salivarius to evaluate the effect on nasopharyngeal colonization of pathogenic bacteria and incidence of OM.

REFERENCES

Autonomic Dysfunction in Chronic Inflammatory Demyelinating Polyneuropathy

Suresh Babu, Mathew Alexander, Sanjit Aaron, Maya Thomas, Murthy
Department of Neurological sciences

INTRODUCTION

Autonomic neuropathy, although common in Guillain-Barré syndrome, is considered rare in chronic inflammatory demyelinating polyradiculoneuropathy (CIDP) and has not been systematically investigated. The present study was aimed at determining the prevalence of autonomic dysfunction and investigating the integrity of autonomic nervous system (ANS) reflexes in CIDP.

Aims and Objectives

To assess the frequency, severity and spectrum of autonomic dysfunction in patients with Chronic Inflammatory demyelinating Polyneuropathy.

Materials and Methods

- Prospective Study
- Patients diagnosed to have CIDP by EFNS/PNS 2010 criteria were included in the study
- 23 patients patients full filled criteria for CIDP during study period.
- The autonomic dysfunction was assessed both autonomic symptoms (using COMPASS score) and autonomic function tests (Finpress TM) were done.

RESULTS

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age &lt; mean, min, max ± SD</td>
<td>0.64±1.38</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>26/32(84)</td>
</tr>
<tr>
<td>N/A autonomic testing</td>
<td>0±140±80</td>
</tr>
<tr>
<td>Age, yrs, min, mean ± SD</td>
<td>51±1±46</td>
</tr>
<tr>
<td>Disease duration, min, max ± SD</td>
<td>40±100, 90</td>
</tr>
<tr>
<td>Autonomic CDP, n (%)</td>
<td>0±100 (9)</td>
</tr>
<tr>
<td>Progressive course, n (%)</td>
<td>0±100 (20)</td>
</tr>
<tr>
<td>Repetitive and meeting, n (%)</td>
<td>0±100 (20)</td>
</tr>
<tr>
<td>Micturition, n (%)</td>
<td>0±100 (20)</td>
</tr>
<tr>
<td>Autonomic function test abnormality</td>
<td>0±100 (10)</td>
</tr>
<tr>
<td>RESP</td>
<td>0±100 (20)</td>
</tr>
<tr>
<td>TCD</td>
<td>0±100 (20)</td>
</tr>
<tr>
<td>RESP normal, n (%)</td>
<td>0±100 (20)</td>
</tr>
<tr>
<td>TCD normal, n (%)</td>
<td>0±100 (20)</td>
</tr>
<tr>
<td>Antidepressant, n (%)</td>
<td>0±100 (20)</td>
</tr>
<tr>
<td>COMPAH II score</td>
<td>2±1±8, 4.8</td>
</tr>
<tr>
<td>Total amyotrophy score (TSS)</td>
<td>2±1±8, 4.8</td>
</tr>
<tr>
<td>CIDP amyotrophy score (C2)</td>
<td>0±100 (20)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Adrenergic score</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>12/21(57)</td>
</tr>
<tr>
<td>1</td>
<td>6/13(13)</td>
</tr>
<tr>
<td>2</td>
<td>5/13(13)</td>
</tr>
<tr>
<td>3</td>
<td>4/13(13)</td>
</tr>
<tr>
<td>4</td>
<td>3/13(13)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cardiovascular score</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>5/23(21.7)</td>
</tr>
<tr>
<td>1</td>
<td>12/23(52.2)</td>
</tr>
<tr>
<td>2</td>
<td>6/23(26)</td>
</tr>
<tr>
<td>3</td>
<td>0/23(0)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sympathetic score</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>9/23(39.2)</td>
</tr>
<tr>
<td>1</td>
<td>12/23(52.2)</td>
</tr>
<tr>
<td>2</td>
<td>0/23(0)</td>
</tr>
</tbody>
</table>

| Conclusion |

1. There is a high Prevalence of autonomic dysfunction in patients with CIDP (80%).
2. The parasympathetic arm (70%) was more involved than sympathetic (60%).
3. Spectral analysis of HRV showing abnormality in sympathetic arm.
4. In our study 37% had mild degree of autonomic dysfunction and 47% had moderate degree of autonomic dysfunction.
**Autonomic Dysfunction in Neuromyelitis Optica and Spectrum Disorders**

**Murthy, Mathew Alexander, Sanjit Aaron, Maya Thomas, Suresh Babu**

**Department of Neurological sciences**

**INTRODUCTION**

In NMO and spectrum disorders which has predilection for the critical areas in the brain and spinal cord causing Autonomic disturbances leading to varied autonomic dysfunction.

**Aims and Objectives**

To assess the frequency, severity and spectrum of autonomic dysfunction in patients with Neuromyelitis optica and spectrum disorders.

**Materials and Methods**

- Prospective Study
- 20 patients studied by using Revised Criteria for NMO and spectrum disorders (2006).

**INCLUSION CRITERIA**

- Revised diagnostic criteria for Neuromyelitis Optica 2006
- Optic Neuritis
- Acute Myelitis
- At least 2 of 3 supporting criteria
  1. Contiguous spinal cord lesions on MRI involving >3 vertebral segments
  2. Brain MRI not meeting the diagnostic criteria for Multiple sclerosis
  3. NMO IgG seropositive status

**EXCLUSION CRITERIA**

- Post or para infectious encephalomyelitis- ADEM
- Multiple sclerosis diagnosed by McDonald’s Criteria
- Causes other than inflammatory conditions diagnosed by radiological, biochemical and microbiological tests.
- Diabetes Mellitus
- Hypertension

**RESULTS**

<table>
<thead>
<tr>
<th>TABLE 2: Baseline Characteristics</th>
<th>Value (percentage)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean (SD)</td>
<td>42.58 (10.95)</td>
</tr>
<tr>
<td>Duration of Disease</td>
<td>2.85 (1.70)</td>
</tr>
<tr>
<td>Autonomic testing</td>
<td>5.20 (25)</td>
</tr>
<tr>
<td>In Remission</td>
<td>45.20 (20)</td>
</tr>
<tr>
<td>TCD% during the ANS testing</td>
<td></td>
</tr>
<tr>
<td>Mild</td>
<td>16 (20)</td>
</tr>
<tr>
<td>Moderate</td>
<td>20 (25)</td>
</tr>
<tr>
<td>Severe</td>
<td>20 (25)</td>
</tr>
<tr>
<td>Autonomic indices &amp; (%)</td>
<td>14 (20)</td>
</tr>
<tr>
<td>Presence of Vasculitis, vessel (%)</td>
<td>10 (14)</td>
</tr>
<tr>
<td>Involvement of brain and (auto) &amp; (6%)</td>
<td>17 (11)</td>
</tr>
<tr>
<td>Normal</td>
<td>17 (11)</td>
</tr>
<tr>
<td>Involvement of Brain (transverse, hypothalamic, autonomic region, peripheral nerve, peripheral nerve)</td>
<td>12 (8)</td>
</tr>
<tr>
<td>Involvement of Spinal (Segment)</td>
<td>6 (4)</td>
</tr>
<tr>
<td>Involvement of Both brain and Spinal</td>
<td>6 (4)</td>
</tr>
<tr>
<td>NYHA</td>
<td>12 (6)</td>
</tr>
<tr>
<td>NYHA, normal</td>
<td>12 (6)</td>
</tr>
<tr>
<td>NYHA, abnormal</td>
<td>6 (3)</td>
</tr>
<tr>
<td>NYHA, severe</td>
<td>6 (3)</td>
</tr>
</tbody>
</table>

**SEVERITY AND DISTRIBUTION OF AUTONOMIC DEFICIT**

<table>
<thead>
<tr>
<th>NYHA</th>
<th>Severity and Distribution of Autonomic Deficit</th>
</tr>
</thead>
<tbody>
<tr>
<td>NYHA</td>
<td>Cerebrospinal (mild)</td>
</tr>
<tr>
<td>NYHA</td>
<td>Sympathetic (mild)</td>
</tr>
</tbody>
</table>

**Conclusion**

1. The Prevalence of Autonomic Dysfunction is high in NMO and spectrum disorders found to be 80% during remission and 95% during active disease.
2. Parasympathetic cardiovascular dysfunction (80%) is more common than Sympathetic cardiovascular Dysfunction (65%)
3. Cardiovascular, gastrointestinal, sudomotor, genitourinary, sexual, sudomotor and secretemotor dysfunction due to damage to ANS centre in brain and spinal cord
Utility of loop-mediated isothermal amplification (LAMP) assay and ELISA in confirmation of leptospirosis: A pilot study

Mallika Sengupta, K P P Abhilash, Sowmya S, O C Abraham, Thambu David, Dolly Daniel, V Balaji, John Antony Jude Prakash

1 Department of Clinical Microbiology, 2 Department of Medicine, 3 Department of Transfusion Medicine and Immunohaematology, Christian Medical College, Vellore

BACKGROUND

- Leptospirosis is a zoonosis which occurs as outbreaks during monsoon
- Being a cause of acute febrile illness, the differentials considered are malaria, dengue, scrub typhus, rickettsial fever and typhoid fever
- Laboratory diagnosis is essential for confirmation of leptospirosis
- Culture and MAT, the reference standard are difficult to perform
- IgM ELISA is the mainstay of diagnosis
- LAMP assay for leptospirosis has been evaluated by only four research groups

OBJECTIVES

1. To revalidate the diagnostic cut-off of ELISA used for confirming a clinical diagnosis of leptospirosis
2. To establish a LAMP assay for detection of leptospirosis
3. To compare the utility of LAMP, PCR and ELISA for diagnosis of leptospirosis
4. To correlate clinical features with the diagnosis of leptospirosis

RESULTS

Validation of ELISA

<table>
<thead>
<tr>
<th>Test</th>
<th>Cut-off</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leptospira PanBio IgM (PanBio units)</td>
<td>20</td>
</tr>
<tr>
<td>Leptospira Virion serien IgM (OD)</td>
<td>0.7</td>
</tr>
<tr>
<td>Leptospira Virion serien IgG (OD)</td>
<td>2.7</td>
</tr>
<tr>
<td>Leptospira InBios IgM (OD)</td>
<td>1</td>
</tr>
<tr>
<td>Scrub typhus InBios IgM (OD)</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Positives by different tests

- IgM ELISA & modified Faine’s criteria: 40
- PCR: 3
- LAMP: 7

Performance of tests

- Prevalence: 2%
- Sensitivity: ELISA 100%, PCR 100%, LAMP 100%
- Specificity: ELISA 72.79%, PCR 98.64%, LAMP 95.24%

Correlation of clinical features

- Decreased urine output (n=28): 15 (58.92%)
- Bilirubin >3mg/dl (n=56): 31 (55.36%)
- Creatinine >2.5mg/dl (n=43): 23 (53.49%)
- Decreased platelet (n=97): 57 (58.63%)

- p-value: 0.020, 0.012, 0.044, 0.002

LIMITATIONS

- IgG antibody testing could not be done on acute febrile illness serum samples
- Convalescent sera could be collected only in 32 patients
- Molecular assay for scrub typhus was not performed
- Many patients came after initial treatment outside

CONCLUSIONS

- Nail PCR, LAMP assay and IgM ELISA was performed and 52 were identified as cases of leptospirosis
- Specificity - good for PCR and LAMP
- LAMP assay performed better in first week

Reference
INTRODUCTION

- Gestational diabetes mellitus (GDM) is considered to result when the genetic predisposition is triggered by increased insulin resistance during pregnancy. Whilst for most women glucose intolerance resolves after child birth, there is up to 50% chance of developing diabetes within 5 years of delivery.
- In subjects with GDM in our population, little is known about the genetic basis of MODY and its potential clinical significance.

OBJECTIVE

- To Screen pregnant women with diabetes for a comprehensive panel of ten MODY genes [HNF1A, HNF4A, GCK, PDX1, HNF1B, NEUROD1, KLF11, CEL, PAX4 and INS] utilizing next generation sequencing (NGS).
- Screen the family members for the presence of the detected genetic variant.

METHODS

- We included 50 south Indian women with hyperglycemia complicating their pregnancy to screen for mutations utilizing a novel multiplex polymerase chain reaction (PCR) based target enrichment established earlier followed by NGS on the Ion Torrent Personal Genome Machine (PGM).
- The detected rare variants which were of pathogenic significance were confirmed by Sanger sequencing and genotype-phenotype correlation was done.

RESULTS

Table 1: Genetic details of MODY positive pregnant women with diabetes

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Type of GDM</th>
<th>BMI (kg/m²)</th>
<th>MODY Gene</th>
<th>Maternal Age (Mean ± SD)</th>
<th>Gestational Age (Mean ± SD)</th>
<th>Random Blood Glucose (mmol/L)</th>
<th>Amino Acid Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>OVERT</td>
<td>24.6</td>
<td>M-2</td>
<td>28.1 ± 6.2</td>
<td>39.3 ± 1.7</td>
<td>7.5 ± 2.1</td>
<td>p.670G&gt;A</td>
</tr>
<tr>
<td>2</td>
<td>PRE GDM</td>
<td>30.1</td>
<td>M-3</td>
<td>32.8 ± 5.3</td>
<td>38.5 ± 1.9</td>
<td>8.2 ± 3.0</td>
<td>p.221L&gt;G</td>
</tr>
<tr>
<td>3</td>
<td>OVERT</td>
<td>26.2</td>
<td>M-5</td>
<td>29.7 ± 6.3</td>
<td>37.2 ± 2.1</td>
<td>7.9 ± 3.2</td>
<td>p.221L&gt;G</td>
</tr>
<tr>
<td>4</td>
<td>PRE GDM</td>
<td>30.2</td>
<td>M-6</td>
<td>31.8 ± 5.4</td>
<td>38.6 ± 1.8</td>
<td>8.5 ± 3.6</td>
<td>p.221L&gt;G</td>
</tr>
<tr>
<td>5</td>
<td>OVERT</td>
<td>28.3</td>
<td>M-8</td>
<td>30.5 ± 5.9</td>
<td>36.0 ± 2.0</td>
<td>7.8 ± 3.1</td>
<td>p.221L&gt;G</td>
</tr>
<tr>
<td>6</td>
<td>PRE GDM</td>
<td>30.4</td>
<td>M-9</td>
<td>32.1 ± 5.6</td>
<td>38.2 ± 1.9</td>
<td>8.3 ± 3.7</td>
<td>p.221L&gt;G</td>
</tr>
<tr>
<td>7</td>
<td>GDM</td>
<td>25.5</td>
<td>M-10</td>
<td>29.3 ± 5.2</td>
<td>37.4 ± 2.1</td>
<td>7.6 ± 2.7</td>
<td>p.221L&gt;G</td>
</tr>
<tr>
<td>8</td>
<td>OVERT</td>
<td>27.6</td>
<td>M-12</td>
<td>28.9 ± 5.3</td>
<td>36.8 ± 1.8</td>
<td>7.9 ± 3.2</td>
<td>p.221L&gt;G</td>
</tr>
</tbody>
</table>

Table 2: Comparing clinical details of pregnant women with diabetes

<table>
<thead>
<tr>
<th>Parameters</th>
<th>MODY Positive [N = 8]</th>
<th>MODY Negative [N = 42]</th>
<th>P-VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>29.7 ± 6.3</td>
<td>28.9 ± 5.1</td>
<td>0.722</td>
</tr>
<tr>
<td>Age at Diagnosis (weeks)</td>
<td>32.5 ± 5.3</td>
<td>29.5 ± 4.9</td>
<td>0.748</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>153.9 ± 8.5</td>
<td>154.0 ± 9.5</td>
<td>0.894</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>70.0 ± 12.3</td>
<td>64.1 ± 14.5</td>
<td>0.246</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>28.5 ± 5.7</td>
<td>21.8 ± 3.7</td>
<td>0.461</td>
</tr>
<tr>
<td>PPARG</td>
<td>2</td>
<td>2</td>
<td>0.662</td>
</tr>
<tr>
<td>KCNJ11</td>
<td>3</td>
<td>3</td>
<td>0.627</td>
</tr>
<tr>
<td>GCK</td>
<td>3</td>
<td>2</td>
<td>0.153</td>
</tr>
<tr>
<td>TCF7L2</td>
<td>2</td>
<td>2</td>
<td>0.153</td>
</tr>
<tr>
<td>G protein</td>
<td>5</td>
<td>3</td>
<td>0.153</td>
</tr>
</tbody>
</table>

SUMMARY

- 50 pregnant women screened for 10 MODY genes
- 16 PRE-GDM
- 16 OVERT GDM
- 8 GDM
- 8 positive for MODY genes [GCK, PDX1, NEUROD1, CEL, INS]
- 3 PRE-GDM
- 4 OVERT GDM
- GCK, PDX1, NEUROD1, CEL
- 1 GDM
- CEL
- Parents of 6 of the 8 women (75%) and 7 of their 15 babies (47%) screened for MODY genes

CONCLUSIONS

- MODY contributes to 16% of the pregnant women with diabetes.
- Pregnant women of Asian Indian origin harbor a higher frequency of NEUROD1 and PDX1 mutations, a pattern that differed from the western literature.
- The NGS platform provides an accurate, rapid and cost effective method for parallelized genetic testing.

REFERENCES

**INTRODUCTION**

Intensive glycemic control that forms the benchmark in the management of type 1 diabetes mellitus (T1DM) is limited by the risk of potentially dangerous hypoglycemia. The annual prevalence of severe hypoglycemia among individuals with T1DM is 30-40%.

**OBJECTIVES**

Aim: To objectively estimate the prevalence of hypoglycemia unawareness in subjects with T1DM.

**STUDY DESIGN**

Subjects with T1DM (18-50 years)

MODIFIED CLARKE'S QUESTIONNAIRE (n=164)

40 subjects with least 4 episodes of documented hypoglycaemia without symptoms in the preceding month and/or a score of >8 on the questionnaire selected for CGM

**METHODS**

We recruited 164 subjects with type 1 diabetes mellitus between 18-50 years over 3 months and administered the modified Clarke’s questionnaire.

Forty subjects (24%) with documented severe hypoglycemia, or hypoglycemia unawareness underwent a seventy two hour continuous glucose monitoring (CGM) study using the Medtronic-ipro2 CGM device-Minimed, Sylmar, CA providing 288 blood glucose measurements every 24 hours.

Subjects also self monitored blood glucose with a glucometer (6-8 times a day: pre & post meal blood glucose, 3 AM and whenever symptomatic), and maintained a symptom diary. Data was obtained using ipro2 software on the Medtronic website.

**RESULTS**

The mean HbA1c of subjects without hypoglycemia unawareness, with partial unawareness and complete hypoglycemia unawareness did not differ significantly.

**Conclusions**

Hypoglycemia unawareness was seen in 20% of subjects with T1DM, and majority of the episodes were nocturnal.

CGM identified 42% more hypoglycemic episodes in comparison to SMBG.

**Recommendations**

Glycemic Targets in T1DM need to be individualized based on severity of hypoglycemia and its awareness.

CGM is an useful tool for objective assessment of Hypoglycemia unawareness

**References**


**Acknowledgements**

Ms Banu, Mrs. Jaya, Mr. Vinod and Mr. John.
Diagnostic value of Procalcitonin for differentiation between bacterial infection and non-infectious inflammation in febrile children with systemic autoimmune disease

L. SATHISH KUMAR, T. SATHISH KUMAR, INDIRA AGARWAL

Pediatric Rheumatology and Department of Pediatrics Unit II, Christian Medical College, Vellore, India

INTRODUCTION

Septic related mortality remains high in autoimmune diseases, so rapid and accurate differential diagnosis between infection and a flare of the underlying autoimmune disease is very important. Procalcitonin (PCT) is more specific than CRP and white blood cell count for inflammatory processes due to bacterial or fungal infection and it may be helpful in distinguishing between infectious and non-infectious causes in acutely ill patients.

AIM

To determine the clinical value of procalcitonin (PCT) in differentiating bacterial infection from disease flare in children with systemic autoimmune disease.

OBJECTIVES

To determine the diagnostic accuracy of PCT in children with systemic autoimmune disease
To evaluate whether procalcitonin is useful to differentiate the disease flare and infections in children with systemic autoimmune disease.
To identify procalcitonin cut off value in children with systemic autoimmune diseases.

METHODOLOGY

Study design: Prospective cross sectional study
Setting: Pediatric Rheumatology Division, Christian Medical College, Vellore
Study period: Mar 2013- Nov 2013

Inclusion criteria
Children with systemic autoimmune diseases like SLE, JIA and JDM presenting with fever (> 38°C) to Pediatric Rheumatology Clinic and Paediatric Emergency Services

Exclusion criteria
Children already on antibiotics before admission or with chronic infections like tuberculosis, HIV or acute pancreatitis or Children with SLE, JIA and Dermatomyositis referred from outside with proven infections.

Methodology: All consecutive children with diagnosis of systemic autoimmune disease (like SLE, JIA, Dermatomyositis, vasculitis) who presented with fever > 38°C were enrolled in this study after consent. All enrolled children were carefully screened for infection by classical methods including CBC, ESR, CRP, chest radiograph and cultures of blood, urine and stool taken before antibiotic treatment. Procalcitonin estimation was also done. Children with systemic autoimmune disease presented with fever were divided into two groups (infection and disease flare group) after final diagnosis.

DATA ANALYSIS

Analyzed using SPSS software. Chi square tests of significance and coefficient of regression were used.

RESULTS

24 children with systemic autoimmune disease presented with fever were recruited
19 had disease flare and 5 had infection

Comparison of CRP and PCT in SLE both groups

CONCLUSIONS

• Our results suggested that the procalcitonin level was the most discriminatory parameter between disease flare and infection followed by CRP in children with systemic autoimmune disease.
• Procalcitonin levels > 1.2 ng/ml in febrile SLE patients should point to bacterial infection, whereas procalcitonin levels less than < 1.2 ng/ml might indicate non-infectious inflammation that could reduce unnecessary antibiotic use.
• Our study evaluated for the first time only patients with fever and previous diagnosis of systemic autoimmune disease. In this setting, PCT determination could be regarded as the better available test in identifying a possible infectious etiology and a useful laboratory tool in therapeutic decision.
A RARE CASE OF PRIMARY SIGMOID CHORIOCARCINOMA

Abhilash Cheriyan, Vimalin Samuel, Suchita Chase, Sukria Nayak.
Department of General Surgery Unit IV, Christian Medical College, Vellore

INTRODUCTION
• Primary colorectal choriocarcinoma – a rare and aggressive tumor.
• Only 14 cases reported in literature and 10 out 14 were metastatic at presentation. Median survival in reported cases of 9 months.
• We report a case of a young lady with primary sigmoid choriocarcinoma

CASE PRESENTATION
• 33 year old lady presented with generalized abdominal pain, constipation and vomiting for five days. She had altered bowel habits and associated loss of weight and appetite for 1 month.
• On examination - tachycardic and tachypneic. Abdomen was distended with tender mass in the left iliac fossa. Hb was 4.2 g/dL.
• Clinical picture was suggestive of large bowel obstruction which was confirmed by plain radiograph. She was transfused blood, however anemia was refractory.
• Underwent emergency laparotomy

DISCUSSION
• Median survival is only 4 months.
• Clinical presentation mimics colorectal adenocarcinoma.
• Retro-differentiation of adenocarcinoma and abnormal migration of primordial germ cells are possible theories to explain extra-genital choriocarcinoma.
• Typical gonadal choriocarcinoma is highly chemo sensitive, whereas for primary colorectal choriocarcinoma there is no standard regimen for chemotherapy.
• Most cases in literature had surgical excision followed by chemotherapy based on etoposide and cisplatin similar to our patient.

INTRAOPERATIVE FINDING
• Intraoperative finding- large ulcerated mass arising from the sigmoid infiltrating ureter, uterus and anterior abdominal wall, bleeding into the peritoneal cavity.
• Debulking, sigmoid colectomy and end-colostomy.
• Biopsy report was surprisingly sigmoid choriocarcinoma with normal ovaries.
• Metastatic workup - lesions in the lung, liver and adrenals.
• b-HCG -71530 mIU/ml.
• Chemotherapy with cisplatin and etoposide was initiated.
• Serial bHCG - a downward trend following initiation of chemotherapy.

CONCLUSIONS
• Primary colorectal choriocarcinoma is an aggressive tumor and is often metastatic at diagnosis with poor survival.
• Chemotherapy after surgical debulking may offer the best chance of prolonging survival in these patients

References
GOBLET CELL CARCINOID OF THE APPENDIX: CASE REPORT

A. Benjamin, S. Chase, S. Nayak
Department of General Surgery Unit IV, Christian Medical College, Vellore

INTRODUCTION
- Goblet Cell Carcinoid (GCC) is a rare appendiceal tumour, constituting 5% of all appendiceal neoplasms.
- Most commonly presents as acute appendicitis, abdominal pain, or abdominal mass.
- Prognosis is intermediate between the more benign appendicular carcinoid to adenocarcinoma.

CASE PRESENTATION
- A 70 year old man presented with complaints of colicky, central abdominal pain for 1 month.
- Intermittent melena for 1 month.
- Subacute intestinal obstruction for 10 days.

HPE
- Goblet Cell Carcinoid, with tumor reaching up to serosa.
- Perineural and lymphovascular invasion was present.
- No nodal involvement.

DISCUSSION
- GCC is unique to appendix.
- Originates from a single undifferentiated pluripotent intestinal epithelial crypt base progenitor cell.
- Dual neuroendocrine and mucinous differentiation.

DEMOGRAPHICS
Age: 18-89 years, commonly seen between 5th-6th decades.

OPERATIVE TREATMENT
- Surgical resection
- Stage I: appendicectomy alone
- Higher stages: Right hemicolectomy
- More extensive resection for locally advanced tumors may include TAH – BSO in female patients.
- Prophylactic oophorectomy to be considered due to high incidence of ovarian metastasis.
- Appendicectomy recommended in patients with Krukenbergs tumour with unknown primary.

ADJUVANT THERAPY
- Lack of evidence Standard Chemotherapy options usually followed: FOLFOX / FOLFIRI
- Intraperitoneal chemotherapy with aggressive cytoreduction (HIPEC) in selected individuals.

METASTASIS
- Most common: Transcoelomic, peritoneal. Most common site: ovary.
- Half of female patients present with ovarian metastasis.
- Mesenteric nodal involvement restricted to locally advanced tumors.

<table>
<thead>
<tr>
<th>Localized disease</th>
<th>Regional disease</th>
<th>Distant mets</th>
<th>Overall Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 year Survival</td>
<td>86%</td>
<td>74%</td>
<td>18%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Stage I</th>
<th>Stage II</th>
<th>Stage III</th>
<th>Stage IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 year survival</td>
<td>100%</td>
<td>76%</td>
<td>22%</td>
</tr>
</tbody>
</table>

INDICATORS OF POOR PROGNOSIS
- High mitotic count (>2/10 hpf)
- High Ki-67 index (>3%)
- Serosal / mesoappendiceal extension
- Nodal involvement
- Increased mucin secretion
- Tumor size not a predictor of outcome in GCC

FOLLOW UP
In labeled octreotide scintigraphy, FDG PET, Plasma Chromogranin A and Urinary 5HIAA

IMPULSE OSCILOMETRY IN THE DIAGNOSIS OF ASTHMA
Dr. S. Jebin Roger, Dr. T. Balamugesh, Dr. D.J. Christopher, Dr. Visalakshi J, Ms. Manjula R
Department of Pulmonary Medicine, Christian Medical College, Vellore, INDIA

Background
- Spirometry is a very important and helpful tool in assessing lung function and response to treatment in patients with asthma.
- Disadvantages of spirometry: 1) requires active cooperation, 2) difficult for pediatric and geriatric age group to perform 3) requires forced expiratory maneuvers.
- Impulse oscilometry (IOS) is a convenient and effort free method of testing pulmonary function which is based on the forced oscillation technique.

Objectives
- Primary objective: To find out a reference value for significant reversibility of R5 after bronchodilator by impulse oscilometry with spirometry as the Gold standard.
- Secondary objective: To grade severity of airway obstruction by impulse oscilometry by correlating with FEV1 by spirometry.

Methods
- A total of 636 patients and 177 healthy individuals were included.
- IOS followed by spirometry before and after bronchodilator was performed after filling asthma questionnaire.
- Correlation coefficients were calculated between FEV1 and R5 and ROC analysis was done to obtain cutoff values at the best sensitivity and specificity for IOS parameters.

Fig: 4 IOS parameters in patients with and without obstruction

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Obstruction*</th>
<th>No obstruction</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
<td>Median</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td>FEV1% predicted</td>
<td>13.6 – 109.1</td>
<td>53</td>
<td>52.7 ± 17.66</td>
</tr>
<tr>
<td>(Pre bronchodilator)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FEV1 (LITER)</td>
<td>0.26 – 3.46</td>
<td>1.36</td>
<td>1.44 ± 0.63</td>
</tr>
<tr>
<td>(Pre bronchodilator)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R5% predicted*</td>
<td>0.19 – 2.28</td>
<td>0.72</td>
<td>0.81 ± 0.38</td>
</tr>
<tr>
<td>(Pre bronchodilator)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R5 kPa l (L/s)</td>
<td>72.7 – 674.4</td>
<td>232.5</td>
<td>250.34 ± 106.19</td>
</tr>
</tbody>
</table>

Fig: 5 Airway obstruction by IOS

Correlation coefficient between FEV1% and R5% was -0.583 (p<0.001)

<table>
<thead>
<tr>
<th>Sensitivity</th>
<th>Specificity</th>
<th>AUC</th>
<th>Cut off value</th>
</tr>
</thead>
<tbody>
<tr>
<td>R5% predicted*</td>
<td>72.7%</td>
<td>70%</td>
<td>0.776</td>
</tr>
</tbody>
</table>

Fig: 6 Severe airway obstruction by IOS

<table>
<thead>
<tr>
<th>Sensitivity</th>
<th>Specificity</th>
<th>AUC</th>
<th>Cut off value</th>
</tr>
</thead>
<tbody>
<tr>
<td>R5% predicted*</td>
<td>63.2%</td>
<td>70.1%</td>
<td>0.739</td>
</tr>
</tbody>
</table>

Conclusions
This study to our knowledge is the first Indian study which has evaluated IOS parameters in patients with asthma. We suggest using:
1. A value of > 20% change in R5% post bronchodilator to be considered as significant bronchodilator reversibility by IOS
2. A value of > 180% for pre bronchodilator R5% predicted to diagnose airway obstruction in asthma
3. A value of > 230% for pre bronchodilator R5% predicted to diagnose severe airway obstruction in asthma.
INTRODUCTION

Klinefelter syndrome (KS) is the most common cause of male hypogonadism and is characterized by the presence of an additional X chromosome. It is found in 1/800 live birth in males and is associated with androgen deficiency, small testes, azoospermia and infertility. Klinefelter syndrome is difficult to diagnose until adolescence or adulthood because the clinical features are subtle and vary with age. Therefore, cytogenetic analysis is the gold standard for diagnosis.

In the majority of patients, the karyotype is 47,XXY. Mosaic karyotypes with a neoploidy and normal cell lines (47,XXX/46,XY) or variants with two or more additional X chromosomes or a structurally abnormal X chromosome may also be seen.

PATIENTS AND METHODS

Patients: All patients seen whose karyotypes showed Klinefelter syndrome were included in the analysis.


Method:
- 25 G-banded metaphases from peripheral blood culture for 72hrs with phytohemagglutinin stimulation were analysed for each case.
- The clinical and laboratory findings including results of polymerase chain reaction for microdeletions of the AZF region on the Y chromosome were recorded.

RESULTS

- There were 90 patients with Klinefelter syndrome.
- Sixteen were below 18 years of age and 74 were adults.
- KS was diagnosed or suspected clinically in only 58 out of 90 patients.
- The median age of adults at presentation was 26 years, mean height was 172 cm, mean arm span was 176.75 cm and mean testicular volume was 3 ml.
- The majority of patients (88%) had a 47,XXY karyotype. Mosaic 47,XXY/46,XY karyotypes were seen in five patients and variants 48,XXX,49,XXXX,48,XXXY in seven.
- Plasma levels of LH, FSH and testosterone were consistent with hypogonadotrophic hypogonadism.
- Submicroscopic deletion of the azoospermia factor (AZF) region on the Y chromosome was seen in 1 out of 14 adults tested.

Clinical Features

<table>
<thead>
<tr>
<th>Clinical features</th>
<th>Prepubertal (n=88)</th>
<th>Pubertal (n=17)</th>
<th>Adult (n=74)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gynecomastia</td>
<td>0</td>
<td>6 (35.3%)</td>
<td>27 (36.5%)</td>
</tr>
<tr>
<td>Hypogonadotropic Hypogonadism</td>
<td>0</td>
<td>4 (25%)</td>
<td>26 (35.1%)</td>
</tr>
<tr>
<td>Small testes</td>
<td>0</td>
<td>0</td>
<td>21 (28.4%)</td>
</tr>
<tr>
<td>Infertility</td>
<td>0</td>
<td>0</td>
<td>8 (10.3%)</td>
</tr>
<tr>
<td>Azoospermia</td>
<td>0</td>
<td>0</td>
<td>18 (24.3%)</td>
</tr>
<tr>
<td>Obesity</td>
<td>0</td>
<td>2 (25%)</td>
<td>8 (10.3%)</td>
</tr>
<tr>
<td>Underdeveloped secondary sexual character</td>
<td>0</td>
<td>1 (12.5%)</td>
<td>7 (9.5%)</td>
</tr>
<tr>
<td>Tall stature</td>
<td>0</td>
<td>0</td>
<td>7 (9.5%)</td>
</tr>
<tr>
<td>Microopen</td>
<td>0</td>
<td>2 (25%)</td>
<td>5 (6.8%)</td>
</tr>
<tr>
<td>Horseback body habitus</td>
<td>0</td>
<td>1 (12.5%)</td>
<td>5 (6.8%)</td>
</tr>
<tr>
<td>Brachydactyly</td>
<td>0</td>
<td>0</td>
<td>5 (6.8%)</td>
</tr>
<tr>
<td>Loss of libido</td>
<td>0</td>
<td>0</td>
<td>5 (6.8%)</td>
</tr>
<tr>
<td>Speech delay</td>
<td>0</td>
<td>0</td>
<td>5 (6.8%)</td>
</tr>
<tr>
<td>Mental retardation</td>
<td>0</td>
<td>0</td>
<td>5 (6.8%)</td>
</tr>
<tr>
<td>Dysmorphism</td>
<td>6 (75%)</td>
<td>1 (12.5%)</td>
<td>2 (2.7%)</td>
</tr>
<tr>
<td>Developmental delay</td>
<td>5 (62.5%)</td>
<td>1 (12.5%)</td>
<td>0</td>
</tr>
</tbody>
</table>

CONCLUSION

Karyotyping is essential for the diagnosis of Klinefelter syndrome because the clinical features are variable.

A definitive diagnosis of Klinefelter syndrome is essential for the proper management of these patients especially with respect to the development of normal body proportions in young boys.

It also helps to counsel those with infertility appropriately so that informed decisions may be made.

REFERENCES

INTRODUCTION

- Rare and highly aggressive mesenchymal tumor.
- 200 cases reported in literature.
- Mainly adolescents and young adults.
- Belongs to the group of childhood tumors – PNETs, Ewing’s, alveolar and embryonic rhabdomyosarcoma and synovial sarcomas.
- Median survival: 17-25 months.
- Five year survival: < 20%
- Mean age at diagnosis: 22.
- Male: Female = 4:1

OPERATIVE FINDINGS

A 15x15 cm solid tumor was seen in the infracolic omentum. There were omental deposits adjacent to right gastroepiploic vessels.

DISCUSSION

- Typically develops in the abdominal cavity, invading omentum with multiple peritoneal implants. Primary in testes, ovaries and pleura have been reported.
- Liver and lung are common sites of metastasis.
- Clinical Features: Mainly due to size: Abdominal pain and distension, ascites, vomiting, weight loss, constipation and bowel obstruction.

HISTOLOGY

- Small round blue cells in nests separated by abundant desmoplastic stroma.
- IHC markers – keratin, epithelial membrane antigen, NSE, Desmin.
- Chromosomal translocation – t(11;22)(p13;q12) that involves EWSR1 and WT1 gene.

MANAGEMENT

- Treatment is aggressive and multimodal.
- Surgical debulking is the mainstay.
- P6 regimen – 7 cycles of cyclophosphamide, doxorubicin, vincristine, alternating with ifosfamide and etoposide.

<table>
<thead>
<tr>
<th>Tumour Location</th>
<th>Treatment options</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peritoneal disease</td>
<td>Surgery, whole abdominal RT, HIPEC</td>
</tr>
<tr>
<td>Liver metastasis</td>
<td>Surgery, Stereotactic radiosurgery, RFA, Y90 Microspheres</td>
</tr>
<tr>
<td>Lung metastasis</td>
<td>Surgery, Stereotactic radiosurgery</td>
</tr>
<tr>
<td>Mediastinal lymph nodes</td>
<td>RT</td>
</tr>
<tr>
<td>Bone metastasis</td>
<td>RT</td>
</tr>
</tbody>
</table>

REFERENCES:

**Introduction**

- Tuberculous fistula in ano occurs in 5-7% patients in India
- There are very few clinical predictors for diagnosis of tuberculous fistula in ano
- Diagnosis is mainly by
  - Histopathology (HPE)- demonstration of caseating granulomas
  - Microbiology- Ziehl Neelsen staining / Culture (LJ medium/MGIT)
- Xpert TB PCR is a recently introduced diagnostic test for TB
  - Provides results within 2 hours
  - Also provides sensitivity towards Isoniazid and Rifampicin

**Clinical presentations of tuberculous fistula in ano**

**Aim**

- To determine accuracy of TB PCR in diagnosing tuberculous fistula in ano, keeping either culture positivity or histological evidence of TB as gold standard

**Methodology**

- Retrospective study July 2012-Dec 2013
  - Single colorectal unit of a tertiary care teaching hospital
  - All new cases of fistula in ano
  - Data collected from prospectively maintained database
  - Chi square test used for analysis

**Results**

- 237 new patients of fistula in ano
- Results of HPE, culture and TB PCR were available for 187 patients
- 169 (90.4%) males
- Mean age = 42 years (S.D. = 11.4 years)
- TB was diagnosed in 7 patients (3.7%)

**Number of patients with tuberculous fistula in ano**

- HPE positive in 2 (1.1%)
- Culture was positive in 7 (3.7%)
- TB PCR was positive in 3 (1.6%)

**Correlation between tests**

- TB PCR when compared to histological positivity or culture positivity showed a sensitivity of 42.9% and specificity of 100%

<table>
<thead>
<tr>
<th></th>
<th>HISTOPATHOLOGY/ AFB CULTURE POSITIVE</th>
<th>HISTOPATHOLOGY/ AFB CULTURE NEGATIVE</th>
</tr>
</thead>
<tbody>
<tr>
<td>POSITIVE</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>NEGATIVE</td>
<td>4</td>
<td>173</td>
</tr>
</tbody>
</table>

**Conclusions**

- TB PCR has low sensitivity but a high specificity to diagnose tuberculous fistula in ano
- TB PCR alone cannot be used as a diagnostic tool for tuberculous fistula in ano
- Larger studies with more patients are required to further validate these results
Pharmacokinetics of Fludarabine in patients with Aplastic Anemia Undergoing Haematopoietic Stem Cell Transplantation


Department of Hematology, Christian Medical College, Vellore, *St. Jude’s Children’s Research Hospital, Memphis, TN, USA

BACKGROUND
- Hematopoietic stem cell transplantation (HSCT) is the best treatment option for young patients with aplastic anemia
- Conditioning with fludarabine (Flu) and cyclophosphamide (Cy) has been associated with improved long-term survival in patients undergoing HSCT.
- Graft versus host disease (GVHD), graft failure and infection associated mortality are some of the major hurdles towards a successful outcome.
- Limited data available on the association between plasma flu levels and HSCT outcome showed higher plasma Flu AUC levels to be a risk factor for non-relapse mortality.
- Fludarabine given intravenously as fludarabine monophosphate is readily converted to Flu by the enzyme ecto-5’-nucleotidase or NTSE and then is taken up into the cells by nucleoside transporters.
- NTSE mRNA expression and gene polymorphism in the lymphoblastoid cell lines have been shown associated with cytotoxicity to thiorpurine drugs; but its role on Flu pharmacokinetics (PK) is not known.

OBJECTIVE
- To evaluate the population PK of Fludarabine in patients with aplastic anemia undergoing HSCT and to test their influence on transplant outcome

PATIENTS AND METHODS
- Thirty patients diagnosed with aplastic anemia and undergoing HLA identical sibling HSCT using a Flu/Cy based conditioning regimen between January 2012 and February 2014 at the Department of Hematology, Christian Medical College, Vellore were included in the study after informed written consent (Table).

SAMPLING
- Pre-HSCT DNA was used to screen polymorphisms in the NTSE gene.
- Plasma was separated from the peripheral blood collected before (0hr) and 1, 2, 3, 5, 7 and 24 hrs after the infusion of fludarabine (30mg/m2/day x 6 days over 1 hr infusion) on days 1 and 4 and stored at -80°C until further analysis.

FLUDARABINE PHARMACOKINETICS
- Plasma fludarabine was analyzed using an in-house developed and validated LC-MS/MS based method.
- The concentration was expressed as ng/ml.
- Flu PK was estimated using the 2-compartment model with linear elimination.

POP PK MODELING
- Inter-occasion variability (between dose 1, 4, 5, and 6 PK studies) on the volume (V) and elimination rate (k) was accounted for in the model.
- The covariates tested were: age, sex, body weight, BSA, disease type, and a polymorphism in NTSE gene.
- The population PK was analyzed using Monolix 4.3.2.
- The PK parameters AUC, CL, V and k were calculated for day 1 and day 4 of sampling.
- The PK and PG parameters estimated are listed in Table.

OUTCOME ANALYSIS
- The influence of flu PK on clinical outcome parameters including overall survival, GVHD and rejection were estimated using Cox regression analysis.

RESULTS
- Flu PK showed wide inter-individual variation in patients with aplastic anemia receiving fludarabine based conditioning regimen (Table), which could be explained by the polymorphism in the gene converting the prodrug to active form of fludarabine.
- The NTSE 5’UTR variant rs2295890 was in complete linkage disequilibrium with 4 other SNPs namely: rs9450278, rs9450279, rs4559602, rs44458647 as seen both in patients and in normal healthy volunteers.
- Patients with variant genotype for rs2295890 showed significantly lower plasma Flu clearance compared to those with wild type genotype (p = 0.0244) (Figure).
- Comparison of Flu PK parameters with previous studies is not possible due to heterogeneous population of patients, Flu dose and donor type included.

FLUDARABINE PK ON CLINICAL OUTCOME
- Of the 30 patients, 26 (86.6%) engrafted at a median of 14 days post BMT (range: 11-18) while 2 (6.6%) had primary graft failure and 2 died within the first 2 weeks of transplant.
- Grade 2-4 acute GVHD was seen in 6 (25%) with chronic GVHD in 28.5%.
- One patient had secondary graft failure. The Day 100 mortality was 26.6% and at present 19 (63.3%) are alive.

None of the Flu PK parameters showed any significant association with engraftment, GVHD, rejection and mortality.

<table>
<thead>
<tr>
<th>Table: Patient demographics, PK, and PG parameters</th>
<th>Figure: NTSE 5’UTR rs2295890 vs. Flu Clearance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient demographics</td>
<td>N=30</td>
</tr>
<tr>
<td>Median (range)</td>
<td></td>
</tr>
<tr>
<td>Age, yrs</td>
<td>25 (15-57)</td>
</tr>
<tr>
<td>Body wt, kg</td>
<td>58 (12-89)</td>
</tr>
<tr>
<td>BSA, m²</td>
<td>1.56 (0.56-1.83)</td>
</tr>
<tr>
<td>Sex ratio, M/f</td>
<td>21.9</td>
</tr>
<tr>
<td>Day 1 AUC, ng/ml/L</td>
<td>4000 (1020-17430)</td>
</tr>
<tr>
<td>Day 4 AUC, ng/ml/L</td>
<td>3860 (1220-11340)</td>
</tr>
<tr>
<td>Day 1 CL, L/ml</td>
<td>7.91 (1.22-29.83)</td>
</tr>
<tr>
<td>Day 4 CL, L/ml</td>
<td>7.58 (2.47-24.79)</td>
</tr>
<tr>
<td>Day 1 V, mL</td>
<td>31.60 (10.48-76.53)</td>
</tr>
<tr>
<td>Day 4 V, mL</td>
<td>28.79 (11.07-74.72)</td>
</tr>
<tr>
<td>Day 1 L/C</td>
<td>0.23 (0.01-0.44)</td>
</tr>
<tr>
<td>Day 4 L/C</td>
<td>0.24 (0.03-0.46)</td>
</tr>
<tr>
<td>rs2295890 genotype</td>
<td>GG = 20</td>
</tr>
<tr>
<td>r2295890 genotype</td>
<td>GC = 09</td>
</tr>
<tr>
<td>r2295890 genotype</td>
<td>CC = 01</td>
</tr>
<tr>
<td>Variant allele frequency</td>
<td>0.18</td>
</tr>
</tbody>
</table>

CONCLUSION
- This first study on Flu PK in a uniform cohort of patients with aplastic anemia undergoing HSCT shows that the PK of Flu is highly variable and there is genetic basis for this variation.
- The clinical significance of the variation in the Flu PK needs to be studied in a larger cohort of patients.

REFERENCES
2. Long-Boyal J et al., Bone Marrow Transplant. 2011 Jan;46(1):20-6

ACKNOWLEDGEMENT
Department of Nephrology, GDI, for funding the project grant No. 84/PR/1387/NED/12/2013
Masked hypoglycemia in women with gestational diabetes on insulin - A study from a tertiary care center in South India

Rita Gupta Patil1, Dukhabandhu Mallick2, DM Mahesh1, HS Asha1, Mercy Inbakumar1, Flory Christina1, Thomas Vizhali Paul1, Jiji Mathew1, Ruby Jose1, Jessie Lionel1, Annie Regi1, Visalakshi2, Nihal Thomas1
1 Department of Endocrinology, Diabetes & Metabolism, 2 Department of Obstetrics and Gynaecology, 3 Department of Biostatistics

CONTEXT
Gestational diabetes mellitus [GDM] is defined as any degree of carbohydrates intolerance with onset or first recognition during pregnancy. Optimal glycemic control in women with gestational diabetes is essential to prevent adverse maternal and fetal outcomes, but hypoglycemia is a major hindrance.

OBJECTIVE
To estimate the prevalence of masked hypoglycemia in women with gestational diabetes on insulin therapy using continuous glucose monitoring system [CGMS].

METHODS

STUDY PERIOD: 3 months
Thirty pregnant women [20 subjects with gestational diabetes (cases) and 10 age matched pregnant women with normal oral glucose tolerance test (OGTT) (controls)] between 24 to 36 weeks of gestation with singleton fetuses were recruited.

INCUSION CRITERIA:
1. Age: > 18 years
2. Gestational diabetes according to ADA (2011) criteria
3. Gestational age between 24-36 weeks of pregnancy
4. Literate

EXCLUSION CRITERIA:
1. Multiple pregnancy
2. Chronic hypertension
3. Pregestational diabetes

The glycemic profile was studied using Medtronic CGMS device [ipro2 MiniMed, Sylmar, CA] over a period of 72 hours. This measures interstitial glucose every 5 minutes providing 750 readings per subject.

Blood glucose was self monitored with a glucometer (6-8 times a day: pre & post meal blood glucose, 3 AM and if symptomatic). They followed standard dietary advice and performed their regular activities.

Data was obtained using ipro2 software on the Medtronic website.

RESULTS
Hypoglycemia was noted in 40% (8/20) of cases and 50% (5/10) of controls. The number of masked hypoglycemic episodes among cases and controls were 3.7 and 2.6 per subject respectively.

- The proportion of time spent at glucose 41-50mg/dl was similar in the two groups (1.95% vs. 1.7%).
- At glucose ≤40mg/dl it was significantly longer in cases as compared to controls (5.65% vs. 1.2%).

CONCLUSIONS

- Women with normal pregnancy as well as those with GDM have masked hypoglycemia.
- The proportion of time spent at glucose ≤40mg/dl was significantly longer in women with GDM on insulin.
- The impact of this on maternal and fetal outcomes needs to be further studied in a larger population, as a separate outcome measure.
- We recommend bedtime snacking for all pregnant women to prevent hypoglycemia.

References

Isolation of human skeletal muscle satellite cells from rectus abdominis muscle

David Livingstone *,†, Albert Kota #, Karthikeyan Rajagopal *,†, Sanjay Chilbule *, Sukria Nayak #, Vrishka Madhuri *,†
*Paediatric Orthopaedics Unit, †Department of Surgery Unit IV, CSCR

Background

- Satellite cells (SC) are muscle progenitor cells responsible for postnatal growth and maintenance.
- These cells can be used to treat for muscle disorders like sphincter dysfunction and muscle loss due to dystrophy or trauma.
- Human studies are very limited due to lack of well established protocols for isolation of SC

Aim

To isolate and characterize the human skeletal muscle satellite cells from human rectus abdominis muscle.

Methodology

Sample Collection

- Muscle biopsy from patients undergoing abdominal surgeries after informed consent.
- Tissue stripped off connective tissues.
- Collagenase type II digestion for 40 minutes.
- Serially filtered in 100μg & 70μg and washed.

Isolation of Cells

- Plated on extracellular matrix coated plates.
- Cultured till near confluence in DMEM + 10% FBS.

Culturing of Cells

- Immunocytochemistry analysis for day 3, day 8 and day 12 cultured cells.
- RT-PCR for day 8 after normalizing with day 3 culture.
- Analyzed for Satellite cell markers.

Characterization of cells

Day 8 cultured cells showing positive for satellite cell marker Pax7, Myf5.

Day 12 cultured cells showing positive for satellite cell marker Pax7, Myf5.

Results

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Age of patient (years)</th>
<th>Sample weight (gram)</th>
<th>Cell yield at confluence</th>
<th>Day of confluence</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>30</td>
<td>1.194</td>
<td>3.04 x 10⁵</td>
<td>12</td>
</tr>
<tr>
<td>2</td>
<td>35</td>
<td>0.781</td>
<td>1.02 x 10⁵</td>
<td>08</td>
</tr>
<tr>
<td>3</td>
<td>76</td>
<td>0.270</td>
<td>0.96 x 10⁵</td>
<td>08</td>
</tr>
<tr>
<td>4</td>
<td>58</td>
<td>0.063</td>
<td>1.95 x 10⁵</td>
<td>09</td>
</tr>
<tr>
<td>Mean</td>
<td>49.74 ± 21.32</td>
<td>0.802 ± 0.39</td>
<td>1.54 ± 0.93 x 10⁵</td>
<td>0.23 ± 0.09</td>
</tr>
</tbody>
</table>

Cell Culture

Phase Contrast image of Cells Cultured on extracellular matrix coated plates on different dates.

Day 3

Day 8

Day 12

Immunocytochemistry Analysis

Day 3 cells in supernatant showing positive for satellite cell marker Pax7, Myf5.

RT-PCR Analysis

After normalizing with day 3, Cells cultured till Day 8 showed 4 and 5 fold increase in Satellite cell markers Pax7 and Myf5.

Discussion

By day 8 isolated cells start to express myogenic marker Myf5, MyoD suggestive of differentiation to myocytes.

Day 12 cultures show typical multinucleated myotube like structures. It is therefore advisable to use the isolated cells for transplantation on day 8 of culture.

Conclusion

Thus a modest, reproducible protocol for isolating satellite cells from human muscle was standardized. This can be adopted to provide GMP grade satellite cells for clinical use.

Acknowledgement

We acknowledge Center for Stem Cell Research for providing required facilities for carrying out this work.
Clinico-pathological profile and outcomes in Metastatic Malignant Phyllodes tumors of the breast- case series of six patients.

Pooja Ramakant, Marie Theresa, Selvamani, MJ Paul,
Endocrine Surgery Department, Pathology, Radiation Therapy Department,
Christian Medical College, Vellore

Introduction
Phyllodes tumors are biphasic tumors with broad pathological spectrum ranging from benign, borderline malignant to malignant phyllodes tumors. Malignant phyllodes tumors (PT) of the breast behave aggressively and have varied patterns of distant metastases resulting in poor survival.

Aim
To assess clinic-pathological outcome of patients with distant metastases in malignant PTs.

Method
A retrospective analysis of patients (167) with Phyllodes tumors of the breast was done from January, 2001 to January, 2014.

Result
Detailed analysis of these 6 patients showed that mean age was 34.67 years (±2.6), 2 patients had recurrences post lumpectomy. Regarding surgical options, 1 patient had wide local excision with muscle reconstruction and other 5 had mastectomy with nodal clearance. The distant metastases were synchronous in 2 patients and other 4 had metastases developing within 2 months to 13 years.

Conclusion
Patients with malignant PT of the breast with distant metastases have a dismal outcome and behave aggressively. They need multidisciplinary approach and close follow-up. Novel targeted therapies may have a role to improve the outcome.

References
A Prospective Observational Study Comparing Cardiac Function of Small for Gestational Age with Appropriate for Gestational Age Babies Using Serial Echocardiographic Studies.

Aim:
To evaluate the cardiac functions of SGA babies by serial echocardiographic measurements and compare this with appropriate for gestational age (AGA) babies during the early postnatal period.

Methodology:
Design: A prospective observational study.
Period: March 2013 to February 2014.

Inclusion: 36 babies born between 34 and 44 weeks of gestation with birth weight + 2 Standard deviation (around 3rd centile) and per WHO growth standards (FENTON) were included as study group. For each baby in the study group a baby of similar gestational age but with birth weight between 10% to 90% centile was selected to form the comparison group.

Exclusion: Obstetric babies, infants of consent, severe perinatal asphyxia, babies with major congenital anomalies, congenital heart disease, infants of diabetic mother, maternal chromosomal and annemia.

Study was approved by the Institutional Research Board and ethics committee, and individual parental consent was obtained for all infants.

Three consecutive echocardiography (Vivid 8, 68 probe, GE Healthcare) were performed by a single observer at 0-4 hours, 24-36 hours and 48-72 hours on all babies who fulfilled the criteria cited in the primary outcome being to compare cardiac function, measured primarily by the myocardial performance index (MPI). (1) Following are the other cardiac parameters (2):

Cardiac Dimension: left ventricle internal diameter during diastole (LVIDd), left ventricle internal diameter during systole (LVIDs) and left ventricle end systolic ratio (LVESR).

Systolic Function: fractional shortening (FBS), ejection fraction (EF), area shortening (AS), left ventricle output (LVO), stroke volume (SV), ejection time (EVT), and ICT. PWD was used to measure A/T and I/C/T.

Diastolic Function: TAVI, A-V ratio, E-V deceleration time (EDT), at both the AV valves, and deceleration time (DRT) of both left and right ventricle. The ratio of the mitral E wave to the isovolumic relaxation time (E/I).

Superior vena cava (SVC) flow (3):

Y max, resitivity index and pulsatility index in anterior cerebral artery, superior mesenteric artery and anterior tibial artery.

Statistics and Sample Size Calculation:
The sample size was to show a difference in left ventricle MPI across SGA and AGA infants was found to be 35 infants in each group with 80% power and 5% level of significance when the difference in MPI was expected to be 0.04 and a variation of 0.05 units across the infants in both the groups.

The analyses were done separately for AGA babies and SGA babies using SPSS v16.0. All the quantitative variables were summarised using mean and standard deviation for parametric data and median and range for non-parametric data. All categorical variables were summarised using frequencies and percentages. Depending upon distribution of the continuous variable Student's t-test or Mann-Whitney U test were used for determining significance. We used Chi-squared test or Fisher's exact test for categorical variables. P value < 0.05 was considered as significant.

Table 1: Demographic and Clinical Findings of Study Population

<table>
<thead>
<tr>
<th>Parameter</th>
<th>SGA (n=18)</th>
<th>AGA (n=18)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth weight (g)</td>
<td>1712±62</td>
<td>3248±417</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Length (cm)</td>
<td>48±2</td>
<td>50±2</td>
<td>0.01</td>
</tr>
<tr>
<td>Birth weight/age</td>
<td>1.8±0.8</td>
<td>1.8±0.8</td>
<td>0.70</td>
</tr>
<tr>
<td>Mode of delivery</td>
<td>0.05</td>
<td>0.05</td>
<td>0.85</td>
</tr>
<tr>
<td>External cephalic</td>
<td>43%</td>
<td>43%</td>
<td>0.85</td>
</tr>
<tr>
<td>Delivery site</td>
<td>43%</td>
<td>43%</td>
<td>0.85</td>
</tr>
<tr>
<td>Antenatal steroids</td>
<td>43%</td>
<td>43%</td>
<td>0.85</td>
</tr>
<tr>
<td>Intravenous steroids</td>
<td>43%</td>
<td>43%</td>
<td>0.85</td>
</tr>
<tr>
<td>Ventilation</td>
<td>43%</td>
<td>43%</td>
<td>0.85</td>
</tr>
</tbody>
</table>

Table 2: Index of Myocardial Performance (MPI)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>SGA (n=18)</th>
<th>AGA (n=18)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVID d</td>
<td>18±1</td>
<td>12±1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LVID s</td>
<td>8±1</td>
<td>7±1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LVESR</td>
<td>0.7±0.1</td>
<td>0.7±0.1</td>
<td>0.20</td>
</tr>
<tr>
<td>EF</td>
<td>50±10</td>
<td>60±10</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>EF s</td>
<td>30±10</td>
<td>40±10</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>AS</td>
<td>50±10</td>
<td>60±10</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>AS s</td>
<td>30±10</td>
<td>40±10</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Stroke volume</td>
<td>50±10</td>
<td>60±10</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Stroke volume s</td>
<td>30±10</td>
<td>40±10</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Table 3: M mode measurements: Cardiac dimension, Fractional shortening and Ejection fraction

<table>
<thead>
<tr>
<th>Parameter</th>
<th>SGA (n=18)</th>
<th>AGA (n=18)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVID d</td>
<td>18±1</td>
<td>12±1</td>
<td>&lt;0.001</td>
</tr>
<tr>
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</tr>
<tr>
<td>LVESR</td>
<td>0.7±0.1</td>
<td>0.7±0.1</td>
<td>0.20</td>
</tr>
<tr>
<td>EF</td>
<td>50±10</td>
<td>60±10</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>EF s</td>
<td>30±10</td>
<td>40±10</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>AS</td>
<td>50±10</td>
<td>60±10</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>AS s</td>
<td>30±10</td>
<td>40±10</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Stroke volume</td>
<td>50±10</td>
<td>60±10</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Stroke volume s</td>
<td>30±10</td>
<td>40±10</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Figure showing LVOG and SVC flow (ml/kg/min)

Results:
- We found significantly higher values of MPI in both ventricles in SGA babies compared to AGA babies on all days except MPI of right ventricle on day 3.
- SGA babies had significantly increased heart rate in comparison to AGA babies during first 3 days of life.
- Left ventricular internal diameter index during diastole and systole (LVDD index and LVDS index), LVAD ratio were significantly increased in SGA babies in comparison to AGA babies in the first 72 hours.
- Fractional shortening, ejection fraction, area shortening, cardiac output or superior vena cava flow were similar in two groups.
- SGA babies had lower vascular resistance in anterior cerebral artery and higher vascular resistance in celiac and superior mesenteric artery in comparison to AGA babies.
- There was no difference in timing of closure of ductus arteriosus in both the groups.

Conclusion:
- We have observed in our study that : volume independent parameters of cardiac function like MPI, ejection time and mitral valve E1 were significantly abnormal in SGA babies as compared to AGA babies. Volume dependent parameters like fractional shortening, ejection fraction, area shortening and stroke volumes were however similar in both the groups.
- We noted higher value of left ventricular internal diameter index during diastole and systole (LVDD index and LVDS index) and LVAD ratio in SGA babies in comparison to AGA babies which could be because of volume overload in SGA babies.
- It may be postulated that SGA babies have compromised myocardial function which is detected only by volume independent parameters as the volume overload causes an increased preload and cardiac contractility (Starling's law)

Limitations:
- Antenatal doppler studies are not available for all the babies.
- First trimester dating scan was not available in all the babies. Date are not analyzed separately for term and late preterm babies.

References:
PREDICTING PATHOLOGICAL COMPLETE RESPONSE IN RECTAL CANCER USING CLINICAL VARIABLES

Rajat Raghunath, Gigi Varghese, Rohin Mittal, M R Jesudason, Benjamin Perakath
Surgery Unit 2 (Colorectal Surgery), Christian Medical College, Vellore

Background
- The standard of care in locally advanced carcinoma rectum is neoadjuvant chemoradiotherapy followed by surgery.
- Preoperative imaging is believed to be a good tool in assessing response to neoadjuvant therapy.
- Pathological complete response (pCR) after neoadjuvant therapy is associated with improved 5-year survival rates.
- pCR is achieved in only 20%.
- Ability to predict pCR could help in prognostication

Objectives
- To assess the correlation between MRI regression grade and tumour regression grade
- To study clinical variables which can help predict pathological complete response

Methods
- A cross sectional study was done among 62 patients with locally advanced carcinoma rectum over a period of one year (January 2012-February 2013)
- All patients received long course radiation therapy with 5040cGy over duration of 28 days with concurrent chemotherapy.
- After an interval of approximately 6 weeks they had a repeat pelvic MRI and then underwent curative surgery.
- Risk factors studied were: age, sex, serum CEA, type of chemotherapy, presence of mucin, histopathological grade, T stage, location of tumour and preradiation absolute lymphocyte and leucocyte count.

Results
- 62 patients with locally advanced carcinoma rectum were included with the mean age being 49 years.
- Forty six patients (74.2%) were male and 16 (25.8%) were female.
- MRI regression grade was found to correlate with pathological regression grade. (Spearman’s rho 0.374)
- Pathological complete response was seen in 13 patients (20.9%)
- Absolute lymphocyte count was found to be a significant predictor of pCR (p=0.003)
- pCR was seen more frequently in men (OR=2.2) and in those with T3 disease (OR=3.3)
- No other factor was found to significantly affect complete pathological response.

Response to neoadjuvant therapy

Conclusions
- MRI tumour regression grade correlates well with pathological response grade
- Patients with complete pathological response had a significantly higher preradiation absolute lymphocyte count.
**INTRODUCTION**

- Chronic myeloid leukemia (CML) is a paradigm for neoplasias
- Most extensively studied malignancy - accounts for ~15% of adult leukemia
- Myeloid malignancy characterized by presence of Philadelphia chromosome (t(9;22)) resulting in production of a constitutive tyrosine kinase BCR-ABL
- Competitive tyrosine kinase inhibitor - occupies ATP-binding pocket of ABL kinase domain - prevents activation of BCR-ABL fusion protein.

**OBJECTIVES**

- To determine association between trough plasma imatinib and Desmethyl imatinib levels with molecular response in CML.
- To evaluate the role of polymorphisms in imatinib transporters (HCO1, ABCB1, and ABCC2) and metabolizing enzymes (CYP3A4, CYP3A5) on molecular response to imatinib therapy in newly diagnosed patients with CML.

**PATIENTS AND METHODS**

- **Imatinib naive CML patients**
  - FISH analysis
  - RT QPCR
  - Cytogenetics/Karyotyping
  - Peripheral blood sampling
  - DNA extracted from peripheral blood
  - Screening the polymorphism of drug transporter and metabolizing enzyme by sequencing or RFLP
  - Measurement of trough plasma imatinib and Desmethyl imatinib levels using HPLC with UV detection

**RESULTS**

<table>
<thead>
<tr>
<th>Patient Demographics</th>
<th>Molecular Response at 12 months</th>
<th>Patients (n)</th>
<th>Molecular Response at 12 months predicts Overall survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Imatinib naive CML</td>
<td></td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>In CML</td>
<td></td>
<td>38</td>
<td></td>
</tr>
<tr>
<td>Lost to follow-up</td>
<td></td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Evaluated from further</td>
<td></td>
<td>38</td>
<td></td>
</tr>
<tr>
<td>Plasma</td>
<td></td>
<td>113</td>
<td></td>
</tr>
<tr>
<td>Chronic phase</td>
<td></td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Acute phase</td>
<td></td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Blast phase</td>
<td></td>
<td>10</td>
<td></td>
</tr>
</tbody>
</table>

**CONCLUSION**

- High trough plasma imatinib levels correlate with achievement of MMR/CMR at 12 months.
- Trough plasma imatinib concentration required to achieve MMR/CMR seems higher in initial population.
- The NOCT1 gene polymorphisms were associated with molecular response and overall survival.
- ABCB2 promoter and ABCB1 variants influence the Day 29 plasma imatinib concentration.
- This study suggests genetic polymorphism in drug transporter and metabolizing enzyme contributes to interindividual variations in response and plasma imatinib concentration in CML patients.
- Therapeutic monitoring of imatinib and molecular monitoring for BCR-ABL are important for management of CML and to early intervention in terms of increasing the dose or switching to 2nd or 3rd generation TKIs.

**ACKNOWLEDGEMENT**

- Dept of Biotechnology, India Grant no: BT/02/40/2009 for financial support to Dr. Preetha Srinivasan.
De Barsy Syndrome type B presenting with cardiac and genitourinary abnormalities

Dutta AK¹, Thomas N², Ekbote A¹, Omprakash S¹, Danda S¹

¹Department of Clinical Genetics and ²Department of Neonatology, Christian Medical College, Vellore, India

PURPOSE
- To describe a child with genetically proven De Barsy syndrome type B.
- To highlight the cardiac and genitourinary abnormalities in the index child which had not been documented in earlier reports.
- To showcase the utility of clinical exome sequencing in the molecular confirmation of rare inherited disorders in a cost effective manner.
- To present the putative biologic effect of the detected mutation.

BACKGROUND
- De Barsy Syndrome type B (EC - 10 Q87.7, OMIM 614328) is a rare cutis laxa syndrome with progeroid appearance due to a defect in Pyrroline-5-Carboxylate Reductase (PYCR1) gene.
- Pyrroline-5-carboxylate reductase (EC 1.5.1.2) catalyses the NAD(P)H-dependent conversion of pyrroline-5-carboxylate to proline.
- PYCR1 deficiency leads to an altered mitochondrial membrane potential (MMP), an increased fragmentation of the mitochondrial network and higher apoptosis rates upon oxidative stress.
- Prevalence: < 1 / 100,000
- Inheritance: Autosomal recessive
- Age of onset: Infancy, neonatal
- Less than 100 cases reported so far, three from India (Dimopoulos et al).

CASE REPORT
- A female preterm neonate, first born to fifth degree consanguineously married parents was admitted under department of Neonatology.
- Concerns: prematurity, intra uterine growth restriction and loose skin folds

Table 1
Clinical features (up to three months of follow up)

<table>
<thead>
<tr>
<th>Clinical features in decreasing frequency (after Dimopoulos et al)</th>
<th>Features in the index case</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wrinkled skin</td>
<td>Present</td>
</tr>
<tr>
<td>Joint hyperlaxity</td>
<td>Present</td>
</tr>
<tr>
<td>Typical facial gestalt</td>
<td>Present (figure 1)</td>
</tr>
<tr>
<td>Ruga</td>
<td>Present</td>
</tr>
<tr>
<td>Psychomotor retardation</td>
<td>Under follow up</td>
</tr>
<tr>
<td>Oligoencephaly</td>
<td>Present (figure 2)</td>
</tr>
<tr>
<td>Thigh translucency skin</td>
<td>Present</td>
</tr>
<tr>
<td>Microcephaly</td>
<td>Present</td>
</tr>
<tr>
<td>Postural growth delay</td>
<td>Present</td>
</tr>
<tr>
<td>Wormian bones</td>
<td>Not evaluated</td>
</tr>
<tr>
<td>Hypopitina</td>
<td>Present</td>
</tr>
<tr>
<td>Late fontanel closure</td>
<td>Under follow up</td>
</tr>
<tr>
<td>Finger contractures</td>
<td>Present</td>
</tr>
<tr>
<td>Meningo</td>
<td>Absent</td>
</tr>
<tr>
<td>Binocular</td>
<td>Absent</td>
</tr>
<tr>
<td>Bilateral strabismus</td>
<td>Absent</td>
</tr>
<tr>
<td>Atrophic movements</td>
<td>Absent</td>
</tr>
<tr>
<td>Cataract / corneal clouding</td>
<td>Present</td>
</tr>
<tr>
<td>*Cardiac anomalies</td>
<td>Aneurysmal dilatation of inter atrial septum</td>
</tr>
<tr>
<td>*Genitourinary anomalies</td>
<td>Enlargement of clitoris</td>
</tr>
</tbody>
</table>

*Not described previously

CLINICAL PHOTOGRAPH

Figure 1

INFANTOGRAM

Figure 2

METHODS OF GENETIC CONFIRMATION
- Based on the presenting features De Barsy Syndrome type A (ALDH1A1 gene related) or type B (PYCR1 gene related) were suspected.
- As the genetic aetiology of a proportion of children with De barsy syndrome remains to be delineated, clinical exome sequencing was planned.
- Clinical exome sequencing was performed in a commercial laboratory with Illumina MiSeq platform using TruSight One Sequencing kit.
- Variants were analysed with VariantStudio software.

CLINICAL PHOTOGRAPH

Figure 3

RESULT AND DISCUSSION
- Clinical exome sequencing revealed a homozygous variant C>T (GRCH37, location chr17:79892986C>T, cDNA.799G>A, R119H) (Figure 3).
- This is a known pathogenic variant (rs121918377) associated with De Barsy Syndrome type B (Rev coursework et al).
- The mutation was confirmed by Sanger sequencing in Clinical Genetics Department, Christian Medical College, Vellore and both the parents were found to carry the mutation in heterozygous state (Figure 4).
- This substitution leads to gain of a donor splice site (CCAC togt) inside exon 4, which will lead to the loss of 116th to 121st amino acids and the downstream amino acids might also get lost.

Figure 4

CONCLUSION
- Here we have described a child with De Barsy Syndrome type B who presented with cardiac and genitourinary anomalies which were not documented in earlier reports.
- As De Barsy syndrome have two implicated genes (ALDH1A1 and PYCR1) and has overlapping features with other Autosomal Recessives Cutis Laxa syndromes (Fibulin5, EFEMP2, LTB4R, GORAB, ATP8V0A2 gene related) genetic confirmation is necessary.
- Clinical exome sequencing is a powerful diagnostic tool for cost effective genetic diagnosis however the low coverage and read depth are the limitations necessitating confirmation by Sanger sequencing.
- Being an Autosomal Recessive disorder this disease has a 25% recurrence risk in future pregnancies. Genetic diagnosis also paves the way for the option prenatal diagnosis for the family.

REFERENCES

ACKNOWLEDGEMENT
- The parents of the affected child for their kind consent.
- Dr Gautam Arunkachal, Clinical Genetics Unit, CMC, Vellore.
- Gevothop Technology.
Quality of mesorectal excision is improved by extralevator abdominoperineal excision

Rohin Mittal, Amrit Pipara, Mark Ranjan Jesudason, Benjamin Perakath
Surgery Unit 2 (Colorectal Surgery), Christian Medical College, Vellore

Introduction
- Abdominoperineal excision is the primary modality of treatment for low rectal cancer
- Conventional APE
- Leads to 'waisting' of the specimen
- High CRM positivity
- High intraoperative perforation rates
- High local recurrence
- Extralevator APE (ELAPE)
- Does not lead to 'waisting' of the specimen
- Lower CRM positivity
- Lower intraoperative perforation
- Decreases local recurrence
- Problems with ELAPE
- Higher wound complications
- Larger perineal defect
- Perineal hernia

Aim
- To compare short-term oncological outcomes between ELAPE and conventional APE

Methodology
- Retrospective review of prospectively collected data
- Single colorectal unit of a tertiary care teaching hospital
- From September 2012 to April 2014
- Inclusion Criteria
  - All patients undergoing APE for adenocarcinoma rectum
- Exclusion Criteria
  - Pathology other than adenocarcinoma
  - Perineal dissection in prone for ELAPE and supine for conventional APE
  - Follow up from hospital visits and electronic medical records
  - Statistical analysis using t-test, Chi square test and Fishers exact test

Results
- 44 patients
- 24 in conventional arm
- 20 in ELAPE arm

Baseline Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Conventional APE</th>
<th>ELAPE</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>45 yrs.</td>
<td>51 yrs.</td>
<td>0.19</td>
</tr>
<tr>
<td>Sex (M:F)</td>
<td>18:6</td>
<td>11:9</td>
<td>0.16</td>
</tr>
<tr>
<td>Stage of disease</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>T2</td>
<td>1</td>
<td>0</td>
<td>0.13</td>
</tr>
<tr>
<td>T3</td>
<td>17</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>T4</td>
<td>5</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Neoadjuvant therapy</td>
<td>19/20</td>
<td>24/24</td>
<td>0.28</td>
</tr>
<tr>
<td>Laparoscopy</td>
<td>13/20</td>
<td>13/24</td>
<td>0.68</td>
</tr>
</tbody>
</table>

Outcomes
- No difference in CRM positivity, intraoperative perforation or wound infection rates

<table>
<thead>
<tr>
<th></th>
<th>Conventional APE</th>
<th>ELAPE</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRM positivity</td>
<td>29%</td>
<td>30%</td>
<td>0.95</td>
</tr>
<tr>
<td>Intraoperative perforation</td>
<td>17%</td>
<td>5%</td>
<td>0.36</td>
</tr>
<tr>
<td>Wound infection</td>
<td>50%</td>
<td>25%</td>
<td>0.09</td>
</tr>
</tbody>
</table>

- Completeness of mesorectal excision (Quirke's) was better in the ELAPE group
- This difference was seen more in T3 tumours and not in T4 tumours

Completeness of mesorectal excision (All cases / T3 disease / T4 disease)

<table>
<thead>
<tr>
<th></th>
<th>Conventional APE</th>
<th>ELAPE</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complications</td>
<td>25%</td>
<td>40%</td>
<td>0.29</td>
</tr>
<tr>
<td>Lymph nodes harvested</td>
<td>10.7</td>
<td>10.3</td>
<td>0.45</td>
</tr>
<tr>
<td>Positive lymph nodes</td>
<td>2.3</td>
<td>1.9</td>
<td>0.32</td>
</tr>
<tr>
<td>Hospital stay</td>
<td>7 days</td>
<td>10.8 days</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Conclusions
- ELAPE results in better quality mesorectal excision in T3 rectal cancer
- There is no difference in rates of circumferential margin positivity, intraoperative perforation and postoperative perineal infections between ELAPE and Conventional APE
A Study to Determine if the Concentration of Phenobarbitone in Serum can be Accurately Predicted from that Measured in Dried Blood Spot Specimens

Aswathy R. Mathew 1, Denise Fleming 1, A.T. Prabhakar 2, Visalakshi Jeyaseelan 1, Kalpana Ernest 1, Mathew Alexander 2

1 Department of Pharmacology & Clinical Pharmacology, 2 Department of Neurological Sciences, 3 Department of Biostatistics
Christian Medical College, Vellore, 632004, Tamil Nadu, India

Introduction

Being the most cost-effective pharmacological therapy for epilepsy makes phenobarbitone (PHB) the most widely prescribed anti-epileptic in the developing world (1). It is well accepted that phenobarbitone requires therapeutic drug monitoring (TDM) due to its variable pharmacokinetics under the influence of age and clinically relevant drug interactions (2).

Rural hospitals do not have the facility to monitor PHB. The conventional serum or plasma specimens used to measure phenobarbitone pose the problems of requiring a cold chain facility to be transported long distances thereby increasing the cost of transport, as well as being a biohazard risk to handlers.

An alternative method to measure drug concentrations, which is gaining popularity is the use of the dried blood spot (DBS). The dried blood spot specimens are stable, easy to store and transport and are less of a biohazard risk when compared to the whole blood, plasma or serum (3,4). Thus, they can be used as a safer and cheaper alternative for small rural hospitals to send specimens for phenobarbitone monitoring.

Results

• Corrected DBS concentration (CDBS) = Final DBS concentration * (Standard plasma % / Patient plasma %)
• Statistical analysis was done using R – program (version 3.1.5). Descriptive statistics, Spearman rank correlation test and Wilcoxon Signed Rank test was used for analysis.

Figure 1: Cutting out the whole dried blood spot (20 µl)

The Spearman correlation coefficient (p) after removing the outlier = 0.9867

The Wilcoxon Signed Rank Test showed that there was no statistically significant difference between the concentration of phenobarbitone in serum and the corrected DBS concentration (p = 0.3919).

The imprecision and the bias between the predicted and the measured serum concentrations were found to be 8% and 0.49% respectively.

Conclusion

The concentration of PHB in serum can be accurately predicted from that measured in a dried blood spot without the need of an equation. Thus, a simple, cheap and easily accessible dried blood spot TDM facility is available for rural hospitals in India.

Table 1: Baseline Characteristics

<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th>VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>**38.11 (18 - 63)</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>**39.60 (29.5 - 51.3)</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>22/14</td>
</tr>
</tbody>
</table>

** = Mean (lower value - upper value)

Table 2: Measured Concentrations of Phenobarbitone

<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th>MEAN (INTERQUARTILE RANGE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum Concentration (mg/l)</td>
<td>17.13 (10.15 - 28.67)</td>
</tr>
<tr>
<td>Corrected DBS Concentration (mg/l)</td>
<td>20.49 (10.24 - 28.98)</td>
</tr>
</tbody>
</table>

The Histogram with the Normal distribution curve and the Shapiro-Wilk test for Normality showed that both the serum and the CDBS concentrations were not normally distributed.

Fib. 2: Correlation between Serum and CDBS concentrations

p = 0.9878

Aim

To determine if the concentration of phenobarbitone in serum can be accurately predicted either directly or with an equation from that measured in dried blood spot specimens.

Methods

• 36 patients between the ages of 18 to 65, who were on phenobarbitone, were prospectively enrolled into the study.
• The brand and the dose of phenobarbitone prescribed were based on the clinician’s discretion and were of no consequence to the study.
• The timing of the specimen collection could be at any time point provided the patient was compliant with the medication.
• The DBS specimens were prepared by spotting 20µl of whole blood on a standard Whatman filter paper using a calibrated micropipette.
• Separate validated (developed in our laboratory) High Pressure Liquid Chromatography (HPLC) assays were used to measure the concentration of phenobarbitone in serum and dried blood spot.

References


Acknowledgements

We would like to thank Mrs. Anita for her guidance during the development and validation of the assay. We express our sincere gratitude to the Fluid Research Grant – CMC for funding this study.
BIOLICAL MESH REPAIR IN PARASTOMAL HERNIAS: AN INITIAL EXPERIENCE
Department of Surgery Unit 4. Christian Medical College, Vellore
S Chase, B Roopavathana, R Selvakumar, A Benjamin, S Nayak

BACKGROUND
- 33% prevalence of parastomal hernia after stoma formation
- With increasing aperture size and age, incidence of hernia is higher
- Most need surgical intervention
- Risk of mesh infection and mesh erosion high with conventional prosthetic repair
- Biological mesh repair reduces risk of mesh infection and mesh erosion into the bowel

AIM
- To study the outcome and complications of using a biological mesh to repair parastomal hernias

METHODS
- Retrospective analysis
- All cases of parastomal hernia repair done using biological mesh in a single unit
- Between September 2011 and January 2014
- Data collected from hospital records

RESULTS
- 9 cases studied
- 5 males and 4 females
- Mean age: 56 years
- Mean follow up: 18 months
- All patients repaired using the open Sugarbaker technique

COMPLICATIONS
- Immediate
  - Conduit obstruction: 11.1%
  - Parastomal abscess: 11.1%
- Late
  - Recurrence: 22.2%
  - Mesh Infection: NIL

CONCLUSIONS
In Patients with parastomal hernia, repair with biological mesh appears to be safe with low recurrence and low infection rates.

FUTURE TRENDS
- Prophylactic mesh placement during stoma formation to lower the rate of parastomal herniation
Background: Differentiated thyroid carcinomas usually have good prognosis but presence of distant metastasis significantly decreases the survival of patients. Bone metastasis is a major cause of impaired quality of life. Review of recent literature suggests that combined modality therapy using surgery, radioiodine ablation & radiotherapy improves outcome.

Aim: To study the clinico-pathological characteristics and outcome of patients with metastatic differentiated carcinoma.

Methodology: We retrospectively reviewed 1488 patients with differentiated thyroid carcinoma, among which 106 patients had distant metastasis. These patients treated and followed up over a period of 10 years from 2004-2013 were analyzed.

Results

- **Detection of Metastasis**
  - At presentation
  - On First Radioactive Iodine Scan
  - On Follow Up

- **Site Of Metastasis**
  - Bone: 25%
  - Lung: 30%
  - Combined: 23%
  - Brain: 5%
  - Soft tissue: 1%
  - Liver: 0%

- **Pathology**
  - Papillary Ca- 85%
  - Follicular Ca- 15%

- **Response to Radioactive Iodine Therapy**
  - Complete Response: 38%
  - Progression: 32%

- **Patient Outcome**
  - Alive: 55%
  - Expired: 40%
  - Default: 5%

- **Follow up period**: 0 to 7.5 years

Conclude: Metastasis in DTC is detected often (63%) at presentation, with bone and lung being the commonest sites (98%). Majority (89%) had uptake in radioiodine scan with 68% showing resolution, response or stability with therapy.
Investigation into celiac disease in Indian patients with portal hypertension
Rakhi Malwadi1, Ashish Goel1, Anna B. Pullmood1, Sudhakar Zadak2, Pratibha Chhatre2, Shrikumar Kankabadi2, Balasubramanian K.A., Banumarti Ramakrishna2, Susy Kurian3, G. John Fletcher3, Priya Abraham4, Gajanan Apte5, B.S. Ramakrishna3, Ewyn Elias3, G.E. Enfield3

Departments of Gastroenterology, Pathology and Virology, Christian Medical College, Vellore, India

Introduction
'Cryptogenic' chronic liver disease is the most common cause of portal hypertension at our center currently. Non-cirrhotic in hepatic portal hypertension (NCPH), a common cause in this group, is caused by a microangiopathy of portal vein branches. Increased prevalence of celiac disease (16%) was noted in NCPH patients. This study aimed to investigate prevalence of celiac disease in portal hypertensive patients with cryptogenic chronic liver disease and NCPH.

Methods
- Design: Prospective, observational
- Period: Jan 2009-Dec 2010
- Cases: Cryptogenic chronic liver disease as per criteria (n=61; 46 males, age: 42(7-67) years); includes 14 patients with NCPH
- Controls: Hepatitis B/C related liver disease (n=59; 53 males, age: 46(21-67) years)

Evaluation of gut pathology
Serology: IgA human anti-tTG (tissue transglutaminase) antibody by ELISA (AESKULISA, Celcheik, Germany). Tiers >20 U/ml interpreted as positive.

Histology: Multiple biopsies from 2nd part of duodenum (D2Bx) were taken when possible. In TTG positive patients the biopsy was interpreted as per Marsh criteria.

Gut permeability: L/M (Lactulose/manitol) ratio, relative % time excretion of the ingested dose of lactulose and manitol in the urine. Ratio > 0.07 was interpreted as an evidence of increased gut permeability.

Anti-celiadon antibody: IgM, IgG and IgA antibody by ELISA (Varelisa).

Serology by other kits: As we not unexpectedly high rate of tTG positivity in both cases and controls, we assayed serum samples (stored at -20°C) by two other test kits - EUROIMMUN (EUROIMMUN AG, Germany) and INOVA QUANTA Lite™ (INOVA diagnostics, U.S.A).

Follow up evaluation: All patients with celiac disease were followed up on gluten free diet.

Variables were expressed as median and range (continuous) or as numbers with percentage (discrete). Non-parametric tests were used to compare.

Results
Table 1: Baseline characteristics in 120 portal hypertensive study patients

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Cases (n=61)</th>
<th>Controls (n=59)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>42.7(21-67)</td>
<td>46(21-67)</td>
<td>0.3</td>
</tr>
<tr>
<td>Sex (M:F)</td>
<td>46:15</td>
<td>53:6</td>
<td>0.05</td>
</tr>
<tr>
<td>Jaundice</td>
<td>13</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Ascites</td>
<td>28</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>Presentation</td>
<td>7</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Encephalopathy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Variceal bleed</td>
<td>22</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Serum total bilirubin (mg/dl)</td>
<td>1.04(3.0-30.8)</td>
<td>1.20(4.17-16)</td>
<td>0.85</td>
</tr>
<tr>
<td>Serum total protein (g/dl)</td>
<td>7.7(5.4-9.1)</td>
<td>7.65(2.0-10)</td>
<td>0.78</td>
</tr>
<tr>
<td>Serum albumin (g/dl)</td>
<td>3.6(1.8-5.2)</td>
<td>3.61(6.5-1)</td>
<td>0.04</td>
</tr>
<tr>
<td>INR for Prothrombin time</td>
<td>1.2(0.9-2.9)</td>
<td>1.39(0-3.4)</td>
<td>0.01</td>
</tr>
<tr>
<td>Serum Creatinine (mg/dl)</td>
<td>0.9(0.5-1.6)</td>
<td>1.0(4-6)</td>
<td>0.002</td>
</tr>
<tr>
<td>Haemoglobin (g/dl)</td>
<td>10.4(2.5-15.1)</td>
<td>11.2(6.17-17.3)</td>
<td>0.26</td>
</tr>
<tr>
<td>Platelet count (X10^9/mm)</td>
<td>0.6(0.2-3.2)</td>
<td>0.67(0.06-5.5)</td>
<td>0.5</td>
</tr>
<tr>
<td>Child's class (A/B/C)</td>
<td>39/15/7</td>
<td>29/19/11</td>
<td>0.25</td>
</tr>
</tbody>
</table>

Table 2: Duodenal mucosal biopsy in portal hypertensive subjects who had cryptogenic chronic liver disease (cases) or hepatitis B/C related cirrhosis (controls)

<table>
<thead>
<tr>
<th>Variables</th>
<th>In all study patients who had duodenal biopsy</th>
<th>In Child's A patients who had duodenal biopsy</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cases (n=53)</td>
<td>Controls (n=33)</td>
<td>p-value</td>
<td>Cases (n=24)</td>
</tr>
<tr>
<td>Crypt hyperplasia</td>
<td>19</td>
<td>6</td>
<td>0.09</td>
</tr>
<tr>
<td>Villous atrophy (mild/moderate)</td>
<td>18/1</td>
<td>6/0</td>
<td>0.05</td>
</tr>
<tr>
<td>Raised Ige</td>
<td>6</td>
<td>1</td>
<td>0.17</td>
</tr>
</tbody>
</table>

Table 3: Celiac serology and duodenal mucosal biopsy in portal hypertensive subjects who had NCIPH or hepatitis B/C related cirrhosis (controls).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>NCIPH (n=14)</th>
<th>Controls (n=59)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Celiac serology (anti-tTG positive/negative)</td>
<td>6 (43%)</td>
<td>17 (29%)</td>
<td>0.35</td>
</tr>
<tr>
<td>Anti-tTG titre (U/ml)</td>
<td>11 (0-300)</td>
<td>7.5 (0-300)</td>
<td>0.38</td>
</tr>
<tr>
<td>Anti-tTG titre &gt;100 U/ml</td>
<td>1</td>
<td>1</td>
<td>0.36</td>
</tr>
<tr>
<td>Duodenal histology (NCIPH: 12; Controls: 33)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crypt hyperplasia</td>
<td>4</td>
<td>6</td>
<td>0.4</td>
</tr>
<tr>
<td>Villous atrophy</td>
<td>5</td>
<td>6</td>
<td>0.13</td>
</tr>
<tr>
<td>Raised Ige</td>
<td>2</td>
<td>1</td>
<td>0.17</td>
</tr>
<tr>
<td>Lamina propria inflammation</td>
<td>11</td>
<td>23</td>
<td>0.2</td>
</tr>
<tr>
<td>Fibrosis</td>
<td>0</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 4: Results of tTG antibody testing in 92 study patients in whom sera were tested by all 3 ELISA kits.

<table>
<thead>
<tr>
<th>Aeskulisa</th>
<th>Euroimmun (positive/negative)</th>
<th>Inova (positive/negative)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls (n=52)</td>
<td>Positive (33)</td>
<td>13/10</td>
</tr>
<tr>
<td>Negative (19)</td>
<td>9/16</td>
<td>1/18</td>
</tr>
<tr>
<td>Controls (n=40)</td>
<td>Positive (12)</td>
<td>5/3</td>
</tr>
<tr>
<td>Negative (28)</td>
<td>9/22</td>
<td>2/26</td>
</tr>
</tbody>
</table>

Figure 1: Flow chart depicting results of celiac serology (tissue transglutaminase antibody) and duodenal biopsy (D2Bx) in study patients.

Intestinal permeability: 9/30 cryptogenic chronic liver disease had increased gut permeability.

IgM cardiomial antibody was negative in 28 cases and 32 controls tested while IgA cardiomial antibody was borderline positive in 4 cases and 4 controls.

Follow up:
- 1/6 celiac disease patients had mild classical symptoms of celiac disease.
- 3/6 patients with celiac disease on gluten free diet had a follow up of > 1 year. On follow up there was improvement in serology and histology with no improvement in liver disease severity.

Conclusions
Celiac disease was over-represented in cryptogenic chronic liver disease patients (6/61) and NCIPH (2/14) as compared to Hepatitis B/C related cirrhosis (0/59).

Much higher prevalence of duodenal mucosal inflammation and architectural changes in patients with cryptogenic chronic liver disease as compared to Hepatitis B/C cirrhosis controls.

This suggests need to investigate etiology of enteropathy and to assess if these mucosal changes in gut have a causative role in development/progression of portal hypertension in these patients.
ADAMTS13 deficiency, despite well compensated liver functions in patients with non-cirrhotic portal hypertension

Ashish Goel1, Alagamma P.R.1, Sakeh G. Hai1, Ian Macfarlane2, Banumathy Ramakrishna3, Jayaprakash Murthi4, Shyamkumar N. Keshava5, C.E. Easen6, Elwyn Elias7

Departments of Hepatology6, Transfusion medicine & Paracrinology3, Pathology5, Community Medicine and Radiodiagnosis5, Christian Medical College, Vellore, India
Haematology Research Unit5, Haematology Department, University College Hospital, London, UK; Emeritus Professor Liver Unit, University Hospital Birmingham, Birmingham, UK.

Introduction
NCIPH (non-cirrhotic intrahepatic portal hypertension) continues to be a significant cause of portal hypertension in India. It is secondary to microvasculocclusion of small portal vein radicles, i.e. localised form of microangiopathy. Deficiency of ADAMTS13 (A disintegrin and metalloprotease with thrombospondin type 1 motif, member 13; vWF cleaving protease) is implicated in pathogenesis of thrombotic microangiopathy. We aimed to study ADAMTS13-von Willebrand factor (vWF) imbalance in Indian patients with NCIPH.

Methods

- Case-Control study
  - Cases: NCIPH (as per standard defining criteria): 29 patients (22 males; age: 29 (13-58) years)
  - Healthy controls: Healthy volunteers - 17 (14 males; age: 32 (27-45) yrs)
  - Disease controls: Cryptogenic chronic liver disease (as per standard definition): 22 (15 males; age: 46 (18-74) years)
  - All discrete variables were expressed as numbers and all continuous variables as median with range. Non-parametric tests were used for comparison.

Assessing ADAMTS13-vWF balance

ADAMTS13
- Antigen (Ag) test by ELISA
- Activity by fluorescence resonance transfer (FRET) assay
- Activity by estimating residual collagen binding activity of purified vWF (CBA-Collagen binding assay)

Normal values: ADAMTS13 activity by CBA (55-160%), ADAMTS13 activity by FRET (60-123%), and ADAMTS13 antigen (64-136%)

vWF: Antigen by automated coagulation analyzer using an immunoturbidimetric method (Normal range: 50-150IU/dl)
vWF: ADAMTS13 ratio: vWF (IU/dl) divided by ADAMTS13 antigen.

Results

Table 1: Baseline characteristics of patients with NCIPH and cryptogenic chronic liver disease (CLD)

<table>
<thead>
<tr>
<th>Patient characteristics</th>
<th>NCIPH (n=29)</th>
<th>Cryptogenic (n=22)</th>
<th>CLD</th>
<th>Healthy controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>MELD score</td>
<td>10 (6-13)</td>
<td>11.5 (6-23)</td>
<td>11.5 (6-23)</td>
<td>11.5 (6-23)</td>
</tr>
<tr>
<td>Child’s score</td>
<td>5 (5-8)</td>
<td>7 (5-13)</td>
<td>7 (5-13)</td>
<td>7 (5-13)</td>
</tr>
<tr>
<td>Child’s class (A/B/C)</td>
<td>23/6/0</td>
<td>9/9/4</td>
<td>9/9/4</td>
<td>9/9/4</td>
</tr>
<tr>
<td>Platelets (10^12/μl)</td>
<td>0.53 (0.93-3.44)</td>
<td>0.33 (0.15-3.16)</td>
<td>0.33 (0.15-3.16)</td>
<td>0.33 (0.15-3.16)</td>
</tr>
<tr>
<td>HEPG (mm)</td>
<td>7 (1-12)</td>
<td>11.5 (7-20)</td>
<td>11.5 (7-20)</td>
<td>11.5 (7-20)</td>
</tr>
<tr>
<td>ADAMTS13-CBA</td>
<td>32% (5-100%)</td>
<td>36% (5-144%)</td>
<td>36% (5-144%)</td>
<td>36% (5-144%)</td>
</tr>
<tr>
<td>ADAMTS13-FRET</td>
<td>50% (14-114%)</td>
<td>49.5-125%</td>
<td>49.5-125%</td>
<td>49.5-125%</td>
</tr>
<tr>
<td>vWF (IU/dl)</td>
<td>172 (59-288)</td>
<td>216 (189-318)</td>
<td>216 (189-318)</td>
<td>216 (189-318)</td>
</tr>
<tr>
<td>vWF-ADAMTS13 ratio</td>
<td>2.7 (0.92-9.7)</td>
<td>0.6 (0.2-1.7)</td>
<td>0.6 (0.2-1.7)</td>
<td>0.6 (0.2-1.7)</td>
</tr>
</tbody>
</table>

*Values are either number (diabetes) or median range (continuous). MELD: model for end-stage liver disease; HEPG: hepatic venous pressure gradient

ADAMTS13 activity was deficient in 19 NCIPH patients (8-mild, 8-moderate, 3-severe) and 15 disease controls (7-mild, 2-moderate, 6-severe) as compared to none of the healthy controls.

Table 2: ADAMTS13 activity and vWF levels in patients with NCIPH and cryptogenic chronic liver disease (CLD)

<table>
<thead>
<tr>
<th>Child’s Class</th>
<th>ADAMTS13 (CBA)%</th>
<th>vWF antigen</th>
<th>vWF-ADAMTS13 ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCIPH</td>
<td>A</td>
<td>23</td>
<td>30% (5-100%)</td>
</tr>
<tr>
<td>B</td>
<td>6</td>
<td>46% (16-69%)</td>
<td>46% (16-69%)</td>
</tr>
<tr>
<td>Cryptogenic</td>
<td>C</td>
<td>9</td>
<td>100% (5-114%)</td>
</tr>
<tr>
<td>CLD</td>
<td>C</td>
<td>6</td>
<td>35% (5-70%)</td>
</tr>
<tr>
<td>Healthy controls</td>
<td>C</td>
<td>4</td>
<td>15% (5-63%)</td>
</tr>
</tbody>
</table>

Salient points
1. Low ADAMTS13 was noted in NCIPH patients despite preserved liver functions.
2. There was no correlation of ADAMTS13 to severity of liver disease or portal hypertension in patients with NCIPH.
3. ADAMTS13 activity as assayed by Collagen Binding Assay (CBA) was disproportionately low.
4. vWF was higher in all portal hypertensive patients as compared to healthy controls.

Conclusions
This study validates the finding of ADAMTS13 deficiency in NCIPH despite preserved liver functions in an Indian population suggesting its involvement in pathogenesis of NCIPH.

References
Goel A, Ramakrishna B, Madhu K et al. Idiopathic noncirrhotic intrahepatic portal hypertension is an ongoing problem in India. Hepatology 2011;54:2274


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Revealing the hidden dimension of the Henderson-Hasselbalch equation

Vinay Oommen, Gnanasenthil Ganesh, Kamalakannan Vadivel and Pragabalathan Kanthakumar

Department of Physiology, Christian Medical College, Vellore

Background

The Henderson-Hasselbalch equation describes the relationship between arterial blood pH, arterial PCO₂ (PaCO₂) and plasma bicarbonate concentrations. The commonly used acid-base nomogram (Fig 1) depicts pH on the horizontal axis, [HCO₃⁻] on the vertical axis, and shows PCO₂ as isopleth lines on this 2D surface.1,2 Siggard-Anderson and Müller-Plathe have also used 2D nomograms to describe acid base disorders.1,3

Plotting the Henderson-Hasselbalch equation, using pH, PaCO₂, and [HCO₃⁻], generates a surface in three dimensional space. However, for simplicity, nomograms that are widely used, depict this surface on a two dimensional plane. This hides one of the dimensions and renders the three dimensional surface plot into a two dimensional contour plot (Fig 1). The axis that is not shown on the graph is represented using isopleth lines. Two dimensional graphs are easy to draw on a paper or a blackboard and are therefore routinely used for teaching acid-base balance. Here, we show two models that represent Henderson-Hasselbalch equation in its original three dimensional form. These 3D models represent all three variables of the Henderson-Hasselbalch equation on separate axes, and can be used as a supplement to the standard acid-base nomograms.

Aim

To represent the Henderson-Hasselbalch equation in its original three dimensional form to help students visualize its true nature.

Materials Required

Colored beads, thin metal rods (brass spokes) and plywood, polystyrene sheets, instant glue, white cement and paints.

Discussion

Both the models described above depict the surface generated by the Henderson-Hasselbalch equation in three dimensional space. Model 1 (Fig 28) can be used to illustrate the arrangement of PCO₂ isopleth lines. Model 2 (Fig 38) can be used to mark the regions of various acid-base disorders.

A two dimensional contour plot of the Henderson-Hasselbalch equation though easy to use, is in reality a simplification of its true three dimensional nature. The 3D form of this equation is brought out in the models we describe and enables the viewer to develop a richer understanding of the topic and the rationale behind the construction of the two dimensional plot. The inter-relationship of all three parameters that contribute to acid base balance can be well demonstrated using these models. It is easy to understand how and why a change in one parameter results in a change in the others.

Construction of the Models

Model 1 depicts the Henderson-Hasselbalch surface as a collection of discreet points marked by colored beads. The pH and PCO₂ axes were marked on a 30cm x 30cm plywood base. Thin metal rods with colored beads on one end were inserted on the base (Fig 2A). The height of these metal rods represented plasma [HCO₃⁻] concentration on the vertical axis. HCO₃⁻ concentrations were calculated for different sets of pH and PCO₂ values using the equation shown below:

\[ [\text{HCO}_3^-] = 0.380 \times \frac{[\text{PaCO}_2]}{10^\text{pH} - 6.1} \]

The final assembly of the model is shown in Fig 2B.

Model 2 depicts the Henderson-Hasselbalch equation as a continuous surface. This surface can be used for writing on while teaching. The linear relationship between [HCO₃⁻] and PCO₂ was exploited to ensure the accuracy of the various points of the surface. Fifteen pieces of poly styrene were stuck together as shown in Fig 3A to provide a step-like three dimensional structure. The gaps between these steps were filled with white cement to create a smooth surface representing the Henderson- Hasselbalch equation. The surface was then painted over. The completed model with a few reference markings is shown in Fig 3B.

Presentation of the models

The models were presented during didactic lectures on acid-base balance to the undergraduate students and the physiology post-graduate students. These models were used to explain various types of acid-base disorders. Figure 4 shows the various regions of acid-base imbalances marked on one of the 3D models. The compensatory responses were also explained. The fact that, the Henderson-Hasselbalch equation restricts freedom of movement to a small surface on a vast three dimensional space was largely appreciated by the students.

The 3D models were used as a supplement to the two dimensional acid-base nomogram. Presenting the 3D models facilitated discussions on the acid-base nomogram.

References

BACKGROUND

- Impaired balance of excitatory and inhibitory mechanisms in the brain lead to epileptic activity and requires detailed understanding.
- Temporal Lobe Epilepsy is a common type of epilepsy with hippocampus as usual site of origin.
- Involvement of hippocampal CA3 subfield in temporal lobe epilepsy has high clinical relevance.
- Loss of dendritic inhibition leading to epileptic activity has been observed in many studies.

AIM

- To study how impaired dendritic inhibition in CA3 pyramidal cells leads to epileptic activity in the network, using computational modelling.

METHODS

CA3 Computation Network Model Details

- There are 800 pyramidal cells, 200 basket cells and 200 OLM (Olivo-Lucomonom Molecular) interneurons with inputs from medial septum and other extra-hippocampal regions.
- The free software NEURON with Python interpreter is used (www.neuron.yale.edu).
- All cells contain leak current, transient sodium current I_{Na}, and delayed rectifier current I_{Kdr} to allow for action potential generation.
- The pyramidal cells contain potassium type A current I_{K-A} for rapid inactivation, and hyperpolarization-activated current I_{h}.
- The OLM cells had calcium-activated potassium current I_{Ca/K} to allow long lasting inactivation after bursting, high-threshold calcium current I_{H} to augment bursting and to activate I_{Ca/K}, hyperpolarization-activated current I_{h} for bursting, and intracellular calcium dynamics.

Synaptic Connectivity

- The pyramidal cell activity drives the basket cells and O-LM cells.
- The basket cells and O-LM interneurons connect to pyramidal cells and produce inhibitory responses in the network, preventing hyperactivity.
- The basket and O-LM interneurons receive inhibitory inputs from Medial Septum.
- Basket and O-LM cells also received external random inputs.
- The pyramidal cells receive external inputs at distal dendritic compartments representing inputs from entorhinal cortex. Some cells also receive random external inputs.
- The pyramidal cell to O-LM connectivity, the pyramidal cell to basket cell connectivity and the recurrent connectivity between pyramidal cells are modeled through NMDA & AMPA synapses.
- The O-LM cells, basket cells and some of pyramidal cells receive background random excitatory and inhibitory inputs through AMPA & GABA receptors.
- Similar inputs received at the distal dendritic compartment of pyramidal cells are through AMPA, NMDA & GABA receptors.
- The O-LM to pyramidal cell, basket to pyramidal cell, basket to basket recurrent connections and Medial Septum to O-LM and basket cell connections are through GABA receptors.
- The baseline normal network generated theta-modulated gamma oscillations (Fig. 1).

Stimulating epileptic activity generation

- Different connectivity changes that lead to generation of epileptic activity were simulated.
- To arrive at conditions that simulate an ictal–tonic activity which is a clinical correlate of full blown seizures, step-wise changes were made in the connectivity between neurons.
- The significant changes that generated the ictal-tonic pattern were
  1) decreasing weight of connectivity between O-LM and pyramidal cells and additionally increasing external inputs received by pyramidal cells at the distal dendritic compartments (Fig. 2)
  2) simulating overall network changes in the connections between all neuron types (Fig. 3).

RESULTS

- Figure 1: A normal network with inputs from Medial Septum (MS). B: Baseline activity – theta modulated gamma oscillations (90-40 Hz). C: Local Field Potential (LFP) along with individual cell responses (PYR – Pyramidal Cells, BC – Basket Cells, OLM – OLM Interneurons).
- Figure 2: A: Network with 10% of baseline OLM to PYR connectivity and 15 times increment in external excitatory inputs. B: LFP shows a typical ictal-tonic pattern as basket cells enter a state of depolarization block (DB) due to high excitation received from pyramidal cells. C: Raster plot showing depolarization block of basket cells.
- Figure 3: A: Network with change of strength of connections between all neurons in the network when OLM – Pyr connectivity was reduced to 30% (0.3x) to 70% (0.7x). The network showed the extent of change in strength between the connected neurons as compared to baseline strength, e.g. 4X means 4 times the normal baseline. B: LFP shows a typical ictal–tonic pattern as basket cells enter a state of depolarization block due to high excitation received from pyramidal cells. C: Raster plot showing depolarization block of basket cells. The epileptic activity set at an intermediate time point compared to Fig. 2.

CONCLUSION

- We were able to simulate a typical ictal-tonic activity which is a clinical correlate of epilepsy and also determine at what levels of dendritic inhibition and changes in the network leads to such a state.
- Our study suggests that greater synaptic plasticity occurring in the network due to increased external excitatory inputs leads to earlier generation of epileptic activity.

REFERENCES

Correlation between clinical scores and antibody titres in pemphigus

Ann Anna George¹, Leni George¹, Dinee Peter¹, Susanne Pulimood¹, Victoria Job²
Department of Dermatology, Venereology and Leprosy¹, Department of Clinical Biochemistry²

Introduction

- Pemphigus is a rare disease
- In pemphigus→ production of auto antibodies against Desmoglein 1 (Dsg 1) and Desmoglein 3 (Dsg 3)
- Dsg ELISA: highly sensitive and specific diagnostic test for pemphigus
- Controversial evidence on correlation between Dsg ELISA and disease activity

Aim

To assess the correlation of the two clinical scores for pemphigus namely, ABSIS (Autoimmune Bullous Skin Disorder Intensity Score) and PDAI (Pemphigus Disease Area Index) score with the Physician’s Global Assessment (PGA) and serum anti-desmoglein antibody titres in patients with pemphigus.

Methodology

Study design: Cross sectional study
Setting: Outpatient Department of Dermatology, Venereology and Leprosy, Christian Medical College
Duration of the study: October 2010 to August 2012
Methodology
- Sample size: 43
- Measurement of clinical severity of disease at initial presentation: PDAI score, ABSIS and PGA
- Serum anti-Dsg 1 and 3 antibody titres obtained
- At follow up (6 months, 1 year): clinical severity measured, serum anti-Dsg 1 and 3 antibody titres obtained

Results

- Age: 6-69 years, mean age: 43 +/- 12 years
- Male to female ratio: 1:1.38
- Duration of lesions: 0.5 - 120 months
- Clinical variants: Pemphigus vulgaris 40 (93%), Pemphigus foliaceus 2 (4.7%), Pemphigus vegetans 1 (2.3%)

Correlation of Dsg 1 & 3 titres with the scores: 1st presentation

<table>
<thead>
<tr>
<th>Dsg</th>
<th>Correlation coefficient</th>
<th>Significance (p-value)</th>
<th>PGA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dsg 1</td>
<td>0.469*</td>
<td>0.003</td>
<td>0.274</td>
</tr>
<tr>
<td>Dsg 3</td>
<td>0.205</td>
<td>0.386</td>
<td>0.307</td>
</tr>
</tbody>
</table>

Correlation of Dsg 1 & 3 titres with the scores: at follow up

<table>
<thead>
<tr>
<th>Dsg</th>
<th>Correlation coefficient</th>
<th>Significance (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dsg 1</td>
<td>0.862*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Dsg 3</td>
<td>0.121</td>
<td>0.174</td>
</tr>
</tbody>
</table>

PDAI | ABSIS | PGA
--- | --- | ---
0.834 | 0.928 | <0.001 | <0.001

Spearman’s correlation of PDAI and ABSIS with PGA scores

Discussion

- ABSIS correlated better with PGA than PDAI
- In contrast; Rosenbach et al had found a better correlation between PDAI and PGA than between ABSIS and PGA (1)
- Serum Dsg 1 & Dsg 3 at presentation correlated with pemphigus scores as in previous studies (2,3)
- The correlation of serum anti-Dsg 1 antibodies with disease activity with a lack of correlation between serum anti-Dsg 3 antibodies and disease activity as observed in our study has been noted earlier (2,5)
- As suggested by Daneshpazhooh et al, it is possible that Dsg 1 antibodies are the 1st to disappear in patients with remission followed by a later fall in anti-Dsg 3 (6)

Conclusion

- PDAI and ABSIS are excellent outcome measures in pemphigus
- In our study Serum anti-Dsg 1 correlates with disease activity as measured by PDAI and serum anti-Dsg 3 correlates with disease activity as measured by ABSIS and PGA
- Serial values of serum anti-Dsg 1 may be useful in assessing and predicting clinical disease activity

References

Clinical Spectrum of Yellow Phosphorous poisoning in a tertiary care centre in South India: A case series

*Department of General Medicine Unit III, ** Department of General Medicine Unit I, *** Department of General Medicine Unit V

Introduction
- Rodenticides, also known as rat poisons, are highly toxic compounds but are commonly used in farms and households.
- Owing to their easy availability, these compounds are being increasingly used with suicidal intent and compared rodenticides in India.
- While toxicity by warfarin like anticoagulants is common in the Western literature, metal phosphides and heavy metals are common in India.
- Literature regarding yellow phosphorous poisoning from India is scanty.
- We report a case series of patients who presented to us with yellow phosphorous poisoning in the time period between 2012 to 2014.

Aim and objectives
To assess the clinical features, mortality and predictors of mortality among inpatients with yellow phosphorous poisoning in a tertiary care centre in South India.

Materials and methodology

Study design: Retrospective cross sectional study.
Setting: Inpatient from the Departments of General Medicine Unit I and III, Christian Medical College.
Duration of the study: January 2012 to August 2014.

Inclusion criteria: All patients who were confirmed to have ingested yellow phosphorus.
Exclusion criteria: Patients who had ingested other rodenticides.
Details on demographics, clinical symptoms, signs, and laboratory parameters were recorded in proforma.
Total Number of patients with rodenticide poisoning: 29
Confirmed yellow phosphorus poisoning: 9
Variables statistically analysed

Results

- Age: 17 years to 42 years, mean age: 28.5 years
- Male to female ratio: 1.23:1 (Male: S/Female: 4)
- Mean Duration of Presentation to CMC: 6 days [min: 1d/maximum: 14d]
- Volume consumed: 3 patients (>10mg)

Baseline

- Clinical feature: Most common: Constitutional symptoms-abdominal pain and vomiting: 100% [6]
- Alteration in sensorium: 66% [6]
- Alteration in posturing: 100% [9]
- Toxic manifestations: [100%]
  - Liver injury: 9 [100%]
  - Coagulopathy: 9 [100%]
  - Hematological: 8 [88.8%]
  - Metabolic acidosis: 4 [44.5%]
  - Renal failure: 33.3%
  - Respiratory failure: None

Clinical

- Toxicity

Management

66% [6] were managed in ward
100% received lactulose
89% received Vitamin K [6]
56% [5] under went gastric decontamination
56% [5] were treated with NAC
Mean duration to initiation of NAC: 3.8 days [min: 2d/maximum: 5d]

Duration to outcome and outcome

Mean/median duration to outcome: 8
Mean/median duration of stay: 8
Days to improve: Mean: 12/Median: 10.5
Poor outcome: 5 [55.6%]

Predictors of outcome

- Younger age
- Gastric lavage
- Initiation of NAC within 48 hours
- Absence of renal failure

Discussion

- Yellow phosphorous poisoning is more common among the younger age group patients [Mean age of 28.5]
- In contrast to another study, which found that the mean duration of presentation to a tertiary care hospital after consumption of any poison was 12 hours, in our study the duration was 6 days which shows the lack of awareness among medical practitioners regarding its lethality [1]
- The common clinical manifestations were abdominal pain and vomiting (100%) which were followed by alteration in sensorium (66%) which was similar to the previous case series [2]
- All the patients had features of acute hepatic failure and coagulopathy which has been described as the toxicrome following ingestion [3]
- Most patients were managed with supportive management in the ward [66%] and all were treated with anti-coma measures [4]
- Death or poor outcome was seen in most of the patients as described in literature [5 out of 9] [5]
- Cytopenia was the first lab abnormality and occurred within 2 days, peaked within 5 days and recovered the earliest within 6.5 days.
- Mean duration to outcome was 8 days and median duration to improvement: was 11 days.
- Factors with a high risk of mortality were renal failure, acidosis, raised alkaline phosphatase, isolated thrombocytopenia and delayed initiation of NAC.

Conclusion

- Unlike others yellow phosphorous poisoning is associated with significant mortality and morbidity.
- Early referral to tertiary care centre, early initiation of NAC, meticulous monitoring and supportive measures are the key to management of patients as there is no specific antidote available at present.
- This poisoning poses a high economic burden for the family in view of longer stay in hospital.
- Further studies are crucial in view of the high morbidity and associated economic burden.

References
Introduction

- There are several host factors that can affect the susceptibility to HIV and progression of the disease in an infected individuals.
- These included natural defenses that act at different points in the viral life cycle by the APOBEC and TRIM3-alpha, 322 deletion at the CCR5 genes and SNPs in different genes encoding the chemokine receptors.
- Recently polymorphism on the IFN-λ3 gene (IL-28B) has been shown to have an impact on the on IL-28B secretion and thereby by its antiviral activity against several viruses.
- The wild type genotypes CC at rs12979860 and TT at rs8099917 have been shown to be associated with better treatment response of HCV infection

Materials and Methods

- Flow cytometry (BD FACS Count) + Absolute CD4+ and CD8+ and average CD4 and CD8 ratio
- IL-28B Polymorphism detection - PCR-RFLP using Restriction Enzymes BanII and BslII (New England BioLabs, UK) for rs12979860 and rs8099917 respectively
- IL-28B in-house quantitative (pg/ml) ELISA (R&D Systems) as per manufacturer’s instructions.
- Statistical analysis: was done using Med-Calc software and p-value < 0.05 was considered significant. Categorical and the median of continuous variables were correlated using Kruskal-Wallis test. The association of haplotypes with continuous variables were analyzed using Mann-Whitney U test

Impact of IL-28B polymorphism on IFN-λ2/λ3 secretion in viral infections

Gene  | SNP  | Genotype  | Impact on expression (heterozygous) | Impact on virus replication and immunity (heterozygous) |
-------|------|----------|-------------------------------------|-----------------------------------------------|
IFN-λ3 | rs12979860 | Major: C/C  | Reducing FNL3 | Lower rates of spontaneous clearance  
         | Minor: C/T, T/T  |                      | Complete CMV: reduced replication |
IFN-λ3 | rs8099917  | Major: T/T  | Reducing FNL3 | Lower rates of spontaneous clearance  
         | Minor: T/C, C/C  |                      | Complete CMV: high ISG production and reduced replication less pricking of CMV-specific T cells |

IFN-λ2/λ3 and IL-28B polymorphism in HIV

There are contradictory reports on the effect of IFN-λ2/λ3 on HIV
- The expression of the CD4, CXCR4, and CCR5 genes increased when the PBMC pretreated with IFN-λ2 and is associated with enhanced HIV-1 binding and replication (Serra et al. 2008)
- IFN-λ3 enhances APOBEC3G and APOBEC3F expression at both the mRNA and protein levels in macrophages (Hou et al., 2009)
- No association of the IL-28B SNP with HIV disease progression or HIV protection (Kallon et al., 2011, Sajadi et al., 2011)

Discussion

- There was a significant increase (p < 0.0001) in the absolute CD4+ T cell count, CD4/CD8 ratio and reversal of CD4/CD8 ratio following 6-9 months of ART
- There is no significant difference (p > 0.05) in the IL-28B level in HIV-1 infected individuals and healthy controls (p > 0.05).
- There is no correlation (p > 0.05) between IL-28B polymorphism and IL-28B plasma level among treatment naive HIV infected individuals
- There is significant association of GT genotype at rs12979860 (p = 0.03) and CT/TT haplotype (p = 0.03) with higher CD4+ T cell count among treatment naive HIV infected individuals
- There is significant (p < 0.05) association of CT/TT haplotype with increase in CD4/CD8% following 6-9 months of ART

Conclusion

Our preliminary data showed significantly higher CD4+ T cells in HIV infected individuals with wild haplotype (CT/TT) prior to ART and significantly high CD4+ T cells and CD4/CD8% following ART. However, this preliminary study failed to show any association between IL-28B polymorphism and IL-28B plasma level. It is better to undertake a study with larger sample size to confirm these findings.

Acknowledgement: We would like to thank the CMC, Fluid Research Fund and Department of Clinical Virology for the funding of the study. Correspondence: lamangara@cmcellora.ac.in

References

Association of genotypes with CD4+ T cell counts and CD4/CD8% before and after 6-9 months of ART

Association of genotypes with CD4+ T cell counts before and after 6-9 months of ART

Association of genotypes with CD4+ T cell counts and CD4/CD8% before and after 6-9 months of ART

Association of genotypes with CD4+ T cell counts before and after 6-9 months of ART
Background

- Mutations present at the polyadenylation site of a β-globin gene result in aberrant cleavage and results in production of elongated unstable mRNA transcripts.
- So far, five such point mutations and two minor deletions have been reported in the conserved AATAAA sequence at the polyadenylation site of β-globin gene resulting in mild β-thalassaemia.
- Polyadenylation site T→C (poly A T→C) mutation was previously reported in heterozygous and compound heterozygous state with other β-thalassaemia mutations in Black American and Turkish families.
- Functional analysis of RNA in these patients indicated that an intact AATAAA signal is not an absolute requirement for correct cleavage of the transcript.
- Heterozygous state for this mutation has been recently reported in 8 individuals from Indian population and they have reported variable haematological and clinical presentation of the patients with this mutation.
- However, haematological, clinical and molecular profiles of patients with homozygous poly A T→C mutation have not been studied extensively.

Aim

- To study the molecular basis for the variability in the haematological parameters and cause for heterogeneity in clinical presentation of the patients with poly A T→C mutation in the β-globin gene.

Methods and Materials

- Peripheral blood samples were collected after informed consent from 35 unrelated families who were diagnosed as poly A (T→C) mutation in the β-globin gene with phenotype of β-thalassaemia major or intermedia.
- Haematological parameters were assessed by automated cell counter (Sysmex KX2100) and haemoglobin variant analysis was performed using VARIANT haemoglobin testing system (Biorad, CA).
- Haplotype analysis in the β-globin cluster was done by restriction fragment length polymorphism (RFLP) method as describe previously.
- Copy numbers of α-, γ-, δ- and β-globin genes were measured by a gene dosage quantitative multiplex fluorescent-PCR (GD-QF-MPCR).
- Multiplex ligase-dependent probe amplification (MLPA) was performed using the SALSA MLPA Kit P102 HBB (MRG Holland, Amsterdam, Netherlands) according to the manufacturer's protocol to detect the rearrangements in the β-globin cluster.

Conclusions

- Patients with poly A (T→C) mutation in the β-globin gene showed remarkable variations in the haematological parameters and clinical phenotype.
- Haplotype analysis (fig 2) showed that this mutation has originated at least twice in this population and one of these haplotypes is similar to the one reported in African population.
- Both the haplotypes are associated with defective α-globin gene, which is the reason for decreased levels of HbF resulting in relative increase in HbA2 in some cases during variant analysis.
- Previous studies showed that point mutation at conserved AATAAA results in milder forms of thalassaemia but we observed heterogeneity in the phenotype in patients with poly A (T→C) mutation from intermediate form to transfusion dependent severe phenotype.
- The patients with intermediate phenotypes have high HbF levels and further screening for earlier reported high HbF SNPs in β-globin locus and intergenic (HBS1-L1 and HBEGF1) regions showed that there is high HbF transfusion dependent phenotype in one of the cases with high HbF levels which suggests that this might be the possible reason for increased levels of HbF.
- Studies using ex-vivo erythropoiesis in patients with this mutation will provide exact mechanism for elevated HbF and heterogeneity in the phenotype.

Acknowledgments

This study was supported by Department of Biotechnology, India.
INTRODUCTION: There are many number of orthoses developed for various upper and lower limb deformities. One of the major site of deformity is lower extremities, the main goal of lower extremities orthoses for muscular weakness of foot and ankle are to increase the functional performance of the patient during gait and to prevent further complications. The innovative design of orthosis for both dorsi flexors and plantar flexors weakness will be discussed.

BACKGROUND: The existing orthosis available for this deformities are PLS AFO, solid AFO, SMO, AAFO, FES with AFO, Hemi posterior leaf spring AFO, Spiral AFO, & etc., there are a number of orthoses available for all foot and ankle complex weaknesses but energy storing designs are very less. So this design will help and increase the functional rehabilitation of the patient with foot and ankle muscular weakness.

AIM & OBJECTIVE: The aim of this project is to design and develop indigenous energy storing, offloading orthosis, applying it over patients and to study and investigate the gait analysis with and without orthosis.

DESIGN: This orthosis consists of two parts: upper and lower end, the upper end with PTB design off loads the weight from the patellar tendon when the heel strikes to the ground, the lower end controls and prevent the foot deformities and its consisting of toe rocker so as to easily clear the ground at the time of push-off. In between this two shells a stainless steel flat is fixed and it will produce a dynamic function at the time of gait cycle.

SIGNIFICANCE: This design is going to act as a dynamic orthosis and it will reduce the energy consumption of the client by assisting weaker muscles during gait.

CONCLUSION: This orthosis is an effective option for foot and ankle weaknesses and a thorough analysis is required to check its effectiveness.

ADVANTAGES OF DEO BRACE
• Energy consumption
• Cosmetically good while sitting
• Allowing knee flexion movement at mid stance
• Simple in construction
• Easily accommodate with in a sandal no need of shoes
• More effective and comfortable than other orthosis
• More Natural gait
• Low profile design & low cost
• Easy to fabricate

DISADVANTAGES OF DEO BRACE
• Weight may be more than the other orthosis

REFERENCES
foot orthotics by kent, k. wu. Clinical and principle application
Role of pleural fluid biomarkers- ADA, ADA2 & Interferon gamma in the diagnosis of Tuberculous pleural effusion.

1 Dr.A.Ashwin Oliver 2 Dr.D.J.Christopher 3 Dr.Victoria Job
1 Assistant professor, Department of Pulmonary medicine, 2 Professor, Department of Pulmonary medicine, 3 Professor, Department of Clinical biochemistry

BACKGROUND:
➢ TB pleural effusion is the commonest cause of pleural effusion in many countries including India.
➢ TB pleural effusion is the one of the common sites for extrapulmonary tuberculosis.
➢ Due to the diagnostic challenges in the diagnosis of tuberculous pleural effusion, newer tests and biomarkers are employed to improve the diagnostic yield.

AIMS & OBJECTIVES OF THE STUDY:
➢ To study the role of pleural fluid biomarkers- ADA, ADA2 and Interferon gamma in the diagnosis of tuberculous pleural effusion.
➢ To study the combination of biomarkers in the diagnosis of pleural tuberculosis.

METHODS:
➢ Between May 2012 and July 2013, 154 patients with exudative pleural effusion were included in the study after informed consent.
➢ All patients underwent pleural fluid aspiration and closed pleural biopsy and these samples were sent for analysis.
➢ The pleural fluid was analyzed for biomarkers in addition to routine tests.
➢ The frequencies and percentages are calculated for the detection of biomarkers and the sensitivity, specificity; positive predictive, negative predictive values were calculated and an ROC analysis was performed.
➢ The area under the curve and cutoff values for biomarkers were calculated

RESULTS:
➢ The mean age of the patients was 44.8 years with a male predominance (74%) and 94% were unilateral effusions.
➢ Tuberculous effusion was commonly seen in age group less than 40 years with male predominance and 80% of the patients had lymphocytic effusion.
➢ Also those with TB had higher protein levels (mean=5.4mg/dl).
➢ A combination of ADA >24U/L with Interferon gamma >3.12IU/L in pleural fluid improves the diagnostic yield in tuberculous pleural effusion but may not replace the gold standard, culture and histopathology.

<table>
<thead>
<tr>
<th>Causes of pleural effusion</th>
<th>No of patients</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tuberculosis</td>
<td>68</td>
<td>44%</td>
</tr>
<tr>
<td>Uncertain aetiology</td>
<td>36</td>
<td>23%</td>
</tr>
<tr>
<td>Malignancy</td>
<td>33</td>
<td>20%</td>
</tr>
<tr>
<td>Lymphoma</td>
<td>7</td>
<td>4.5%</td>
</tr>
<tr>
<td>Benign mesothelial proliferation</td>
<td>2</td>
<td>1.2%</td>
</tr>
<tr>
<td>Mesothelioma</td>
<td>2</td>
<td>1.2%</td>
</tr>
<tr>
<td>Total patients</td>
<td>154</td>
<td></td>
</tr>
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</table>

Table 1: Causes of pleural effusion

<table>
<thead>
<tr>
<th>Pleural sample</th>
<th>No. of patients positive</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pleural biopsy culture</td>
<td>17</td>
<td>25%</td>
</tr>
<tr>
<td>Pleural fluid culture</td>
<td>4</td>
<td>5%</td>
</tr>
<tr>
<td>Histopathology</td>
<td>47</td>
<td>69%</td>
</tr>
<tr>
<td>Total patients with TB effusion</td>
<td>68</td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Yield of pleural cultures

RESULTS: Biomarkers:
➢ ADA had a sensitivity of 70.1% with a specificity of 71.1%.
➢ ADA2 had a better sensitivity than ADA but lacks specificity.
➢ Interferon gamma had a better specificity and specificity than ADA and ADA2.
➢ The sensitivity was 80% and specificity was 87%.
➢ The combination of biomarkers improved the sensitivity but failed to improve the specificity.

Biomarkers | Sensitivity | Specificity | AUC | Cut-off |
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>ADA</td>
<td>70.4</td>
<td>71.1</td>
<td>0.76</td>
<td>24</td>
</tr>
<tr>
<td>ADA2</td>
<td>78</td>
<td>62.7</td>
<td>0.70</td>
<td>12.6</td>
</tr>
<tr>
<td>INF gamma</td>
<td>82.1</td>
<td>86.2</td>
<td>0.88</td>
<td>3.12</td>
</tr>
</tbody>
</table>

Table 3: Sensitivity, specificity, AUC & cutoff for biomarkers

<table>
<thead>
<tr>
<th>Biomarkers</th>
<th>ADA</th>
<th>ADA2</th>
<th>Interferon gamma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tuberculosis</td>
<td>47</td>
<td>15.9</td>
<td>34.7</td>
</tr>
<tr>
<td>Malignancy</td>
<td>15.4</td>
<td>7.6</td>
<td>10.6</td>
</tr>
<tr>
<td>Lymphoma</td>
<td>13.3</td>
<td>31.0</td>
<td>22.3</td>
</tr>
</tbody>
</table>

Table 4: Showing pleural fluid biomarkers levels in tuberculosis, malignancy and lymphoma

CONCLUSION:
➢ Tuberculous pleural effusion is one of the common causes for pleural effusion with a diagnostic challenge.
➢ The pleural fluid biomarkers and combination of tests in diagnosing TB effusion but are not sufficient to replace the conventional gold standard methods for diagnosis.
Serum growth differentiation factor 15 levels in patients with ulcerative colitis

Chinmoy Jana
department of gastroenterology, Pondicherry Institute of Medical Sciences, Pondicherry, India

BACKGROUND

Hepcidin is the central regulator of iron homeostasis. Previous work has shown that serum hepcidin levels were decreased in patients with ulcerative colitis (UC). Growth differentiation factor 15 (GDF-15) is one of the known negative regulator of hepcidin. Other known regulators of hepcidin are shown in the figure below.

TABLE 1

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Patients with ulcerative colitis</th>
<th>Control patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Males/females</td>
<td>13/7</td>
<td>13/7</td>
</tr>
<tr>
<td>Mean age (years) (± SD)</td>
<td>40.45 (8.17)</td>
<td>39.75 (7.55)</td>
</tr>
</tbody>
</table>

TABLE 2

<table>
<thead>
<tr>
<th>Correlation coefficient</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum GDF-15 vs Age</td>
<td>0.416</td>
</tr>
<tr>
<td>MCV (n=15)</td>
<td>0.55*</td>
</tr>
<tr>
<td>Serum ferritin</td>
<td>0.391</td>
</tr>
<tr>
<td>Haemoglobin</td>
<td>-0.375</td>
</tr>
</tbody>
</table>

Control patients (n=20)

| Serum GDF 15 vs Age     | 0.569*  | 0.009  |
| MCV (n=14)              | -0.616* | 0.019  |

Using total data (UC + control) (n=40)

| Serum GDF 15 vs Age     | 0.446*  | 0.004  |
| Haemoglobin             | -0.379* | 0.018  |

Serum GDF 15 levels did not show significant correlations with serum iron or ferritin.

HYPOTHESIS

Serum GDF-15 levels may be increased in patients with ulcerative colitis (UC), leading to down-regulation of hepcidin in such patients.

AIM

To test the hypothesis that serum GDF-15 levels may be increased in patients with ulcerative colitis (UC).

MATERIALS AND METHODS

Forty patients, aged between 18-80 years, were recruited from the Department of Gastroenterology at CMC, Vellore.

CASES

Patients diagnosed to have ulcerative colitis (UC).

Either on or off treatment and had active or quiescent disease.

Not on iron supplementation or treatment with erythropoietin.

Patients diagnosed to have dyspepsia, with no abnormalities detected on upper gastrointestinal endoscopy.

Non-anemic (hemoglobin ≥12 g% in females and ≥13g% in males).

Serum C-reactive protein (CRP) levels < 6 mg/L.

Controls

Data were analyzed by unpaired T-test and Mann-Whitney U test, for iron and ferritin respectively.

ACKNOWLEDGEMENT

CMC Fluid Research Funds for the financial support for the study (IRB Min No.8150 dated 09.01.2013).

BIBLIOGRAPHY

Effect of polymorphisms in **DRD4** and **DAT VNTR** on clinical response to clozapine in patients with treatment-resistant schizophrenia (TRS).

Susan Philip1, Veetaranikandand R1, Lakshminirupa S2, Anto P Rajkumar1, C Chitra1, Anju Kurup1, Alok Srivastava1, B Poonkuzhal1, Moly Jacob1 and K S Jacob1

Departments of Psychiatry1, Biochemistry1 and Hematology1, Christian Medical College, Vellore, India and 2Centre for Psychiatric Research, Aarhus University Hospital, Rigshospitalets, 8240, Denmark

**Background**

The use of clozapine in treatment-resistant schizophrenia (TRS) is associated with adverse effects and variable clinical responses (1). Genetic factors such as polymorphisms play an important role in determining its treatment efficacy and adverse effects profile (2). Polymorphisms in dopamine receptor D4 (DRD4) and dopamine transporter (DAT) genes have been implicated in the pathophysiology of schizophrenia and pharmacogenetics of clozapine (3,4).

**Aim**

The aim of this study was to determine whether a 120-base pair duplication in **DRD4** and a variable number tandem repeat (VNTR) in DAT genes influence clinical response and adverse effects of clozapine in TRS.

**Methodology**

Subjects of this study were patients diagnosed to have TRS and on stable doses of clozapine for at least 12 weeks. After informed consent, patients were interviewed to obtain clinical and socio-demographic information, using standard instruments. Genomic DNA isolated from patients' peripheral blood samples were used for genotyping the polymorphisms of interest, using polymerase chain reaction (PCR), restriction digestion and agarose gel electrophoresis (5). Clinical responses to clozapine were assessed using Brief Psychiatric Rating Scale (BPRS), where a score ≤ 35 indicated a good response and vice versa (6). Appropriate statistical methods were employed for the analysis (Chi-squared test, Kruskal Wallis test and logistic regression).

**Table-2 Association statistics**

<table>
<thead>
<tr>
<th>Response</th>
<th>Genotype</th>
<th>OR</th>
<th>CI</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DRD4</strong></td>
<td>DD (240/240)</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>SD (240/120)</td>
<td>2.12</td>
<td>0.61-7.38</td>
<td>0.96</td>
</tr>
<tr>
<td></td>
<td>SS (120/120)</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>DAT VNTR</strong></td>
<td>AA (80/80)</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>AB (60/40)</td>
<td>0.26</td>
<td>0.05-1.35</td>
<td>0.16</td>
</tr>
<tr>
<td></td>
<td>BB (40/40)</td>
<td>0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Results & Discussion**

Sixty four patients, comprising 41 males and 23 females, with a mean age of 31.45 ± 9.42 years were studied. The BPRS scores of patients ranged from 6 to 159 with a mean of 53.84 ± 23.8. Clinical characteristics of patients and incidence of adverse effects in responders and non-responders are shown in Table-1. The genotype frequencies of the studied polymorphisms (DRD4 [SS-5; SD-22; DD-37] and DAT-VNTR [AA-43; AB-20; BB-1]) were within limits of Hardy-Weinberg equilibrium.

The polymorphisms interest were not significantly associated with clinical response to or adverse effects of the drug (Table-2). These data are preliminary in nature. Further follow-up of the study participants will be done. In addition, more patients will be recruited. The additional data that will be gathered is expected to provide more insights to the study.

**Conclusion**

The **DRD4** 120-bp duplication and **DAT VNTR** polymorphisms were not significantly associated with either clinical response to or adverse effects of clozapine, in patients with treatment-resistant schizophrenia.

Acknowledgement

The study was approved by the institutional review board (IRB) of Christian Medical College, Vellore and funded by the Department of Biotechnology, Government of India, New Delhi.

**References**


**Table-1 Clinical characteristics of study participants**

<table>
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**Acknowledgement**

The study was approved by the institutional review board (IRB) of Christian Medical College, Vellore and funded by the Department of Biotechnology, Government of India, New Delhi.

**References**

Effect of bloom strength on radiochromic gel dosimeters

Ebenezer Suman Babu and Paul Ravindran
Christian Medical College, Vellore, India.

Objective

The purpose of this study was to analyze the spectrophotometric response of Fricke gel dosimeter prepared with gelatin (300 bloom) and to compare the outcome with the cost effective 240 and 200 bloom gelatin locally available, belonging to Type B category.

Introduction

Bloom strength is defined as the ability of the gelatin to swell when dissolved in water to form a matrix. The dose response of Fricke gel prepared with 270 bloom strength of gelatin has been reported [1] as good performance when compared with 300 bloom gelatin that is usually recommended for preparation of gels. The non-availability of Type A gelatin at this bloom strength requires the use of Type B gelatin as an alternative. Studies on gelatin have shown that the source of gelatin (Type A, Procine) or (Type B, Bovine) did not alter the quality of gel prepared but bloom strength did affect the outcome [2]. Various researchers have investigated Fricke gel dosimeter, the role of various combinations of components and their influence on the optical properties [3–6]. The non-availability of high bloom strength gelatin in India has pushed the need to investigate the use of different bloom strengths and their limitations when radiochromic gels are prepared with them.

Materials and Methods

Three different bloom strengths of gelatin 300 (Sigma), 240 (SDfine), 200 (Raymon) were chosen for the experiments.

Gel Preparation

- 5% gelatin by weight added to triple distilled water and stirred well for 1 hour inside a water bath maintained at 45°C.
- After 1 hour the gelatin solution was allowed to cool down to 25°C and required quantities of Xylenol Orange, Ferrous Ammonium Sulphate and 1 M H2SO4 were added.
- The final gel consisted of 50 mM H2SO4, 0.05 mM Xylenol Orange and 0.3 mM Ferrous Ammonium Sulphate.
- The gel solution was then transferred to cuvettes of 3.5 ml capacity and path length 1cm and kept in the refrigerator at 4°C.

Irradiation

- Bulk gel phantom – 3 Gy to 10 cm depth by 2 cm diameter, stereotactic cone (figure 1a) attached to linear accelerator (Siemens, USA).
- Cuvettes in a water bath, irradiated on a 60Co teletherapy unit (figure 1b).

Results and Discussion

Fricke Gel – 240 Bloom:

- Stable at room temperature (28°C), similar physical condition of the samples of 300 bloom gelatin during irradiation and measurements.
- The dose response (Figure 3) was linear with transparency little lower than the 300 bloom gelatin when prepared as a bulk gel (1 L) but comparable transparency (figure 4) for using it as a 3D dose verification tool, not affected by temperature till 30°C.

Fricke Gel – 200 Bloom:

- Fricke gel dosimeters prepared with 200 bloom gelatin were less transparent than the 300 and 240 bloom gelatin.
- Physically stable at (28°C) but showed signs of melting at 30°C.
- The gel samples prepared with 200 bloom showed linearity in dose response (figure 3) but poor optical transmittance when a larger quantity (~ 1 L) was prepared.

Conclusion

- The obtained results indicate that the gels prepared with 240 bloom gelatin exhibit similar linearity in dose response compared to 300 bloom and could be a potential substitute that is locally available at a low cost.
- Further studies on improving the gel strength of locally available 240 bloom gelatin should be performed to remove the limitations of its use.

References


Read out systems

- An in-house optical Cone Beam CT scanner with an aquarium (figure 2a), turntable (figure 2b), matrix of yellow LEDs as light source and a high resolution camera (1024 x 768, Point Gray Fly Capture Camera) as a detector (figure 2c).
- Cuvettes were read at a wavelength of 585 nm in a spectrophotometer (UV-1800, Schimadzu, Japan).
INTRODUCTION

- Acute myeloid leukemia is a heterogeneous disease characterised by the increased proliferation of immature cells called blasts.
- Conventional chemotherapy with cytosine arabinoside (Ara-C) and Daunorubicin (DNR) eradicate blasts but less effective on leukemic stem cells (LSC).

![Fig 1: Intracellular metabolism of Ara-C and Dnr](image)

We hypothesised that quantitative differences (constitutive or acquired during leukaemogenesis) in expression of candidate drug metabolising/transporter genes in LSCs among patients explain the differences in the chemotherapy outcome.

AIM

- To compare the pattern of expression of these enzymes and transporters between total cells vs. putative LSC (pLSC) fraction of the same patient.

PATIENTS AND METHOD

- Bone marrow samples from AML patients at diagnosis (n=25) were collected after obtaining informed consent.
- Mononuclear cells (MNCs) were isolated by ficoll density gradient centrifugation; a proportion of these cells were enriched for putative LSC (CD34+CD38-123+) by FACS or magnetic sorting methods.
- The cells were then incubated with fluorescently tagged antibodies: CD34-FITC, CD38-APC and CD123-PE. The samples were sorted based on serial gating strategies and CD34+CD38-CD123+ (pLSC fraction) was collected.
- Total RNA from MNCs as well as from pLSC fractions were extracted by kit based method or chloroform based method depending on cell count.
- cDNA synthesis was done using ABI high capacity cDNA method.
- TaqMan pre-amplification method (ABI) was done to amplify the target genes in an unbiased way.
- Expression of various AraC and Dnr metabolizing genes Densycyclidine kinase (DCK), Cytidine deaminase (CDA), Equilibrative nucleoside transporter 1 (ENT1), 5' Nucleotidase NT5C2, CBR1(carbonyl reductase 1), CBR3 (carbonyl reductase 3), Ribonucleotide reductase (RRM1) and various drug transporters ABCB1, ABCG2 in the sorted fractions were analysed by Real time quantitative PCR and was normalized against housekeeping gene GAPDH to determine the relative expression.

The expression of these genes in the pLSC fraction was compared with the expression from total cells and the statistical significance was identified by paired t test using graph pad prism software.

RESULTS

- Representative flow sort of AML bone marrow MNCs conjugated with fluorochrome tagged antibodies CD34 FITC,CD38 APC,CD123 PE.
- Expression of Ara-C influx transporter hENT1 was identified to be significantly lower (p<0.0003) in pLSC fraction compared to the total cells. Meanwhile the expression of efflux transporters ABCG2, ABCB1 were significantly higher in pLSC fraction compared to the total cells.
- Daunorubicin metabolising genes CBR1, CBR3 expression in the CD34+CD38- fraction was found to be significantly higher with p value of 0.01 and <0.0001 respectively when compared with the total expression.

CONCLUSION

- This study has identified the differential expression of several candidate genes involved in transport and metabolism of chemotherapeutic drugs in pLSC fraction compared to total cells.
- The functional role of these differentially expressed genes in pLSC which contribute to resistance need to be further confirmed by additional studies.
- This study suggests that identification of these factors may not only provide the biomarkers predictive of treatment outcome but also allow designing patient specific treatment strategies.

ACKNOWLEDGEMENT

ICMR (INDO/FRC844/19-HD) and Dept. of Biotechnology (DBT) COE/08/01) for supporting this study. University grants commission (UGC) India for providing Research Fellowship to SK.
Objectives

* To design and develop a Balance Assessment Tool using Inertial Measurement Units

* To establish normative data of young and old people and quantify differences between them

Introduction

* Balance involves coordination of multiple systems in the body
  * Visual, Vestibular and Proprioceptive feedback
  * Extrapyramidal system and the basal ganglia

* Need to distinguish age related postural instability from neurological impairment

* Current clinical assessment uses visual assessment and subjective classification based on broad rules

* A new IMU based device is presented here that provides an alternative, quantitative and objective method that can potentially be used clinically

Methodology

* Pilot study on two age groups
  * Group I: 20-35 years (5 subjects)
  * Group II: 50-50 years (5 subjects)

* Subjects did 14 activities
  * Rising from a seat
  * Walking on level ground and ramps
  * Bending to pick up objects
  * Rolling on a bed, etc.

Standing to Sitting

The Balance Assessment Tool

System Block Diagram

Summary of Results

* The parameters which showed significant differences between the two age groups:

<table>
<thead>
<tr>
<th>Test</th>
<th>Parameter</th>
<th>Normalized Pitch Length</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standing to sitting</td>
<td>Pitch</td>
<td></td>
</tr>
<tr>
<td>Standing Unsupported</td>
<td>Pitch</td>
<td></td>
</tr>
<tr>
<td>with Legs Apart</td>
<td>Roll</td>
<td></td>
</tr>
<tr>
<td>Climbing Staircase</td>
<td>Pitch</td>
<td></td>
</tr>
<tr>
<td>Standing on One Leg</td>
<td>Pitch</td>
<td></td>
</tr>
<tr>
<td>Roll</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tandem Walking</td>
<td>Pitch</td>
<td></td>
</tr>
<tr>
<td>Roll</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Walking Down a Slope</td>
<td>Yaw</td>
<td></td>
</tr>
<tr>
<td>Pull Test</td>
<td>Pitch</td>
<td>Shoulder &amp; Neck</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Show definite age related changes in balance control

* A mild degree of balance dysfunction due to age has been brought out in “Normal Subjects” in 50% of the tests

Conclusion

* Using a novel IMU based balance assessment tool we have shown age related changes in stability

* Future studies on neurological impairment can be used to distinguish age related effects from neurologically treatable changes

* We hope this will be a useful clinical tool
Molecular basis of Von Willebrand Disease in 67 patients from India
Sumitha E, Eunice S.Edison, Viswabandya A, Abraham A, George B, Mathews V Srivastava A.
Department of Haematology, Christian Medical College, Vellore-632004, Tamil Nadu, India.

INTRODUCTION

- Von Willebrand disease, a disorder of blood coagulation caused by heterogeneous mutations in the VWF gene.
- Detection of these mutations is important for the precise genetic diagnosis in families affected by this disorder as well as for studying the molecular basis of this disease.
- Information on the molecular lesions underlying type 3 VWD (~474 mutations) is scarce due to the rarity of this disorder, that affects 0.1-5.3 per million in the general population.
- In this report, we describe the molecular abnormalities in Indian patients with inherited type 3 von willebrand disease.

PATIENTS AND METHODS

Patients
Sixty-seven patients with clinical symptoms suggestive of type-3 von willebrand disease were evaluated at the Department of Haematology CMC, Vellore between 2012 and 2014.

Hematologic and coagulation assays
The diagnosis was based basis of prolonged clotting times, reduced ristocetin co-factor activity, FVIII levels and reduced antigen levels.

Mutation detection
- Genomic DNA was screened for mutations in VWF gene by PCR amplification, conformation sensitive gel electrophoresis (CSGE) and DNA sequencing.
- Samples displaying abnormal CSGE profiles were sequenced by Big Dye Terminator cycle sequencing kit on an ABI 310 genetct analyzer (Figure 1).

Gene Dosage Analysis
Specific region of DNA was amplified using fluorescently labelled primers, for an exonic deletion and quantified using capillary electrophoresis (Gene mapper V4 software).

RESULTS AND DISCUSSION

Patient characteristics
The clinical presentation of 52 patients with von Willebrand factor deficiency is given in Figure 2.

Gene scan Analysis
The VWF peak was absent in the proband confirming their deletion status

Figure 2. Clinical manifestations of patients

Mutations in patients with type-3 von Willebrand disease
Mutations were identified in 52 patients. A total of 27 mutations including included frame shifts (44.4%), missense (29.6%), non sense (14.8%) and splice site mutations (11.1%) mutations. Nineteen of them were novel (Figure 3).

Table 1. In silico analysis of novel missense mutations

Common mutations
Two nonsense mutation p.Trp2107* and Arg373* was seen in in 9 unrelated patients of which one being novel and a splice site mutation c.2443-1G>C was identified in 7 patients in this study (Figure 3, 5).

Figure 3. Schematic representation of the mutations identified in the present study

Frameshift mutations (n=12)
The molecular pathology of twelve novel frame shift mutations are self-evident as they result in truncated von willebrand factor proteins.

Splice site mutation (n=2)
Splice site mutations (VWF: c.2443-1G>C, n=9; c.3675+1G>C, n=1) abolished the donor splice sites possibly leading to exon skipping or exon retention.

REFERENCES
Acute myeloid leukemia with a rare t(4;12)(q12;p13)
Suganya S 1, Angi M 3, Sitar Mu 1, Fouzia NA 1, Ganapule A 1, Abraham A 1, Viswabandya A 1, George B 1, Mathews V 1, Srivastava A 1, Srivastava VM 3.
Departments of 1 Haematology, 2 Transfusion Medicine and Immunohaematology and 3 Cytogenetics Unit, Christian Medical College, Vellore.

**INTRODUCTION**

- The t(4;12)(q12;p13) is a rare translocation in AML, with only about 20 cases reported till date. 1
- It involves the CHIC2 gene at 4q12 and ETV6 gene at 12p13 and is associated with ectopic expression of the homeobox gene, G5X2 on chromosome 4 with or without the formation of a CHIC2/ETV6 fusion gene. The 4q12 breakpoints are heterogeneous.  2
- The marrow in t(4;12) AML has been reported to show dysplastic changes, basophilia and blasts which may resemble lymphoid cells with low/absent myeloperoxidase activity. However, the blasts express myeloid antigens as well as CD7. It is associated with a poor prognosis. 3, 4
- We describe the cytogenetic and clinicopathological features of three patients with AML and the t(4;12)(q12;p13).

**PATIENTS AND METHODS**

- Patients: All patients with AML and the t(4;12) seen in the Department of Haematology, Christian Medical College, Vellore
- Method: Unstimulated overnight cultures of bone marrow, trypsin G banding.
- 20 metaphases analysed for each case, reported according to ISCN.
- Karyotypes were correlated with blood and bone marrow findings.

**RESULTS**

- Number of AML seen during the period of study: 1975
- Total number of patients with t(4;12) in this study: Three
- 0.15% (3/1975) of total AML
- Two of the three patients had a complex karyotype (> 2 abnormalities), with abnormalities of 17p in two, and the t(8;21)(q22;q22) in one. Follow up details are not available.

**CONCLUSIONS**

The t(4;12)(q12;p13) was seen in 0.15 % of AML. All three patients were below 60 years of age. An unusual finding is its association with the t(8;21) in one patient. These three cases will add to the reports of this rare entity in AML.

**REFERENCES**

Proteasome activity is dispensable for the degradation of PML-RARA: Efficacy of bortezomib along with arsenic trioxide in the treatment of ATO sensitive and resistant acute promyelocytic leukemia

Saravanan Ganesan, Ansu Abu Alex, Ehilsarasi Chandamurari, Nithya Balasundaram, Hamenth Kumar Palani, Sachin David, Jayendharan G Rao, Aby Abraham, Auro Viswabandaya, Biju George, Alok Srivastava, Poornakshi Balasubramanian, Rose Ann Padua, Christine Chomienne and Vikram Mathews
Department of Haematology, Christian Medical College, Vellore-632004, Tamil Nadu, India
*University Paris-Diderot, INSERM UMR-51131- Institut Universitaire d’Hématologie, Hôpital Saint Louis, Paris

2014

Background
Degradation of PML-RARA upon treatment with ATO is predominantly mediated by the proteasome complex.

Results
The combination of ATO and BO induced a significant apoptosis in all the resistant cells similar to naïve N84 cells (Figure 1A; n=4, p<0.02).

- The mechanism of inducing apoptosis in the resistant cell lines was similar to naïve N84 cells, as previously reported by us, and involved an increased level of ROS, decreased mitochondrial membrane potential, induction of UPR (CHOP expression) and activation of caspase-3 (Figure 1B).

Figure 1. Synergistic effect of BO(80ng/ml) and ATO in inducing apoptosis on ATO resistant cell lines (n=4).

![Figure 1: Synergistic effect of BO and ATO in inducing apoptosis](image)

- In an APL transplanted mice model, combination of ATO and BO prolonged the life span of the mice as illustrated in Figure 3a.
- A reduction in the LIC was demonstrated by secondary transplants. We also observed that transplanting bone marrow cells from the long term surviving mice post ATO+BO therapy did not induce leukemia (Figure 3b) and no transcripts of PML-RARA were detected in the recipients.

Figure 3. Efficacy of ATO and BO therapy in pre-clinical mice model

- 3A) ATO and BO induces prolonged survival in mice
- 3B) No leukemia was induced when secondary transplantation was done from long term surviving mice.

![Figure 3: Efficacy of ATO and BO therapy](image)

Methods

- Cell lines: Naive N84 cell line, N84-EV-Asx1, N84-EV-Asx2, HS-5
- Stromal cell line.

- Cytotoxicity Assay: In-house standardized in vitro cytotoxicity assay was done using MTT viability kit.

- Western Blot and Co-immunoprecipitation (Co-IP)

- Real time PCR

- Real-time PCR was performed using SYBRgreen method for ATG5, Beclin1, Chop and GAPDH. For PML-RARA transcripts copy number determination Taqman probes were used. Data were generated by the comparative threshold cycle (ΔCt) method by normalizing to GAPDH (SYBRgreen method).

- Immunofluorescence assay

- N84 cells either treated with drugs or without drugs were subjected to cytospin, fixed with Acetone & methanol (1:1) followed by blocking for 1 hour and incubating with primary antibody (PML-Santa Cruz, p62, LC3, Ubiquitin-Abcam) for over-night. The slides were probed with secondary antibody (Alexafluor or Invitrogen). The slides were then washed and counter stained with DAPI along with DAPI-mountant.

Mice Models
A transplantable mice model of APL was established in our centre.

- Briefly, 0.5-1x10^6 mice APL blast cells (A kind gift from Pr.Christine Chomienne, INSERM, France) were intravenously (i.v.) Injected in FVB/N mice (6-8 weeks) followed by treatment with ATO (5mg/kg) on day 7 for up to 28 days. BO was given as weekly dose (0.5 mg/kg) for 4 weeks. For serial transplantation experiments, the 10^9 cells were injected (i.v.) followed by treatment with ATO and BO and the mice were sacrificed on day 20. One million bone marrow cells were serially transplanted into FVB/N mice as mentioned earlier and were observed for their survival.

![Figure 2: ATO and BO induces autophagy which degrades PML-RARA through interacting with p62 protein](image)

- A phase II clinical study combining BO with ATO and chemotherapy has been initiated for patients with relapsed APL (NCT01950611). In this ongoing study 12 patients have been enrolled. The median age was 32 years. 7 were males. All patients achieved hematological remission and the median time to complete molecular remission was 42 days (one patient still on induction therapy). The addition of BO was well tolerated. None of the cases had evidence of significant neuropathy, worsening of coagulopathy, K bleed or a cerebrovascular syndrome. None of the patients have relapsed so far. Long term follow up is awaited to comment on the efficacy of this combination.

![Figure 4: Conclusion](image)

- In conclusion, the mechanism of ATO+BO synergy is multi-factorial and appears to be predominantly due to increase in ROS activity and apoptosis. PML-RARA degradation is mediated by up-regulation of autophagy with this combination. This synergism was further confirmed in a pre-clinical model. This combination is also effective in ATO resistant cell lines with high levels of synergism.

Reference
A Dosiometrical Comparison Between Intensity Modulated Radiotherapy and 3D Conformal (Tangential) Radiotherapy

A. Judith, B. Sasiadharan, S. Ebenezer, B. Thangakumari, B. Antoniam, B. Selvamani
1. CARITAS Cancer Institute, Kottayam, Kerala, India
2. Christian Medical College, Vellore, Tamil Nadu, India

Introduction

- Post-mastectomy radiotherapy (PMRT) is known to improve local control as well as overall survival in locally advanced cancers of breast [1-3].
- Tangential beam radiotherapy is the standard technique used in PMRT.
- It has been long assumed that respiratory motion does not significantly affect dose distribution within the target (chest wall) with standard tangential beam radiotherapy.
- However, the impact of respiration on dose distribution within target (chest wall) while using newer techniques such as intensity modulated radiotherapy is not clearly known.

Aim

To quantify the effect of breathing motion on post mastectomy radiotherapy with 3D conformal tangents and intensity modulated radiotherapy (IMRT).

Objectives

- To determine the change in target coverage that can occur with respiratory motion when treated with IMRT and 3D CRT.
- Assess whether the Lung & Heart doses are being under reported as compared to actual when plans are finalised on FB scan.

Methods

- PATIENT IMMOBILISED ON BREAST BOARD
- PLANNING CT SCAN OF THORAX WITH RT MARKERS IN
- FREE BREATHING (FB SCAN)
- 2 MORE CT SCANS IN THE SAME POSITION AT NORMAL RESPIRATION (NI SCAN) 
- NORMAL EXPIRATION (NE SCAN)
- CT SCAN IMAGES WERE TRANSFERRED TO 
- CONTOURING WAS DONE ON ALL 3 CT SCANS AS PER RTDG GUIDELINE
- CTV = CHEST WALL + ORGANS AT RISK + LUNG + HEART
- DVH OF THE 3 CT SETS WERE COMPARED (IMRT AND 3D-CRT)

Results

- A total of 10 patients were recruited.
- The mean respiratory rate was 20.3 breaths/minute ranging from 15 to 30.
- The mean tidal volume was 0.66 litres ranging from 0.44 to 0.85 litres. Median tidal volume was 0.67 litres.
- Mean chest expansion was 0.67 cm ranging from 0.5 to 1.2 cm.

Discussion

- From literature, it is known that the mean amplitude of chest wall movement with respiration is 8-10 mm. This was confirmed by this study [1].
- The effect of respiratory motion has been studied in whole breast IMRT and great dose inhomoeggeneities within the clinical target volume was found, with the minimal clinical target volume dose being decreased by 6-9% with respiration [5].
- However, the effect of breathing on chest wall radiotherapy is poorly understood.

This study found that:

- There is significant reduction in target volume coverage in different breathing phases with IMRT technique (Figure 1).
- Greater dose inhomoeggeneity occurs within target volume. The minimal clinical target volume is decreased by 20-25% with respiration in IMRT technique and only 10-12% with 3D conformal tangents plan (Figure 2).
- Lung dose increases with respiration in both IMRT and 3D conformal tangents technique. However, it does not cross tolerance limits (Table 1).
- Heart dose also increases with respiration in both techniques. However, it crosses the tolerance dose (V25 > 10%) with respiration in 3D conformal tangents plan for patients with left sided breast cancer (Table 2).
- There was more than 5% under coverage with different breathing phases in IMRT in comparison to 3D tangents technique for patients with tidal volume more than the median value of 0.67 litres (Figure 3).

Conclusions

- Diomension coverage of target chest wall is sensitive to chest wall motion with respiration for IMRT technique when compared to 3D tangents technique.
- There is definitely a trend found for 3D CRT technique for chest wall motion in patients with large tidal volume or chest wall expansion. However, it needs to be tested in a larger sample.
- However, in patients with left sided breast cancer there might be a benefit with IMRT technique.

Acknowledgements

We gratefully thank all our colleagues for their contribution towards this study as well as all the participants who willingly consented.

References


INTRODUCTION

• Radiation therapy has an important role in the treatment for Acute Lymphoblastic Leukemia (ALL).

• In ALL, cranial radiation is given in either therapeutic or prophylactic doses.

• Exposure to ionising radiation is one of the predisposing factors for gliomas.

• Radiation induced Glioblastoma Multiforme (GBM) is rare, its frequency is about 1-2% among all GBMs.(1)

CASE REPORT

• 6 year old boy presented with multiple episodes of fever and petechial rashes in 2002.

➢ Diagnosed to have Pre B cell ALL.

➢ After remission with induction chemotherapy, received prophylactic cranial radiation, 12 Gy in 9 fractions, in March 2002.

➢ CNS relapse in May 2003.

➢ Total body irradiation, 12 Gy in 6 fractions, 2 fractions per day, 6 hours apart, followed by peripheral blood stem cell transplant in July 2003.

➢ In August 2012, presented with holocranial headache and weakness of right upper and lower limbs of one month duration.

➢ On evaluation, well defined heterogenous enhancing mass in the left inferior frontal gyrus with significant perilesional oedema.

➢ Underwent subtotal excision of the lesion which was reported as SMALL CELL GBM.

➢ Received post op concurrent chemo irradiation, 54 Gy in 30 fractions with Concurrent Temozolomide 75mg/sq.m followed by adjuvant Temozolomide.

➢ After 3 cycles of adjuvant Temozolomide he had documented disease progression with significant deterioration in activities of daily living.

➢ He succumbed to his illness  8 months after the diagnosis of GBM.

DISCUSSION

➢ Radiation and its oncogenic effects in brain were reported for first time by Jones in 1960.

➢ Sarcomas and meningiomas are the commonest radiation induced tumors.

➢ Radiation induced GBM has also been reported.

➢ Salvati et al. analysed radiation induced gliomas and measured the frequency of radiation induced GBM (RIGBM) among all GBMs to be 1.3%

➢ Male/female ratio - 11 : 2

➢ Mean duration from the brain radiation therapy to the development of GBM was 72.3 months (range 11-132)(1)

➢ Radiation Induced GBMs have shorter overall survival(OS) than the de Novo GBMs. Mean OS for RIGBM was 10.5 months compared to 15.1 months in other GBMs.(1,2)

➢ No dose incidence relationship in the occurrence of RIGBM, this being a stochastic effect.

➢ In radiation induced GBM the clinical course was more aggressive and treatment refractory than de novo cases.

➢ No histopathological or genetic factors have been identified till now to differentiate radioinduced gliomas from de novo gliomas.

CONCLUSION

➢ GBM developed after irradiation have been known to have less tumor control rate and more aggressive behavior compared to that of the de novo GBM, even after aggressive surgical and medical management.

➢ Severity of the progress of gliomas, when developed after irradiation, is not related to radiation dose received.

➢ The management of RIGBM remains same as that of de novo GBMs.

REFERENCES


Intracranial Primitive Neuroectodermal Tumour with Extracranial Metastasis

Solly Thomas, Thomas S Ram, Selvamani B
Department of Radiation Therapy, Christian Medical College, Vellore

Definition

The World Health Organization (WHO) defines supratentorial primitive neuroectodermal tumour as an embryonal tumour in the cerebrum or suprasellar region consisting of undifferentiated or poorly differentiated neuroepithelial cells or neuroepithelial cells with divergent differentiation.

Introduction

- The Primitive Neuroectodermal Tumor (PNET) is rare tumour among adults, <1% of the primary CNS malignancy.
- The tendency of supratentorial PNET to spread within the CNS is well-known, but few cases of extracranial metastases of supratentorial PNET have been reported.
- The reported median survival was found to be 16 months inspite of the aggressive management.
- We report a case of 37-year-old man with a supratentorial PNET, metastasized to his left scapula, 3½ years after completion treated for primary.

Clinical Presentation

- 37 yr old gentleman presented with holocranial headache, vomiting and dizziness in October 2007.
- There was no history of trauma.
- Clinical examination revealed features suggestive of raised intracranial tension with papilloedema.

Imaging

- Well defined intensely heterogeneously enhancing right frontal dural based mass causing significant edema, midline shift to left and mass effect on ipsilateral frontal horn and brain stem & sclerosis of frontal bone.

MRI showed cystic enhancing mass with a large dural tail

Surgery and Post op Imaging

- He underwent Right frontal Craniotomy with total tumour excision on 30/01/2008.
- No evidence of residual disease post op imaging.

Brain Biopsy

Sheets of spindle and round cells with pale eosinophilic cytoplasm showing mitotic activity and necrosis with interfacing fascicles of spindle shaped cells.

Biopsy from the scapular lesion

Large cells with prominent nuclei, pale eosinophilic cytoplasm showing mitotic activity and necrosis with interfacing fascicles of spindle shaped cells.

IHC positive for Vimentin, Cytokeratin, EMA, S-100 and weakly positive for GFAP and negative for CD117 and CD99.

Radiotherapy details

- He received postoperative craniospinal irradiation (36Gy in 20 fractions) and tumour bed boost of (19.8 Gy in 11 fractions) in April 2008.

CT thorax showed large enhancing lesion with sun burst appearance with sclerotic foot in the left scapula infiltrating into the muscles

Imaging of the brain showed gliotic changes

Brain Biopsy (Contd...)

- These cells were positive for Vimentin and Cytokeratin 7 and S100 protein & negative for EMA, Synaptophysin and GFAP with focal Vimentin positivity of spindle cells. IN-1 expression was retained which ruled out atypical Teratoid Rhabdoid Tumour.
- Finally reported as High grade Malignant tumour with divergent differentiation suggestive of PNET.

Comparison of IHC markers

<table>
<thead>
<tr>
<th>IHC Tumour Markers</th>
<th>Brain Biopsy</th>
<th>Scapular Biopsy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vimentin</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Cytokeratin</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>S100</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>EMA</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Synaptophysin</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>GFAP</td>
<td>-</td>
<td>Weak +</td>
</tr>
<tr>
<td>NF</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Further treatment

- Was advised palliative radiotherapy to left scapula followed by palliative chemotherapy.
- Patient wished to take further treatment at hometown and was lost to follow up.

Discussion

- CNS PNET is a rare tumour in adult and systemic metastases are even rarer as Cerebrum does not have a lymphatic system.
- The intracerebral veins are thin-walled and would probably collapse early from compression by an expanding tumour.
- Immunological response of the host organ to tumour cells may prevent their growth outside the CNS.
- The life span of patients with intracranial tumours tends to be short.
- However bone is a frequent site for metastases and also the lesion is mostly osteoblastic in nature as in our case.

Risk Stratification

- Standard Risk: If the residual tumour is <1.5 cm³ there is currently no randomised data available supporting the routine use of adjuvant or neo-adjuvant chemotherapy in adult patients with primary PNETs.
- High Risk: Metastatic disease or a residual tumour is >1.5 cm³ Chemotherapy is accepted as having an important role in treatment of metastatic, although the optimal chemotherapy regimen is yet to be defined.
- Combination of Platinum and Vincristine based regimen can be tried.
- Other indications for chemotherapy includes bone marrow tumoral cells, spinal seeding and VP shunt

Conclusions

- The treatment of adult CNS PNET requires surgical resection as complete as possible followed by adjuvant therapy.
- Radiation therapy is recommended for all patients after surgical procedure as prophylactic treatment to prevent local and distant CNS recurrence.
- The benefits of chemotherapy are yet to be defined in standard risk PNET.
- Further studies on molecular biology and the gene expression of the PNET may shed light on the biological properties of the tumours that metastasize and the comprehension of the pathophysiology may guide new forms of treatment.

References

[References provided if available]
Persistent Mullerian Duct Syndrome
A case series of eight patients from South India

Stephen SF\(^1\), Shobha HB\(^2\), Kumar RM\(^2\), Anuradha C\(^3\), Mathai J\(^4\), Karl IS\(^4\), Srivastava VM\(^1\)
Cytogenetics Unit\(^1\), Departments of Pathology\(^2\), Radiology\(^3\) and Pediatric Surgery\(^4\), CMC, Vellore.

**Introduction**

- A rare disorder of sex development characterized by the persistence of mullerian derivatives, uterus and tubes, in otherwise normally virilized males.\(^1\)
- Usually diagnosed incidentally during surgical repair of cryptorchidism or inguinal hernia.\(^2\)
- PMDS results from either defects in AMH (antimullerian hormone) on chromosome 19 or its receptor AMHR-II (chromosome 12).\(^3\)

**Normal development**\(^3\)

**Testicular Biopsy**

![Images of histological sections showing normal male karyotype and ultrasound of the pelvis of a 15 year old boy with PMDS showing uterus and prostate gland.]

**Methods and Results**

Retrospective review of 8 patients with PMDS diagnosed on the basis of a combination of clinical, radiological, histopathological and cytogenetic findings between 2001-13

**Clinical features**

- Undescended testis
- Inguinal hernia
- Seminoma

**Karyotype and Radiology**

- \(46, XY\)
- Progenital ducts

**Histopathology**

- Sertoli cell only
- Leydig cell +
- Atrophic testis
- Tumor

**Conclusions**

- PMDS is a rare cause of DSD and most patients are incidentally detected during herniotomy.
- Correlation of examination with radiological, histopathological and karyotyping is important.
- The cryptorchid testes in PMDS are at risk of developing malignancy.

**References**

The Indispensable role of Sitting posture and Anterior fibreoptic bronchoscopic approach in facilitating awake nasal intubation in a practically impossible oral intubation.

Rajasekar A, Divya Issac, Raj Sahajanand.
Department of Anaesthesia, Christian Medical College, Vellore.

INTRODUCTION

- We present a case of Giant (7+5 cm sized) tonsillar tumour in a 47-year-old male with symptoms of airway obstruction. He presented with the history of progressively increasing swelling in the throat since 3 months, change in voice and mild breathlessness.
- He complained of worsening dyspnea, stridor and upper airway obstruction during sleep for two weeks before admission.
- He had history of dysphagia and regurgitation of food.
- He was unable to lie flat, owing to the aggravation of breathlessness by supine position and during sleep, hence he required elevation of head end and adapted lateral position while sleeping.
- An attempt at indirect laryngoscopy by an otolaryngologist provoked stridor and breathlessness.

FIG.1. Arrow pointing at the giant tonsillar mass

FIG.2. Tomographic sections of the oropharynx

FIG.3. Coronal section

Procedure
Awake Nasal Fiberoptic intubation was planned.

Challenges
- Unachievble oral intubation,
- Distorted anatomy,
- Inadequate visualization during fibreoptic intubation,
- Possibility of complete upper airway obstruction on induction of anaesthesia,
- Inability to ventilate with Bag and Mask,
- Difficulty in threading the Endotracheal tube over the scope,
- Hazards of trauma and haemorrhage,
- Risk of aspiration with blood and tumor fragments,
- Difficulty in suctioning.

Advantages of Sitting position
- Unobstructed patent airway,
- Preservation of the functional residual capacity,
- Comclusive for the patient as there are no airway intubations in the supine position,
- Prevent posterior displacement of the mass secondary to the gravitational force,
- Enables better drainage of the secretions away from the field of vision,
- Reduced risk of aspiration of the gastric contents.

Advantages of Awake Fiberoptic Intubation
- Maintains upper airway tone and thereby prevents airway obstruction,
- Preserves spontaneous respiration and functional residual capacity,
- Avoids intubation technique. Can’t intubate scenario,
- Being fiberoptic scope the posterior aspect of the tumor could be assessed.

Technical Considerations
- The procedure was explained in detail to the patient. Sedative premedications were avoided and the patient was sufficiently fasted.
- All the necessary equipment for the management of difficult airway were arranged.
- Provisions for securing an emergency surgical airway were arranged.
- Right nostril was selected for intubation as the patient could breathe better through the right nose.
- Demethylcaine and glycopyroate 0.4mg were administered.
- Maximum allowable dose of lignocaine was calculated.
- Oxygenator nasal drops were applied to both the nostril. The following Airways block were performed.
- Cotton pledgets soaked with 4% lignocaine was used to anesthetize the anterior ethmoidal nerve and branches of posterior ethmoidal ganglion on either sides.
- The superior laryngeal nerves were blocked bilaterally.
- Trans-cricothyroid recurrent laryngeal nerve block was performed.
- Patient was placed in the sitting position and sedated with 4% topical lignocaine solution.
- A well lubricated, Rusch nasal airway (size 3) was inserted in the left nostril for supplementing oxygen.
- Patient was adequately preoxygenated for 5 minutes.
- Considering the reverse fibreoptic orientation of the structures during anterior approach, the black arrow portion of the fibreoptic field was positioned at the 12’0 clock position Refer (Fig. 5).
- 4% lignocaine was sprayed once the vocal cords were visualized.
- Awake nasal fibreoptic intubation through 5.5 mm size nasal RAE tube, approaching from the anterior aspect of the patient was performed successfully, preserving spontaneous respiration.
- Patient was comfortable during the intubation process.
- Fiberoptic visualization of the structures were satisfactory.
- The surgical procedure was uneventful.
- At the end of the procedure, patient was extubated, fully awake and comfortable with the nasal airway in place.
- Biopsy revealed a B-cell lymphoma, patient was further followed up by medical oncology.

Discussion
- Proper assessment of the patient and adequate preparation with adaptation of Sitting posture and Anterior approach for introducing the Endotracheal tube, overcomes the shortfall of supine position and preserves the airway patency.
- Mastering anterior approach requires understanding of the reverse structural orientation in contrast to the conventional approach.

FIG.4. Conventional view
FIG.5. Anterior approach view

Suggestions
- Proper attention to the patient’s symptoms in relation to the position and planning could prevent airway disasters.
- Adequate airway anaesthesia is the cornerstone for successful awake intubation.
- Establishing good rapport with the patient is imperative for better cooperation of the patient.

Conclusion
The Anterior fibreoptic bronchoscopic approach and Sitting posture in a compromised upper airway provides a dual benefit of unobstructed airway and improved visualization during intubation.

Reference
NYSORA - The New York School of Regional Anesthesia - Regional & Topical Anesthesia for Endotracheal Intubation.
BACKGROUND

Cerebral palsy is the commonest physical disability in children, with no cure till now. An important goal in management is to improve motor function of hand. Existence of sensory inputs to the motor cortices raises the potential of using sensory stimuli such as vibration, applied on palms, to induce neuroplasticity to improve hand dexterity in cerebral palsy (CP) children.

AIM

To investigate the effect of local palmar vibration therapy in improving motor hand function of children with cerebral palsy.

MATERIALS AND METHODS

Study Design: Single blinded, randomized controlled trial (Registry number CTRI/2013/05/003700) with target sample size of 50

Subjects: Hemi-/di-/tri-/quadriplegic CP children, 6-15 years, of either sex, who were able to follow 2-step commands, were recruited, randomized and allocated to intervention and control groups.

Control group: Received standard conventional therapy.

Intervention group: Received standard therapy + Palmar Vibration therapy

Intervention:

- Commercially available portable vibrator
- Bilateral palmar vibration, 5 min daily, 5 days/week, for 4 weeks

Primary Outcome: Box & Block test (BBT) for hand dexterity

Secondary Outcome: Modified Barthel Index to assess Activities of Daily living

Pre- and Post-assessments were done.

DISCUSSION

- Efficacy of vibration therapy not confirmed by analysis of small data at time of interim analysis
- Evidences indicate a role of vibration therapy in improving hand dexterity in CP children, as only in the intervention group non-dominant hand dexterity improved, and 2 patients (20%) were reclassified to a higher stage (as per MBI scores).
- We postulate that vibration therapy may be producing this effect through neuroplasticity.
- Our findings are also in line with studies that report vibration attenuating inter-hemispheric inhibition of weaker cortex.

CONCLUSION

While interim analysis gave no conclusive proof for a positive effect of vibration therapy over and above standard therapy, evidences point towards a positive role of vibration therapy in improving dexterity of the weaker hand in the CP children of our study population. Findings are indicative of a useful role of vibration therapy in improving motor function.

REFERENCES

INTRODUCTION

In the acromegaly patient, macroglossia, prognathism and hypertrophy of soft tissue structures pose a challenge to the anesthetist. Various methods have been used to overcome these difficulties in order to secure an advanced airway. We describe a case where such a patient with a difficult airway was intubated using GlideScope with adjunctive use of Magill forceps.

CASE DESCRIPTION

The patient was a forty-five-year-old man scheduled for bimaxillary transphenoidal excision of growth hormone secreting pituitary macroadenoma. He had acromegaly with airway features of prognathia, soft tissue hypertrophy and macroglossia; there was no hoarseness of voice or stridor evident. He gave history of snoring but no other symptoms suggestive of obstructive sleep apnoea.

After establishing peripheral venous access, invasive blood pressure monitoring, application of standard monitors and pre-oxygenation, anaesthesia was induced with 1 mcg/kg of fentanyl, sevoflurane 2%, propofol 2mg/kg and thiamarinium 1,5mg/kg.

Introduction of GlideScope into the oral cavity was difficult owing to macroglossia, and revealed a Cormack and Lehane grade IIIb view which did not improve with external laryngeal pressure. An attempt to manipulate a bougie past the glottic was unsuccessful.

PROCEDURE

On recognising that the enlarged tongue and soft tissue hyperterophy were obstructing optimal visualisation, a Magill's forceps was used to apply traction on the tongue (Fig. 1).

The laryngoscopy view improved to grade IIIb and a size 8 cuffed oral endotracheal tube was secured (Fig. 2).

At the end of surgery, following adequate reversal of muscle relaxation, the patient was brought to spontaneous ventilation and extubated. The Magill's forceps was used to apply gentle traction on the tongue and displace it from the airway (Fig. 3), thus preventing airway obstruction.

Traction was not associated with any significant hemodynamic changes, discomfort or pain to the patient.

CLINICAL IMPLICATION

- Although routinely used to pack the throat and aid in the insertion of endotracheal tube and nasogastric tubes, use of Magill's forceps to dynamically retract the tongue to improve laryngoscopic view and thus enable intubation has not been described.
- Use of the Magill's forceps rather than the laryngoscope blade alone to retract the enlarged tongue allows better visualisation of the glottis. Traction on the tongue will also result in the epiglottis being lifted anteriorly and hence aid intubation.
- It may also be used to prevent airway obstruction after extubation, where the introduction of an oral airway may not only be difficult due to the enlarged tongue, but may also be insufficient to prevent obstruction as a result of the prognathism. (Fig.3 ) Moreover, it isatraumatic and does not cause hemodynamic changes or discomfort to the patient.
- Use of tongue suture has been described as a means to prevent airway obstruction following extubation. This procedure is traumatic and does not enable dynamic retraction of the tongue as is possible with a Magill's forceps.
- Some reports have suggested nasal intubation followed by fibreoptic oral intubation in acromegaly. In a patient undergoing bimaxillary surgery, nasal intubation impairs surgical access and is to be considered only if all other options fail.
- Use of Magill forceps to pull out the tongue has been reported to aid fibreoptic intubation (6), but its use as an adjunct to intubation with GlideScope has not been described. Although it is a part of the intubatin kit and is widely used in guiding intubation and placing throat packs, its use in improving laryngoscopic view is new.

CONCLUSION

The use of the Magill forceps to retract the tongue during laryngoscopy improves the laryngoscopic view in patients with macroglossia.

REFERENCES

INTRODUCTION

- Haemoglobinopathies refer to a diverse group of inherited disorders characterized by a reduced synthesis of one or more globin chains (thalassemias) or the synthesis of a structurally abnormal haemoglobin (Hb).

- Haemoglobinopathies are the commonest monogenic disease affecting the world's population.

- Diagnosis of haemoglobin disorders is based on clinical features, haematological parameters, peripheral smear examination and quantification of haemoglobins F (HbF), A₂ (HbA₂) and haemoglobin variants.

- There is limited data on levels of HbF, Hb A₂ and haemoglobin variants in various haemoglobin disorders in Indian population.

- In normal individual the level of HbA is 96-98%, HbA₂ < 3.5 % and HbF is < 1.5%.

MATERIALS AND METHODS

- We did a retrospective analysis of 9000 samples for which the levels of HbF, Hb A₂ and variant haemoglobins were quantified between 2004 and 2013.

- Haematological parameters were measured by standard methods.

- Haemoglobin analysis was performed using a Cation exchange High Performance Liquid Chromatography (VARIANT™; Bio Rad Laboratories, Hercules, CA, USA).

- Statistical analysis were done by SPSS Software v.17

RESULTS AND DISCUSSION

- Out of the 9000 samples, 2400 had heterozygous β thalassemia and the median age of the cohort was 33 years (2-82 years) with 1067 males and 1333 females.

- The haematological parameters and the hemoglobin analysis are listed in Table-1.

- Mutation data was available only for 124 heterozygous β thalassemia patients and the distribution of mutations and hematological parameters are shown in Figure 1 and Table 2.

- We have diagnosed 275 heterozygous sickle patients in this cohort. The age ranged between 3 to 76 and sickle percentage between 22.90% to 44.90% and hematological parameters are shown in Table 3.

- We have diagnosed 417 heterozygous HbE patients with their age range between 2 to 76 and HbE percentage between 19.70% to 35.90% and hematological parameters are shown in Table 4.

CONCLUSION

- This is a retrospective analysis with a large sample size.
- This data gives an overview of HbF, HbA₂ and variant Hb levels in heterozygous β-thalassemia, heterozygous HbE and heterozygous sickle (HbS) in the Indian population.
- This study will aid in interpreting the HbF and HbA₂ levels in diagnostic laboratories.

Table 1: Hematological parameters of Heterozygous beta-thalassemia patients

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Hb (g/dl)</th>
<th>MCV (fl)</th>
<th>HbF(%)</th>
<th>HbA₂(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>2395</td>
<td>2286</td>
<td>2400</td>
<td>2400</td>
</tr>
<tr>
<td>Mean±SD</td>
<td>10.87±1.6</td>
<td>63.78±5.5</td>
<td>0.87±1.1</td>
<td>4.97±0.59</td>
</tr>
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</table>

Table 2: Hematological parameters of 124 Heterozygous β-thalassemia patients

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Hb (g/dl)</th>
<th>MCV (fl)</th>
<th>HbF(%)</th>
<th>HbA₂(%)</th>
</tr>
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<tbody>
<tr>
<td>N</td>
<td>120</td>
<td>119</td>
<td>124</td>
<td>124</td>
</tr>
<tr>
<td>Mean±SD</td>
<td>11.078±1.40</td>
<td>64.17±4.8</td>
<td>0.710±0.93</td>
<td>4.94±0.62</td>
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</table>

Table 3: Hematological parameters of Heterozygous Sickle cell patients

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Hb (g/dl)</th>
<th>MCV (fl)</th>
<th>HbF(%)</th>
<th>HbA₂(%)</th>
<th>HbS(%)</th>
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<tbody>
<tr>
<td>N</td>
<td>248</td>
<td>240</td>
<td>275</td>
<td>275</td>
<td>273</td>
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<tr>
<td>Mean±SD</td>
<td>12.32±1.98</td>
<td>80.19±8.64</td>
<td>1.01±1.15</td>
<td>5.23±0.42</td>
<td>34.70±4.27</td>
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Table 4: Hematological parameters of Heterozygous HbE patients

<table>
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<tr>
<th>Parameter</th>
<th>Hb (g/dl)</th>
<th>MCV (fl)</th>
<th>HbF(%)</th>
<th>HbA₂/E(%)</th>
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<tr>
<td>N</td>
<td>415</td>
<td>401</td>
<td>417</td>
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<tr>
<td>Mean±SD</td>
<td>11.67±1.88</td>
<td>75.00±5.84</td>
<td>0.81±0.84</td>
<td>27.45±2.26</td>
</tr>
</tbody>
</table>

REFERENCES

Arsenic Trioxide Resistance: More to It than Mutations in PML-RARA

Ansul Abu Alex, Ezhilarasi Chendamaraj, Saravanan Ganesan, Nithya Balasundaram, Hameenth Kumar Palani, Sachin David, Arun Jose Nillichal*, Biju George, Alok Srivastava and Vikram Mathews

Department of Haematology, Christian Medical College, Vellore-632004.
*Department of Clinical Biochemistry, Christian Medical College, Vellore-632004.

Background

• Arsenic trioxide (ATO) has proven efficacy as upront therapy in the treatment of acute promyelocytic leukemia (APL).
• There has been a recent concern of ATO resistance in patients treated with ATO. The focus of ATO resistance has centred on mutations in PML-RARA gene (Blood 2011, NEJM 2014).
• In our laboratory, we generated 3 resistant NB4 sub-clones. NB4EV-ASR1, ASR2 and ASR3. In addition we have also established an ATRA resistant APL cell line U11 (kind gift from Dr. Christine Chomienne, Hôpital Saint Louis, Paris).

Aim

• To study the mechanisms of ATO resistance in the in-house generated ATO resistant NB4 cell lines.

Methods

Generation of ATO resistant cell line
NB4 cells in our laboratory were exposed to serial increasing concentrations of ATO over a period of 1 year and when stable at 1μM concentration of ATO, clonal populations were further isolated by limiting dilutions and methylcellulose plating.

Cell viability assay
in-house standardized in vitro cell viability assay was done using MTT viability kit and cytotoxicity of different drugs was measured by flow cytometry using Annexin V and 7AAD.

Intracellular arsenic trioxide concentration
The IC-ATO level was measured by treating the cells with 0.5μM ATO for 24 hours and the cell pellet was digested with suprapur nitric acid and hydrogen peroxide (2:3 v/v) at 45°C for 16 hours and analyzed using Atomic Absorption Spectrometer (AAS).

Detection of PML-RARA by Real-time RT-PCR, Immunofluorescence and Western blotting
RTQPCR: Immunofluorescence probing for PML and Western blotting was done for detecting PML-RARA using standardized protocols.

Measurement of ROS and GSH in cell lines
Baseline ROS levels and GSH were measured using Cell Ox green reagent and o-phthalaldehyde (OPT) respectively by flow cytometry (BC Gallios).

Microarray analysis and Exome sequencing
Gene expression profiling of NB4 and NB4 resistant primary clone was done with the help of Genotypic Technology, Bengaluru using 44k human microarray chip analysis (Agilent technologies).

Whole Exome sequencing (NGS) was done by Ion TargetSeq Exome Enrichment by Ion Proton System and Ion PGM for PML B2 domain mutation.

Results

• MTT assay shows that the resistant cell lines had a higher IC50 to ATO and to other therapeutic drugs such as Daunorubicin and Cytoxan arabinoside and a reduced differentiation effect on exposure to ATRA when compared to naive NB4 (Table 1).
• Whole exome sequencing revealed that only NB4 EV-ARSI had ATO resistance causing mutation (A216V) in the PML B2 domain (VAF-91.7%) while NB4EV-ASR2 and ASR3 did not have this mutation in PML B2 domain.
• Expression array reveals that 1490 genes were differentially regulated (2-fold) between naive and resistant cell lines. The pathways significantly enriched for differentially expressed genes were cell survival, ABC transporters, glutathione synthesis, ubiquitin-proteasome degradation system etc.
• Validating the micro-array data by real time PCR, we found that there is an increased expression of ABC transporters such as MRPI, AQP5 (arsenic efflux proteins) (n=3) in resistant cell lines.

• The up-regulation of these transporters also correlated with decreased levels of intracellular ATO(ASR)(n=6).
• There is a reduction in the basal reactive oxygen species level in resistant cell lines when compared to NB4.

• There is a varying reduction in the basal reduced glutathione levels in the resistant cell lines (n=3, table 1).
• Depletion of GSH by treating the cells with 100μM BSO significantly sensitized the resistant cells to ATO (n=3).

Discussion

• We have observed that in addition to ATO-RARA mutations, variations in the Redox system, ATP transporters, intracellular ATO concentration and anti-apoptosis pathways are likely to be altered in ATO resistance and it is likely that ATO resistance is multi-factorial.
• It is also important to note the heterogeneity of malignant cell line sensitivity to other conventional agents used to treat APL.

• Novel agents and strategies based on these observations are required to address the issue of ATO resistance in patients with relapsed APL.

Table 1: Characteristic features of NB4 naive, NB4 resistant cell lines and U11

<table>
<thead>
<tr>
<th>Characteristic features</th>
<th>NB4 naive</th>
<th>NB4 EV-ARSI</th>
<th>NB4 EV-ARSI</th>
<th>NB4 EV-ARSI</th>
<th>U11</th>
</tr>
</thead>
<tbody>
<tr>
<td>Presence of PML B2 domain mutation (A216V)</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>NA</td>
</tr>
<tr>
<td>Sensitivity to ATO (IC 50 - μM)</td>
<td>0.9</td>
<td>3.09</td>
<td>3.44</td>
<td>2.86</td>
<td>4.1</td>
</tr>
<tr>
<td>Differentiation ability by ATRA(μM for 72hrs)(n=4)</td>
<td>CD11b expression(measure=50°C)</td>
<td>0.92±0.3</td>
<td>0.15±0.03</td>
<td>12.6±2.5</td>
<td>30.5±2.6</td>
</tr>
<tr>
<td>Immunophenotypic profile</td>
<td>CD13</td>
<td>++</td>
<td>++</td>
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<tr>
<td>CD38</td>
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<tr>
<td>CD4</td>
<td>+++</td>
<td>++</td>
<td>++</td>
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<td>++</td>
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<tr>
<td>Sensitivity to other chemotherapies(μM)</td>
<td>Daunorubicin(μM)</td>
<td>0.14</td>
<td>0.22</td>
<td>0.19</td>
<td>0.2</td>
</tr>
<tr>
<td>Cytosine arabinoside(μM)</td>
<td>8.3</td>
<td>16.5</td>
<td>4.7</td>
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<tr>
<td>Reactive oxygen species (ROS) levels (MF-Fold difference normalized to NB4 cells)(n=3)</td>
<td>1</td>
<td>0.74</td>
<td>0.96</td>
<td>0.68</td>
<td>0.3</td>
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<tr>
<td>Glutathione levels (MF-Fold difference-normalized to NB4 cells)(n=3)</td>
<td>1</td>
<td>1.37</td>
<td>1.45</td>
<td>1.39</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Reference

1. Goto et al., Missense mutations in PML-RARA are critical for the lack of responsiveness to arsenic trioxide treatment, Blood, 2011; 118(6):1600-6
Electro-Convulsive Therapy for Refractory Epilepsy and Organic Psychosis-A Case Report

Nanma Livingstone, Jibi Achanma Jacob, Saumil Dholakia, Deepa Braganza.
Christian Medical College, Vellore, Tamilnadu.

INTRODUCTION

- Psychiatric disorders like Psychosis, Drug Resistant Epilepsy/Refractory Epilepsy (RE) is known to be a chronic illness with a myriad of psychiatric manifestations.
- and Personality Change are common complications.
- Current Evidence for management of Epilepsy resistant to two or more antiepileptic drugs are Surgery, Vagus Nerve Stimulation, investigational drugs, or devices like Deep Brain Stimulation and aggressive combination of available Anti-Epileptic Drugs.
- Electro Convulsive Therapy (ECT) has been used to treat Epilepsy since the 1930s and the first case reports of its efficacy in RE were published in early 1990s.

CASE REPORT

Mr. B, a 23 year old single male with normal birth and developmental history presented with 4 constellation of symptoms:

- Generalized Tonic Clonic Seizures with EEG documented Left Temporal focus in a frequency of 4-5 episodes per month following Viral Encephalitis and subsequent Bilateral Mesial Temporal Sclerosis since the age of 12.
- Increased sexual drive and socially disinhibited behavior.
- Paranoid and referential beliefs with delusions of persecution and black magic with an alternating pattern with episodes of seizures (forced or Paradoxical Normalization).
- Pervasive Personality Change with Viscosity, Hypergraphia, salience to details with a tendency to give detailed background information (Gestaut-Geschwind Syndrome).

INTERVENTION

- Drug Resistance was confirmed with Mr. B failing to show response to a therapeutic doses of combination of Zonisamide, Carbamazepine, Phenobarbitone, Clobazam.
- Mr. B's psychotic symptoms also showed drug resistance to therapeutic dosages of Risperidone, Quetiapine and Aripiprazole.
- ECT was considered based on the rationale of efficacy of ECT in psychosis, seizures and possibility of raising the seizure threshold. The patient had history of delayed recovery from anexthesia in the past and hence modified ECT was started after discussing with the family and obtaining informed written consent. He received 19 sessions of ECT including 3 as maintenance ECT without any major post ECT complications.

RESULTS

- The Seizure frequency reduced to about once a month following ECT. Psychosis persisted and the antipsychotic had to be changed to Zuclopenthixol-decanate. EEG 4 hour telemetry done three months later was normal.

DISCUSSION

- The efficacy and safety of ECT in Status Epilepticus (SE) refractory to medications is reported in scientific literature, however its efficacy and place in the protocol of treatment for RE is not clear.
- ECT is known to increase the Seizure threshold in patients with seizure disorder by modulating intrinsic neurotransmitters like GABA, Neuropeptide Y and certain monoaminergic systems in the Brain.
- ECT affects EEG seizure pattern in several different stimulation parameter-dependent ways:
  - modulation to different pattern;
  - increased inter-ictal time and thereby achieve seizure cessation.

CONCLUSION

- While the first line of therapy for epilepsy is medications, many patients who are considered "refractory" to medical therapy have often failed multiple medications. This suggests that certain electrical activity is simply refractory to the Neurochemical/Electrical changes induced by those medications. However, a seizure with a different electrical pattern may not be so refractory. Hence, a change in the seizure pattern induced by ECT may well allow a seizure to no longer be as medically refractory, leading to seizure cessation.
- This Case report attempts to propose ECT as a definitive treatment option before considering empirical or surgical invasive procedures for patients suffering from Epilepsy which is resistant to pharmacological interventions.

REFERENCES

Protecting pacemakers in prone position - an inexpensive alternative

Bernice Theodore, Georgene Singh
Department of Anaesthesiology, Christian Medical College, Vellore

Background

Pacemakers have two components: electrodes and generator.

The electrodes are introduced trans-venous and placed within one or more chambers of the heart according to the indication, while the generator is implanted in the subcutaneous plane inferior to the clavicle.

Though convenient from the point of view of access, this position poses certain challenges for anaesthesiologists when patients undergo operations in the prone position, namely:

- risk of pressure necrosis of the skin over the generator
- risk of pacemaker malfunction

Most operating table manufacturers also supply gel attachments that may be placed beneath the patient so as to protect the pacemaker generator. These cost approximately 25,000 INR.

Method

We would like to describe an alternative method for protecting the pacemaker generator in a patient who required to be in prone position for an operation predicted to last 4 to 6 hours. The patient had a pacemaker sited inferior to his right clavicle. He was thin built and the pacing generator was prominent.

![Generator position marked](image1)
![Sponge pads taped together](image2)

We placed a piece of commercially available sponge, which is normally trimmed to be used as a face pillow for patients in prone position. The sponge accommodates for the shape of the generator, thus preventing any pressure on the overlying skin.

![Pads positioned on patient](image3)
![Patient positioned prone](image4)

Another identical piece of sponge was used on the opposite side so that the patient remained square on the operating table, without any tilt.

This cost us approximately 500 INR.

Conclusion

Leading operating table manufacturers supply table accessories like gel rings which may be used for pacemaker protection. However these are expensive. Our alternative method has been shown to be both inexpensive and effective.
Prenatal diagnosis of Haemophilia A in twin pregnancies
Department of Haematology, Christian Medical College, Vellore-632004, TamilNadu, India.

INTRODUCTION
Haemophilia (HA) is an X-chromosome linked coagulation disorder with an incidence of 1 in 5,000 males worldwide.

The major molecular defects in the F8 gene causing HA are intron 222 and intron 1 inversions2 responsible for ~50% of HA.

Prenatal diagnosis in carriers with twin pregnancies is more challenging than in women with singleton pregnancies3.

Here we report prenatal diagnosis performed to three couples (families A, B, and C) with twin pregnancies who were at risk for hemophilia A.

In this report, we describe the molecular abnormalities found in the f8 gene in Indian patients with twin pregnancy.

PATIENTS AND METHODS

Patients
Three families with clinical symptoms suggestive of haemophilia A were evaluated at the Department of Haematology and Reproductive Medicine Unit, CMC Vellore between 2006 and 2014.

Mutation detection
Mutations identified in the index cases were further screened in twin chronic villus sample (CVS) obtained at 10-12 wks of gestation.

Maternal contamination in CVS samples were ruled out by using five VNTR markers.

RESULTS AND DISCUSSION

Patient characteristics
Family A: The propositus, a 25 year old female, had lost one child due to scalp haematoma in the occipital region following a fall and spontaneous ecchymoses. (FVIII:C<1%) (Figure.1).

Family B: The propositus is a 25 year old female, her brother is affected with haemophilia A (FVIII:C<1%) (Figure.2).

Family C: The propositus is a 19 year old female, her brother is affected with haemophilia (FVIII:C<1%) (Figure.3).

Figure.2 Pedigree of family B.

In family B and C:
- In family B one of the two fetuses was positive for intron 22 inversion and the other foetus was normal. In family C among the two fetuses one was carrier for intron 22 inversion while the other was normal.

Mutations in patients with Haemophilia A
We performed PCR based screening for intron 1 and intron 22 inversions and point mutations were screened by CSSE and DNA sequencing using big Dye Terminator cycle sequencing kit on an ABI 3130 genetic analyzer.

In family A:
The causative mutation is a novel single nucleotide insertion ‘A’ at codon 713 of F8 gene. This mutation creates a frameshift possibly resulting in truncated factor VIII protein. (Figure.4).

CONCLUSIONS

Prenatal diagnosis is the major way of prevention of the genetic disorders including hemophilia A.

Collection of CVS samples and precise genetic identification of affected fetus in twins can be challenging.

VNTR analysis is a useful tool for confirmation of identity and detection of maternal contamination of CVS.

This is the first report from India, on molecular analysis in patients with twin pregnancies.

REFERENCES
Single dose Recombinant TSH injection in remnant and metastatic thyroid cancer ablation – CMC experience

Mithun Raam, Julie Hephzibah, David Mathew, Nylia Shanthly, Regi Oommen
Department of Nuclear Medicine, Christian Medical College Hospital, Vellore

Background
- Recombinant human TSH (rhTSH) is being used as an alternative to levothyroxine withdrawal, for stimulated diagnostic thyroid whole body scintigraphy (TWBS) and remnant thyroid radioactive iodine ablation (RAIA) following total thyroidectomy for Papillary carcinoma thyroid.

- The current protocol used is two doses of rhTSH (0.9 ml IM) on Day 1 and Day 2. TWBS dose is given on day 3 followed by TWBS on day 5. Two similar doses of rhTSH is given followed by RAI.

Aim
To evaluate the effectiveness of single dose rhTSH stimulation in remnant as well as metastatic thyroid cancer RAIA.

Methods
- We retrospectively analyzed the patients who underwent rhTSH stimulated TWBS and RAIA from 2011 to 2014.

Results
- Total of 8 patients (10 RAIA) underwent single dose rhTSH injection.

- Mean TSH levels, 24 hours after rhTSH administration was 140.66 µU/ml (Range 78.3 - 268.25 µU/ml).

- All post therapy scans showed good uptake in the thyroid bed or in metastatic lesions.

- Exposure rates measured at 24 hours after ablative dose at 1 meter distance were comparable with randomly chosen controls matched for age, sex and disease stage who underwent TWBS and RAI following levothyroxine withdrawal.

Conclusions
- rhTSH is comparable to levothyroxine withdrawal for stimulated TWBS and RAIA - It reduces the 1-month waiting period and the morbidity associated with hypothyroidism.

- Single dose rhTSH protocol is a cost-effective alternative to the current standard protocol in affordable patients both for remnant as well as metastatic thyroid cancer ablation.

References
1. Recombinant Thyroid-Stimulating Hormone in Differentiated Thyroid Cancer: Yodphat Krausz et al; IMAJ 2001;3:843-9
2. Recombinant Human TSH (rhTSH); Use in Papillary and Follicular Thyroid Cancer; Furio Pacini et al; Practical Management of Thyroid Cancer
3. Recombinant Human Thyrotropin is Helpful in the Follow-Up and 131I Therapy of Patients with Thyroid Cancer: A Report of the Results and Benefits Using Recombinant Human Thyrotropin in Clinical Routine; Susanne Kohlfuerst; THYROID; Volume 15, Number 4, 2005
4. Rh-TSH aided Radiodine ablation and treatment of differentiated thyroid carcinoma: a comprehensive review; Markus Luster et al; Endocrine-related Cancer 2005

Fig 1. Single-dose protocol for TWBS
Fig 2. TWBS following rhTSH stimulation
Fig 3. Post therapy scan following rhTSH stimulated RAIA
Is Lichen Planus a marker for Dyslipidemia and Metabolic Syndrome?

Manoj Kumar Agarwala1, Dincy Peter1, Nihal Thomas2, Thomas V Paul2, Meera Thomas3
Leni George1, Visali Jeyaseelan4, Susanne Pulimood1

Departments of Dermatology1, Endocrinology2, General Pathology3 and Biostatistics4, Christian Medical College, Vellore, Tamilnadu, India

BACKGROUND
Lichen planus (LP) is a distinct auto-immune inflammatory, self-limiting papulosquamous disorder of unknown cause that affects the skin, mucous membranes, nails and hair.
Previous studies have established a link between LP and diabetes mellitus (1).
Recent studies have suggested that patients with LP have higher risks for development of cardiovascular diseases (2).
There is a paucity of studies looking at the correlation between LP and Metabolic syndrome.
The present study is the first of its kind in India with a limited sample size.

AIM OF THE STUDY
To compare the prevalence of metabolic syndrome and dyslipidemia in patients of biopsy proven lichen planus and healthy controls.

IRB APPROVAL
Approval of the Institutional Review Board was obtained prior to the recruitment (IRB Min No. 7815 dated 18.04.2012).

METHODS

| CASES - biopsy proven Lichen Planus patients of ≥6 months duration |
| CONTROLS - Age and gender matched subjects - 2 controls for each case |
| Informed consent sought |
| Patients were called after 8-12 hrs of overnight fast |
| Samples for fasting plasma glucose, lipid profile, ESR and CRP |
| Anthropometric measurements |
| Dual-energy x-ray absorptiometry (DXA) scan |

RESULTS

Demographic and clinical characteristics of the study population

<table>
<thead>
<tr>
<th>CHARACTERISTICS</th>
<th>PATIENTS WITH LP</th>
<th>CONTROLS</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median Age</td>
<td>58 (77-19)</td>
<td>56 (74-18)</td>
<td>0.882</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>21 (53.8)</td>
<td>42 (53.84)</td>
<td>1.00</td>
</tr>
<tr>
<td>Female, n (%)</td>
<td>18 (46.2)</td>
<td>34 (46.1)</td>
<td>1.00</td>
</tr>
<tr>
<td>Smoking, n (%)</td>
<td>6 (15.38)</td>
<td>11 (14.10)</td>
<td>0.644</td>
</tr>
<tr>
<td>Alcohol, n (%)</td>
<td>7 (17.94)</td>
<td>7 (8.97)</td>
<td>1.00</td>
</tr>
<tr>
<td>Chewing tobacco n (%)</td>
<td>4 (10.25)</td>
<td>7 (8.97)</td>
<td>0.618</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.06 ± 3.58</td>
<td>24.95 ± 4.89</td>
<td>0.903</td>
</tr>
<tr>
<td>Central Obesity</td>
<td>29 (74.35)</td>
<td>40 (51.28)</td>
<td>0.018</td>
</tr>
<tr>
<td>Waist/ Hip Ratio</td>
<td>0.95 ± 0.11</td>
<td>0.91 ± 0.11</td>
<td>0.048</td>
</tr>
</tbody>
</table>

Prevalence of Met S (IDF 2006)

Prevalence of Dyslipidemia (ATP III)

INFLAMMATORY MARKERS

No difference among cases and controls

<table>
<thead>
<tr>
<th>CHARACTERISTICS</th>
<th>CASES (LP)</th>
<th>CONTROLS</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ESR (mm)</td>
<td>25.64 ± 22.09</td>
<td>27.14 ± 20.25</td>
<td>0.715</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>4.80 ± 3.96</td>
<td>5.19 ± 4.66</td>
<td>0.559</td>
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</tbody>
</table>

CONCLUSION

The higher prevalence of metabolic syndrome in patients with lichen planus as compared to healthy controls suggests that lichen planus may be associated with metabolic syndrome and dyslipidemia.

The mechanism underlying this increased risk is unknown, and additional randomized and controlled studies are required for clarification.

LIMITATIONS

The small sample size was a limitation in the study.
Since there was no follow-up, we could not comment on the prevalence of diabetes mellitus and hypertension.

REFERENCES:

RECOMMENDATIONS

Screening for the metabolic syndrome is advisable keeping in view the long term complications associated with it.

REGIONAL BODY FAT DISTRIBUTION (DXA SCAN)
Total Intravenous Anaesthesia with propofol for a child undergoing a Wada Test.

Dr George P Kurian, Dr Ekta Rai
Department of Anaesthesia
Christian Medical College, Vellore.

What is the Wada Test?
- It is used to establish cerebral language and memory representation of each hemisphere of the brain, by anaesthetizing one hemisphere of the brain and then testing for whether the other hemisphere is able to carry out language and memory function on its own.
- Named after Japanese-Canadian neurologist and epileptologist John Atsushi Wada who first performed this test.
- It needs active cooperation between the different departments of anaesthesia, neurology, and interventional radiology during the test.
- It is often not used in children because of the difficulties in performing the angiographic portion of the procedure in conscious children because of the attendant pain and the high degree of cooperation that is required from the child.

Indications:
- It provides important information for planning of epilepsy surgery in patients with epilepsy refractory to medical therapy.
- A patient's inability to perform a functional MRI due to agitation, mental disablement, or perceptual impairment.
- Evaluation of propagation of ongoing interictal bilateral epileptiform EEG activity.

Components of the Wada Test
- Initial EEG probes are placed to measure brain wave activity.
- The radiologist will achieve vascular access through the femoral artery in the leg. A catheter is threaded upwards through the blood vessels till it reaches the internal carotid artery of one side of the brain and an angiogram of that side of the brain is performed.
- That side of the brain is anaesthetised by injecting an anaesthetic drug through the catheter eg, etomidate, amobarbital. The subject is then asked a battery of questions to assess the memory and language function of the awake side of the brain. The drug will wear off in 5-7 minutes. The catheter can then be redirected to the other side of the brain to repeat the test again and hence assess the function of both hemispheres.

Anaesthetic challenges

Patient related challenges:
Developing a rapport with patient during the preanaesthetic visit to elicit cooperation during the test.

Interdepartmental Communication:
The conduct of the test needs cooperation and active participation of the anaesthesiologists, interventional radiologists, neurologists, and neurosurgeons.

Anaesthesia related challenges:
- Adequate anaesthesia and analgesia to prevent patient pain and movement during vascular access and insertion of catheter.
- TIVA is required so that speech function can be assessed.
- Attaining a good recovery profile so that patient is wakes up rapidly and smoothly and is awake enough to assess cognition.
- Other implications of remote location anaesthesia like hypothermia, monitoring etc.

Discussion
- The use of propofol ensured rapid induction of anaesthesia without requiring endotracheal intubation to secure the airway.
- There were no significant changes in cardiorespiratory parameters requiring treatment.
- Propofol anaesthesia supplemented with fentanyl for analgesia facilitated the smooth conduct of the angiographic portion of the test without patient discomfort or movements.
- Recovery from anaesthesia was smooth and rapid and the patient was awake enough to perform all the neurological tests successfully within five minutes (average of 2-3 minutes).
- The use of propofol did not interfere with neurophysiological monitoring.
- The addition of lignocaine prevents pain on injection of propofol.
- Other alternatives to propofol are dexmedetomidine, an alpha 2 agonist. We chose to use propofol in view of the higher cost of dexmedetomidine.
- The use of a BIS monitor to assess depth of anaesthesia might help to fine tune the requirement of propofol and hence enable faster wake up time and a more conscious patient.
Analysis and comparison of surrogate measures of insulin sensitivity with hyperinsulinemic euglycemic clamp study in an Indian population.

Padmanabhan Venkatesan1,2, Ridhhi Das Gupta1, Mohan Jambugolu1, Mercy Inbakumari1, Flory Christina1, Finney S Geetanjali2, Joe Fleming3, Meredith Hawkins4, Nihal Thomas1

1Department of Endocrinology, Diabetes and Metabolism, Christian Medical College, Vellore, India
2Department of Endocrinology, Christian Medical College, Vellore, India
3Albert Einstein College of Med, Bronx, New York, USA

Introduction

- Insulin resistance is a key factor in development of Type 2 diabetes mellitus and obesity related disorders
- Measuring insulin resistance is significant in both research and clinical practice
- The euglycemic hyperinsulinemic clamp technique is the gold standard method to measure insulin sensitivity but is rarely performed in India because of its cost and complexity
- Over the years surrogate measures of insulin sensitivity from fasting samples and oral glucose tolerance tests have been proposed as an alternative to clamp technique. These surrogate measures of insulin sensitivity were developed and validated against clamp technique in western populations
- As Asian Indians have an unique diabetic phenotype, it is imperative to find and validate the surrogate measure that would be appropriate in Indian population

Objective

To find the most appropriate surrogate measure of insulin sensitivity calculated from fasting samples in Indian population by comparing them with euglycemic hyperinsulinemic clamp technique.

Methods

Study design: Cross sectional
Study subjects: 36 healthy South Indian males

- Subject recruitment
- Informed consent
- Screening
- Medical history
- Anthropometric measurements

Euglycemic hyperinsulinemic clamp study

Test Results:

<table>
<thead>
<tr>
<th>Variable(s)</th>
<th>Area</th>
<th>Std. Error</th>
<th>95% CI</th>
<th>Asymptotic 95% Confidence Interval Lower Bound</th>
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<td>0.03</td>
<td>0.09</td>
<td>0.03</td>
</tr>
<tr>
<td>FBR</td>
<td>0.06</td>
<td>0.01</td>
<td>0.03</td>
<td>0.09</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Results

Table 2: Baseline characteristics

<table>
<thead>
<tr>
<th>N</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>Std. Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>22.00</td>
<td>49.00</td>
<td>29.81</td>
<td>5.81</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>16.70</td>
<td>26.80</td>
<td>29.19</td>
<td>6.36</td>
</tr>
<tr>
<td>Waist hip ratio</td>
<td>0.90</td>
<td>1.07</td>
<td>0.97</td>
<td>0.09</td>
</tr>
<tr>
<td>Fasting glucose (mg/dl)</td>
<td>78.00</td>
<td>110.00</td>
<td>93.75</td>
<td>8.54</td>
</tr>
<tr>
<td>Fasting insulin (μU/mL)</td>
<td>3.69</td>
<td>9.55</td>
<td>6.95</td>
<td>2.56</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>18.00</td>
<td>19.00</td>
<td>18.50</td>
<td>1.25</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>20.00</td>
<td>25.00</td>
<td>23.00</td>
<td>3.50</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>18.00</td>
<td>30.00</td>
<td>24.00</td>
<td>4.37</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>18.00</td>
<td>25.00</td>
<td>21.00</td>
<td>3.00</td>
</tr>
</tbody>
</table>

Table 3: Correlation of M value of low phase with surrogate markers of insulin resistance

- Spearman’s correlation coefficient r = 0.13, p = 0.05
- p = 0.10

Table 4: Area under the curve of ROC curve of surrogate measures to diagnose insulin resistance

- Fasting insulin I: 0.06
- HOMA-IR: 0.06
- QUICKI: 0.06
- FBR: 0.06
- GIP: 0.06
- QUICKI: 0.06
- FBR: 0.06
- QUICKI: 0.06
- FBR: 0.06
- QUICKI: 0.06
- FBR: 0.06

Conclusion

- HOMA-IR is the most commonly used surrogate measure of insulin sensitivity both in Indian and western population but our study show HOMA-IR is not better than other surrogate measures in Indian population.
- Among the surrogate measures evaluated, fasting glucose to insulin ratio (FIR) had the strongest correlation with clamp derived M value (r = 0.13, p < 0.05) and also it had greater AUC of ROC curve to diagnose insulin resistance (AUC = 0.05).
- These findings suggests that FIR is the most appropriate surrogate measure of insulin sensitivity in normoglycemic Indian subjects.
CLASSICAL AND ATYPICAL MUTATIONS IN ACVR1 GENE IN FIBRODYSPLASIA OSSIFICANS PROGRESSIVA IN INDIAN POPULATION

Mona Santhanam2, Legasri K Sugumar2, Karthikeyan Rajagopal2, Sanjay K Chilbule1, Vrisha Madhuri1,2
Paediatric Orthopaedics Unit1, Centre for Stem Cell Research2, Christian Medical College, Vellore, Tamil Nadu, India

AIM
To identify the mutations in the ACVR1 gene which is responsible for Fibrodyplasia Ossificans Progressiva in Indian population.

BACKGROUND
➢ Fibrodyplasia Ossificans Progressiva (FOP) is a very rare disabling skeletal disorder with ectopic bone formation in anatomical sites, usually in soft tissues resulting in restricted movement of affected joints.
➢ FOP is believed to be inherited in an autosomal dominant pattern with an estimated incidence of one in two million.

METHODS
Fourteen patient’s and parent’s blood sample were collected and direct DNA sequencing was performed for ACVR1 exons using ab3130 Genetic Analyzer.

RESULTS
Twelve patients were positive for the common recurrent mutation c.617 G>A; p.R206H in the exon 6 using restriction Fragment Length Polymorphism(RFLP)

DISCUSSION
➢ This is the largest series of FOP from India.
➢ Even though autosomal dominant pattern is suggested, all our patient’s had sporadic mutation.
➢ Mutation profile of Indian patients corresponds with international literature.
➢ Our diagnosis of one child in early infancy before the manifestation of heterotopic ossification phenotype shows the importance of diagnosing this disease at the beginning and the value of the genetic diagnosis.
➢ This is the first report from India to present the mutations for atypical FOP.

CONCLUSION
➢ The recurrent mutation c.617 G>A; p.R206H is common in our population and once it is found negative, screening of other exons of ACVR1 gene will be beneficial.

ACKNOWLEDGEMENTS
The authors thank the patients and their families for participating in the study and we acknowledge the assistance provided by the Centre for Stem cell Research at Christian Medical College. The study was funded by Department of Biotechnology (DBT).

REFERENCE
Inversion(14)(q11.2q32) in T cell acute lymphoblastic leukemia

Angi M 1, Sifaram U 2, Fouzia NA 1, Ganapule A 1, Abraham A 1, Viswabandya A 1, George B 1, Mathews V 1, Srivastava A 1, Srivastava VM 3.
Departments of 1 Haematology, 2 Transfusion Medicine and Immunohaematology and 3 Cytogenetics Unit, Christian Medical College, Vellore.

INTRODUCTION

> The inversion(14)(q11.2q32) is a distinct T-cell neoplasia-associated abnormality which is usually seen in T-prolymphocytic leukemia and ataxia telangiectasia-associated T cell leukemia.1

> It has also been reported in about 25 patients with T-ALL and less commonly, in B-ALL and AML expressing lymphoid-associated antigens.2,4

> The inversion 14 is associated with activation of the TCL1 (T cell leukemia 1) gene on 14q32 due to its juxtaposition to the TRA/D (T cell receptor alpha/delta) gene on 14q11.2. Its prognostic impact in T-ALL is not well defined.1,5

PATIENTS AND METHODS

> Patients: All patients with T-ALL and the inversion 14 seen in the Department of Haematology, Christian Medical College, Vellore.


> Method: Unstimulated overnight cultures of bone marrow, trypsin G banding.

> 15-20 metaphases analysed for each case, reported according to ISCN.

> Karyotypes were correlated with blood and bone marrow findings.

RESULTS

> Total number of ALL seen during the study period: 1450

> Number of T-ALL seen during the study period: 290

> Number of patients with inversion 14 in this study: Four

> 0.3% (4/1450) of total ALL

> 1.4% (4/290) of T-ALL

> Three patients had a complex karyotype with two or more additional abnormalities (deletion 12p in two, tetraploidy in one).

> Two patients were in remission at 5 and 8 years of follow up. The third patient who presented in 2014 is in remission at 7 months of follow up. The fourth patient who had a complex karyotype relapsed within one year of treatment.

<table>
<thead>
<tr>
<th>Patient details, haematological and cytogenetic findings</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pr</strong></td>
</tr>
<tr>
<td>-------</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>4</td>
</tr>
</tbody>
</table>

Karyotype

89.XXY.Y-4,9,12.inv(14)(q11.2q32)x2-18,+mar

CONCLUSIONS

> The inversion (14)(q11.2q32) was found in 0.3% of all ALL and 1.4% of T-ALL. It is important to be aware of this rare abnormality because it can be easily overlooked, especially if the morphology is suboptimal. A larger number of cases need to be studied to understand its impact on prognosis in ALL.

REFERENCES

ACUTE LIVER INJURY INDUCED BY LOW DOSE DIMETHYLNITROSAMINE ALTERS MEDIATORS OF HEPATIC VASCULAR FLOW


The Wellcome Trust Research Laboratory, Department of Hepatology, Division of Gastrointestinal Sciences and Center for Stem Cell Research, Christian Medical College, Vellore-632004, India.

INTRODUCTION

- Alterations in vascular tone play an important role in chronic liver disease
- Mediators such as nitric oxide, hydrogen sulphide and ADAMTS13 can modulate vascular tone

AIM

To study the vascular mediators in response to single dose of hepatotoxin dimethylnitrosamine (DMN)

METHODOLOGY

- Intraperitoneal injection of DMN to C57 black mice
- Mice sacrificed at 24, 48 and 72 hours after DMN administration
- Liver histology and plasma ALT activity
- Liver oxidative stress parameters and nitrate
- Measurement of hydrogen sulfide metabolism
- Immunofluorescence for nitrotyrosine, GFAP and ADAMTS13

RESULTS

1. Plasma ALT
   - Controls 24 h, 48 h, 72 h
   - Liver histology

2. Liver nitrate
   - Hours after DMN: 0, 10, 20, 30, 40, 50, 60, 70, 80
   - Controls 24 h, 48 h, 72 h

3. Western blot - Liver iNOS
   - Hours after DMN: 24, 48 h
   - Controls 24 h, 48 h, 72 h

4. Liver eNOS mRNA
   - Controls 24 h, 48 h
   - 48 h, 72 h

5. Liver Hydrogen sulphide synthesis
   - Hours after DMN: 0, 10, 20, 30, 40, 50, 60, 70, 80
   - Controls 24 h, 48 h
   - 48 h, 72 h

6. Liver Hydrogen sulphide degradation
   - Hours after DMN: 0, 10, 20, 30, 40, 50, 60, 70, 80
   - Controls 24 h, 48 h
   - 48 h, 72 h

CONCLUSIONS

ACKNOWLEDGEMENTS: FLUID RESEARCH, CMC
**Introduction**

Early detection of skull base invasion in patients with nasopharyngeal carcinoma (NPC) is important for staging, assessing the treatment response, and RT planning. Computed tomography (CT) provides an intricate level of anatomical detail but cannot detect initial bone involvement whereas even a 5% bone turnover can be detected by planar bone scintigraphy (PBS).

**Aim**

To explore the diagnostic utility of PBS in the detection of skull base infiltration.

**Methods and Materials**

- Retrospective analysis of PBS and CT of 93 patients with histology proven NPC
- Study duration: 2009 to 2013.

**Imaging:**

- PBS, lateral views of the skull base and CT

**Interpretation:**

- Bone invasion on PBS: When skull base demonstrated increased tracer uptake.
- Bone invasion on CT: When contrast-enhanced tumour tissue was visible outside the cortical bone or when the cortical bone was partially eroded or totally destroyed.

**Results**

- Positive PBS and CT: 45% (42/93 patients)
- Negative PBS and CT: 30% (28/93 patients)
- Positive PBS & negative CT: 11% (10/93 patients)
- Positive CT & negative PBS: 14% (13/93 patients)
- Of the 10 patients with positive PBS and negative CT, 4 were found to be positive on follow up CT

**Discussion**

- CT is the most widely used tool in the assessment of skull base involvement. However, CT may appear normal in the early stage of skull involvement.

- False positives produced by PBS include bone injury, acute osteomyelitis, ischemic osteonecrosis, metabolic bone diseases, benign tumors and radiation osteitis.

- Even a 5% bone turnover can be detected by bone scan, whereas CT and radiographs require a minimum mineral loss of 30% and 50% respectively before a lesion is visualized.

- False negatives can be due to:
  - Lack of osteoblast activity which causes insufficient uptake of MDP
  - Endarteritis and vascular thrombosis caused by the tumour infiltrating local blood vessels.
  - Radiation-induced vascular insufficiency and/or microcirculatory disturbance.

**Conclusions**

- PBS could accurately detect or rule out skull base infiltration in 70 patients (75%).
- Early detection of skull base infiltration was observed in 4 cases by PBS which was missed initially by CT.
- PBS is an excellent modality for early detection of skull base infiltration and disseminated skeletal metastases.

**References**

1. Comparison of SPECT/CT, MRI and CT in diagnosis of skull base infiltration in nasopharyngeal carcinoma; Shu Xu Zhang et al
2. Assessment of skull base involvement in nasopharyngeal carcinoma: comparison of SPECT, planar bone scintigraphy and X ray computed tomography; Chiang Hsuan Lee et al
INTRODUCTION

- Ulcerative colitis is an inflammatory condition of colon with unknown etiology.
- Hydrogen Sulphide (H₂S) is a gas transmitter known to cause inflammation at high concentrations.
- Synthesis of H₂S is mediated by cystathionine β synthase (CBS) and cystathionine γ lyase (CSE).
- Rhodanese is a mitochondrial enzyme involved in H₂S detoxification.
- Impaired detoxification of H₂S in colonic mucosa is associated with mucosal injury.

AIM

- To evaluate enzyme activities involved in H₂S metabolism in the colon of patients with ulcerative colitis and in mice colitis.

METHODOLOGY

ANIMAL MODEL

- Mice model of colitis induced by Dextran Sodium Sulphate (DSS) administration (5% in drinking water).
- Mice body weight noted and sacrificed on day 2, 4 and 7, followed by colonocyte isolation & enzyme activity measurements.

HUMAN STUDIES

- Colon biopsy sample collected from UC & Control patients after informed consent.
- H₂S Synthesis and Rhodanese Assay.

RESULTS

Mouse Model

- Colon Length
- Body Weight
- H₂S Synthesis
- Rhodanese Activity

HUMAN STUDY

Table 1. Characteristics of UC Patients & Controls

<table>
<thead>
<tr>
<th>Baseline Characteristics</th>
<th>Control</th>
<th>UC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>22</td>
<td>19</td>
</tr>
<tr>
<td>Female</td>
<td>8</td>
<td>16</td>
</tr>
<tr>
<td>Mean Age</td>
<td>47±10</td>
<td>40±13</td>
</tr>
<tr>
<td>Clinical Measures</td>
<td></td>
<td></td>
</tr>
<tr>
<td>UCDAI Score &lt; 3</td>
<td>N/A</td>
<td>1</td>
</tr>
<tr>
<td>3-10</td>
<td></td>
<td>32</td>
</tr>
<tr>
<td>&gt; 10</td>
<td></td>
<td>1</td>
</tr>
</tbody>
</table>

Table 1. Characteristics of UC Patients & Controls

- In the animal model, elevated H₂S synthetic capacity accompanied by increased rhodanese was seen at later stages.
- However, in human biopsies, elevated H₂S synthetic activity was accompanied by decreased rhodanese activity.

Conclusion: Changes in enzymatic activities involved in H₂S metabolism may play a role in pathogenesis of ulcerative colitis.
Clinical and Molecular Characterization of Fanconi Anemia: an Indian Perspective

Nancy Beryl Janet Arthur1, Abhishek Ganapule1, Dhavapriya Palani2, Auro Viswabandya1, Vikram Mathews1, Abhy Abraham1, Vivi M. Srivastava1, Alok Srivastava1, 2, Biju George1, Srijay R. Velayudhan1, 2

1Department of Hematology, 2Centre for Stem Cell Research, 3Cytogenetics Unit, Christian Medical College, Vellore, Tamil Nadu, India.
Correspondence: rsvijay@cmcvellore.ac.in

5th ANNUAL RESEARCH DAY

Introduction

Fanconi anemia (FA) is an inherited bone marrow failure disease caused by a genetic defect in the Fanconi anemia pathway of DNA repair.

Clinical features:
- Progressive bone marrow failure: ~90%
- Congenital malformations: 65-70%
- Increased risks of malignancies: 25-30%
- AML, MDS: 10-30%
- Sensitivity to DNA cross-linking agents: ~100%

Materials and Methods

Patient recruitment:
Collection of peripheral blood and fibroblast.

Complementation analysis:
Novel inducible lentiviral plasmids
- FA fibroblast
- FA-A transduced fibroblast

Mutation screening:
MLPA

Results

n=101

With physical abnormalities c/w FA
n=76

Without physical abnormalities c/w FA
n=25

Figure 6: Classification of patients based on clinical presentation.

Comparison of sensitivity of FANCD2 western blot and chromosome breakage analysis

Table 1: Correlation between chromosome breakage analysis and physical abnormalities.

Table 2: FANCA mutations and polymorphisms.

Conclusions

>90% of patients showed defective mono-ubiquitination of FANCD2 due to a non-functional protein in the FA core complex.

Transduction with the novel inducible FANCA lentiviral vector showed efficient complementation and restoration of FANCD2 ubiquitination.

Complete correction of G2M phase cell cycle arrest was seen after transduction with FANCA inducible vector.

Complementation analysis showed high frequency of FANCA defects (~80%) in patients with FA Indian population.

Acknowledgements

This research work is supported by the Department of Biotechnology, Government of India.

References

ACQUIRED NEONATAL CHIKUNGUNYA ENCEPHALOPATHY

DR DULARI GUPTA*, DR ANURADHA BOSE, Dr WINSLEY ROSE
Department of Community Medicine and Child Health III,
Christian Medical College, Vellore, India

INTRODUCTION

- There has been a re-emergence of Chikungunya infection worldwide
- We present 3 neonates with fever and seizures, and a characteristic rash due to acquired neonatal Chikungunya encephalopathy
- This is the first description of postnatal rather than vertical transmission of Chikungunya, in neonates whose mother have not been previously exposed to Chikungunya infection

METHODOLOGY

- According to the National Institute of Communicable Diseases New Delhi, a confirmed case of Chikungunya can be diagnosed by the presence of virus specific IgM antibodies in a single serum collected five days after onset of illness (1)
- Chikungunya IgM was performed using the On Site Chikungunya IgM Rapid Test commercial chromatographic immunochromatographic assay (sensitivity 90.3% and relative specificity 100%)

CASE SERIES

<table>
<thead>
<tr>
<th>Age</th>
<th>Neonate A</th>
<th>Neonate B</th>
<th>Neonate C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days</td>
<td>10</td>
<td>23</td>
<td>25</td>
</tr>
<tr>
<td>Fever</td>
<td>Fever for 2 days</td>
<td>Fever for 2 days</td>
<td>No fever</td>
</tr>
<tr>
<td>Seizure</td>
<td>One episode of tonic seizures</td>
<td>Nine episodes of multifocal clonic seizures</td>
<td>Five episodes of multifocal clonic seizures</td>
</tr>
<tr>
<td>Rush</td>
<td>Day 3 developed blistery erythematous hyperpigmented rash involving the face, limbs, trunk and back. The rash progressed to involve the tip of nose (Figure 1.)</td>
<td>Day 4 developed a macular, erythematous blistery rash with characteristic hyperpigmentation involving the whole body with per-nodal hyperpigmentation</td>
<td>Day 3 developed hypopigmented blistery rash over the face and trunk. The rash resolved by desquamation</td>
</tr>
<tr>
<td>Other Signs</td>
<td>Poor feeding</td>
<td>Vomiting</td>
<td>Poor feeding, irritability</td>
</tr>
<tr>
<td>Sodium (mEq/L)</td>
<td>141</td>
<td>123</td>
<td>134</td>
</tr>
<tr>
<td>Calcium (mg/dL)</td>
<td>8.5</td>
<td>8.5</td>
<td>8.7</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>78</td>
<td>86</td>
<td>-</td>
</tr>
<tr>
<td>Cerebrospinal Fluid Analysis</td>
<td>Total count 3 (lymphocytes), Protein 40 mg/dL, Glucose 50 mg/dL</td>
<td>Total count 70 (lymphocytes 13), Protein 35 mg/dL, Glucose 52 mg/dL</td>
<td>Total count 11 (neutrophils 41, lymphocytes 5, mononucleosis 4), Protein 155 mg/dL, Glucose 48 mg/dL</td>
</tr>
<tr>
<td>Blood Culture</td>
<td>No growth</td>
<td>No growth</td>
<td>No growth</td>
</tr>
<tr>
<td>Chikungunya IgM for baby</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>Chikungunya IgM for mother</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Condition at discharge</td>
<td>Well, no further seizures on day 28</td>
<td>Well, no further seizures on day 38</td>
<td>Well, no further seizures on day 35</td>
</tr>
<tr>
<td>Follow up</td>
<td>At 50 days hypopigmented rash persisting</td>
<td>At 8 months developmentally normal and no rash</td>
<td>At 4 months developmentally normal and no rash</td>
</tr>
</tbody>
</table>

DISCUSSION

- Vertical transmission of Chikungunya was first recorded in 2005 in Reunion Islands. Eighty-four pregnant women were affected and ten neonates had congenital Chikungunya fever (2,3)
- Neonatal Chikungunya was reported from India in 2007 in 4 neonates (4). The authors highlighted the fact that symptom onset 12 days or more postnatally almost confirms acquired infection as it is more than twice the incubation period of the virus
- In the neonates that we reported, symptom onset was more than 12 days after birth. Whereas in reports of vertical transmission, symptom onset is within 3-5 days
- In neonates the commonest features of Chikungunya are fever (79%), pain (100%), rash (82%), peripheral edema (58%), seizures (15.8%), hemorrhagic syndrome (15.8%), hemodynamic disorders (26.9%), thrombocytopenia (76%), lymphopenia (47%) hepatic involvement (83%) (5)
- Neurological manifestations like febrile seizures, encephalopathy or acute encephalitis are also seen in children with Chikungunya (4)
- Maculopapular hyper-pigmented rash in Chikungunya is very distinctive of the disease (6). Since the rash postdates the febrile illness it is helpful in diagnosis only in the recovery period
- These are the first reported cases of neonates with acquired neonatal Chikungunya encephalopathy

REFERENCE

ROLE OF INOSITOL 1,4,5-TRIPHOSPHATE ON HEART RATE IN ISOLATED HEART MODELS OF WISTAR RATS
Jesi.W, Anand Bhaskar, Sathya Subramani
Department of Physiology, Christian Medical College, Vellore.

BACKGROUND
Do you know calcium is released from sarcoplasmic reticulum (SR) during diastole in pace-maker cells? This calcium is extruded by the sodium calcium exchanger (NCX) - 3 sodium ions for one calcium – resulting in an inward current - causing depolarization. This is emerging as the primary pacemaking mechanism in heart. Ryanodine receptor (systolic calcium release channel on SR membrane) is thought to be responsible for diastolic calcium release too. However, we hypothesize that the other calcium channel on SR membrane, the IP3 receptor, is equally, if not more important for diastolic calcium release.

AIM
To study the role of Inositol 1,4,5-triphosphate (IP3) in rhythm generation in isolated Wistar rat hearts

OBJECTIVES
To change intracellular IP3 concentration using an IP3 agonist (Phenylephrine) and an IP3 antagonist (Neomycin) and record the change in heart rate.

MATERIALS AND METHODS
Isolated heart preparation of Wistar rats (n=18) perfused with normal extracellular solution in Langendorff mode was used for this study.*

Surface ECG was recorded with surface ECG electrodes using CMCdaq computerized data acquisition system. Heart rate was calculated from ECG recordings.

The heart was perfused with normal extracellular solution for 15 minutes to record basal heart rate. Then the heart rate was recorded for further 15 minutes with some intervention (Control group: no intervention, Group1- Phenylephrine 10µM, Group2- Neomycin 2mM+Phenylephrine 10µM). The drug was then washed with normal extracellular solution for further 15 minutes and heart rate calculated.

*Approved by the Institutional Animal Ethics Committee

RESULTS

CONTROL GROUP

INTERVENTION GROUP 1

INTERVENTION GROUP 2

Figure 1: Heart rate over time in all the three groups (Mean ± SD).

Control group shows that the heart rate does not change with time (p=0.345). With Phenylephrine there is increase in heart rate (p=0.028). With Neomycin, the increase in heart rate seen with Phenylephrine is blocked and in fact a decrease in heart rate is seen (p=0.028).

DISCUSSION
Phenylephrine (PE) increases IP3 levels (known already) and therefore increases heart rate (known already). We interpret this result as follows: calcium release through IP3 receptors is important in catecholamine-induced tachycardia. Neomycin reduces IP3 levels (known) and also reduced heart rate. Blocking of PE-induced increase in heart rate by neomycin confirms its action through inhibition of IP3 generation. However, since neomycin had direct negative chronotropic effect, our interpretation is that IP3 receptors are involved in normal rhythm-generation too. They must have equal contribution to diastolic calcium release as compared to Ryanodine receptors.

CONCLUSION
IP3 has a role in pacemaking of heart.
This opens a whole new portal of phamaco-therapeutics in the management of cardiovascular pathologies like arrhythmias.

REFERENCE

Acknowledgement: CMC Fluid Research grant for funding.
Alcohol-induced effects on iron-related proteins cause dysregulation of iron homeostasis

Jithu James¹, Joe Varghese¹, Sree Rohini S¹, Visalakshi Jayaseelan², Abitha Sukumaran² and Molly Jacob¹
Departments of Biochemistry¹ and Biostatistics², Christian Medical College, Vellore, India

Background to and aim of study
Hepatic iron overload induced by chronic alcohol intake has been postulated to play an important role in the pathogenesis of alcoholic liver disease. The mechanisms involved in such iron accumulation have not been clearly elucidated. We carried out a time-course study to determine changes in iron-related proteins in mice, in response to alcohol ingestion.

Methodology
- Male Swiss albino mice were pair-fed with Lieber–DeCarli alcohol (20% of total calories provided as ethanol) or isoalchoric control diets, for periods varying from 2 to 12 weeks; mice were sacrificed at the end of these periods of feeding.
- The following parameters were studied: hepatic cytochrome P450 2E1 (CYP2E1), liver iron levels, expression of hepcidin, heme oxygenase 1 (HO-1), ferritin, ferroportin (Fpn), divalent metal transporter (DMT1), transferrin receptors 1 (TfR1) and 2 (TfR2) in the liver; Fpn and DMT1 in the duodenum and serum levels of hepcidin and iron (n = 3 to 6 for each parameter).
- Statistical analysis was done using Statistical Package for Social Scientists (SPSS), version 16.0. Data from alcohol-fed and pair-fed control mice at each time point were compared using generalized estimating equations (GEE). Correlation analysis was done using Spearman correlation coefficient. A p value < 0.05 was taken to indicate statistical significance in all cases.

Results
- The average daily alcohol consumption per mouse was 12.4 ± 1.8 grams per kg per day and was similar in mice in all the time periods studied. CYP2E1 protein levels and activity in the liver were significantly increased in response to alcohol, at the time points studied.

Summary of results
- Hepatic iron levels tended to be higher after 4 weeks of alcohol ingestion and then fell to significantly lower levels at 12 weeks.
- Changes in ferritin (F) protein levels in the liver mirrored changes in hepatic iron levels. TfR1 and HO-1 mRNA levels in the liver were significantly higher at 4 and 8 weeks after alcohol feeding; these fell by 12 weeks. TfR1 protein levels showed similar trends but the changes seen were not statistically significant. HO-2 activity was significantly increased at 4 weeks but not at the other time points studied.
- Hepatic TR2 protein levels were significantly lower at 4 and 8 weeks. TR2 mRNA showed similar trends but changes were not statistically significant.
- Hepatic hepcidin mRNA levels and serum hepcidin levels were significantly lower at 8 and 12 weeks. This was associated with increased protein levels of duodenal ferroportin (Fpn) (at 8 and 12 weeks) and DMT1 (at 8 weeks), in response to alcohol.
- Serum iron levels increased progressively to become significantly elevated at 12 weeks.
- There was a tendency for liver iron to be correlated positively with hepatic and serum hepcidin and TfR1 protein levels. Duodenal ferroportin tended to correlate positively with serum iron and negatively with serum hepcidin. These correlations, however, were not statistically significant.
- There were no significant effects on expression of hepatic ferroportin and DMT1.

Conclusions
- Chronic alcohol ingestion
  - Tendency to increase hepatic TR1 and HO-1 at early time points
  - Decreased hepatic TR2
  - Decreased hepcidin
  - Tendency to increase hepatic iron at an early time point
  - Iron mobilization from liver
  - Increased expression of duodenal ferroportin and DMT-1
  - Decrease in hepatic iron at later time points
  - Increased serum iron levels
  - Increased intestinal iron absorption

Acknowledgements - This study was funded by grants from the Department of Biotechnology, Government of India awarded to J (BT/PR1078/GBD/27/133/2008 and MJ (BT/PR12738/ Med/30/214/2009) and a fluid research grant from CMC, Vellore (IRB Min. No. 7714).

* Alcohol produced effects in iron-related proteins in the liver and duodenum; such changes may contribute to dysregulation of iron homeostasis seen in response to alcohol.
* Further experiments will be required to confirm the sequence of events suggested.
Stochastic and Molecular Events Governing Mouse Somatic Cell Reprogramming
Kannan V.M.1,2, Syed Muhammed Musheer Aalam1, Sumithra P. Bharathan3, Alok Srivastava1,2, Shaji R.V.1,2.
1Centre For Stem Cell Research, 2Department of Haematology, Christian Medical College Vellore, India 632 002. Correspondence: rsvzhaji.csrr@cmcvellore.ac.in

Introduction
Somatic cells can be reverted back into a pluripotent state called Induced Pluripotent Stem Cells (iPSCs) by overexpressing four transcription factors, namely Oct4, Sox2, Klf4, and c-Myc (OSKM). (1)

The dynamic and stochastic expression of various markers during this process revealed three stages namely: early, intermediate and late stages.

Limitations of somatic cell reprogramming:
- Reprogramming is slow and inefficient.
- Mechanism of reprogramming is largely unknown.
- Molecular and functional heterogeneity of the clones generated.

Stochastic epigenetic events drive very few and rare somatic cells into a pluripotent state suggesting the involvement of cellular barriers impeding the generation of iPSCs.

NANOG: Transcription factor critically required to establish naïve pluripotency in ESCs and iPSCs. The induction of Nanog expression is reported to occur in the final stages of reprogramming.

Retroviral silencing is a gradual process that is completed towards the final stages of reprogramming.

Pre-iPSCs are ESC like iPSC colonies that have failed to express critical pluripotency genes during the late events of reprogramming.

Vitamin C is a cofactor for many enzymatic reactions involving iron and alpha-ketoglutarate-dependent dioxygenases.

Vitamin C accelerates and enhances somatic cell reprogramming by enhancing Mesenchymal-to-Epithelial Transition (MET).

Objectives
To study the dynamics of Nanog induction in mouse embryonic fibroblasts (MEFs) undergoing reprogramming.

To demonstrate the role of Nanog in Mesenchymal-to-Epithelial transition (MET).

To evaluate the role of Vitamin C in converting pre-iPSCs into fully reprogrammed iPSCs.

Methods
Generation of miPSCs—Mouse iPSCs were generated using lentiviruses or retroviruses encoding the CDNA of Oct4, Sox2, c-Myc and Klf4. Mouse iPSC colonies were picked on day 12 and expanded on MEF feeders.

Characterization of iPSCs and pre-iPSCs:
- Morphological evaluation.
- Retroviral expression status (Red fluorescent protein: RFP).
- Real-time PCR.
- In vitro differentiation.

Immunofluorescence (IMF): Fixing was performed using 4% paraformaldehyde and ICC was performed using appropriate anti-bodies following standard protocol. Imaging was done using Leica FL Microscope.

Flow cytometry (FCM) was performed using BD Calibur.

Vitamin C (Vc; L-ascorbate-sigma) was used at a final concentration of 50 μg/ml.

Expression analysis was performed using QuantStudio Real time PCR machine (Life technologies).

Generation of Nanog Lentiviral Reporter construct: 2.5Kb Nanog promoter DNA was amplified from RIESCs and cloned into pRRLSIN.PPT.GFP.PWP.WPRE vector.

Results
Subpopulation of cells expression NANOG and SSEA1 during early stages reprogramming

Figure 1. a. Reprogramming was initiated using STEMCCA based lentiviral vector. IMF showing the induction of Nanog (green) as early as day 4. b. The co-localization and the presence of SSEA1 cells (green) in and around the clustered Nanog cells (red) were also observed. These cells were small when compared to MEFs; few were round.

Mesenchymal-to epithelial transition initiates (MET) on day 4.

Figure 2. a. IMF demonstrating a-Cadherin (a-Cdh1, green) expression and CD44 down-regulation of CD44 (red) in the epithelial like clustered cells and b. FCM demonstrating fibroblast specific marker CD44 down-regulation and SSEA1 upregulation from day 4. C. A sub-population of Nanog (green) expressing epithelial like cells showing down-regulation of CD44. The onset of Nanog induction along with CD44 down-regulation marks the transition of cells into mesenchymal phase of reprogramming.

Nanog-GFP lentiviral reporter construct: A reprogramming marker

Figure 3. a. Vector map of Nanog-GFP lentiviral reporter construct. b. Phase contrast (left) and fluorescent microscopy image (right) showing Nanog-GFP cells (green). IMF done using anti-Nanog antibody further confirmed the induction of Nanog (left, below, red). Cells were fixed and stained on the day 7 of reprogramming.

Nanog expressing iPSCs down-regulate lineage specific marker: CD44

Figure 4. Quantitative RT-PCR analysis of CD44 mRNA in the pre-iPSCs (Nanog-RFP+) and iPSCs (Nanog-iPSCs). Pre-iPSCs demonstrate high levels of CD44 expression (yellow bars) when compared to Nanog-iPSCs fully reprogrammed iPSC clones.

Conclusion
Schematic representation of molecular events during somatic cell reprogramming

Figure 6. Real time PCR of Vitamin C treated pre-iPSC clone demonstrating reduced expression of hK-de, Arf and p21.

Vitamin C establishes pluripotency in pre-iPSCs by abrogating the expression of reprogramming barriers hK-de, Arf and p21.

Nanog-GFP cells and CD44-cells are parent markers to study the transition stages of reprogramming. Enriching these cells can enable us to study the mechanistic insights of induced pluripotency.

The observed induction of Nanog and its functional role during the early to intermediate stages of reprogramming needs to be validated.

Vitamin C promotes the conversion of pre-iPSCs to iPSCs by silencing the expression of hK-de, Arf and p21.

References
Effect of human umbilical cord vein derived endothelial cells on muscle satellite cell culture.

Karthikeyan Rajagopal 1, Jeeva Sellathurai 2, Henrik Daa Schröder 2, Belavendra Antonisamy 3, Vrisha Madhuri 1
1. Paediatric Orthopaedics Unit, Department of Orthopaedics, Christian Medical College, Vellore
2. Department of Clinical Pathology, Odense University Hospital, Odense, Denmark
3. Department of Biostatistics, Christian Medical College, Vellore

Introduction

During muscle regeneration, myogenesis and angiogenesis take place simultaneously and the complete restoration of vascular network is essential for successful muscle repair. In ischemia condition, the process of muscle regeneration is impaired and tissue is replaced by fibrotic tissues. In addition the density of capillaries regulates the proportion of satellite cells in the muscle.

Satellite cells:
Cryopreserved satellite cells, isolated from vastus lateralis muscle of the volunteer for the previous experiment, were used for this study.

Indirect Co-culture.

Satellite cells cultured without endothelial cells acted as a control.

Indirect Co-culture: Cell proliferation assay

![Figure 3: Cell count was higher in the co-cultured group compared to control (P<0.001).]

Gene expression analysis:

![Figure 4: Markers specific to satellite cell proliferation (Pax7 and MyoD) were higher in co-cultured group than the control. Quiescent (Pax3 and CALCR) and differentiation (Desmin, MYH1 and SMA) markers were inhibited in co-cultured.]

Discussion

- Endothelial cells isolated from the human umbilical cord vein were confirmed by both phenotypical and functional characterisation.
- During co-culture, paracrine factors of endothelial cells stimulated the proliferation of satellite cells.
- Gene expression analysis showed that satellite cells were activated from quiescence and underwent proliferation; however the differentiation was inhibited.
- This finding may have implication in cell therapy by maintaining the satellite cell phenotype during ex vivo expansion.

Aim

To study the in vitro effect of endothelial cells on satellite cell culture.

Methods

Isolation of endothelial cells:
After informed consent, human umbilical cord was harvested from a patient in labour room and the endothelial cells were isolated from umbilical cord (UC) vein.

Characterization
Endothelial specific markers such as Von Willebrand factor (vWF) and CD31 markers were used to characterize the isolated cells by immunocytochemistry.

Tube formation assay
Cells were seeded in a 96 well plate, pre-coated with 50μl Gelvetx™ Reduced Growth Factor Basement Membrane Extract (Invitrogen). After overnight incubation with HUVEC media, cells were observed under microscope.

Results

Characterization of HUVEC

CD31

vWF

![Tube formation assay]

Figure 2: Cells were stained positive for endothelial cell specific markers (A) CD31 (B) vWF and (C) formed capillary network like structure on matrigel.

Conclusion

Endothelial cell’s paracrine factors induces proliferation and maintains the phenotype of satellite cells

References

- Davis G. et al. Cir Res. (2005)

Acknowledgement

We acknowledge Department of Biotechnology (India) and Danish Council for Strategic Research (Denmark) (a Indo-Danish Research Collaboration program) for funding.
MINIMUM LETHAL DOSE OF ISOLATED TOXINS AND WHOLE AQUEOUS EXTRACTS OF CLEISTANTHUS COLLINUS IN RATS

Neetu Prince, Benjamin Jebaraj, Soosaimanickam Amirtham, Abirami.V, Sathya Subramani
Department of Physiology, Christian Medical College, Vellore.

INTRODUCTION

Cleistanthus collinus poisoning is common in rural South India. Victims consume either a boiled decoction or fresh leaf juice.

Mortality rate is 30% and is stated to be higher with consumption of boiled decoction.

We estimated lethal doses of the two types of extracts in rats and found that both were equally toxic.

3 fluorescent compounds were isolated from the extracts. Two were identified at IIT Mandi as Cleistanthin A and Cleistanthin C.

Cleistanthin C is reported as a toxin by us for the first time.

AIMS

To see if the boiled decoction is more toxic than fresh leaf juice in rats.

To see if the three isolated compounds are toxic; and if so estimate their lethal doses.

EXTRACTION AND IDENTIFICATION OF TOXINS

Whole aqueous extracts: Two types: C. collinus leaves were either boiled in water for 10 minutes and filtered (FLB, fresh leaf boiled) or ground with tepid water and filtered (FLG, fresh leaf ground).

When FLB and FLG were chromatographed on silica plates (TLC), many fluorescent compounds were seen.

Chloroform was added to FLB and FLG. Chloroform layer formed at the bottom.

Top water layer was devoid of fluorescence when observed on TLC. (non-fluorescent fraction, NFF).

Chloroform sequestered all fluorescent compounds. (fluorescent fraction, FF). (Our finding).

Chloroform fraction was concentrated to a dry powder. It was subjected to preparatory Thin Layer Chromatography to isolate 3 prominent fluorescent bands. The bands were scraped and the compounds eluted.

Top band is Cleistanthin A and the bottom one Cleistanthin C (identified by IIT Mandi).

Middle band is yet to be identified (putatively, Diphyllin).

TOXICITY TESTING IN RATS BY ORAL ADMINISTRATION

FLG is as toxic as (if not more toxic than) FLB in rats:
NFF of both extracts are non-toxic

Cleistanthin A and C are highly toxic
Diphyllin is non-toxic

Table 1

<table>
<thead>
<tr>
<th>Fraction tested</th>
<th>Minimum lethal dose MLD（per 100 gram BW）（n=4）</th>
<th>LD10（n=4）</th>
<th>LD50（n=4）</th>
<th>Non-lethal dose（n=4）</th>
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<tbody>
<tr>
<td>FLB</td>
<td>75 mg</td>
<td>50 mg</td>
<td>25 mg</td>
<td>200 mg</td>
</tr>
<tr>
<td>FLG</td>
<td>50 mg</td>
<td>25 mg</td>
<td></td>
<td>200 mg</td>
</tr>
<tr>
<td>FLB-NFF</td>
<td></td>
<td></td>
<td></td>
<td>200 mg</td>
</tr>
<tr>
<td>FLG-NFF</td>
<td></td>
<td></td>
<td></td>
<td>200 mg</td>
</tr>
</tbody>
</table>

Table 2

<table>
<thead>
<tr>
<th>Compound tested</th>
<th>Minimum lethal dose MLD（per 100 gram BW）（n=4）</th>
<th>LD10（n=4）</th>
<th>LD50（n=4）</th>
<th>Maximum non-lethal dose（per 100 gram BW）（n=4）</th>
</tr>
</thead>
<tbody>
<tr>
<td>FLB</td>
<td>3 mg</td>
<td>1 mg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FLG</td>
<td>3 mg</td>
<td>1 mg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FLB-NFF</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FLG-NFF</td>
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</tbody>
</table>

In a parallel study (Soosai Manickam Ph.D Thesis) concentrations of the pure compounds in FLB and FLG were worked out.

Based on that study, we have estimated the total amounts of toxins present in MLD（100）of FLB and FLG.

In Table 2, Cleistanthin C has the highest toxicity, followed by Cleistanthin A and then Diphyllin.

Table 3

<table>
<thead>
<tr>
<th>Compound tested</th>
<th>Minimum lethal dose for whole leaf extracts for 100 gram BW</th>
<th>Cleistanthin C</th>
<th>Cleistanthin A</th>
</tr>
</thead>
<tbody>
<tr>
<td>FLB 75 mg</td>
<td>9.6 – 10.3 mg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FLG 50 mg</td>
<td>0.23 – 0.61 mg</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

DISCUSSION AND CONCLUSION

Though in patients the boiled decoction (FLB) is stated to be more toxic than the fresh leaf juice (FLG), we found no such difference in rats. Both were equally toxic.

Cleistanthin C was identified as a toxin in the boiled decoction for the first time. Only Cleistanthins A and B were considered to be toxins in C. collinus thus far. We could not isolate Cleistanthin B from the aqueous extract however. It is probable that it is present in the plant, but does not elute in water extracts and therefore not clinically relevant.

Cleistanthin A is toxic. Diphyllin is not toxic.

Quantity of Cleistanthin C in MLD（100）of FLB (table 3) is very high and can account for all of the toxicity of FLB.

The concentrations of both Cleistanthins is very low in FLG to account for its high toxicity, therefore we have to explore for new toxins in FLG.

REFERENCES

1. K.S. Annapporani - Ph.D Thesis (held at forensic sciences division, chennai)

Acknowledgement: CMC Fluid Research grant; Dr. K.S. Annapporani for help with chromatography; Dept of Clinical pharmacology, CMC, Vellore for technical support.
Role of parathyroid hormone related peptide (1-34) during periosteal mesenchymal stem cell differentiation to chondrocytes

Sowmya Ramesh1, Karthikeyan Rajagopal1, Vrisha Madhuri1,2
1Paediatric Orthopaedics Unit, Department of Orthopaedics, Christian Medical College, Vellore
2Centre for Stem Cell Research, Christian Medical College, Vellore

INTRODUCTION

One of the major reasons for chondrocyte hypertrophy is the presence of collagen X, Runx-2. Parathyroid hormone related peptide (PTHrP 1-34) plays a crucial role in the suppression of hypertrophy in bone marrow (BM) MSCs. The effect of PTHrP on the hypertrophic markers during the course of periosteal MSC differentiation has been discussed in this study.

METHODS

• Periosteal MSCs from 2
human patient donors.

• Cells were differentiated using TGF-
β3 (25 ng/ml) and PTHrP 1-34 (100 ng/ml) from day 3.

• RNA isolation at 1, 3, 6, 9, 12, 18, 24 h.

• Typing, biochemical

• Immunohistochemistry

• RT-PCR

RESULTS

Cell were characterized positive for MSC markers

Matrix accumulation and cellular hypertrophy were observed in PTHrP treated pellet

CONCLUSIONS

Unlike in BMMSCs, PTHrP (1-34) does not suppress hypertrophy in PDMSCs. The time of introducing the growth factors to the culture is therefore crucial.

ACKNOWLEDGEMENTS

• This study was supported by a grant from the Science and Engineering Research Board (SR/SC/HS/190/2012), Department of Science and Technology, Government of India.
• The authors thank Centre for Stem Cell Research for providing the facility to carry out the experiments.
INTRODUCTION

- During somatic cell reprogramming, genome-wide chromatin reorganization occurs that results in decondensation of heterochromatin structure of somatic cells to a disassembled state similar to that of embryonic stem cells (ESCs) [1,2].
- The transition in the structure of chromatin is mediated by the activity of chromatin modifying enzymes, many of which act as facilitators and others as repressors of reprogramming [1,2].
- Histon deacetylases (HDACs) are recognized as the major class of histone modifying enzymes involved in chromatin transition during somatic cell reprogramming [1,2].
- Perturbation of histone deacetylase activity during mouse somatic cell reprogramming with small molecule inhibitors such as Vapreic acid, Trichostatin A, Sodium butyrate or SAHA has been shown to promote reprogramming and translation of pre-IPSCs to fully reprogrammed iPSCs [1,3,5].
- Vapreic acid is a major component of the chemical cocktail that was found sufficient to induce reprogramming of mouse somatic cells [3].
- Mouse somatic cells derived from Hott2 knockout could be efficiently reprogrammed by transfection of microRNA-1302-3p sh-Cluster [1].
- Class Ia HDACs including Hott2 were shown to regulate mesenchymal epidermal transition (MET) during mouse somatic cell reprogramming by perturbing the activity of ME2 proteins [1].

OBJECTIVES

- To determine the expression kinetics of different classes of histone deacetylases (HDACs) during the course of reprogramming.
- To understand role of specific histone deacetylases (HDACs) in regulating mouse somatic cell reprogramming.

METHODS

- Screen for differentially expressed histone deacetylase genes by qRT-PCR.
- Cloning of shRNA's targeting regulatory HDACs into inducible lentivectors.
- Validation of knockdown efficiency in SNL cell lines.
- Injection of MEFS with GSMM and dox inducible shRNA lentivectors.
- Induction of shRNA mediated knockdown at defined time points during reprogramming.
- Scoring of colonies based on Alkaline phosphatase and Nanog immunostaining.

RESULTS

- Correlation between HDAC expression and pluripotency.
- Inverse correlation between Hott2 and Nanog gene expression.
- Determination of knockdown efficiency of Inducible Hott2 and Hott2 shRNA Vectors.

CONCLUSION

- The specific expression of Hott2 in RIESCs, iPSCs, pre-IPSCs and ESAt-1 reprogramming intermediates compared to that of MEFS is suggestive of its role as a facilitator of reprogramming. In contrast the higher expression of Hott2 in MEFS and pre-IPSCs compared to RIESCs and iPSCs is indicative of its role as a barrier to reprogramming.
- The knockdown of Hott2 during early stages of reprogramming was found to impede the reprogramming process by reducing the alkaline phosphatase and Nanog positive colony numbers. However knockdown at the later stages of reprogramming did not have any significant effect, indicating its role during early stage of reprogramming.
- Cell cycle progression and proliferation are considered important events during somatic cell reprogramming and HDACs has been implicated to regulate these events in mammalian cells. Our further studies will focus on highlighting these roles of HDACs during somatic cell reprogramming.
- The knockdown of Hott2 at any stage did not have any significant effect on alkaline phosphatase positive colony numbers. There were two possible explanations for this observation, the most prominent knockdown achieved with Hott2-shRNA might not be sufficient to influence the reprogramming process. As an alternative explanation the presence of Hott2 in reprogramming factor cocktail might mask the beneficial effect of Hott2 knockdown by accelerating MET process.

ACKNOWLEDGEMENTS

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IDH mutations and 1p/19q status in tumours with an oligodendrogial morphology – clinical relevance

Dr Tulasi Geevar1, Dr Bimal Patel1, Dr Karthik Molepathi2, Dr Jacob John1, Dr Rekha Pai1, Dr Ari G Chacko3, Dr Rajesh B4, Dr Subhashini John4, Mrs Grace Rebekah5, Dr Geeta Chacko5, Departments of General Pathology1, Neurosurgery1, Community Medicine1, Radiotherapy1 and Biostatistics1, Christian Medical College, Vellore

INTRODUCTION

- Oligodendrogial tumours are a subset of glial tumours with distinct histological and molecular features.
- IDH1/2 mutations and 1p/19q co-deletions are frequent genetic alterations in oligodendrogial tumours, associated with good prognosis (1-3)

AIMS AND OBJECTIVES

- To determine the frequency of IDH1 and IDH2 mutations by PCR sequencing, and IDH1 mutations by immunohistochemistry among 50 cases of oligodendrogial tumours.
- To calculate the performance indicators of IDH1 immunohistochemistry as a diagnostic marker.
- To correlate 1p/19q status and IDH1/IDH2 mutations with clinico-pathological variables and measures of outcome.

MATERIALS AND METHODS

- 50 oligodendrogial tumours diagnosed from January 2009 to January 2012 were studied.
- These included 11 Grade II Oligodendroglioma, 15 Grade III Oligodendroglioma, 7 Grade II Oligoastrocytoma, 10 Grade III Oligoastrocytoma and 7 Grade IV Glioblastoma with an oligodendrogial component (GBMO).
- 1p/19q status was determined by FISH. IDH1 mutational status was determined by PCR and immunohistochemistry (IHC).

RESULTS

- 1p/19q co-deletion and polyomavirus of chromosome 1/19 was seen in 46% (23/50) and 36% (18/50) of the cases respectively.
- 1p/19q co-deletion was significantly associated with pure oligodendrogial tumours, classical histology and frontal lobe location.
- Polyomavirus 1/19 was associated with mixed oligoastrocytotic tumours.
- IDH1/2 mutations by PCR were seen in 46/50 cases, which included 42 cases of IDH1R132H, 1 case of IDH1R132G and 3 cases of IDH2R172H mutations.
- IDH1 immunohistochemistry was positive for all 42 cases with IDH1R132H mutation. 4 cases showing other types of IDH mutation were negative by IHC.
- Sensitivity and specificity of IDH1 immunohistochemistry was 91.3% and 100%.
- 23 of 46 cases with IDH mutation showed 1p/19q co-deletion and 4 cases that were negative for IDH mutation were also negative for 1p/19q co-deletion.
- On univariate analysis, necrosis, WHO grade and IDH mutation were found to have increased risk of recurrence and death.
- On multivariate analysis, using the following variables, 1p/19q co-deletion, polyomavirus 1/19, classical histology and WHO grade, only WHO grade was found to be associated with increased risk of recurrence.

CONCLUSION

- 1p/19q co-deletion was significantly associated with pure oligodendrogial tumours and classical histology.
- Classical histology may be used as a surrogate marker for 1p/19q status in centres where genetic testing is not readily available.
- Polyomavirus of chromosome 1/19 was commonly seen in mixed oligoastrocytotic tumours.
- 1p/19q status may be used to distinguish mixed oligoastrocytotic tumours from pure oligodendrogial tumours.
- IDH1 and IDH2 mutations were seen in 80% and 6% of the cases respectively.
- IDH1 IHC can be used as a simple and inexpensive laboratory test to determine IDH mutational status.
- IDH1 IHC can be supplemented by IDH PCR in all immunonegative cases.
- Determination of IDH mutational status is imperative in addition to assessment of 1p/19q status in order to identify patients with better prognosis.
- On univariate analysis, necrosis, WHO grade and IDH mutation were found to have increased risk of recurrence and death. Whilst in multivariate analysis only WHO Grade had an increased risk of recurrence.
- Further studies on larger cohorts are indicated to assess the prognostic relevance of these genetic changes in our population.

REFERENCES

ELECTROPHYSIOLOGICAL STUDY OF FRESHLY ISOLATED ARTICULAR CHONDROCYTES vs. CRYOPRESERVED CHONDROCYTES
Upasana Kachroo, Praghalathan Kanthakumar, Sathy Subramani
Department of Physiology, Christian Medical College, Vellore.

BACKGROUND
Cryopreservation of articular chondrocytes has recently gained ground because of its applications in cell culture, tissue engineering and reconstructive surgery. Questions may be raised as to the use of cryopreserved chondrocytes since this intervention may cause changes in chondrocyte phenotype and biology. One way of assessing phenotype maintenance is by studying the electrophysiological profile using the patch clamp technique.

AIM
To compare freshly isolated articular chondrocytes and cryopreserved chondrocytes based on an electrophysiological study

OBJECTIVES
Primary objective was to compare ion channel expression in freshly isolated and cryopreserved chondrocytes using patch clamp technique. Secondary objective was to assess chondrocyte viability after a specific period of cryopreservation

MATERIAL AND METHODS
Goat articular chondrocytes were isolated from cartilage shavings by enzymatic digestion. Cell aliquots were transferred to liquid nitrogen after overnight gradual cooling to -80°C. Cells were cryopreserved for a period of 7-15 days (2 study groups-Day 7-10 and Day 11-15). Percentage viability was checked upon rapid thawing.

Cells were subjected to patch clamp analysis. Ionic currents were recorded in whole cell configuration using depolarizing potentials (V_Hold = 80 mV; Test pulses: 80 mV to +70 mV at 10 mV increments).

DISCUSSION
Cryopreservation yielded chondrocytes with good although varying viability which may be improved by optimization of cryopreservation protocol. Patch clamp analysis revealed a family of outwardly rectifying currents which were slowly activating and non inactivating, in both freshly isolated as well as cryopreserved chondrocytes. 10 mM TEA^- (a potassium channel blocker) decreased current magnitude thus confirming that currents originated from voltage gated potassium channel sub family. These results indicate that cryopreservation does not alter channel expression.

CONCLUSION
It is seen that chondrocytes remain viable and show similar ion channel expression even after 15 days of cryopreservation. Cryopreserved chondrocytes may be utilized as an alternative to freshly isolated chondrocytes.

STATISTICAL ANALYSIS
The results were analyzed using Kruskal-Wallis Test. A P value of less than 0.05 was considered statistically significant.

RESULTS
Figure 1. Graph expressing percentage viability over time of retrieval from cryopreservation. Day 0 represents freshly isolated chondrocytes (Data from 4 individual experiments)

REFERENCE

Acknowledgement: CMC Fluid Research grant for funding the study.
The Story of an infertile male with 45 chromosomes...

Pujari GP, Kamath MS, Meena J, Kumar RM, Srivastava VM

Cytogenetics and Reproductive Medicine Units, Haematology and Pathology

Departments, Christian Medical College, Vellore

Introduction

The 45,X karyotype is typically associated with a female phenotype (Turner syndrome). We present here a 23-year-old male with a 45,X karyotype detected during investigation of infertility.

Clinical narrative
- Married for three years
- No phenotypic abnormalities or obvious intellectual disability
- Androgenisation appeared normal
- Testicular size about 10cc bilaterally
- Epididymides were not distended

Investigations
- Semen analysis: Azoospermia
- TESA/PESA: No mature sperms
- Serum FSH: 8.79 mIU/ml
- Testicular biopsy: Sertoli cells only
- Other biochemical parameters: WNL

FISH analysis
- To detect a 45,X/46,XY mosaic cell line
- The presence of the SRY gene (sex-determining region of the Y chromosome)
- There was no mosaicism, but part of the short arm of chromosome Y bearing the SRY gene appeared to be inserted into the terminal part of the long arm of chromosome 1
- Sequences from the long (q) arm of chromosome Y were not present nor was there any translocation of chromosome 1 material

Final karyotype 45,X,der(1)(1pter-1q44::Yp11.2-Ypter)

Polymerase chain reaction

References

Phenotypic and molecular characterization of the carbapenem resistant
*Klebsiella pneumoniae* and *Escherichia coli* causing blood stream
infections
A Sharma¹, Shalini A¹, Jones D¹, Yamuna B¹, Thambu D², Santhanam S³, Winsley R³, V Balaji¹
⁰Department of Clinical Microbiology, ¹Department of General Medicine ³Department of Paediatrics, Christian
Medical College, Vellore

**Introduction**
- Gram negative enteric bacilli are a prominent cause for sepsis in children and adults
- The infections caused by *Enterobacteriaceae* are community and hospital-acquired as these bacteria have the potential to spread in the hospital environment and also across continents
- Irrational use of antibiotics coupled with rapid emergence of drug resistance in bacteria is a major problem faced by health care setting around the world
- The resistance to carbapenem groups of drugs is on the rise.
- It is especially of concern in the nosocomial pathogens which are multidrug resistant
- The most common mechanism of carbapenem resistance in *Enterobacteriaceae* is the production of carbapenemase enzymes
- These genes coding for these enzymes are carried on mobile plasmids and have a greater propensity to cause outbreaks
- The prevalence of carbapenem resistant *Enterobacteriaceae* in India has been reported to vary from 5.3% to 51%

**Objectives**
- To characterize the different carbapenem resistance mechanisms in *Klebsiella pneumoniae* and *Escherichia coli* isolated from blood stream infections using phenotypic and genotypic methods by performing the following tests:
  a. Phenotypic differentiation of carbapenemase and non-
  carbapenemase mediated mechanisms by the CarbaNP test
  b. Molecular characterization of bla*NDM*, bla*OXA*, bla*KPC*, bla*OXA-48* like, bla*PA
  - To determine the susceptibility pattern of the isolates for first line and second line drugs
  - To identify the enzyme variants of a proportion of the isolates by PCR- sequencing

**Methods**
- Observational study conducted in the Department of Clinical Microbiology, Christian Medical College, Vellore from January 2013 to December 2013
- 122 consecutive, non repetitive *Klebsiella pneumoniae* and *Escherichia coli* isolates from blood stream infections, showing resistance to imipenem and meropenem by disk diffusion were included in the study

**Blood cultures positive for gram negative bacilli**

**Identification of by Klebsiella pneumoniae and Escherichia coli by culture and biochemical tests**

**Screening test for carbapenem resistance by imipenem, meropenem disk diffusion**

**Phenotypic CarbaNP test to detect carbapenemase production**

**Multiplex PCR for bla*NDM*, bla*OXA*, bla*KPC*, bla*OXA-48* like, bla*PA**

**Sequencing for identification of enzyme variants of carbapenemases**

**Results**
- CarbaNP test was positive for 96% of the isolates indicating carbapenemase production as the mechanism of resistance (Fig. 1)

![CarbaNP test](image1)

**Fig.1 CarbaNP test**

![Gel electrophoresis picture showing various carbapenemase genes detected by multiplex PCR](image2)

**Fig.2 Gel electrophoresis picture showing various carbapenemase genes detected by multiplex PCR**

**Fig.3 Susceptibility profile of the study isolates for common antimicrobials**

**Fig.4 Prevalence of carbapenemase enzymes**

- On sequencing for identification of enzyme variants all the 20 (100%) of the bla*OXA-48* like genes were found to be bla*KPC*, a variant of the bla*OXA-48* like gene. The 7 bla*NDM* positive genes were found to be the bla*OXA-48* variant

![Sequence chromatogram for bla*KPC* gene](image3)

**Figure 5. Sequence chromatogram for bla*KPC* gene**

![Sequence chromatogram for bla*NDM* gene](image4)

**Figure 6. Sequence chromatogram for bla*NDM* gene**

**Conclusions**
- NDM was found to be the most prevalent carbapenemase enzyme among *K. pneumoniae* and *E.coli* isolates closely followed by OXA-48 like enzymes
- The isolates show resistance to most antimicrobials
- Good in vitro susceptibility to colistin is observed but the drug needs to be used with precaution to prevent emergence of resistance

**References**
INTRODUCTION

• Consumption of pesticides worldwide have been increasing dramatically as human population has increased and to increase crop production.

• Organophosphorus pesticides are used widely for agriculture and vector control.

• The potential adverse impact on human health from exposure to pesticides is likely to be higher in countries like India due to easy availability of concentrated and highly hazardous products, and low risk awareness among users.

• The majority of agriculture pesticide sprayers are not trained in safe handling of OP pesticides making them prone for chronic effects of poisoning. So this study is an attempt to find out the factors influencing outcomes in chronic OP poisoning.

AIM

• To document the biochemical changes in pesticide sprayers in after long term exposure to organophosphates

SUBJECTS AND METHODS

• Fifty five male pesticide sprayers (age>18 years) with a minimum exposure of 2 years from Krishnagiri district, Tamilnadu were included after informed consent.

• Data on work practices, duration of exposure, neurological symptoms like tingling and burning sensations in the hands and feet, exercise intolerance and handwriting deterioration was assessed using a questionnaire.

• Butyryl Cholinesterase (BChE) was measured by Ellman’s method. Acetyl cholinesterase (AChE) was assayed using acetyl thiocholine iodide as a substrate. Liver function and renal function were assessed by standard biochemical parameters.

• Creatine Kinase, Amylase, Lipase were also measured.

RESULTS

• The mean age of the whole group was 38.44 ± 10 years. The median duration of exposure - 10 years (2 – 25 years).

• Thirteen percentage of subjects were smokers and consumption of alcohol was about 27.3%. Usage of protective equipments among sprayers like gloves were observed in 29% mask 60% Boots 38%. Exercise intolerance (65%) was another finding.

• Inhibition of cholinesterases due to long term exposure was reflected in lower BChE and AChE levels in the patients when compared with controls was statistically significant (p<0.0001) (Fig 1).

• Total protein and albumin levels in the chronic cohort did not differ significantly when compared to controls (Fig 2).

DISCUSSION

• Pancreatic enzymes were elevated in the sprayers, as were liver enzymes, and total CK in serum.

• Use of personal protective gear was not prevalent even though every-one was aware of the need to wear gloves, masks and boots and they were easily available in local shops. Inconvenience and the hot climate were suggested as the reasons for not using protective clothing.

• Long term exposure to low levels of OP compounds may be responsible for abnormal biochemical values in workers. Education and motivation to follow safe work practices need to be instigated.
Intrinsic characteristics of human Wharton jelly derived mesenchymal stem cell (hWJMSC) induces cancer cell apoptosis in vitro.

Balasubramanian Sundaram and Sanjay Kumar.
Centre for Stem Cell Research, Christian Medical College, Vellore, India-632002

**INTRODUCTION**

- The traditional chemotherapy and radiation treatments for breast cancer become ineffective in cancer treatment as evidenced by the recurrence of metastatic disease years after the primary treatment.
- Mesenchymal stem cells (MSCs) have the inherent ability to migrate and home into the tumor microenvironment.
- Several in vivo animal studies utilized the tumor homing ability of MSCs in cancer therapies by overexpressing the anti-tumorigenic cytokines and suicide gene products.
- The application of MSCs in cancer therapy remain controversial as contradicting results of both pro-tumorigenic and anti-tumorigenic results have been reported for MSCs from different sources in several in vivo animal models.
- It has been shown earlier that human Wharton jelly derived MSCs (hWJMSC) do not transform into tumor associated fibroblasts unlike MSCs derived from Bone Marrow (BM-MSCs) and Adipose tissue (AD-MSCs).
- Moreover the rare incidence of fetal tumorigenesis in pregnant cancer patients led to the hypothesis that WJ-MSCs act as the natural defence against the migrating cancer cells from the maternal circulation.
- WJ-MSC considered as better and safer source for cancer therapy as they do not transform in in-vitro long term expansion, possess high proliferative potential and anti-tumorigenic potential than MSCs derived from adult tissues.

**OBJECTIVES.**

- To isolate and characterize MSC's from human Umbilical-Cord Wharton's Jelly tissue.
- To assess the effect and interaction of hWJ-MSC's on human breast adenocarcinoma cell line MCF-7 by confocal live cell time lapse imaging.
- The isolation and characterization of MSC's from human umbilical cord is carried out as per the protocol described by Alp can and Deniz Balezi with minor modifications (1).
- The MCF-7 and hWJMSC stably labelled with fluorescent proteins dRed Express and enhanced green fluorescent protein (EGFP) respectively by the second generation lentivirus and positive population enriched by Fluorescence Activated Cell Sorting.
- Equal number (2 x 10^5) of hWJMSC-EGFP and MCF-7 dRed Express cells co-plated in glass bottom confocal dish and subjected to confocal live cell time lapse imaging for overnight.

**METHODS AND MATERIALS**

**RESULTS**

1. Isolation and characterization of MSC's human Umbilical Cord.
   A) Immuno phenotypic characterization.

   **RESULTS**

   - Fig. 1: A) Flow cytometry analysis of WJ-MSCs
   - B) In-vitro multilineage differentiation.

   **DISCUSSION**

   - Mesenchymal Stem Cells derived from the Wharton’s jelly of human umbilical cord fulfilled the minimum criteria laid down by International Society of Cellular Therapy as they are adherent to the plastic surface, express CD29, CD54, CD105 and negative for CD45, CD14 and CD11b on their cell surface. Showing an in vitro plasticity MSCs differentiates not only to the classic mesenchymal lineages but also differentiated into cell types of mesodermal lineages and endodermal lineages, and neural cells and pancreatic progenitors respectively.
   - Human Wharton’s Jelly MSCs labelled with EGFP co-cultured with dRed Express expressing MCF-7 breast cancer cells. The interaction and fate of both cells monitored through confocal live cell time-lapse imaging. Upon co-culture MSCs migrates towards the cancer cells and interacts through filopodia and induce the cancer cells apoptosis. The migration of MSCs to the cancer cells may be due to the inflammatory factors secreted by cancer cells.
   - Apart from the role of direct cell to cell contact which leads to the cancer cell death, MSC secreted factors which contains exosomes and other micro vesicles will also have a role in cancer cell death. Further studies required to assess the role of secreted factors in MSC - Cancer interactions.
   - In this current study we demonstrated that MSCs isolated from the Wharton’s jelly of human Umbilical Cord has the potential to inhibit the proliferation of MCF-7 breast cancer cells through direct cell-to-cell contact. The exact mechanism may be through apoptosis which required further extensive study.

**REFERENCES**


**ACKNOWLEDGEMENTS.**

We acknowledge University Grants Commission, New Delhi for UGC-REF-NET fellowship to BS and Department of Biotechnology, New Delhi for Ramalingaswami Fellowship to SK.
Role of Aquaporin-4 (AQP-4) levels in brain oedema and neurological function in an automated cortical cryoinjury model in mice

Edmond Jonathan G. Ranjith K. Moorthy, Keesvan Narasimhan, Prabhakar V. Anna Oommen, Vedantam Rajshekhar
Department of Neurological sciences, Christian Medical College, Vellore

Introduction

- AQP-4, a transmembrane water channel, has been found to have a role in water homeostasis.
- Previous studies confirm its role in generation of brain oedema.
- No studies to date correlating AQP-4 levels with neurological function post injury.

ROLE OF AQP-4 IN TRAUMATIC BRAIN INJURY

Objectives

- To study the spatial and temporal profile of AQP-4 in the murine brain following a cortical cryoinjury.
- To correlate the AQP-4 levels with neurological function after an automated cortical cryoinjury in mice.

Materials and Methods

- 42 inbred Swiss Albino Mice
- 3-6 months, 25-35 grams

Anesthesia (IP injection of kg/ratazine 100mg/kg + kg/ratazine 10mg/kg)

Experimental Group N=18
- Cortical cryoinjury at Wk. 15 days, C for 4 hours in a hot air oven.

Control Group
- No sedation procedure

Brain water content was assessed by dehydration of brain at 120 deg. C for 48 hours in a hot air oven.

Brain water content = [Wet wt – dry wt / wet wt] x 100

Determination of AQP-4 by Western blotting

Brain tissue processed for protein estimation
- SDS electrophoresis (20 microgram protein with loading control)
- Protein transfer to polyethylene fluoride (PVDF) membrane
- Addition of primary and secondary antibodies immunoblot preparation
- Diaminobenzidine (DAB) color staining and measurement of intensity by Electrochemical luminescence (ECL)

Results

Significantly higher percentage water content noted in the experimental group compared to sham group at 24 hours, but not at 48 and 72 hours.

NSS and RR scores demonstrated significantly higher functional impairment in the experimental group with maximal deterioration in neurological function in experimental group being seen at 24 hours. There was a trend for a gradual recovery at the end of 72 hours.

Conclusion

- There was 1.4 fold increase in AQP-4 in injured brain at first 24 hours. AQP-4 expression was highest at the site of injury and in the hemisphere adjacent to the site of injury.
- This correlated to worse neurological function at site 24 hours post injury.
- There was gradual decline in AQP-4 expression with partial functional recovery at 72 hours.

References

Serum hepcidin levels in normal pregnancy

Gnanaprabha P, Joe Varghese, Jasmine Prasad, Visalakshi Jeyaseelan, and Molly Jacob

Departments of Biochemistry, Community Medicine, and Biostatistics, Christian Medical College, Vellore

Background
Systemic iron levels are regulated by hepcidin. Animal studies have shown that hepcidin levels decrease during pregnancy, to provide adequate iron for fetal development, and are raised in the post-partum period, when iron demands are reduced. Literature in this area on human subjects is limited. No data is available from India.

Aim
The aim of this study was to determine serum hepcidin levels in women with uncomplicated pregnancies.

Materials and methods

Subjects
Pregnant women (10 in each trimester), who attended the antenatal clinic at the Community Health and Development (CHAD) Hospital, CMC, Vellore

Controls
Non-pregnant women staff (10 subjects) of the pre-clinical departments at CMC, Vellore

Inclusion criteria
Pregnant women with
1. hemoglobin levels equal to or more than 11 g/dL in the first and third trimesters, and more than 10.5 g/dL in the second trimester
2. serum levels of CRP less than 6 mg/L
3. no complication of pregnancy
4. those who were not willing to participate in the study

Exclusion criteria
Pregnant women with
1. hemoglobin levels less than 11 g/dL in first or third trimesters, or less than 10.5 g/dL in the second trimester
2. serum levels of CRP more than 6 mg/L

Blood obtained from each subject, after getting informed consent, was used to estimate the following:
1. Serum ferritin (indicator of iron status)
2. Hematological parameters (hemoglobin, hematocrit, mean corpuscular volume [MCV])
3. Serum CRP (marker of inflammation)
4. Serum hepcidin

Results

Table 1: Clinical characteristics of the subjects

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Non-pregnant subjects</th>
<th>Pregnant women in the first trimester (&lt;18 weeks)</th>
<th>Pregnant women in the second trimester (18-26 weeks)</th>
<th>Pregnant women in the third trimester (&gt;26 weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Subjects</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Age (years) (±SD)</td>
<td>28.10 ± 2.28</td>
<td>22.20 ± 3.39</td>
<td>22.20 ± 1.64</td>
<td>21.70 ± 1.64</td>
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<td>Mean gestational age (weeks) (±SD)</td>
<td>38 ± 2.13</td>
<td>39 ± 2.13</td>
<td>39 ± 2.13</td>
<td>39 ± 2.13</td>
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<tr>
<td>Parity</td>
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<td>9</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>Primary / Second pregnancy</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Height (cm) (±SD)</td>
<td>160.8 ± 2.82</td>
<td>153.4 ± 3.05</td>
<td>156 ± 6.34</td>
<td>152 ± 6.34</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg) (±SD)</td>
<td>115.6 ± 6.34</td>
<td>110.8 ± 7.08</td>
<td>107 ± 12.77</td>
<td>106 ± 12.77</td>
</tr>
<tr>
<td>Dystolic blood pressure (mm Hg) (±SD)</td>
<td>72.2 ± 5.99</td>
<td>66.6 ± 9.75</td>
<td>64 ± 10.29</td>
<td>64 ± 12.59</td>
</tr>
</tbody>
</table>

Table 2: Correlational analysis

- Serum ferritin levels in pregnant women were found to be lower than in control subjects. The levels showed a tendency to decrease with increasing gestational age. However, the decreases seen were not statistically significant.

Correlation | r-value | p-value
--- | --- | ---
All subjects (non-pregnant and pregnant subjects) | 0.39 | 0.02
Ferritin vs. hemoglobin | 0.31 | 0.06
Hemoglobin vs. hematocrit | 0.82 | <0.001
Haptoglobin vs. MCV | 0.52 | 0.06
Non-pregnant women | 0.66 | 0.04
Ferritin vs MCV | 0.94 | <0.001
Hemoglobin vs. hematocrit | 0.82 | <0.001
Pregnant women | 0.32 | 0.08

* p < 0.05

Hemoglobin correlated positively with hematocrit in both non-pregnant and pregnant women.

No correlation was found between serum levels of hepcidin and ferritin in the groups studied.

Conclusion
Serum hepcidin levels tended to decrease with increasing gestational age. They were not significantly correlated with any of the parameters measured.

Since the sample size in this study was small, further studies in this area are warranted to confirm these findings and to further study the complex biological relationships between hepcidin and iron-related proteins, in pregnancy.

Acknowledgement
The study was approved and funded by the Institutional Review Board (IRB) at Christian Medical College (CMC), Vellore, India (IRB Min. No. 8151 dated 09.01.2013).
Clinicopathological Correlates Of Primary Central Nervous System Lymphoma Of The Cerebral Parenchyma

Patel B1, Chacko G2, Nair S1, Anandan J1, Chacko AG1, Rajeshkumar V1, Turel M2
Department of Pathology1 and Department of Neurosurgery2
Christian Medical College, Vellore

INTRODUCTION

Primary central nervous system lymphoma (PCNSL) are extranodal lymphomas with absence of lymphoma in other systems. The worldwide incidence of PCNSL is 3-4/100,000 while that in India is 0.95-1.4/100,000. Histologically majority are diffuse large B-cell lymphomas (DLBCL). Exact pathogenesis of PCNSL is unclear. Molecular subtypes of DLBCL are germinal centre like B-cell type (GCB) and non-germinal centre like B-cell type (non-GCB), which differ in aggressiveness, treatment and prognosis5, with GCB subtype having a better prognosis5,6. There is limited literature on subtyping of DLBCL of the CNS, with no studies to date from India.

OBJECTIVES

To study the clinical profile and immunophenotype of PCNSL that presented between 2000-2013.

MATERIALS AND METHODS

1. Retrospective study of PCNSL that presented in CMC Hospital, Vellore between 2000 to 2013.
2. Inclusion criteria: PCNSL of cerebral parenchyma only. Exclusion criteria: Lymphomas of spinal cord, meninges and orbit.
3. The clinical data was collected from the hospital database.
4. The paraffin embedded blocks and slides were retrieved from pathology archives and were reviewed. The relevant histological and immunohistochemistry findings were noted.
5. Immunohistochemistry was done using the following antibodies CD3, CD20 and MIB1. Further subtyping was done using CD10, bcl6 and MUM-1 in 51 cases of DLBCL-NOS with sufficient tissue.
6. Hans Algorithm3 was used for further subtyping of DLBCL-NOS.

RESULTS

- Total cases = 73
- All patients were immunocompetent.
- Gender ratio: M:F = 2:1
- Most of the patients opted for treatment elsewhere and none came for follow up.

Age & Sex Distribution

H&E sections showing classical angiocentric arrangement of atypical lymphoid infiltrate, displaying double chromatin and prominent nuclei (inset). The lymphoid cells are CD20 positive. Few CD20 positive reactive T-lymphocytes are seen in the background. The MIB-1 labelling is high 90%.

CONCLUSION

- Majority of the Primary CNS lymphomas (PCNSL) were Diffuse Large B cell lymphomas.
- On subtyping >60% were of the non-germinal centre B-cell type.
- The proportion of Germinal centre B cell type were more than that described in literature.

TAKE HOME MESSAGE

- There is a role for molecular subtyping of DLBCL particularly since the GCB subtype is reported to have a better prognosis.
- Further studies with adequate follow-up information are indicated to corroborate our findings and to assess the prognostic significance of these subtypes in the Indian population.
Does Adenosine 2A receptor (A2AR) have a bi-directional role in Traumatic Brain Injury (TBI)?

Parthiban K V 1, Muthukumar M 1, Victoria Job 2, and Srinivasa Babu K 1
1 Department of Neurological Sciences, 2 Department of Clinical Biochemistry, Christian Medical College, Vellore – Tamilnadu, India

Introduction:
TBI is a prevalent, debilitating health problem around the world. The immediate biomechanical damages the structural damage to the neurons. Secondary injury, consist protective cellular damage.
Potential reasons for poor translation of basic research results to the clinic include,
1) Complexities of multi-system traumas
2) A compromised blood brain barrier
3) Potential drug toxicities and side effects.
The failure of these paradigms indicates that understanding different approaches for the treatment of acute TBI are immensely needed.

Aim: To test the role of adenosine 2A receptor (A2AR) at different time windows in a TBI model

Materials and Methods:
- Wistar Rats were divided into four groups (Total n=96, each group n=24) i.e. Sham control, TBI group, A2AR agonist and A2AR antagonist treated groups.
- The effect of A2AR agonist and antagonist was tested at 15 mins, 1, 12 and 24 hrs post injury.
- Institutional animal ethics committee permission was obtained for this study.

Surgical Procedure:
1. All the animals were anaesthetized (i.p) with ketamine (50mg/kg) and xylazine (10 mg/kg).
2. Using an electric drill, make 4.8 mm circular shallow hole on the bone and affix the female Luer-lock adapter.
3. Animals were housed for 48 hrs to recover from the surgical pain.
4. All the surgical procedure were conducted under aseptic conditions only.

Induction of Injuries:
1. Animals were re-anesthetized and fill the female Luer Lock with sterile saline (no air bubbles).
2. Fluid pressure was set at 2.0 ± 0.5 atm using customized device and fluid delivered in a single shot for 20ms to induce an injury.
3. Animals were removed from the device and postoperative care were given with respect to any swelling, bleeding and close the scalp using sutures.

Therapeutic Procedure:
1. A2AR agonist (CGS 21680, 0.1 mg/kg – i.p) and A2AR antagonist (SCH 58261, 0.01 mg/kg – i.p) were treated post injury and leave it for 2 hrs.
2. Neurological functional score (NFS) and the levels of cAMP, IL-1β, oxidative stress markers and Caspase-3 were studied

Results:
1. Neurological Functional Score (NFS):
Mean NFS was performed in sham control, TBI, A2AR agonist and A2AR antagonist groups using eight point behavioral rating scale. Values expressed as Mean±SD. ** = p<0.01

2. cAMP assay:
A low pH ELISA kit was used to assay the cAMP level by non-acetylated method. Values expressed as Mean±SEM.

3. Inflammatory Reactions:
IL-1β was analyzed by solid phase enzyme linked immunosorbent assay (ELISA) kit based on the sandwich principle. Values expressed as Mean±SEM.

4. Oxidative stress markers:
Lipid peroxidation was seen by analyzing the MDA levels colorimetrically at 532nm by thiobarbituric Acid (TBA) assay kit. Values expressed as Mean±SEM.

5. Anti-oxidant enzyme activity:
Catalase activity was seen by H2O2 reacted with Oxid probe to produce a product which can be measured at 570nm colorimetrically. Values expressed as Mean±SEM.

6. Apoptotic Cell-death Assay:
Caspase -3 activity was seen by amount of cleavage of its substrate using fluorimetric method. The fluorescent will be measured at 480 – 505 nm. Values expressed as Mean±SEM.

Conclusion:
- A2AR agonist showed beneficial effect at early stages of post TBI period and A2AR antagonist showed beneficial effect at later stages.
- This suggests that A2AR agonist and antagonist can be used for beneficial purpose depending on the time at which TBI has occurred.

References:

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