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Scope of the Journal

The Sri Ramachandra Journal of Medicine - a scientific journal, entertains communications on all aspects of original biomedical research contributing to the advancement of knowledge in medical sciences. The scope of the journal allows publication of papers on medical education at undergraduate and postgraduate levels in either medical or paramedical courses; innovations in techniques; epidemiologic investigations and case reports. Readers are encouraged to write comments on papers published in the journal in the form of correspondence. Brief communication containing significant findings will be given priority. Review articles are also invited on topics of current interest. The journal is issued thrice in every calendar year. All papers are subjected to peer review by the Editorial Board and also experts in the field before acceptance for publication. All papers are accepted subjected to editorial changes.

Articles submitted to the journal should abide by the following manuscript submission guidelines.

Submission of Manuscript:

Each manuscript submission should include the following documents.

Part I - Title Page
Part II - Manuscript file
Part III - Acknowledgment, declaration by authors, patient consent and supplemental file.

All contents related to manuscript submission should be in English on a White paper of A4 size (210 x 297) with margins of 25mm (1 inch) wide on all the four sides. Print should be on one side only with double spacing throughout. Pages should be numbered consecutively, beginning with title page. Lettering should be in Times New Roman with a font size of 12. Three copies should be submitted to the editor. A copy of the title page and manuscript file must be emailed (as an attachment) with a covering letter address to the editor.

PART I - Title Page must include:

a) Title of the article
b) Name of each contributor with the highest degree and institutional affiliation.
c) Name, cellphone, e-mail of the corresponding author.

PART II - Manuscript file: Should include the text of the article followed by tables and figures. The table/figure number (eg: Table 1, Figure 1) should be appropriately mentioned in the text. The references should be numbered as they appear in the article and must be written in Vancouver style. The references should be kept after the tables/figures.

PART III - Acknowledgement: May include the names with details of affiliation, if any. They will appear in the article, but before the references.

Declaration by the authors: All the authors should submit a declaration regarding originality of the work, submission to other journals, whether the articles were already published and financial conflicts of interest which might influence the manuscript.

Supplemental file: These articles/texts which might help the review process, they should be relevant to the article submitted.

Nature of Articles - 1. Original articles: Articles of original research are welcome in this category. Articles should not exceed 4000 words. It must include an abstract of 250 words which should be structured as a) Aim of the study, b) Methodology, c) Results and d) Discussion. Minimum of three MesH words to be mentioned at the bottom of the abstract. Upto 50 references may be included in these articles.

2. Review articles: These articles addressing an issue/theme of current interest. They should not exceed 4000 words. Should include an unstructured abstract of 400 words with three MesH words. Article may include up to 100 references.

3. Case reports: Case reports reflecting a major clinical problem are welcome for this section. Word count should be restricted to 300 with references up to 5. May include 2 photographs and 1 table. Photographs having visible identification of patients must have written consent from the patient/close relatives. Case reports having more than 1 case will be given preference. Photographs should be at least 5 by 7 inches. Photographs may be submitted in a digital file, preferable in a JPEG (or) Tiff format. Photographs should be labeled appropriately.

4. Letter to the Editor: Correspondence to the editor regarding an article published in the journal are invited in this category. The content should be restricted to 300 words with references up to five.
On behalf of the editorial board we thank everybody from Sri Ramachandra University for their whole hearted help and cooperation in bringing out this edition of the journal.

We are getting good response from our faculty and in this edition we have four original articles, two brief communications, four case reports.

The articles cover entire spectrum of medical field. The original articles are quite interesting. The original articles are

- Important aspect of Methicillin resistant staphylococcus aureus infection in SRU,

- An Epidemiological study of hypertension in rural community,

- Human Interphase Lymphocyte DNA condensation employing mitotic extracts,

- Fungus as an etiology in Keratitis - our experience in SRMC.

I am sure it will encourage many more departments to send articles. We are not far away in getting this journal indexed.

Once again we thank every ones help, contribution for this journal

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The Editorial Board gratefully acknowledges their contribution.
ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS AT SRI RAMACHANDRA MEDICAL CENTRE

M. Shanthi *, Uma Sekar *

ABSTRACT:

BACKGROUND: Methicillin resistant Staphylococcus aureus (MRSA) is an important nosocomial pathogen causing significant mortality and morbidity. It is associated with a wide spectrum of infections ranging from mild skin and soft tissue infections to life threatening sepsis. Infected and colonised patients are the main reservoirs of infection and hand carriage by health care workers is the predominant mode of transmission.

AIM: This study was undertaken to determine the prevalence of MRSA and their susceptibility pattern at Sri Ramachandra Medical Centre.

METHODS: Forty isolates of Staphylococcus aureus were obtained from different clinical specimens from patients hospitalised for > 48 hours. They were screened for methicillin resistance by standard laboratory procedures. Susceptibility to beta lactams, aminoglycosides, macrolides, fluoroquinolones, glycopeptides and Oxazolidinones were determined by disc diffusion method. RESULTS AND DISCUSSION: Among the 40 Staphylococcus aureus isolates studied, 18(45%) were MRSA. The MRSA isolates were associated with a high degree of co-resistance to other groups of antimicrobial agents. Active screening and compliance with recommended infection control practices play an important role in the control of MRSA.

Key words: MRSA, Antimicrobial tests, infection control;

INTRODUCTION:

Staphylococcus aureus is one of the most common pathogens causing a variety of infections ranging from relatively benign skin infections to life threatening systemic illness such as pneumonia, endocarditis, septic arthritis, subcutaneous or visceral abscesses[1].

Before the introduction of penicillin in the late 1940s, Staphylococcal sepsis was associated with an extremely high mortality rate. Penicillin dramatically improved the prognosis of this infection[2]. However, penicillin resistant strains were discovered shortly and penicillin became ineffective both in the hospital and community settings[3,4]. The development of beta-lactamase resistant penicillins such as methicillin and oxacillin in the early 1960s once again revolutionized the treatment of Staphylococcal infections. Within a year of the use of methicillin, methicillin resistant Staphylococcus aureus(MRSA) strains were reported worldwide and over the next few decades, MRSA has reached epidemic proportions[5,6].

MRSA is a resistant variant of Staphylococcus aureus which has evolved an ability to survive treatment with beta lactam antibiotics which includes penicillin, methicillin and cephalosporins and to various other groups of antimicrobial agents. They are often referred to as super bugs. Most isolates remain susceptible to Glycopeptides (Vancomycin, Teicoplanin), Oxazolidinones (linezolid), Streptogramins (quinupristin-dalfopristin), and polycyclic compounds (tetracycline, tigecycline)[7,8].

MRSA is well recognised now as a major cause of nosocomial infections worldwide and these infections impose a high burden on health care resources[9]. A significant concern now is the spreading of MRSA in the community, possibly because of antibiotic pressure outside the hospital and transfer from hospital settings. Community acquired MRSA (CA-MRSA ) strains differ from health care associated MRSA (HA-MRSA) in that they are more frequently recovered from skin and soft tissue infections and also cause severe pneumonia in otherwise healthy patients[10,11].

Accurate and rapid identification of MRSA and their antimicrobial susceptibility profile is therefore necessary for the selection of appropriate therapy[12]. This study was carried out to determine the prevalence of MRSA and their susceptibility pattern to various antimicrobial agents.

MATERIALS AND METHODS

STUDY DESIGN: Staphylococcus aureus strains isolated from cultures of specimens from patients who have been hospitalised for > 48 hours were included in the study.

S. aureus were characterised by their morphology on Gram staining, growth characteristics and coagulase production.

PERIOD OF STUDY: July to August 2007

SOURCE OF ISOLATES: The source of the isolates were exudative specimens (pus, wound swabs, ear swabs and body fluids), blood, respiratory secretions and urine obtained from cultures of specimens from patients who had been hospitalised for > 48 hours.

SAMPLE EVALUATION: A total of 40 consecutive, clinically significant, nonrepetitive Staphylococcus aureus strains were included in the study.
METHODS: The *Staphylococcus aureus* isolates were subjected to susceptibility testing by disc diffusion technique according to the Clinical Laboratory Standards International (CLSI) guidelines with quality controls (*Staphylococcus aureus* ATCC 29213) [13].

The antimicrobials tested included ampicillin (10µg), cephalaxin(30µg), cefotaxime(30µg), clindamycin (30µg), gentamycin(10µg), amikacin(30µg), netilmicin(30µg), erythromycin (30µg), clindamycin(30µg), ciprofloxacin (5µg), chloramphenicol(30µg), vancomycin (30µg), teicoplanin (30µg) and linezolid(30µg).

Screening for MRSA

Methicillin resistance was screened by disc diffusion method using 30µg cefoxitin disk (Becton Dickinson). The diameter of the zone of inhibition was measured and interpretation was done in accordance with the CLSI guidelines. An isolate was considered to be a MRSA strain if cefoxitin inhibition zone diameter was < 21 mm[13].

Since *Staphylococcus aureus* can be a coloniser [6,7], special emphasis was laid on the clinical significance of all the isolates. This was done by correlating with Gram stained smear examination and ascertaining significance with the clinical history.

RESULTS

A significant proportion of the *Staphylococcus aureus* isolates were obtained from the exudative specimens such as pus(20), wound swabs(7), ear swab(3), Pericardial fluid(1) and drain fluid(1). Five isolates were from blood, two from urine and one from bronchoalveolar lavage(BAL).

Among the 40 *Staphylococcus aureus* isolates 82.5% showed resistance to ampicillin and cephalaxin (Beta lactamase producers), 65% were resistant to gentamycin, 62.5% to erythromycin, 60% to ciprofloxacin. Susceptibility to clindamycin, amikacin and netilmicyn were 65%, 77.5% and 75% respectively.

Of the 40 *Staphylococcus aureus* isolates 18 (45%) exhibited < 21 mm zone of inhibition to cefoxitin (30µg). They were considered as MRSA. All the MRSA were susceptible to vancomycin, teicoplanin, linezolid and chloramphenicol. Of the 18 MRSA isolates 66% (n = 12)were susceptible to clindamycin and 55.5% (n = 10) to amikacin and netilmicyn.

The source of the *Staphylococcus aureus* and MRSA from different clinical specimens is shown in figure 1.

The susceptibility pattern of *Staphylococcus aureus* is shown in Figure 2.

DISCUSSION

MRSA is a major cause of nosocomial infections worldwide. Serious endemic and epidemic MRSA infections occur globally as infected and colonised patients in the health care settings are the reservoirs. Transient hand carriage by the health care workers is the predominant mode for patient-to-patient transmission[7,14].

The risk factors for infection with MRSA that are unique to the hospital environment are well established. The emergence of MRSA as a cause of infection in the community (CA-MRSA) in patients who have never been hospitalised and who have no other risk factors for MRSA infection is a significant concern. CA-MRSA strains carry the gene for Panton -Valentine leucocidin (PVL) which has been associated with heightened virulence[11,15].

The prevalence of MRSA in this study was 45.5%. The prevalence from several centres in India as reported ranges from 20-80% [12,16,17,18]. In a surveillance study conducted simultaneously at three centres across India, using the same methodology, the MRSA isolation rates were 27%, 42.5% and 47% in each of the centres and the overall rate was 32%[19].

The prevalence of MRSA infections as reported by the National Nosocomial Infection Surveillance System (NNIS)
in The United States has been steadily increasing from 2.4% in 1974, 5% in 1981, 29% in 1991 to 43% in 1997[2]. In intensive care units (ICU) the proportion of MRSA isolates is between 59.5%-64.4%. Furthermore, the percentage of hospitals treating patients with MRSA infections is also increasing. In a survey by the Society for Health care Epidemiology of America in 1990, 97%, reported having managed patients with MRSA in their hospitals. An understanding of the magnitude of the problem requires accurate National estimates of incidence[3].

In this study majority of the MRSA isolates were from exudative specimens. There were four isolates from blood and one from bronchoalveolar lavage (BAL). Many investigators have reported an increase in the incidence of MRSA originating from wounds (pus)[14,16,17]. Blood stream infections caused by MRSA is also frequently reported and a cause of concern especially in patients with intravascular catheterisations[14,16]. In one study bacteremia occurred in 27% of patients with microbiologically documented primary sites of MRSA infection [6]. In the same study the body sites that were affected by overt MRSA infections were surgical sites (31.1%), pneumonia (27%), and endovascular catheter infections (20.3%). Approximately 25% of patients with MRSA infections had bacteremia but only 6.5% had overt septic shock[6].

Carriage of Staphylococcus aureus in the anterior nares plays a key role in the epidemiology and pathogenesis of infection. Patients with Diabetes mellitus, those on hemodialysis, IV drug abusers, patients with skin and soft tissue infections and those with HIV infection are at increased risk for carriage of Staphylococcus aureus in their anterior nares [20]. Up to 80% of cases of Staphylococcus aureus bacteremia are due to the strain isolated from the patients anterior nares [21]. A significant reduction in the rate of infection is achieved after nasal decolonisation in surgical and dialysis patients. Therefore it is necessary to screen high risk patients for Staphylococcus aureus carriage because they have a greater probability of infection[7,20].

Measures to control the spread of MRSA include swab sampling of the anterior nares, isolating colonised and infected patients until complete decolonisation and implementing hygienic precautions such as handwashing and antisepsis, the efficacy of which has been well established[7,14]. Application of mupirocin (2%) in the anterior nares twice daily for 5 days is highly efficacious in eliminating Staphylococcus aureus in both healthy carriers and carriers belonging to high risk groups[7].

Protective measures for health care workers against MRSA include contact isolation of the patient, using protective gown, gloves, mask and goggles and most importantly cleaning hands with alcoholic solution at glove removal and between patients. These measures are also of paramount importance to prevent the transmission of MRSA from patient-to-patient[7,20].

Methicillin resistance in Staphylococcus aureus is associated with the production of an altered low affinity penicillin binding protein PBP 2a encoded by the chromosomal mec gene complex. Because of its low beta lactam affinity, PBP 2a can take over the cell wall assembly when the normal Staphylococcal penicillin binding protein are blocked by the beta lactam compounds[7,8]. Expression of the methicillin resistance in the laboratory setting is subject to environmental conditions (ie) temperature, pH, incubation time and salt concentration in the medium. Conditional expression of PBP 2a gene may cause ambiguity in susceptibility testing. To complicate this issue further methicillin resistance is often expressed heterogeneously, masking the genetic information for resistance that the bacteria carry. Cells expressing heteroresistance grow more slowly than the oxacillin susceptibility population and may be missed at temperatures > 35°C. The microbiology laboratory has to take particular care in the identification of MRSA[7,8,12].

The conventional methods to detect MRSA in the Microbiology laboratory include oxacillin agar screen, disk diffusion using 1µg oxacillin disc or by minimum inhibitory concentration (MIC) testing[12,19].

Cefoxitin, a cephamycin is a more potent inducer of the PB2a and several groups of investigators have reported that the results of cefoxitin disk diffusion tests correlate better with the presence of mec gene responsible for methicillin resistance[5,22,23,24].

Errors in the detection of methicillin resistance can have adverse clinical consequences. False susceptibility results may result in treatment failure and the spread of MRSA if appropriate infection control measures are not applied. Conversely, false resistance results in increasing health care costs following unnecessary isolation precautions and overusage of glycopeptides. Hence accurate detection of MRSA is necessary[22].

Emergence of vancomycin and linezolid resistance among Staphylococcus aureus is an alarming threat. The prevalence of vancomycin intermediate Staphylococcus aureus (VISA) strains in India is reported to be 6.3%[25]. This has potential for increasing incidence and rapid spread, further complicating the treatment of Gram positive infections.

The limitations of this study are that, the isolates obtained after 48 hours of admission were included in the study. This could possibly include some strains the patients had acquired before admission. Clinical history was obtained for all the isolates to ascertain its clinical significance.

However risk factor and other contributing factors for acquisition was not obtained or analysed for selection of the isolates in the laboratory.

CONCLUSION
The prevalence of MRSA is 45.5% among clinical isolates of Staphylococcus aureus. Active screening and compliance with recommended infection control practices play an important role in the control of MRSA. Attention...
should be paid to halt the transmission of MRSA by health care workers by meticulous handwashing.

REFERENCES

HUMAN INTERPHASE LYMPHOCYTE DNA CONDENSATION EMPLOYING MITOTIC EXTRACTS
Maddaly Ravi a, Deepa Parvathi. V a, Govind Pai. M a, Sulogna Ghosh b, Preetha. B a, Venkateswaran N a and Solomon F.D Paul a

ABSTRACT

INTRODUCTION: Premature DNA condensation is a powerful cytogenetic tool. Various phosphorylulltically regulated cell cycle protein kinases have been characterized, separated and identified in extracts from mitotic and interphase cells. Interphase DNA condensation employing mitotic extracts might be a tool in not only understanding the condensation mechanisms but also will throw light upon the levels of these factors in the various stages of the cell cycle. In this study, we aimed at and have demonstrated DNA condensation capacities of CHO mitotic extracts in human interphase lymphocytes.

MATERIALS & METHODS: CHO cells at Mitotic and Interphase stages were harvested and cytosolic proteins extracted. Protein estimation of the CHO Interphase and Mitotic extracts was done by Bradford method. Protein profile analysis of the extracts was performed by SDS PAGE. Human Lymphocytes were isolated from peripheral blood by Ficoll density gradient method and were subjected to a range of mitotic extract concentrations (10, 20, 30, 40 and 50%). The slides were prepared and cells with varying degrees of condensation were scored and photo documented. A total of 1000 cells per concentration were analyzed for the degree of condensation.

RESULTS: The quantum of interphase human lymphocyte DNA condensation showed a distinct increase from 20% to 50% mitotic extract treatment. It was observed that maximal condensation and also number of cells with such DNA condensation was most prominent in lymphocytes subjected to 50% mitotic extract.

CONCLUSION: Induction of PCC although is achieved to a great degree by somatic cell hybridization techniques, the same if done through isolated proteins might give us a better understanding of finer molecular factors. This also will eliminate the fusion efficiency limitations as is determined by the ratios of fusion partners. Interference of homologous entities when cells of similar phylogenetic lineages are used either for somatic cell hybridizations or for employing specific condensation factors will also be eliminated. Our results show that mitotic cytosolic extracts have potential PCC properties thus paving a path to circumvent cell hybridizations for the same; having wide applications in genotoxicity studies.

KEY WORDS: Mitotic Extracts, DNA condensation cell cycle proteins, Human lymphocytes

INTRODUCTION:

Premature Chromosome Condensation (PCC) has been shown to be a powerful cytogenetic and cytokinetic tool in understanding cell-cycle analysis(1) and also for diagnostic purposes(2,3). Although PCC is a useful cytogenetic tool, it involves somatic cell hybridizations employing a mitotic partner. Understanding of the factors responsible for chromatin condensation and their mechanism of action can throw better light on the cell cycle kinetics and also the protein repertoire of the cells in the various phases of the cell cycle. A number of proteins involved at various levels of cell cycle have been characterized and a variety of protein kinases have been separated and identified in extracts from mitotic and interphase cells. It is also understood that protein phosphorylation / dephosphorylation may be integral to the mechanism of chromosome condensation(4). The induction of PCC in cells using chemicals was well demonstrated and some of the chemical agents employed are protein phosphatase inhibitors (Okadaic Acid, Calyculin A, Fosteirecin, etc.)(5,6).

Protein profiles in the various stages of the cell cycle revealed that in normal cell-cycle, an acidic protein with a molecular weight 35 KDa was specifically associated with chromosome condensation at the mitotic phase(7). It was also found that the condensation factor was cytoplasmic, which is present throughout the cell cycle, but enters the nuclear membrane causing condensation of chromatin during the mitotic stage(8). These factors are collectively known as the “Structural Maintenance of Chromosomes” (SMC). It was shown that mitotic chromosomes are composed of five histones and a large number of non-histone proteins that maintain the chromosomal structural integrity. It has been demonstrated that chromosome “Scaffold Proteins” are important to maintain the condensed structure of the mitotic chromosomes(9).

Many experiments were reported which have employed cytoplasmic extracts from a variety of mitotic cells and their ability to induce condensation of interphase chromatin. It was also shown that these factors responsible for interphase chromatin condensation do not have cross-species barriers or specificity, but rather being able to induce condensation of interphase chromatin across species boundaries and limitations(10).
Interphase chromatin condensation employing mitotic extracts might be a useful tool not only understanding the condensation mechanisms but also will throw light upon the levels of these factors in the various stages of the cell cycle. Also, demonstration of cross-species condensation induction might be useful in the relative ease of collection of such factors and employment of the same for human health care apart from a better understanding of such phenomena.

MATERIALS AND METHODS:

CHO cells were cultured by standard procedures. DMEM supplemented with 5% Serum was used as the culture medium and were maintained in 5 % CO$_2$ at 37°C. The same were passaged by routine trypsinization protocols and a continuous culture was maintained. At optimal levels of confluence, CHO cells at Mitotic and Interphase stages were harvested and cytosolic proteins extracted. The mitotic cells were collected by gentle agitation of the flasks and interphase cells collected by tripisnization, were washed once in serum-free medium. The cell pellet was agitated by hand homogenization in a Tarson cell grinding pestle and spun again. The supernatant was collected and left for dialysis in Phosphate Buffered Saline. Further to protein estimation, the two were analyzed for their protein profiles by SDS Page.

Protein estimation of the CHO Interphase and Mitotic extracts was done by Bradford method(11). Bovine Gamma Globulin of 0.225, 0.325, 0.75, 1.125 and 1.50 mg/ml concentrations were used as standards. Protein profile analysis of the extracts was performed by SDS PAGE. A 4% stacking Gel and 10% separating gel was used for optimal resolutions of the separated protein components. Protein molecular marker of medium range (14.3 - 66 KDa) was used. The gel was stained by Coomassie blue and was scanned and analyzed.

Heparinised human peripheral blood was collected by vein-puncture and lymphocytes isolated by Ficoll density. The cell aliquots were subjected to a range of mitotic extract concentrations (the original extract was diluted in PBS to obtain various concentrations) and were incubated for 30 minutes in 4°C followed by 2 hours at 37°C. The concentrations of the Mitotic Extracts employed were 10, 20, 30, 40 and 50%. The same were washed and harvested according to standard methods for cytogenetic analysis(12). The slides were prepared and cells with varying degrees of condensation were scored and photo documented. A total of 1000 cells per concentration were analyzed. According to the levels of the DNA condensation, the cells were positioned in four categories [1. No Condensation (NC), 2. Low Condensation (LC), 3. Intermediate Condensation (IC) and 4. Maximal Condensation (MC)]. The differentiation between the categories was based on visual microscopic observation as represented in figure 4. The percentage of cells in the four categories and for each of the concentrations were calculated and the results analyzed.

RESULTS:

Protein quantification of CHO interphase and mitotic extracts showed values of 0.54 and 0.6 mg/ml respectively. Further to SDS PAGE analysis, a comparison of protein profiles was made to identify proteins unique to mitotic cell extracts. 4 distinct proteins of higher molecular weights (between 28 KDa and 50 KDa) and a group of low molecular weight proteins (between 14 – 20 KDa) were observed unique to mitotic extracts (whose corresponding bands were absent in the interphase cell extract). Of these unique mitotic proteins, 2 of molecular weights 38 KDa and 26 KDa were of significant quantities. (Figure 1).

The quantum of interphase human lymphocyte DNA condensation showed a distinct increase from 20% to 50% mitotic extract treatment. A 10 % concentration showed no condensation and values matched control samples. The DNA condensation capacity of the various concentrations of the mitotic extract and levels of condensation are represented in (Figure 2). Maximal condensation was found in lymphocytes treated with 50 % mitotic extract.
It was observed that maximal condensation and also number of cells with such DNA condensation was most prominent in lymphocytes subjected to 50% mitotic extract.

**DISCUSSION:**

Protein profiles during cell cycle are critical for the various cellular mechanisms and proper regulations of the same. Differences in protein repertoire of a cell in its interphase and mitotic phase are important for the various changes necessary for DNA condensation leading to karyokinesis during cell division. This is very clear by the premature chromosome condensation capacity of mitotic extracts in interphase cells and also by the chromosome de-condensation properties of interphase extracts on mitotic cells (13).

Induction of DNA condensation although is achieved to a great degree by somatic cell hybridization techniques, the same if done through isolated proteins will give us a better understanding of the finer molecular factors responsible for the same. This also will eliminate the fusion efficiency limitations as determined by the ratios of the fusion partners (13).

Injection of mitotic extracts into interphase cells and subsequent condensation of interphase DNA has been demonstrated (10). However if large quantities of cells with condensed interphase DNA were required for any study, such an approach would have inherent disadvantages. We therefore have attempted to cause simultaneous DNA condensation of a population of interphase cells by mere subjection of such cells to mitotic extracts with a suitable incubation and processing protocols. Even a maximal condensation index of about 10% of cells would be useful for a variety of reasons. The fact that mitotic extracts of any cell type can induce DNA premature condensation in cells of different phylogenetic lineages becomes an added advantage of this technique. This also eliminates the possible interference of homologous entities when cells of the same phylogenetic lineages are used either for somatic cell hybridizations or for employing specific condensation factors.

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AN EPIDEMIOLOGICAL STUDY OF HYPERTENSION IN A RURAL HOUSEHOLD COMMUNITY

L. Kannan\textsuperscript{a}, T.S. Satyamoorthy\textsuperscript{b}

ABSTRACT

BACKGROUND and OBJECTIVES

Chronic Non-Communicable diseases are important among adult population all over the world. The prevalence of chronic diseases like hypertension, diabetes etc, is showing an upward trend. Hence the study was undertaken, to measure the prevalence of hypertension and identify the risk factors.

METHODS

A cross sectional study was carried out among the adults in the age group of 30 years and above, residing under Mugalivakkam Primary Health Centre (PHC) area of Kancheepuram District, Tamilnadu. The mid-year population for 2002 covered in this PHC area was 40,850, out of which 12,051 were adults in the age group of 30 years and above (29.5%). By using cluster-sampling method, 750 individuals were selected and studied. Data entry was made using SPSS and Epi-info.

RESULTS

189 individuals (25.2\%) were found to have hypertension including 93 known hypertensives. Among 357 adult males, 81 (22.6\%) and among 393 adult females 108 (27.4\%) were found to have hypertension. The odds ratio for hypertension among Smokers were 2.4 (1.52-3.61) and was statistically significant (P<0.003), similarly alcohol use, obesity, tobacco chewing, diabetes and oral contraceptive use were statistically significant.

CONCLUSION

The prevalence rate of hypertension was 25.2\%. The prevalence rate was higher (27.4\%) among females. Increase in age, family size, occupation, alcohol, smoking, chewing tobacco, obesity, oral contraceptives use and diabetes mellitus have been found to have association. Hence health care providers should take note and institute appropriate preventive measures.

Keywords: hypertension, cross sectional study, rural population

INTRODUCTION:

Although blood pressure is easily measurable it has taken several decades to realize that hypertension is a frequent world wide health disorder\cite{1}. Chronic Non-Communicable diseases are assuming greater importance among adult population in developed as well as developing countries. The prevalence of chronic diseases such as hypertension, diabetes mellitus etc, is showing an upward trend in most countries. The main factors responsible for this rising trend are changing life styles, obesity, and behaviour pattern of people etc\cite{2}. The Joint National Committee reports on prevention, detection, evaluation and treatment of high blood pressure (JNC – VI & VII) emphasized the necessity of clinicians’ appropriate judgment of their patients in diagnosis and treatment. Further, these guidelines for primary care physicians have been specified in the report. Therefore this national guideline should serve as a tool to be adopted and implemented in local and individual situation\cite{3}.

Even though 72.2\% of Indian populations live in rural areas\cite{4}, many studies have not been carried out to determine the prevalence of hypertension among rural folks and the contributing risk factors, if any. The present study was undertaken, to measure the prevalence of hypertension and to identify the risk factors responsible with reference to medical, social and individual characteristics, with a view to formulate preventive measures especially applicable to rural population.

MATERIALS & METHODS:

Place of study: The study was conducted in areas under the jurisdiction of Primary Health Centre, Mugalivakkam covering five sub centers namely Mugalivakkam, Mowlivakkam, Manapakkam, Gerugampakkam and Kolapakkam.

Study population: The study population comprised of adult individuals in the age group of 30 years and above residing under Mugalivakkam Primary Health Centre area of Kancheepuram District, Tamilnadu. The mid year population for 2002 covered in this Primary Health Centre area was 40,850, out of which 12,051 (29.5\%) were adults in the age group of 30 years and above residing in this area.

Sampling methods & Sample size: The sampling methods used in the study were cluster-sampling method. This method was used according to their sub divisions of “Nagar” and colony were identified and 30 cluster sampling technique. With the available studies relating to prevalence of hypertension in India, the prevalence of 24\%\cite{5} was taken for estimating the sample size requirement with limit of accuracy as 18\% of prevalence and a design effect of 2, accordingly the minimum sample size required for the study was found to be 750 individuals.
**Data collection:** Data for this study was collected from the community, by visiting the house holds in the selected area. Since the sampling was done during the day time, there may be a sampling bias. It was a household based study and questionnaires were administered to those in the household and the necessary information were collected to meet the objectives of the study. The data were collected from the selected 30 clusters as designated by the probability proportionate to size (PPS) method[6]. So, 25 individuals from the each cluster were selected randomly to attain the required sample size of 750 for the study. On an average, three families were examined during each day of the visit. The medico social history and other required detailed were filled up in the Proforma.

The following techniques were used as per the recommendation of Joint National Committee (JNC) VI Criteria[3].

1. The individual was seated in a chair with his back supported and his arms bared and supported at heart level and was refrained from the use of tobacco in any form or ingestion of caffeine during the 30 minutes preceding the measurement.

2. Under special circumstances measuring blood pressure in the supine and standing position were indicated. The appropriate (RIVA-ROCCI) cuff size was used to ensure an accurate measurement. The inflatable rubber bag within the cuff was encircling at least 80% of the arm. Many adults required a large adult cuff. Measurements were taken preferably with mercury sphygmomanometer. Both systolic and diastolic blood pressure was recorded.

3. The first appearance of sound [phase I] is used to define systolic blood pressure. The disappearance of sound [phase VI] is used to define diastolic blood pressure. Two or more reading was be repeated after 3-5 minutes interval. If the first two readings differ by more than 5 mm of Hg, additional readings were obtained and averaged.

After following the measurement guidelines of blood pressure prescribed by JNC-VI criteria, the classification of hypertension was done as per the JNC-VII guidelines[18], as there was no proper cut-off point for deciding the category of prehypertensives in JNC-VI.

Those found to have hypertension were examined again after 2 days in the similar manner to confirm that hypertension was constant. The individuals were not informed of the results of the previous screening. However the newly diagnosed hypertensive individuals were referred to the primary health center for further investigations and management. The known hypertensive cases were emphasized to continue their regular treatment.

**Limitation of the study:** Investigation of urine and blood analysis and ophthalmoscope examinations could not be carried out due to various constraints.

**STATISTICAL ANALYSIS**

Method of data analysis was done by using SPSS software 8. Version between the two gender groups in relationship to different stages of hypertension and normal individuals, by using appropriate test of significance (Chi Square) with 95% Confidence level and $p<0.05$ level. In case of associated risk factor, subgroup analysis was done for different groups like family size, occupation, smoking, tobacco chewers, alcohol use, obesity, diabetes and oral contraceptive use was done with calculation of odds ratio, 95% CI and $p<0.05$ level. Epi-info 2000 Center for Disease Control software was used for doing trend analysis.

**RESULTS**

Out of 12,051 adults in the age group of 30 years and above residing under Primary Health Centre, Mugalivakkam, 750 individuals (6.2%) were studied.

Out of 750 adults surveyed, 189 individuals (25.2%) were found to be suffering from hypertension including 93 known hypertensives. Among 357 adult males, 81 (22.6%) and among 393 adult females, 108 (27.4%) were found to be suffering from hypertension. The majority of hypertensives (25.2%) had both systolic and diastolic hypertension followed by 20.4% who had only systolic and 19.2% who had only diastolic hypertension. Both systolic and diastolic pressures are important criteria for classification of hypertension.

Among 357 males studied, 233 (65.26%) were prehypertensives (systolic pressure 120-139 mm of Hg and diastolic pressure 80-89) followed by 61 (17.08%) who were stage I hypertensives (systolic pressure 140-159 mm of Hg and diastolic pressure 90-99 mm of Hg) and 20 (5.60%) were stage II (systolic pressure > 160 mm of Hg and diastolic blood pressure > 100 of Hg) hypertensives. Of the 393 females, 196 (49.87%) were prehypertensives followed by 67 (17.04%) in stage I hypertension (systolic pressure 140-159 mm of Hg and diastolic pressure 90-100 mm of Hg) and 41 (10.4%) were stage II (systolic pressure > 160 mm of Hg and diastolic blood pressure > 100 mm of Hg) hypertensives (Table 1).

**Table 1. Distribution of hypertensive cases by sex.**

<table>
<thead>
<tr>
<th>Sex</th>
<th>Normal</th>
<th>Pre-hypertension</th>
<th>Stage-I hypertension</th>
<th>Stage-II hypertension</th>
<th>Total No (%)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>357 (47.6)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>43</td>
<td>233</td>
<td>61</td>
<td>20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>393 (52.4)</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>89</td>
<td>196</td>
<td>67</td>
<td>41</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>132</td>
<td>429</td>
<td>128</td>
<td>61</td>
<td>750 (100)</td>
<td></td>
</tr>
</tbody>
</table>

Note: Figures in parentheses denote percentages.
It has been observed from table 2 that the prevalence rate of hypertension shows an upward trend as age advances in males as well as in females. The difference was found to be statistically significant (p<0.001), which indicates that age and sex have some influence in the association of hypertension.

Table 2. Distribution of hypertensive subjects as per age among both sexes

<table>
<thead>
<tr>
<th>Age in years</th>
<th>Total surveyed</th>
<th>Hypertension No (%)</th>
<th>95%CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30-39</td>
<td>146</td>
<td>9(6.16)</td>
<td>2.26-10.07</td>
<td></td>
</tr>
<tr>
<td>40-49</td>
<td>97</td>
<td>16(16.4)</td>
<td>9.11-23.8</td>
<td></td>
</tr>
<tr>
<td>50-59</td>
<td>58</td>
<td>22(37.9)</td>
<td>25.44-50.42</td>
<td></td>
</tr>
<tr>
<td>60-69</td>
<td>42</td>
<td>24(57.1)</td>
<td>42.18-72.11</td>
<td></td>
</tr>
<tr>
<td>&gt;70</td>
<td>14</td>
<td>10(71.4)</td>
<td>47.76-95.09</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>357</td>
<td>81(22.6)</td>
<td>18.34-27.03</td>
<td></td>
</tr>
</tbody>
</table>

Female

<table>
<thead>
<tr>
<th>Age in years</th>
<th>Total surveyed</th>
<th>Hypertension No (%)</th>
<th>95%CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>30-39</td>
<td>173</td>
<td>23(13.2)</td>
<td>8.24-15.6</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>40-49</td>
<td>85</td>
<td>24(28.2)</td>
<td>18.67-37.8</td>
<td></td>
</tr>
<tr>
<td>50-59</td>
<td>72</td>
<td>23(31.9)</td>
<td>21.17-42.71</td>
<td></td>
</tr>
<tr>
<td>60-69</td>
<td>50</td>
<td>30(60)</td>
<td>46.42-73.58</td>
<td></td>
</tr>
<tr>
<td>&gt;70</td>
<td>13</td>
<td>8(61.5)</td>
<td>35.09-87.99</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>393</td>
<td>108(27.4)</td>
<td>23.07-31.89</td>
<td></td>
</tr>
</tbody>
</table>

It has been observed from table 2 that the prevalence rate of hypertension shows an upward trend as age advances in males as well as in females. The difference was found to be statistically significant (p < 0.001), which indicates that age and sex have some influence in the association of hypertension.

It has been observed from Table 3 that the prevalence of hypertension was higher (41.07%) among those living in house holds having 6 or more members compared to individuals living in house holds having 5 or less members (22.42%). Greater prevalence of hypertension was observed in unemployed (31.3) and unskilled (31.8) category of occupation followed by professional (22.05), semiprofessional (20.0) and skilled labourers (18.7) of the selected population. The higher prevalence in the unemployed and unskilled category is statistically significant as compared to the other three categories of the study population.

**Hypertension and risk factors**

Table 3 shows the prevalence of hypertension to be higher among smokers 48(40.33%) and 141(22.3%) in non-smokers. These differences were found to be statistically significant. The prevalence of hypertension was higher 33.3% among those who were in the habit of chewing tobacco for more than 5 years as compared to 31.6% who had this habit for less than 5 years. The lower prevalence rate of hypertension among non-tobacco chewers was 23.5%. It has been found out of 750 individuals, 79(10.5%) were found to be consuming alcohol. Among the drinkers 41 (51.8%) had hypertension followed by 34(43.0%) who were pre hypertensives, only 4(5.0%) had normal blood pressure. Out of 189 hypertensives, 75 (39.6%) of hypertensive individuals had normal/expected weight, 20 (10.5%) were under weights, 55(29.1%) were pre-obese, 26(13.7%) were class I obese and 11(5.8%) were class II obese. Only 2 individuals were in class III obesity, these difference were highly significant p < 0.001.

**Table 3. The comparative figures of various factors in relation to hypertension.**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Hypertensive</th>
<th>Non hypertensive</th>
<th>Odds ratio</th>
<th>95%CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Family Size</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;5</td>
<td>143</td>
<td>495</td>
<td>0.41</td>
<td>0.27-0.64</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>&gt;6</td>
<td>46</td>
<td>66</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Occupation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Un employed</td>
<td>77</td>
<td>169</td>
<td>25.56-37.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Professional</td>
<td>15</td>
<td>53</td>
<td>12.9-33.76</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Semiprofessional</td>
<td>13</td>
<td>52</td>
<td>11.1-31.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skilled</td>
<td>49</td>
<td>238</td>
<td>12.9-21.94</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unskilled</td>
<td>35</td>
<td>73 (75)</td>
<td>23.7-42.09</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smokers</td>
<td>48</td>
<td>71</td>
<td>2.4</td>
<td>1.5-3.61</td>
<td>&lt;0.003</td>
</tr>
<tr>
<td>Non smokers</td>
<td>141</td>
<td>490</td>
<td>1.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tobacco</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tobacco chewers</td>
<td>47</td>
<td>101</td>
<td>1.0</td>
<td>0.99-2.27</td>
<td>0.040</td>
</tr>
<tr>
<td>Non-Tobacco chewers</td>
<td>142</td>
<td>460</td>
<td>1.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alcohol</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alcoholic</td>
<td>41</td>
<td>38</td>
<td>3.812</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non alcoholic</td>
<td>148</td>
<td>523</td>
<td>1.0</td>
<td>2.36-6.15</td>
<td>0.001</td>
</tr>
<tr>
<td>Obesity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Obese</td>
<td>39</td>
<td>48</td>
<td>2.778</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non obese</td>
<td>150</td>
<td>513</td>
<td>1.0</td>
<td>1.70-4.51</td>
<td>0.001</td>
</tr>
<tr>
<td>Diabetes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetic</td>
<td>48</td>
<td>41</td>
<td>4.32</td>
<td>2.66-7.00</td>
<td>0.001</td>
</tr>
<tr>
<td>Non diabetic</td>
<td>141</td>
<td>520</td>
<td>1.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oral contraceptive</td>
<td>Users</td>
<td>21</td>
<td>23</td>
<td>2.749</td>
<td>1.37-5.47</td>
</tr>
<tr>
<td>Non users</td>
<td>87</td>
<td>262</td>
<td>1.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Figure 1. The comparative figures of hypertension by various risk factors**

were pre hypertensives, only 4(5.0%) had normal blood pressure. Out of 189 hypertensives, 75 (39.6%) of hypertensive individuals had normal/expected weight, 20 (10.5%) were under weights, 55(29.1%) were pre-obese, 26(13.7%) were class I obese and 11(5.8%) were class II obese. Only 2 individuals were in class III obesity, these difference were highly significant p < 0.001.
Among the diabetics, a majority of 48 (26.46%) individuals were found to be in stage I and II of hypertension and were followed by 30 (6.99%) who were prehypertensives. Only 11 (8.33%) were having normal blood pressure.

Among 393 females studied, only 44 (11.9%) were taking oral contraceptives and among these individuals 41 (93.18%) were taking oral contraceptive pills for less than 3 years and the remaining 3 (6.8%) were taking oral contraceptive pills for more than 3 years. The prevalence of hypertension was 66.6% among those who were taking oral contraceptive pills for more than 3 years and it was 46.3% among those taking the oral contraceptive pills for less than 3 years.

Smokers were 2.4 times at a greater risk for hypertension compared to non-smokers (Table 3) and the difference was statistically significant, similarly alcohol use, obesity, tobacco chewing, environmental stress, anxiety, diabetes and oral contraceptive use were found to have statistically significant higher risk for hypertension.

**DISCUSSION:**

Until recently hypertension was considered to be one of the important public health problems in the developed and industrialized countries only. In the developing countries, its impact was not fully felt due to presence of rampant communicable diseases. However with control of communicable disease and increased life expectancy with life style changes, hypertension is becoming one of the emerging problems with its implications for concomitant increase in risk of cardiovascular and renal disease.

In the present study, out of 750 individuals examined 189 individuals (including 93 old hypertensive cases) were found to be suffering from hypertension and over all prevalence rate of hypertension was found to be 25.2 percent among both sexes. However the prevalence of hypertension was 22.6 percent among males (81 cases in 357 males) and 27.4 percent was among females (108 cases in 393 females).

Different studies carried out by Indian workers revealed varying prevalence rate of hypertension among adult population depending upon the criteria taken for classification, age group and type of population studied. The prevalence rate of hypertension reported in the present study 25.2% was the highest as compared to other studies in rural populations of India. The criteria taken for labeling hypertension (that is > 140 / 90 mm of Hg.), the increasing global trend in high prevalence of hypertension including rural areas due to changing life styles, stress and strain of life. The awareness of the problem and the co-operation of rural population for subjecting themselves readily for medical examination might be some of the factors for finding the high prevalence of hypertension in the present study. According to WHO scientific group 10-20 percent of adults in the world have hypertension based on the criteria suggested by their expert committee[7]. In the developing countries[3] the prevalence rate of hypertension varied from 15 to 33 percent[8]. Thus the prevalence rate of hypertension differs from population to population depending upon the cut off point used.

The prevalence rate of hypertension as shown in Table 2 showed an upward trend as age advances in both sexes. This proportionate increase of prevalence of hypertension as age advances in both sexes has been observed in many other studies. Joshi et al[2] observed in a study in Mumbai that there was an increasing trend of hypertension as age advances and even in post menopausal women, as the prevalence rate rose from 4% among young to 17.2% in 60 years of age group and similar findings was also contributed by Shakuntala Chockalingam[9] among both sexes.

Increased family size has got a positive association with hypertension. The difference was found to be highly significant. There was no such observation made in the earlier studies.

The occupational status has been found to have association with hypertension in the present study. Those who were in professional group had lesser prevalence rate as compared to other category of occupation. On the contrary Padmavathi and Guptha[10] at Delhi, Gosh & Joshi et al[11] at Simla found that hypertension was more common in professional group as compared to unskilled and semiskilled groups, but Mill et al[12] did not find any significant association between occupational status and hypertension. Probably, the level of occupation may materially affect physical activity and other aspect of life in relation to hypertension.

In the present study among hypertensives, smoking and tobacco chovers had important risk factor for the association of hypertension and the difference was statistically significant compared to nonsmokers and non tobacco chovers. Studies done by Benovitz Neal L [13] observed that sodium absorption was higher (107 mmol/day) among persons who smoked cigarettes and chewed tobacco as compared to non-tobacco users. This increased sodium absorption in the body due to tobacco use, has got some role in association of hypertension.

Among the over all alcoholics, 4 (5.06%) were normotensives followed by 34 (43.03%) who were prehypertensive and remaining 41 (51.8%) were found to have higher prevalence of hypertension. Statistical analysis was found to be significant indicating that alcohol is one of the risk factors in association with hypertension compared to non-alcoholics. Fried-man[14] reported similar finding that excessive alcohol intake is related to development of hypertension.

Obesity is one of the risk factors for hypertension, and this has been observed in this study, as increased BMI was resulting in hypertension in the selected subjects. Similarly, Friedman et al[14] carried out a 6-year follow-up study on
hypothesis and obesity and found that obesity and weight gain were clear precursors of hypertension.

In the present study (table 3) among hypertensive, 48 (25.3%) were suffering from diabetes mellitus. Among the over all diabetics, 11 (12.3%) were normotensives followed by 30(33.7%) were prehypertensives and 48(53.93%) were in stage I and stage II hypertension. On statistical analysis the difference was found to be significant indicating that hypertension and diabetes mellitus have got an association, similarly Raj B singh[16] et al carried out a study among ethnic groups of rural and urban population of North India and revealed that the diabetes mellitus was risk factor for hypertension.

Oral contraceptive usage was found to be a significant risk factor in causation of hypertension. Among female hypertensive, 44(40.7%) were using oral contraceptives. In their usage 41(93.18%) were taking oral contraceptive for less than 3 years and 3(6.8%) were taking oral contraceptive for more than 3 years. The difference was less significant indicating that long term usage of oral contraceptive had an association. On the contrary, studies carried out in China[17] revealed that prevalence rate of hypertension was significantly higher with long term usage of oral contraceptive and the mean systolic pressure and diastolic pressure in groups on oral contraceptive pills were higher by 6.5 mm of Hg and 3.24 mm of Hg respectively as compared to blood pressure readings in control group.

The over all prevalence rate of hypertension in the rural community studied, among the age group 30 years and above in both sexes was 25.2 percent. The prevalence rate was higher (27.4%) among females than males (22.6%). Increase in age, large family size, occupation, alcohol, smoking, chewing tobacco, obesity, use of oral contraceptives and diabetes mellitus were found to be associated with hypertension. There is a necessity for the health care providers to take note of this trend and institute appropriate preventive measures, including changing life style modifications.

REFERENCES
FUNGUS AS AN ETIOLOGY IN KERATITIS- OUR EXPERIENCE IN SRMC


ABSTRACT

PURPOSE: To evaluate the role of fungus in the etiology in patients with corneal ulcers attending Ophthalmology OPD in SRMC, a tertiary care centre in Chennai.

METHODS: All patients with keratitis who presented from July 2006 to 31st May 2008 were evaluated. They were examined by slit-lamp biomicroscopy and corneal scrapings which were taken for cultures and smears by using standard protocols.

RESULTS: During this period, 300 patients with corneal ulcerations were registered and 45(15%) diagnosed as microbial keratitis were evaluated. Out of 45 corneal ulcers cultured, 10(22%) were found to be bacteria, 20(44%) were found to be fungi, 3(6.7%) were found to be mixed with bacteria and fungi, and the remaining 12(26.7%) were found to be culture negative. Both gram positive cocci and gram positive bacilli were seen, with one case of gram negative bacilli (Pseudomonas spp). The predominant fungal pathogens isolated were Aspergillus fumigatus 8 (40%) followed by Fusarium solani 7 (35%).

CONCLUSION: Fungal infections occurred with an increased frequency when compared to the bacterial infections, the predominant fungal pathogens being Aspergillus fumigatus followed by Fusarium solani. The findings of our study show that there is a region wise variation in the predominance of corneal pathogens. This has an important public health implication for the initiation of therapy.

Key words: Fungal, keratitis, microbial

INTRODUCTION:

Microbial keratitis is the most common serious ocular infection and may be caused by a variety of bacteria, fungi, viruses or parasites.

Corneal infection is a leading cause of ocular morbidity and blindness worldwide. Corneal ulceration is a major cause of monocular blindness in developing countries (1,2). A recent report on the causes of blindness worldwide consistently lists corneal scarring as second only to cataract as the major etiology of blindness and visual disability in many of the developing nations like Asia, Africa and the Middle East (3).

Almost any microorganism can invade the corneal stroma, if the normal corneal defense mechanisms, i.e. lids, tear film and corneal epithelium are compromised (4). A wide spectrum of microbial organisms can produce corneal infections and consequently the therapeutic strategies may be variable (5). One of the key elements in this effort is a proper understanding of the microbiological and clinical characteristics of this disease entity which will enable the ophthalmologist to initiate appropriate antimicrobial therapy (5).

Considering the importance of corneal ulceration as a world wide cause of visual loss, there are surprisingly few studies evaluating the aetiological factors predisposing a population to corneal infection (6,7,8). The majority of bacteria cultured from infections of the cornea are of the same species that normally are present in the conjunctival sac, on the lids or periocular skin, and in the adjacent nasal passages. Their incidence may vary geographically (9,10,11). The purpose of this study was to evaluate the current microbial pathogens of all infectious corneal ulcers seen at a tertiary referral centre in south India, during a period of 23 months and compare these profiles with other series.

MATERIALS AND METHODS:

Clinical specimen and method of collection:

Corneal scrapings taken from patients suffering from keratitis (fig. 1) were subjected to microbial culture when at least one of the following was present:

- Size: infiltrate with > 2mm epithelial defect
- Location: infiltrate > 2 mm from the limbus
- Depth: infiltrate > 20% of the corneal thickness
- Associated findings: anterior chamber reaction > grade 2
- Organic trauma
- Atypical ulcerations: younger individuals and children
- Failure to regress with in 24 hrs

After a detailed ocular examination corneal scrapings were collected under aseptic conditions from each ulcer by an ophthalmologist after instillation of 4% paracaine eye drop without preservative, using a sterile Bard Parker blade (No 15). The procedure was performed under magnification of slit-lamp or operating microscope. The scraping material obtained from leading edge and base of each ulcer was initially directly inoculated onto the surface of solid media such as sheep’s blood agar, chocolate agar and Sabouraud’s...
dextrose agar in a row of C-shaped streaks (6,12). The material obtained by the next scraping was spread onto labeled slides in a thin, even manner for 10% potassium hydroxide (KOH) wet mount (Fig 2) and Gram staining. Meticulous care was taken in the collection of material and transferring it to the appropriate culture media aseptically.

LABORATORY PROCEDURES:

All inoculated media were incubated aerobically. The inoculated media - blood agar, chocolate agar were incubated at 37°C and were evaluated at 24 hours and at 48 hours and later discarded if there was no growth. The inoculated fungal media-Sabouraud’s dextrose agar was incubated at 25°C, examined daily, and discarded at 3 weeks if no growth was seen. Microbial cultures were considered positive only if at least one of the following criteria were met (5).

a. The growth of the same organism was demonstrated on two or more solid media on the C-streak; or there was semiconfluent growth at the site of inoculation on one solid medium, (Fig 3)
b. The same organism was grown from repeated scraping,
c. It was consistent with clinical signs,
d. Smear results were consistent with cultures.

Cultures for Staphylococcus epidermidis and Corynebacterium spp. were considered positive only if there was moderate growth on at least two solid media. The specific identification of bacterial pathogens was based on microscopic morphology, staining characteristics, and biochemical properties using standard laboratory criteria (12).

The etiological agent was identified using KOH mount and gram’s stain and preliminary report was given to the clinician based on which, the treatment was started with the appropriate antifungal agent. The diagnosis was then confirmed with culture on appropriate culture media.

FUNGAL CULTURE IDENTIFICATION:

Macroscopic appearance

The major macroscopic features remarkable in species identification are the growth rate, color of the colony, and thermo tolerance.

Microscopic appearance

The basic microscopic morphology is different for different species. Microscopic structures are unique to certain species and constitute the key features for species identification together with the surface color of the colony.

Results

From July 2006 to 31st May 2008, 300 cornea cases were registered out of which 45 cases were diagnosed as microbial keratitis and evaluated.

Out of 45 corneal ulcers cultured, 10(22%) were found to be bacteria, 20(44%) were found to be fungi, 3(6.7%) were found to be mixed with bacteria and fungi, the remaining 12(26.7%) showed no growth (Fig 4).
Both Gram positive cocci and Gram positive bacilli were seen, with one case of Gram negative bacilli (Pseudomonas spp.).

In fungal corneal ulcers most common organism noted was Aspergillus fumigatus (8) followed by Fusarium solani(7). 2 cases were identified as Curvularia spp, and 1 each of Homonema dermatioidis, Alternaria alternata and Scytallidium infestans.

**DISCUSSION:**

Fungi gain access into the corneal stroma through a defect in the epithelium, then multiply and cause tissue necrosis and an inflammatory reaction. The epithelial defect usually results from trauma (eg, contact lens wear, foreign material, prior corneal surgery). The organisms can penetrate an intact descemet membrane and gain access into the anterior chamber or the posterior segment. Mycotoxins and proteolytic enzymes augment the tissue damage.(5)

Corneal trauma is the most frequent and major risk factor for fungal keratitis. In fact, the physician should have a high level of suspicion in a patient with a history of corneal trauma, particularly with plant or soil matter.

Fungal keratitis is a major blinding eye disease in Asia. One report from South India found that 44% of all central corneal ulcers are caused by fungi (13). This high prevalence of fungal pathogens in South India is significantly greater than that found in similar studies in Nepal(17%), Bangladesh (36%), Ghana (37.6%), and south Florida (35%) (14,15,16,17,18).

In China, the incidence of fungal keratitis has increased during the past decade (19).

In temperate climates, such as Britain and the northern United States, the incidence of fungal keratitis remains very low (18,20,21).

There is a geographical variation in the incidence of fungal keratitis. In our institution the incidence of fungal keratitis was found to be 44%. This is similar to the studies from other parts of South India(22, 23) whereas reported incidence in states of northern India are less (7.3%-32%) (24,25).

A study from Goa which is again in the south western part of India had reported a prevalence of 38.9% (26). This regional variation could be because the climate in the southern part of India is hot and humid for most part of the year.

Aspergillus spp was the predominant etiological agent causing keratitis in our study, similar to the reports from other parts of India (27,28). Other rare isolates reported were Curvularia spp, Homonema dermatioidis, Alternaria alternata and Scytallidium infestans. The dematiaceous fungi are reported as causes of keratitis in many tropical and subtropical regions(29).

Case Report

Of the 45 patients who had microbial keratitis, only 28 had good compliance and were reviewed regularly. Conservative management was successful for 25 patients who got a visual acuity in the range of > 6/60 to 6/9 after best correction. Surgical management for 3 patients who underwent keratoplasty, after the refraction got a visual acuity of CF 3mtr to 6/36.

**CONCLUSION:**

Globally, the incidence of keratomycoses is rising. Fungal infections is occurring with an increased frequency when compared to the bacterial infections, the predominant fungal pathogens being Aspergillus fumigatus and Fusarium solani respectively. The findings of our study show that there is a region wise variation in the prevalence of the corneal pathogens.

Ongoing research towards rapid diagnosis and specific drug therapy could minimize the morbidity caused by this preventable disease. Various antifungal drugs including itraconazole, fluconazole and voriconazole, have been applied recently for the treatment of keratomycosis.

Our study emphasizes the fact that early diagnosis and intervention can significantly decrease permanent corneal scarring and vision loss in patients with infective keratitis.

**REFERENCE:**


THE MOBILE PHONE IN A TROPICAL SETTING – EMERGING THREAT FOR INFECTION CONTROL

Padma Srikanth¹, Ezhilarasan Rajaram², Suchithra Sudharsanam³, Anandhi Lakshmanan⁴, SSM. Umamaheswari⁵, Kalyani J⁶

ABSTRACT:

Introduction: Mobile phones have become an extension of office practice for physicians; they act as perfect substrate for microorganisms, especially in hot humid conditions, and may serve as a vehicle in transmitting nosocomial infections.

Objective: This study was conducted to determine whether mobile phones of healthcare workers (HCWs) and corporate office goers harbour microorganisms since they represent two different environments.

Methods: Swabs were taken from mobile phones surfaces, inoculated in Blood agar and MacConkey agar and thioglycollate medium, and incubated aerobically. Growth was identified as per standard microbiological procedures. Antibiotic susceptibility was determined for S. aureus. Questionnaire was used for data collection on awareness of mobile phones usage and disinfection.

INTRODUCTION

Global burden of healthcare associated infections (HAI) is on the rise, and contributes significantly to morbidity and mortality of patients[1]. Increase in HAI is concomitantly associated with increase in expenditure for healthcare facilities[1] Majority of HAI are inadvertently transmitted through hands of healthcare workers (HCWs), the environment being the source of nosocomial agents occasionally[2].

Inanimate objects in the hospital environment are known to be contaminated with microorganisms[2]. Mobile phones have become an extension of office practice for physicians, and may serve as perfect substrate for microorganisms, especially in high temperature and humid conditions. Extensive use of mobile phones by HCWs acts as a vehicle for transmission of nosocomial agents.

There are few reports on the role of mobile phones in the spread of nosocomial infections[3,4,5,6,7] especially in a tropical setting[8]. This study was undertaken to determine whether mobile phones of HCWs are only contaminated, since they are used in an environment that harbours nosocomial agents or whether mobile phones of corporate office goers are also contaminated since it represents an environment free from contamination.

Materials and methods

Surface samples were taken from mobile phones of HCWs from a tertiary care centre and non-hospital (corporate) personnel in June – July 2007 after obtaining consent. The corporate office that was sampled is located in the centre of Chennai city, in a very urban location, completely air-conditioned, well maintained, dust-free environment, the nature of work being mainly administrative. Sterile swabs moistened with sterile demineralised water were rotated over both the surfaces of the mobile phone or the casing surface for cased mobile phones and collected. Sampled swabs were streaked over Blood agar and MacConkey agar plates, and inoculated in thioglycollate medium (Hi-Media Company Limited, India) for characterization of aerobic bacteria; no anaerobic / fungal cultures were taken. Plates were incubated aerobically at 37 °C for 24 - 48 h. Gram-positive and Gram-negative bacteria were identified as per standard microbiological procedures[9].

Gram-positive cocci were identified by Gram staining, colony morphology, and haemolysis. Staphylococci were further identified based on catalase, slide- & tube-coagulase, and utilisation of O-F-glucose and mannitol. Gram-negative bacilli were identified by Gram staining, colony morphology, lactose fermentation, and motility, and further biochemical tests like indole production, sugar fermentation and H₂S production, urease production, citrate utilization, and MR-VP test for the nature of fermentation. Non-fermenters were further identified using catalase and oxidase tests.

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Results: In all, 51 HCW and 36 corporate mobile phones were sampled. Polymicrobial growth was detected in 71 % HCW and 78 % corporate mobile phones respectively. Pathogens isolated from HCW samples included S. aureus [MSSA (4), MRSA (2)], E.coli (1), K.pneumoniae (1), Ps.aeruginosa (1) and CONS (43). Among 78 bacterial isolates from corporate office samples, 54% were pathogenic. Only 12 % HCWs used disinfectants to wipe their mobile phones.

Conclusion: High level of contamination irrespective of the environment is disturbing. Isolation of MRSA from HCWs mobile phones is a cause for concern, indicating the potential threat of mobile phones spreading infections and the importance of hand hygiene to prevent infection.

MeSH words: Gram-positive cocci, Gram-negative bacteria, cellular phone, handwashing, MRSA
ability to grow on MacConkey agar and growth at 42 °C, and biochemical tests like OF-dextrose utilisation, nitrate reduction and gelatin hydrolysis. Antibiotic susceptibility test was done for Staphylococcus aureus isolates by Kirby-Bauer Disc diffusion method.

RESULTS

In all, 51 mobile phones of HCWs and 36 of corporate office goers were sampled. Among the mobile phones sampled, 94 % were contaminated and only 6 % were free of aerobic bacterial growth. Majority (74 %) of the mobile phones had polymicrobial growth; 48 % had two species and 26 % had three or more species (Figure 1). Polymicrobial growth was documented in a larger number (71 %) of mobile phones belonging to HCWs. A total of 89 bacterial isolates were isolated from mobile phones of HCWs, of which 58 (65 %) were pathogenic. Commonly isolated pathogens from mobile phones were S. aureus (6) [MRSA (2), MSSA (4)], Escherichia coli (1), Pseudomonas aeruginosa (1), Acinetobacter spp. (6) and Klebsiella pneumoniae (1) and Coagulase-negative Staphylococci (CONS) (43) as shown in Table 1. A higher percentage (42 %) of mobile phones of doctors was contaminated.

Among 79 bacterial isolates in 36 samples from corporate personnel, 43 (54 %) were pathogens. Polymicrobial growth was detected in 28 (78 %) mobile phones of office users. MSSA (1), E. coli (2), Pseudomonas aeruginosa (3), Acinetobacter spp. (16) and Klebsiella pneumoniae (1) and CONS (20) were the commonly isolated pathogens.

Analysis of the questionnaire showed that 38 (75%) HCWs and 11 (37%) of corporate users were aware that mobile phones harbour microorganisms and transmit infectious agents. Only 12 % HCWs used disinfectants to wipe their mobile phones. Majority (73%; n = 37) of the HCWs felt the need for restricted usage of mobile phones during working hours, especially in critical areas, while 18% (9) felt it as unnecessary and impractical. It was also found that 29% of HCWs followed restricted usage of mobile phones (less than three times) and 57% handled mobile phones more than three times to a maximum of 20 during working hours; 14% used greater than 20 times.

DISCUSSION

Few reports have documented the contamination of mobile phones among HCWs,[3,4,5,6,7,8] so far no study has ascertained the contamination of mobile phones with microorganisms among the general public external to the hospital environment. The mobile phones of corporate office-goers are representative of a non-hospital environment; they represent a relatively cleaner environment in a tropical country. Results of our study indicate that a high percentage of mobile phones of HCWs and office goers are contaminated. Since majority of the mobile phones are contaminated with microorganisms, it is likely that the contamination occurs via hands irrespective of the environment. The profile of microorganisms isolated from mobile phones in our study is similar to previous reports[3,4,5,6,7,8]. It is indeed surprising to document the presence of pathogenic organisms such as Staphylococcus aureus, Pseudomonas aeruginosa, Acinetobacter spp., Klebsiella pneumoniae, and E. coli in the mobile phones of corporate users. While CONS and Micrococci were the predominant isolates among Gram-positive cocci, the isolation of MRSA in a small percentage (2 %) is a cause for concern. This represents an additional route for cross transmission. The percentage of isolation of Gram-negative bacilli may show some geographic disparity. Acinetobacter was commonest isolate among Gram-negative bacilli. A study from Israel has shown Acinetobacter to be the predominant isolate recovered from cell phones[6]. Most organisms are killed within hours due to drying but bacteria

---

Table 1 : Microbiological Profile of Isolates from Mobile Phones

<table>
<thead>
<tr>
<th>ORGANISMS ISOLATED</th>
<th>HEALTHCARE PERSONNEL</th>
<th>CORPORATE PERSONNEL</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>GRAM-POSITIVE BACTERIA</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Staphylococcus spp.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methicillin Resistant S. aureus</td>
<td>2 (2 %)</td>
<td>0</td>
</tr>
<tr>
<td>Methicillin Sensitive S. aureus</td>
<td>4 (5 %)</td>
<td>1 (1 %)</td>
</tr>
<tr>
<td>Coagulase-negative Staphylococci</td>
<td>43 (48 %)</td>
<td>20 (25 %)</td>
</tr>
<tr>
<td>Micrococci</td>
<td>19 (21 %)</td>
<td>19 (24 %)</td>
</tr>
<tr>
<td>Aerobic spore bearers</td>
<td>12 (14 %)</td>
<td>17 (22 %)</td>
</tr>
<tr>
<td><strong>GRAM-NEGATIVE BACTERIA</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>1 (1 %)</td>
<td>2 (3 %)</td>
</tr>
<tr>
<td>Klebsiella spp.</td>
<td>1 (1 %)</td>
<td>1 (1 %)</td>
</tr>
<tr>
<td>Pseudomonas spp.</td>
<td>1 (1 %)</td>
<td>3 (4 %)</td>
</tr>
<tr>
<td>Acinetobacter spp.</td>
<td>6 (7 %)</td>
<td>16 (20 %)</td>
</tr>
</tbody>
</table>
like *Staphylococcus aureus* and *Acinetobacter* are resistant to drying and can survive for weeks and multiply rapidly in warm environment[10]. It is not clear whether the source of Gram-negative bacilli is from the environment since surface sampling of the environment was not undertaken in this study.

Analysis of the questionnaire has shown that only 12% of the HCWs wipe mobile phones with disinfectant. There is an urgent need to disseminate knowledge among HCWs regarding the possible contamination of mobile phones and the importance of periodic cleaning of the phones, as well as the importance of hand hygiene. One study reported use of 70% isopropyl alcohol to be effective as a disinfectant[8]. Another study reported that restricted usage of the mobile phones during working hours along with proper hand hygiene practices enabled to maintain the mobile phones free of contamination.

This study has certain limitations. The purpose of the study was to determine the presence of aerobic bacteria; cultivation of anaerobic bacteria and fungi was not done. Surface sampling of the environment and cultures from the hands of the personnel were not done. It is not possible to conclude from the study whether the organisms are transient or resident, since only one time sampling was conducted. Further studies on simultaneous surface sampling may enable identification of possible sources of contamination.

To our knowledge, this is the first report that has studied the potential for mobile phones to harbour microorganisms in both hospital and non-hospital settings. The high level of contamination of mobile phones irrespective of the environment is disturbing. Isolation of nosocomially significant pathogens such as MRSA, *Pseudomonas aeruginosa*, *Acinetobacter* spp. in HCWs’ mobile phones demonstrates a hitherto unsuspected source of transmission of nosocomial agents. The presence of pathogenic organisms among mobile phones of corporate users is surprising, raising the concern for the spread of infectious agents to family, especially children. The presence of bacteria in both mobile phones suggests that hands may be the source of contamination of the mobile phones.

Infection control guidelines must target use of suitable disinfectants to avoid mobile phone contamination, and advocate hand-wash prior to and after mobile phone usage. Policy makers of individual healthcare facilities should formulate specific protocols for restricted use of mobile phones in sensitive patient care areas and make recommendations for periodic disinfection. Lack of awareness regarding the possibility of mobile phone contamination occurring in their grooves and keys (though they appear to be clean and shiny) suggest the need for creating awareness and ensuring hygienic practices in its handling.

**ACKNOWLEDGEMENT:**

The authors thank all the participants of the corporate office for their willingness to participate and appreciate the authorities of the corporate office to permit the conduct of study.

Potential conflict of interest : Nil

**REFERENCES:**


ABSTRACT:

Cleft lip or palate (CL/P) is one of most common congenital anomalies. The worldwide incidence of CL/P is 1 in 700 and in India it is 1 in 500 live births. Of the various etiological factors, chromosomal aberrations are reported as one of the major causes. Hence, the main objective of this study was to screen for the presence of chromosomal aberrations in individuals with cleft lip or palate or both of Indian origin. The blood samples were obtained from 10 patients visited the departments of Plastic and Reconstructive surgery and Human Genetics, Sri Ramachandra University, with informed consent. The chromosomes were analyzed from the cultured lymphocytes after GTG banding. The abnormality identified with GTG banding was confirmed using FISH. Of the ten cases screened for chromosome abnormality, nine showed normal karyotype and one with trisomy 18. The trisomy was confirmed with FISH using locus specific probe for chromosome # 18. Since cleft lip and palate belong to the multifactorial group, the influence of other factors in the causation of cleft lip and palate cannot be ruled out in the cases with normal karyotype.

Key words: Cleft plate, Cleft lip, GTG banding, FISH.

INTRODUCTION

Congenital malformations are defect and/or cognitive delays present at the time of birth. The defects may be an isolated or syndromic. Approximately 2% of live births have major congenital malformations. The etiologies for such malformations include single gene defects (20%), chromosomal aberrations (10%), teratogens (10%), environmental factors (30%) and other unknown causes (30%) (1). Cleft lip and palate are one of the most common congenital malformations. While, the incidence of CL/P worldwide is 1 in 700 live births and it is nearly 1 in 500 in India (2). Thus, the incidence of cleft lip and palate varies according to geographical location, ethnicity and socio-economic status (3).

Cleft lip can occur either as unilateral (left or right side) or bilateral anomaly. Furthermore, CLP can be isolated, non-syndromic (70%) or it can be syndromic where more additional anomalies are involved along with CLP. The majority of the CL/Ps is non-syndromic (70%) and the remaining are syndromic cases. Orofacial clefts represent a complex phenotype which can be caused by many etiological factors. In a large series of cases, it has been found that they are caused by single mutant genes, chromosomal aberrations, specific environmental agents or interaction of many genetic and environmental factors, the multifactorial group (4). The high familial aggregation rates, recurrence risks and elevated concordance rates in monozygotic twins provide evidence for a strong genetic component in CL/P (5).

Environmental causes includes Teratogens (Maternal smoking), infections, nutrients (folic acid supplement) and cholesterol metabolism (has role in human facial embryogenesis) (6).

Fogh-Anderson (1942) provided the first population-based evidence that CLP (cleft of the lip or palate) has a strong genetic component (7). Studies have been reported the association between chromosomal anomaly and clefts of the palate in animals and humans. Ingalls (1963) induced cleft palate in mice by administrating 6-amino nicotinamide to pregnant females and found polyploidy and fragmentation of chromosomes in fetuses affected with isolated cleft palate. Gropp et al (1964) reported a patient with cleft palate showed nearly triploid chromosomes with modal number of 72 chromosomes in cells cultivated from palatal mucosa. On contrary, negative association of chromosomal aberration and CL/P is also reported (8&9). In the view of different studies, in the present we have reported the results of chromosomal studies carried out from 10 patients with CL/P of south Indian origin. The chromosomes were analyzed using GTG banding and FISH.

AIM

The main aim of the study was to screen for the presence of chromosomal abnormality in individuals with cleft lip or cleft palate or both by Giemsa staining.

MATERIALS AND METHODS:

The study group involved 10 individuals with cleft lip or palate or both attended the Departments of Plastic and Reconstructive surgery and Human Genetics, Sri Ramachandra University. A pedigree and medical history was charted out from the data provided by the patient and/or guardian. The information and the blood samples were collected from the informed consent of the patient or/and patient’s guardian. About 5ml of peripheral blood was drawn in heparinized vacutainers and used for chromosomal study as explained below.
Chromosome preparation and GTG- Banding

About 1ml of blood was added to 8 ml of RPMI medium, 2ml of fetal bovine serum and 500 microlitre of Phytohaemagglutinin and incubated at 37°C with 5% CO₂ for 72 hours. At 66.5 hour, Ethidium bromide (1mg/ml) was added followed by the addition of Colchicine (0.1mg/ml) at 67th hour and incubated for 1.5 hour. The cells were then harvested by hypotonic treatment (20 minutes with 0.45%KCL at 37°C), washed thrice with Carnoy’s fixative (methanol and acetic acid 3:1) and casted on clean prechilled slides. Multiple slides were casted for each sample and used for chromosomal aberration analysis and Fluorescence in-situ hybridization. The slides were exposed to Trypsin (8mg/50 ml of Nacl) for 20 – 30 seconds and then stained with 10% Giemsa, air dried and mounted with coverslip using DPX for the analysis of chromosomal aberrations. For each sample 25 metaphases were analyzed and interpreted (10).

Fluorescence in-situ hybridization:

The slides with metaphase chromosomes prepared as mentioned above was dehydrated in 70%, 80% and 100% ethanol for 2min each, at room temperature and air-dried. The locus specific probe was mixed with hybridization buffer and deionised distilled water, and applied to the slides. The metaphase chromosomes and the probes were co-denatured using Hybrite at 73°C for 3 minutes. The slides were sealed with coverslip using rubber cement and hybridization was carried out for 24 hours at 37°C. After 24 hours of hybridization, the coverslip was removed and the slides were rinsed in formamide wash solution (0.4X SSC/ 0.3%NP-40) at 45°C and the slides were air-dried. After air drying the slides were counterstained with DAPI (7.5 µl/slide) and covered with coverslip and slides were stored in dark prior to signal enumeration and observed under fluorescent microscope for appropriate signals (11).

RESULTS:

Table-1 gives the details of patient age, sex, consanguinity and type of CLP. The age group varies between 2 days to 19 years. Among the cases screened, 5 patients are with cleft palate, 2 patients with unilateral cleft lip, 2 patients with bilateral cleft lip and palate and 1 patient with complete cleft lip and palate.

Of the 10 patients, 3 were born to the parents of first degree consanguinity marriage and remaining seven of them were non-related. Only one patient’s mother had the medical history of intake of Dolopar (Acetaminophen) during pregnancy. The cases 9 and 10 are examples of cleft related syndromes and the rest are examples of non-syndromic cleft lip and/or palate.

Twenty five G-banded metaphases at 450-550 band resolution were analyzed for each patient. The karyotypes of the patients were given in table-1. The result showed 9 cases with normal karyotype and one with trisomy-18.

<table>
<thead>
<tr>
<th>Code number</th>
<th>Age/sex</th>
<th>Consanguinity</th>
<th>Cleft type</th>
<th>Karyotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case 1</td>
<td>14 yrs/M</td>
<td>1st Degree</td>
<td>Incomplete cleft of soft palate</td>
<td>46,XY</td>
</tr>
<tr>
<td>Case 2</td>
<td>8 yrs/F</td>
<td>NC</td>
<td>Complete cleft of hard palate</td>
<td>46,XX</td>
</tr>
<tr>
<td>Case 3</td>
<td>13yrs/M</td>
<td>NC</td>
<td>Complete cleft of hard &amp; soft palate</td>
<td>46,XY</td>
</tr>
<tr>
<td>Case 4</td>
<td>8yrs/M</td>
<td>NC</td>
<td>Unilateral cleft lip</td>
<td>46,XY</td>
</tr>
<tr>
<td>Case 5</td>
<td>3 yrs/F</td>
<td>NC</td>
<td>Unilateral cleft lip</td>
<td>46,XX</td>
</tr>
<tr>
<td>Case 6</td>
<td>12yrs/M</td>
<td>NC</td>
<td>Bilateral complete cleft lip &amp; palate</td>
<td>46,XY</td>
</tr>
<tr>
<td>Case 7 *</td>
<td>17yrs/F</td>
<td>NC</td>
<td>Bilateral cleft lip &amp; palate</td>
<td>46,XX</td>
</tr>
<tr>
<td>Case 8</td>
<td>19yrs/F</td>
<td>1st degree</td>
<td>Complete cleft of posterior &amp; soft palate</td>
<td>46,XX</td>
</tr>
<tr>
<td>Case 9</td>
<td>11mon/M</td>
<td>1st degree</td>
<td>Cleft palate</td>
<td>46,XY</td>
</tr>
<tr>
<td>Case 10</td>
<td>2days/F</td>
<td>NC</td>
<td>Complete cleft lip &amp; palate</td>
<td>47,XX,+18</td>
</tr>
</tbody>
</table>

* Medical history of drug intake during pregnancy
NC – Non consanguineous

Karyotype of the patient with trisomy 18 was further confirmed by increasing the analysis of G-banded metaphases to 100 and by FISH (Figure 1a & 1b).

Case 10
47,XX,+18

Fig. 1a : Fish with Locus specific probe for chromosome # 18 showing trisomy of Chromosome 18
Fig. 1b : GTG banded metaphase showing Trisomy 18
DISCUSSION

It has been estimated that 6% of all congenital malformations are due to visible cytogenetic abnormalities (12). Of which approximately 5% of congenital defects with cleft lip and/or palate have been reported an association with structural and numerical chromosomal abnormalities (3). Chromosomal aberrations either numerical or structural can be identified by the GTG banding technique with a band resolution of 400-450. This is the widely used cytogenetic method to screen the genetic association for different malformations. In an attempt to screen for chromosomal abnormalities in individuals with CL/P, consequently gains an insight to the possible relation between the two, of the ten cases screened. Nine out of ten cases showed normal karyotype and one with trisomy 18. Earlier, Subrt et al reported ten negative results and one trisomy 21 karyotype out of eleven cases studied.

In the present study, of the ten cases screened, one of them (case – 10) showed trisomy of chromosome 18. Aneuploidies occur due to non-disjunction of chromosomes during meiosis, resulting in an extra chromosome than the usual two copies. Therefore, the presence of an extra copy of genes on these chromosomes, results in multiple malformations leading to a syndrome. Trisomy 18 has been associated with the presence of cleft lip and/or palate (13&14). Clefts of lip or palate or both have also been observed in individuals with ring chromosome 18 (1) and chromosome 18 involved in a complex rearrangement (15). This shows that chromosome 18 might be harbouring a gene (or genes) that have a direct role in lip and/or palate formation, or at least acts as a modifier during embryogenesis.

Case 9 presented with the clinical features like micrognathia, cleft palate, and glossoptosis which are characteristic of Pierre Robin Syndrome (also referred to as Pierre Robin Sequence). He was the child of consanguineously married parents. Pierre Robin Syndrome occurs sporadically, but it may be familial, in which the mode of inheritance is autosomal dominant (16). However, GTG banding technique at 450-550 band resolution may not be sensitive enough to detect complex alterations, submicroscopic deletions or single gene changes, which may be causative reason(s) of CL/P. This could be one of the reasons for failing to identify subtle chromosomal alterations often associated with CL/P.

Individuals, whose karyotypes showed no numerical or visible structural abnormalities, two (cases – 1 and 8) were children of consanguineously married parents. Significant association has been found between clefting and consanguinity (17). CL/P can be due to either an insult as gross as a visible chromosomal alteration or changes in genes as subtle as substitution, deletion, etc which cannot be detected by conventional cytogenetic techniques. Micro deletions or isodisomy may also contribute to clefts as suggested by studies. Hence using techniques like Comparative genomic hybridization (CGH) or mFISH can detect such cross chromosomal abnormalities which were missed by conventional karyotyping in the above individuals. Moreover, it is shown that number of genes has been associated with the regulation and craniofacial morphogenesis (6). Perturbations in the function of any of these genes in the form of mutations can result in haploinsufficiency leading to a cleft lip, or palate, or both depending on the affected gene and its role during embryogenesis (18). Thus the role of genes in regulating the morphogenesis could not be identified in the present study.

Of the other four cases, there were two pairs of siblings (cases – 2, 3 and 4, 5). Neither of the pairs of the siblings had any other family member affected. The manifestation of the disease in these individuals could be due to a possible de novo germ line mutation of any of the related genes in either of the parents or they could have been subjected to some environmental factors during their embryogenesis (life style habits and health of the mother during pregnancy). Also, the contribution of syndromic genes in these non-syndromic cases cannot be ruled out.

The negative results obtained for the cases screened (cases 1-9) could mainly be attributed to the fact that CL/P is a complex anomaly with a multifactorial inheritance. Though there are many genes involved in the formation of the lip and palate during embryogenesis, the intrauterine environmental factors and other environmental factors like maternal smoking, consumption of drugs (teratogens), and nutrition also have an influence on the developing fetus, which should also be considered.

CONCLUSION:

To identify presence of subtle chromosomal alterations in complex disorders like the cleft lip and palate if any, karyotyping has to be combined with new techniques. This would increase the sensitivity of the diagnosis and hence rule out the genetic contribution; as the recurrence risk of cleft lip and palate increases in siblings of affected individuals with chromosomal/genetic abnormalities. Once the genetic contribution is ruled out, the other environmental factors (maternal smoking, maternal nutritional status, uptake of teratogenic drugs) which could have been plausible causes can be tried and identified so that these factors can be modified/avoided in subsequent pregnancies.

In spite of limited efficiency of karyotyping in detecting subtle chromosomal aberrations, it still serves as the basis for identifying gross chromosomal aberrations and hence aids in ruling out chromosomal aberrations as the possible cause for malformation like that of CL/P. Consequently it helps directing individuals with such complex disorders in the right path of diagnosis, by leading them to look at the newer aspects of other causes.
ACKNOWLEDGEMENT:

My special thanks to Dr. P. Venkatachalam for encouraging me at every juncture of this publication and for helping me to put forth my ideas in the form of bright illustration. I would like to thank Ms. Teena Koshy, who donated hours of her valuable time to help me with my experiments and clarification.

REFERENCES:


ABSTRACT:

The new millennium promises to guide in the age of human genome. So far, a different area of biology, the stem cell biology – has captured both scientific and international news headlines. Stem cells are generally very early stage cells that have the ability to differentiate into other specialized types of cells. Stem cells hoist the prospect of regenerating failed body parts and curing diseases that have so far defined drug – based therapy. This review attempts to give an overview of stem cell biology and scientific factors surrounding it.

Keywords: Embryonic stem cells; Adult stem cells; Stem cell markers; Hematopoietic stem cells

INTRODUCTION:

Stem cells bridge the cleft between the fertilized egg that is our origin and architecture that we become. The stem cells supply the cells that construct the adult bodies and as the age, replenish worn out, damaged and diseased tissues. And depending on the source, they have potential to form one, many or all cell types of an organism. Stem cells are isolated from two sources generally, the adult stem cells and embryonic stem cells. Recently, cord blood stem cells are isolated for use in regenerative medicine. Depending on the cell source, stem cell may be totipotent, pluripotent, multipotent or unipotent [Table 1].

<table>
<thead>
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<th>Table 1: Definition of Terms</th>
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<td>Totipotent cell: - able to give rise to all cell types. In mammals, only the fertilized egg and early cleavage stage blastomers are truly totipotent cells of inner cell mass and ES cells are unable to differentiate into cells of the tropoctoderm lineage.</td>
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<tr>
<td>Pluripotent cell:- able to give rise to all cell types found in embryo and adult animal</td>
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<td>Multipotent cell:- able to give rise to more than one differentiated cell type.</td>
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<td>Unipotent cell:- able to give rise to a single cell type.</td>
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<td>Lineage:- The natural progression from an immature cell type to one or more differentiated cell types.</td>
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<td>Lineage restriction:- The inability of one lineage to give cell type of another, that is, to cross lineage boundaries.</td>
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The common characteristics of stem cells include extensive proliferative potential and ability to give rise to one or more differentiated cell types in early mammalian embryos. But embryonic cells lose these properties as differentiation follows and growth promoting signals decline subsequently becoming adult progenitor cell or a differentiated cell (1). The adult progenitor cells can operate at ‘steady state’, i.e., they can generate in an average of one replacement stem cell and one tissue cell at each division with no apparent limit. Adult stem cells are hence controlled by particular microenvironments known as ‘niches’ (2). Unlike embryonic stem cells that are isolated from ‘blastocyst stage’ of embryo for culturing, adult stem cells are isolated from ‘niches’. Cord blood contains hematopoietic stem cells, progenitor cells that can form red blood cells, white blood cells and platelets. However Cord blood stem cells are not embryonic stem cells.

In most tissues, stem cells are rare. As a result, stem cells must be identified prospectively and purified carefully in order to study their properties. Fluorescence Activated Cell Sorter (FACS) and Visual Assessment can aid in separation of stem cells from normal cells. Stem cells are often identified by the presence of markers. The markers may be ligands, cell surface protein receptors, cytoplasmic proteins, transcriptional factors or genes (3). The chance of stem cells becoming cancerous in regenerative medicine has led researchers to focus on three aspects between stem cells and tumor cells. First, the similarities in the mechanisms that regulate self-renewal of normal stem cells and cancer cells; second, the possibility that tumor cells might arise from normal stem cells; and third, the notion that tumors might contain ‘cancer stem cells’ – rare cells with indefinite proliferative potential that derive the formation and growth of tumors (4).

Embryonic stem cells

Human embryonic stem cell (hESC) are non – transformed cells that are self-renewing and pluripotent or multipotent for a tissue type, and highly proliferative with the following characteristics: (i) can be isolated from the inner cell mass (ICM) of the blastocyst, (ii) proliferate extensively in vitro, (iii) maintain a normal euploid karyotype over extended culture, (iv) differentiate into derivatives of all three germ layers, (v) express high levels of transcriptional factor, Oct-4 and (vi) show telomerase activity (5 - 7). The percentage of hESC lines successfully
derived from ICMs ranges from 5%-100%; this wide range reflect differences in number of embryos used for derivation, embryo quality or derivation techniques (8). Recently, cell lines have also been isolated from morula stage embryo or from later stage embryos (7 - 8 days) (9) (Fig. 1 & 2). Therefore, the current hESC lines have been derived from embryos with different characteristics and this was used earlier for conducting diagnostic tests in the normal course of in vitro fertilization (10).

The cell line should reliably differentiate into an appropriate cell population and remain stable during expansion and differentiation as well as after transplantation. Generating and maintaining the cells in defined culture conditions will allow the establishment of more reproducible cultures that can be maintained in multiplex laboratories. Use of cells exposed to these components for cell replacement therapies may thus carry the risk of infection by nonhuman pathogens. Some progress has been made toward the elimination of xenogeneic components in hESC derivation and culture. For instance, one hESC line has now been derived using lysed Mouse Embryonic Fibroblast (MEFs) (11).

Culturing hESC requires an appropriate substratum. The use of a single matrix such as laminin or fibronectin has been successful in the maintenance of hESC. The adherence of hESC to each other, although critical in maintaining cell interactions, has presented several challenges. Regardless of the passaging technique either mechanical or enzymatic, it is important that the hESC remain in clusters to preserve the integrity of the culture. This situation makes it difficult to generate cultures with consistent cell density from passage to passage. However, there are currently no clinically applicable protocols for achieving this goal.

Markers currently used to characterize hESC include glycolipids, glycoproteins and transcriptional factors. These surface markers were identified on human embryonal carcinoma cells or in human preimplantation embryos, such as SSEA-4, TRA-1-60, and TRA-1-81 (12). hESC also express surface antigens such as AC133, c-kit (CD117), flt3 (CD135) and CD9 (13-15). The transcriptional factors that serve as markers have a critical role in maintaining self-renewal e.g. Oct 3/4 (16 - 17).

**Adult stem cells**

The adult stem cells (ASC’s) are unspecialized or undifferentiated cells that are found in differentiated tissues maintained in a stable micro environmental niche and most of the cells are lineage restricted. The niche safeguards against excessive stem cell production that could lead to cancer. Stem cells must periodically activate to produce progenitor or transit amplifying (TA) cells that are committed to produce mature cell lineages (18). Maintaining a balance of stem cell quiescence and activity is a hallmark of a functional niche. ASC’s can be found in the bone marrow, blood stream, cornea and retina of the eye, dental pulp of the tooth, liver, skin, gastrointestinal tract and the pancreas. However, there is not much evidence that ASC’s unlike the ESC’s are pluripotent (19, 20). Although differentiation potential is slightly decreasing in long-term cultures, it is possible to keep cell lines up to passage 140. Adult stem cells reside in various organs in a specific cellular environment called the niche, in which they are kept in an undifferentiated state (21 - 23). These stem cells possess an extensive self-renewal capability bearing an indefinite proliferative potential. Until recently; adult stem cells are believed to be lineage-restricted with limited differentiation potency, compared with embryonic stem cells. Pluripotent embryonic stem cells are blastocyst-derived cells and proliferate unlimited in an undifferentiated state, being capable of giving rise to cells found in all three germ layers (24, 25) However, this stem cell plasticity was recently shown also for adult stem cells by various groups (26). Different examples of the versatility of adult stem cells have been demonstrated from bone marrow (27) umbilical cord blood (28), testes (29) and pancreas (30). They differentiated into various cell types cutting across lineage boundaries.

**Pancreatic stem cell niche**

Adult pancreatic stem cells are able to differentiate spontaneously in vitro into various somatic cell types. Stem cells isolated from rat pancreas show extensive self-renewal
ability and grow in highly viable long-term cultures. Several approaches are being used for isolating and culturing stem cells or islet precursor cells from fetal and pancreatic tissue (Fig.3). These cells were formerly termed pancreatic stellate-like cells because of their morphologic and immunohistochemical similarities to pancreatic stellate cells, which are located within the interlobular septa and interacinar areas of the pancreas (31). The cells can be engineered to avoid immune rejection. Recent studies in mice show that embryonic stem cells can be coaxed into differentiating insulin-producing beta cells, and new reports indicate that this strategy may be possible using human embryonic cells as well (32). Additionally, these cells express typical stem cell markers such as Oct-4, nestin and SSEA-1. We have previously reported a simple but effective method for isolation of stem cells from the exocrine pancreas. Before transplantation, they could be placed into nonimmunogenic material so that they would not be rejected and the patient would avoid the devastating effects of immunosuppressant drugs. Since their discovery three years ago, several teams of researchers have been investigating the possibility that human embryonic stem cells could be developed as a therapy for treating diabetes.

**Intestinal stem cells niche**

Epithelial villus and its surrounding pericryptal fibroblast and mesenchyme in the small intestine make up an anatomical unit that generates four cell lineages: absorptive enterocytes and the goblet, enteroendocrine and paneth cells of secretory lineage. Intestinal stem cells and transit amplifying (TA) cells within the crypt regenerate the entire villus every 3 to 5 days (33). Progeny of activated ISCs migrate upwards to become TA cells. When they reach the top of the crypt, TA cells stop proliferating, differentiate, and assume their appropriate positions within the villus structure (34). Although asymmetric cell division along the vertical crypt axis is an attractive mechanism, this process has yet to be rigorously demonstrated in the ISC system (Fig.4).

**Hair follicle stem cell niche**

Skin epidermis and its associated structures arise from two stem cell populations within the hair follicle and interfollicular regions (35). One, in the basal layer of skin, normally gives rise to stratified skin layers. A second, the hair follicle stem cell, resides in a region of the outer root sheath called the bulge, and it is responsible for the regeneration of hair and sebaceous glands (36 - 39). It had been suggested that bulge stem cells are also responsible for the long-term replenishment of the interfollicular epidermis (Fig.5). It is now clear that bulge stem cells are not required for normal epidermal homeostasis, although they can contribute transiently to this tissue in wound healing (40 - 44).

**Neural stem cell niche**

Neural stem cells are a subtype of progenitor cells in the nervous system that can self-renew and generate both neurons and glia (Fig. 6). Adult neural stem cells have now been found in the two principal adult neurogenic regions, the hippocampus and the sub ventricular zone, and in some non – neurogenic regions, including spinal cord (45, 46). Recent findings in stem cell research indicate the presence of stem cells in the hippocampus-a region in brain, which is important in memory. Curious observations in regeneration of neural cells in foetal and adult brain resemble the undifferentiated cells in a developing embryo that give
rise to nervous tissue (47). Stem cells are under active consideration as a source of donor tissues for neuronal cell therapy for Parkinson’s disease (48), Huntington’s disease (49), spinal cord injury (45), stroke (50) and multiple sclerosis (51).

**Hematopoietic stem cells**

The stem cells that form blood and immune cells are known as hematopoietic stem cells (HSCs). They are ultimately responsible for the constant renewal of blood, the production of billions of new blood cells each day.

**Sources of Hematopoietic stem cells**

**Bone Marrow**

Bone marrow transplants by anesthetizing the stem cell donor, puncturing a bone, typically a hipbone and drawing out the bone marrow cells with a syringe. About 1 in every 100,000 cells in the marrow is a long-term, blood-forming stem cell; other cells present include stromal cells, stromal stem cells, blood progenitor cells, and mature and maturing white and red blood cells (56-59).

**Umbilical Cord Blood**

In the late 1980s and early 1990s, physicians began to recognize that blood from the human umbilical cord and placenta was a rich source of HSCs. This tissue supports the developing fetus during pregnancy, is delivered along with the baby, and, is usually discarded. Since the first successful umbilical cord blood transplants in children with Fanconi anemia, the collection and therapeutic use of these cells has grown quickly (60, 61).

**Peripheral Blood**

It has been known for decades that a small number of stem and progenitor cells circulate in the bloodstream, but in the past 10 years the researchers have found that they can coax the cells to migrate from marrow to blood in greater numbers by injecting the donor with a cytokine, such as granulocyte-colony stimulating factor (GCSF). The donor is injected with GCSF a few days before the cell harvest. To collect the cells, doctors insert an intravenous tube into the donor’s vein and pass his blood through a filtering system that pulls out CD34+ white blood cells and returns the red blood cells to the donor, from which just 5 to 20 percent will be true HSCs (54, 62).

**Heart and Cardiac Muscle**

Heart tissue has a limited regenerative capacity: thus; the use of HSCs for cardiac repair has been found to be of clinical relevance (Fig. 8). Researchers are now exploring ways to save additional lives by using replacement cells for dead or impaired cells so that the weakened heart muscle can regain its pumping power. Like the mouse stem cells, the human hematopoietic stem cells can be induced under the appropriate culture conditions to differentiate into numerous tissue types, including cardiac muscle. In 1998, Kajstura and colleagues (63) discovered the presence of myocytes undergoing mitosis in the failing human heart. Such cells were found to be scarce, explaining the lack of significant myocardial regeneration after myocardial
infarction. These cells have subsequently been demonstrated to be self-renewing, clonogenic and able to give rise to myocytes, smooth muscle and endothelium. These cells have subsequently been defined as Lin- c-kit<sup>+</sup> cells within human hearts (64). A series of animal studies and clinical trials have been used to attempt to determine whether stem cells could be useful in treating myocardial infarction (65, 66). Stem cells injected into the bloodstream leading to the damaged rat heart, these cells prevented the death of viable myocardial cells and reduced progressive formation of collagen fibers and scars (67).

**Cancer Stem cells**

The cancer stem cell (CSC) model of tumor development and progression states that tumors, like normal adult tissues, contain a subset of cells that both self renew and give rise to differentiated progeny. As in other tissues, the stem cells are the minority of the whole organ, and are the only cells that can maintain tumor growth indefinitely. The self renewal properties of the CSCs are thus real driving force behind tumor growth. The identification of markers that allow the prospective isolation of CSCs from whole tumor tissues will allow us to develop an understanding of several important biological properties of CSCs. Cancer stem cells can only be defined experimentally by their ability to recapitulate the generation of a continuously growing tumor. Cancer stem cells share many characteristics with normal stem cells (68-70). A few cancer stem cells could evade treatment and later give rise to a tumor, referred to as cancer relapse. The tumors formed are really the progenies of the cancer stem cells. Like all progenies of stem cells, they multiply rapidly. However, the progenies of cancer stem cells are not like normal progenies, whose growths are tightly controlled.

A well known property of normal stem cells is their dependence upon their microenvironment, or ‘niche’ to maintain their quiescent and undifferentiated state, while maintaining their proliferation and differentiation potentials (71, 72). The discovery of signaling pathways that play a functional role in CSC self renewal is extremely important from a therapeutic perspective, as some of these pathways have known chemical inhibitors (e.g. the Hh pathway can be inhibited by cyclopamine treatment), or function as inducers of differentiation (e.g. BMPs). The development of methods for the prospective isolation of CSCs is thus the first step, which then opens the door to a variety of approaches that could ultimately lead to CSC – specific therapies for cancer treatment (73-75).

**Delivery of stem cell**

Clinical trial is going on delivery of stem cells in animal models as well as in human for regeneration of damaged tissue or organ. Tissue regeneration targets are mostly successful in skin, bone, cartilage. Stem cells are generally implanted or seeded into an artificial structure capable of supporting 3-D tissue formulation called scaffold allowing cells to influence the own microenvironment. Scaffold should be a) biodegradable in nature b) able to allow cell attachment and migrations c) delivers and retain cells and biochemical factors d) enable diffusion of vital cell nutrients e.g. polycaprolactone, polyglycolic acid, collagen or fibrin, glycosaminoglycans etc.

**Ethical Aspects**

Some scientists use ASCs due to the ethical and moral aspects involved in using human ESCs as a less controversial alternative. US Federal funding for stem cell research is restricted to the 64 cell lines. This ethical issue has divided the scientific community into two i) those that believe in the extension of stem cell research and thus do not believe in the ethical questioning, and ii) those who are apprehensive about the prospect and thus take a moral stand on research against using ESCs (76, 77). In India, wasted embryos available from IVF clinics only are permitted to be used by researchers after receiving consent from the donors. However, more recently, Indian Council of Medical Research, New Delhi has formulated guidelines for stem cell research in the country.

The main ethical issues for consideration are as follows:

i. Instead of producing a baby a human embryo, develops only into certain types of cells. Is it acceptable to reprogramme a human embryo?

ii. Are there likely to be viable alternative therapeutic methods, which could avoid using embryos?

iii. If the route to such alternative methods involved some limited embryo research, would such research be permissible?

iv. Would it be acceptable to perform the nuclear transfer of human cells into the enucleated egg of a cow, to produce a non-viable chimera, which would be reprogrammed to produce certain human cells?

v. Would the risks involved in cell replacement therapy be considered acceptable?

If a procedure with discerning ethical difficulties were to be pursued, it is also essential to be honest about its chances of success. There is a formidable list of experimental hurdles to overcome. No one knows how successful cloned cells would be on patients, or what risk there is of cultured cells becoming cancerous (20).

**Concluding Remarks**

Do cells with unpredicted stem cells hide in other parts of the body? Is it possible to induce ASCs to produce cell types other than their standard range of progeny? Stem cells taken directly from adult tissues promise to be useful in many ways for tissue repair. It should be possible to use adult tissues to derive ES cells with the same genome as the adult patient whose body is in need of repair. The cloning of Dolly, the sheep and of other mammals has indicated a way to do this. The nucleus of an egg cell can be artificially replaced by a nucleus derived from adult cell and the hybrid...
cell can then go on to develop into an entire individual whose nuclear genome is identical to that of the adult donor. Serious ethical issues need be resolved and enormous technical problems overcome before such an approach can become a reality. Perhaps other, better ways will be found to restore adult cells to an embryonic state of versatility. But by one route or another, it seems that stem cell biology is beginning to open up new opportunities for improving on nature’s mechanisms of tissue repair.

REFERENCES:


WONDER ANIMAL MODEL FOR GENETIC STUDIES - Drosophila Melanogaster – ITS LIFE CYCLE AND BREEDING METHODS – A REVIEW

Deepa Parvathi V, Akshaya Amritha S, Solomon FD Paul

ABSTRACT:

Drosophila is a genus of small flies, belonging to the family Drosophilidae, whose members are often called “fruit flies”. The entire genus, however, contains about 1,500 species and is very diverse in appearance, behavior, and breeding habitat. One species of Drosophila in particular D. melanogaster, has been heavily used in research in genetics and is a common model organism in developmental biology. Basic genetic mechanisms are shared by most organisms. Therefore, it is only necessary to study the genetic mechanisms of a few organisms in order to understand how the mechanisms work in many organisms, including humans. Drosophila melanogaster, the fruit fly a little insect about 3mm long, is an excellent organism to study genetic mechanisms. The general principles of gene transmission, linkage, sex determination, genetic interactions; molecular, biochemical and developmental genetics, chromosomal aberrations, penetrance and expressivity, and evolutionary change may all be admirably demonstrated by using the fruit fly Drosophila melanogaster. The life cycle of Drosophila is short and completes in about three weeks. Embryonic development, which follows fertilization and the formation of the zygote, occurs within the egg membrane. The egg produces larva, which eats and grows and at length becomes pupa. The pupa, in turn develops into an imago or adult. The duration of these stages varies with the temperature. Drosophila cultures ought to be kept in room temperature where the temperature does not range below 20°C or above 25°C. They are bred on fermenting medium which contains corn, dextrose, sugar and yeast extract. Their breeding ratio is 1:3 (male:female). The common culture contaminants include fungi, mites and bacteria. The male and the female are differentiated (under the microscope) based on their size, markings on their abdomen and presence of sex combs following anesthetization with ether.

Key Words: Drosophila melanogaster, Genetics, Breeding

INTRODUCTION:

Drosophila is a genus of small flies, belonging to the family Drosophilidae, whose members are often called “fruit flies”. One species of Drosophila in particular D. melanogaster, has been heavily used in research in genetics and is a common model organism in developmental biology. The entire genus, however, contains about 1,500 species and is very diverse in appearance, behavior, and breeding habitat. Scientists who study Drosophila attribute the species’ diversity to its ability to be competitive in almost every habitat, including deserts.

Why Drosophila?

Drosophila melanogaster is a fruit fly, of the kind that accumulates around spoiled fruit. It is also one of the most valuable organisms in biological research, particularly in genetics and developmental biology. Basic genetic mechanisms are shared by most organisms. Therefore, it is only necessary to study the genetic mechanisms of a few organisms in order to understand how the mechanisms work in many organisms, including humans. Drosophila melanogaster, a little insect about 3mm long, is an excellent organism to study genetic mechanisms. The general principles of gene transmission, linkage, sex determination, genetic interactions; molecular, biochemical and developmental genetics, chromosomal aberrations, penetrance and expressivity, and evolutionary change may all be admirably demonstrated by using the fruit fly D. melanogaster and its hundreds of related species have been extensively studied for decades, and there is extensive literature available (1).

The extensive knowledge of the genetics of D. melanogaster and the long term experimental experience with this organism together with extensive genetic homology to mammals has made it of unique usefulness in mutation research and genetic toxicology. Many Drosophila genes are homologous to human genes and are studied to gain a better understanding of what role these proteins have in human beings. Much research about the genetics of Drosophila over the last 50 years has resulted in a wealth of reference literature and knowledge about hundreds of its genes.

It is an ideal organism for several reasons:

- Fruit flies are hardy with simple food requirements and occupy little space.
- The reproductive cycle is complete in about 12 days at room temperature, allowing quick analysis of test crosses.
- Fruit flies produce large numbers of offspring to allow sufficient data to be collected. Examination and data collection is easy because the flies can be quickly and easily immobilized for examination.
- Many types of hereditary variations can be recognized with low-power magnification.

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Drosophila has a small number of chromosomes (four pairs), a genome size smaller than the human complement of 23 pairs of chromosomes. The giant ("polytene") chromosomes in the salivary (and other) glands of the mature larvae.

- Show far more structural detail than do normal chromosomes, and
- They are present during interphase when chromosomes are normally invisible

A large number of genetically defined mutants are available which define most aspects of the fly’s biology.

Many Drosophila genes are homologous to human genes and are studied to gain a better understanding of what role these proteins have in human beings. Much research about the genetics of Drosophila over the last 50 years has resulted in a wealth of reference literature and knowledge about hundreds of its genes.

The genome is relatively small for an animal (less than a tenth that of humans and mice).

Mutations can be targeted to specific genes (1,2).

**Life cycle of Drosophila**

**Stages and duration:**

Embryonic development, which follows fertilization and the formation of the zygote, occurs within the egg membrane. The egg produces larva, which eats and grows and at length becomes pupa. The pupa, in turn develops into an imago or adult. (Fig. 1) The duration of these stages varies with the temperature. At 20°C, the average length of the egg-larval period is 8 days; at 25°C it is reduced to 5 days. The pupal life at 20°C is about 6.3 days, whereas at 25°C is about 4.2 days. Thus at 25°C the life cycle may be completed in about 10 days, but at 20°C about 15 days are required. Drosophila cultures ought to be kept in room temperature where the temperature does not range below 20°C or above 25°C. Continued exposure to temperatures above 30°C may result in sterilization or death and at low temperatures the viability of flies is impaired and life cycle prolonged. (2)

**The egg:**

The egg of *Drosophila melanogaster* is about 0.5 of a millimeter long. An outer investing membrane, the chorion, is opaque and shows a pattern of hexagonal markings. A pair of filaments, extending from the anterodorsal surface, keeps the egg from sinking into soft food on which it may be laid. Penetration of spermatozoa into egg occurs through a small opening or micropyle, in the conical protrusion at the anterior end, as the egg passes through the uterus. Many sperms may enter an egg, through normally only one functions in fertilization. The spermatozoa have been stored by the female since the time of mating. Immediately after the entrance of the sperm, the reduction (meiotic) divisions are completed and the egg nucleus (female pronucleus) is formed. The sperm nucleus and the egg nucleus then come into position side by side to produce the zygote nucleus, which divides to form the first two cleavage nuclei, the initial stage of development of the embryo. Eggs may be laid by the mother shortly after they are penetrated by the sperm, or they may be retained in the uterus during the early stages of embryonic development. (2)

**The Larval Stages:**

The larva, after hatching from the egg, undergoes two molts, so that the larval period consist of three stages (instars). The final stage, or third instar may attain a length of about 4.5 millimeters. The larvae are such intensely active and voracious feeders that the culture medium in which they are crawling becomes heavily channeled and furrowed (2).

The larva has 12 segments: the 3 head segments, 3 thoracic segments, and 8 abdominal segments. The body wall is soft and flexible and consists of the outer noncellular cuticula and the inner cellular epidermis. A great number of sense organs are spread regularly over the whole body. (Fig. 2) (2,3)
The larvae are quite transparent. Their fat bodies, in the form of long whitish sheets, the coiled intestine, and the yellowish malpighian tubules, as well as the gonads embedded in the fat body can easily be distinguished in the living larva when observed in transmitted light. The dorsal blood vessel is the circulatory organ of the larva. The larval muscles, segmentally arranged, are transparent but can be made visible when the larva is fixed in hot water. The larva contains a number of primitive cell complexes called imaginal discs, which are the primordia for later imaginal structures.

The primary mechanism by which the larva grows is molting. At each molt the entire cuticle of the insect, including many specialized cuticular structures, as well as the mouth armature and the spiracles, is shed and has to be rebuilt again. During each molt, therefore many reconstruction processes occur, leading to the formation of structures characteristic of the ensuing instar. The growth of the internal organs proceeds gradually and seems to be rather independent of the molting process, which mainly affects the body wall. Organs such as Malpighian tubes, muscles, fat body, and intestine grow by an increase in cell size; the number of cells in the organ remains constant. The organ discs, on the other hand, grow chiefly by cell multiplication; the size of the individual cells remains about the same. In general, one might say that purely larval organs grow by an increase in cell size, whereas the presumptive imaginal organs grow by cell multiplication.

The Pupa:

A series of developmental steps by means of which the insect passes from the larval into the adult organism is called “metamorphosis”. The most drastic changes in this transformation process take place during the pupal stage.

Shortly before pupation the larva leaves the food and usually crawls onto the sides of the culture bottles, seeking a suitable place for pupation, and finally comes to rest. The larva is now very sluggish, everts its anterior spiracles, and becomes motionless. Soon the larva shortens and appears to be somewhat broader, thus gradually acquiring its pupal shape. The shortening of the larval cuticle, which forms the case of the puparium, is caused by muscular action. The puparium, which is the outer pupal case, is thus identical with the cuticle of the last larval instar. When the shaping of the puparium is completed, the larval segmentation is obliterated, but the cuticle is still white. This stage lasts only a few minutes and is thus an accurate time mark from which to date the age of the pupa. Immediately after the cuticle reaches the white prepupal stage, the hardening and the darkening of the cuticle begin and proceed very quickly. About three and a half hours later the puparium is fully coloured. Pigmentation apparently starts in the external surface of the cuticle and proceeds inward.

Four hours after the formation of the puparium, the animal within it has separated its epidermis from the puparium and has become a headless individual having no external wings or legs and known as the “prepupa”. A very fine prepupal cuticle has been secreted and surrounds the prepupa.

Pupation takes place about 12 hours after puparium formation. By muscular contraction the prepupa draws back from the anterior end of the puparium and everts its head structures. This movement also ejects the larval mouth armature, which until now was attached to the anterior end of the prepupa. The wings, halteres and legs are also everted. A typical pupa with head, thorax, and abdomen is thus shaped. In section it is seen that the pupa now lies within three membranes: an outer membrane, the puparium: an intermediate membrane, the prepupal cuticle; and an inner membrane, the newly secreted pupal cuticle.

Now metamorphosis involves the destruction of certain larval tissues and organs (histolysis) and the organization of adult structures from primitive cell complexes, the imaginal discs. It must, however, be realized that some larval organs are transformed into their imaginal state without any very drastic change in their structure. The duration and extent of these transformation processes vary greatly for the different organs involved. Larval organs which are completely histolyzed during metamorphosis are the salivary glands, the fat bodies, the intestine and apparently the muscles. All these organs are formed anew, either from imaginal disc cells already present in the larva or from cells which come visibly into being in the course of pupal reorganization. The Malpighian tubules are relatively little altered during metamorphosis but nevertheless undergo some change in their structural composition. The same situation seems to prevail in the brain, which is not completely histolyzed. The extremities, eyes, mouthparts, antennae, and genital apparatus differentiate from their appropriate imaginal discs, which were already present in the larval stage and which undergo histogenesis during pupal development. The body wall of the imaginal head, thorax, and abdomen is also formed from imaginal disc cells. The body wall of head and thorax is formed by the combined effort of all the imaginal discs in this region, each of which contributes its part. The hypoderm of the abdomen is formed by segmentally arranged imaginal cells which first become visible in young prepupae.

Adult stage

When metamorphosis is complete, the adult flies emerge from the pupal case. They are fragile and light in color and their wings are not fully expanded. These flies darken in a few hours and take on the normal appearance of the adult fly. Upon emergence, flies are relatively light in color but they darken during the first few hours. It is possible by this criterion to distinguish recently emerged flies from older ones present in the same culture.
They live a month or more and then die. A female does not mate for about 10 to 12 hours after emerging from the pupa. Once she has mated, she stores a considerable quantity of sperm in receptacles and fertilizes her eggs as she lays them. Hence, to ensure a controlled mating, it is necessary to use females that have not mated before. These flies are referred to as virgin females (3,4).

6. **Sex organs during larval stage**

During the late larval stage males can be distinguished by the presence of a large, white mass of testicular tissue. This tissue is located at the beginning of the posterior third of the larva in the lateral fat bodies and can be seen through the integument. The corresponding ovarian tissue of the female constitutes a much smaller mass (5).

**Tools for culturing Drosophila**

Basic fly handling equipment includes a binocular microscope with a good light source, an etherizer or a CO$_2$ plate for anesthetization, a fly pusher, an aspirator, a pounding pad, and a morgue. For most purposes flies can be kept at room temperature, but one or two constant temperature rooms, preferably humidified, or incubators are generally useful and are necessary for some techniques(6).

**Microscope and light source:**

A binocular or trinocular microscope with good quality optics, easy access to the magnification changer and a smooth accessible focusing mechanism is ideal (6).

**Anesthetizing flies:**

Ether and CO$_2$ are the fly anesthetics of choice. CO$_2$ requires more setup and maintenance than ether. If ether is chosen an etherizer and a sorting plate is required, on the other hand a CO$_2$ pad serves as both an anesthetizer and a sorting plate(6).

Ether is flammable, has a strong odor and will kill flies if they are over-etherized. Carbon dioxide works very well, keeping flies immobile for long periods of time with no side effects, however CO$_2$ mats (blocks) are expensive and a CO$_2$ source (usually a bottle) and delivery system (vials and clamps) are necessary, increasing the costs. The least harmful to the flies is either carbon dioxide or cooling anesthetizing. Of these two choices, cooling is the simplest, requiring only a freezer, ice and petridishes. In addition, it is the only method which will not affect fly neurology, therefore behavior studies may begin after the flies have warmed up sufficiently.

**Anesthetizing flies by cooling**

In order to incapacitate the flies, place the culture vial in the freezer until the flies are not moving, generally 8-12 minutes. Flies are dumped onto a chilled surface. This can be constructed by using the top of a petridish, adding crushed ice, then placing the bottom of the petridish on top. Adding flies to this system will keep them chilled long enough to do each experiment. Simply place the flies back into the culture vial when finished. There are no long-lasting side effects to this method, although flies left in the refrigerator too long may not recover (7).

**Stock Keeping:**

Most stocks can be successfully cultured by periodic
mass transfer of adults to fresh food. Bottles or vials are tapped on the pounding pad to shake flies away from the plug, the plug is rapidly removed, and the old culture is inverted over a fresh bottle or vial. Flies are tapped into the new vessel, or some are shaken back into the old one, as necessary, and the two are rapidly separated and replugged. Good tossing technique combined with plugs that are easily removed and replaced results in very few escapes. The frequency with which new subcultures need to be established depends on health and fecundity of the genotype, the temperature at which it is raised, and the density of the cultures (6).

It is very good practice to keep the old cultures for 2 weeks (at 18°C) after transfer, so that they can be used as a backup should the new stocks fail for any reason (8).

Culture contaminants:

Drosophila is largely pestilence-free, but mites, fungi, and bacteria can be problems in laboratory cultures. Benchtop and fly pushing equipments must be regularly cleaned. Benchtop and all equipments that come into contact into potentially contaminated stocks should be cleaned with 70% ethanol or soap and water after use. Sharing pounding pads, CO₂ pads, fly pushers, and sorting plates can aid in the spread of contaminants (6).

Mites are egg predators and are the most dangerous contaminating species. Even those that simply feed on the medium can outcompete weak genotypes and compromise experimental observations. Frequent stock transfer, tight plugs and zero mite tolerance by all fly workers in a building are best defenses. Cultures that are grown at 24 – 25°C must never be kept for more than 30 days. If mites are known to be a problem cultures should be checked and discarded after 18 – 20 days. To prevent the import of mites from outside sources, all stocks new to the lab should be quarantined for at least two generations. Any culture found to contain mites should be autoclaved immediately and replaced with a mite free source (6).

Fungi and bacteria can also contaminate the culture. If mould is the problem in isolated cultures, it can usually be eliminated by daily transfer of adults for 7 – 10 days. Visually inspect cultures from the later transfers for hyphae (look around the pupal cases) and use one that appears to be free of fungal growth for further subculture. If fungal contamination is a widespread problem be sure that fungal inhibitor (p-hydroxy-benzoic acid methyl ether) is being added to the medium after it is cooked (boiling destroys the inhibitor) (6).

A variety of bacterial contaminants can occur in fly cultures. Most common problems are caused by mucous-producing bacteria. Although not directly toxic, larva, and to some extent adults become trapped in the heavy layer of mucous that coats the surface of the food. Large numbers of larvae overcome the effect of the bacteria in a healthy stock, but weak stocks or pair matings can be seriously compromised. A wide spread bacterial problem may indicate that the pH of the medium is too high; try lowering the pH (6,7).

Culture conditions:

Timing & Lighting

Fruit flies are “cold-blooded” so rate of growth and development varies with temperature. The duration of the different stages varies with the temperature. At 20°C the average length of the egg-larval period is 8 days; at 25°C it is reduced to 5 days. The flies are attracted to lights. Part of fly courtship behavior is visual, so it is probably a good idea to keep them in an area with good lighting most of the time (9).

Methods of breeding drosophila:

Drosophila melanogaster is found in abundance on soft fruits like grapes, bananas, and plums, especially if they are overripe and have begun to ferment. Adult flies as well as larvae feed on fruit juices: and since yeast is present wherever fermentation is in progress, it is believed that yeast constitutes an important part of their diet. Therefore Drosophila may be raised on any fermenting medium. The different types of medium routinely used for breeding Drosophila include commen medium, banana jaggery medium, sucrose dextrose medium and maltose corn medium. The composition of the food predominantly includes sugar, yeast extract, dextrose and corn flour. They can be bred in glass bottles to obtain large numbers of the progeny (Fig. 5). And most often crosses and experiments are set up in glass vials.
ease of handling, short reproductive cycle allows scientists to analyze test crosses. Also, the offspring are produced in large numbers which provides statistically significant data and phenotypic mutant changes are easily recognizable under the microscope. This review details on the lifecycle of *D. melanogaster*, its importance in genetic studies and also basic tools required for culturing flies in laboratory.

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EOSINOPHILIC CYSTITIS- A DISTINCT CLINICO-PATHOLOGICAL ENTITY

Shalinee Rao\textsuperscript{a}, Sandhya Sundaram\textsuperscript{a}, Sunil Shroff\textsuperscript{b}, K. Kulothungan\textsuperscript{a}, Prathiba D\textsuperscript{a}

Eosinophilic cystitis is an uncommon poorly understood clinicopathological condition first described in 1960\cite{1,2}. It usually cause irritative voiding symptoms and can sometimes simulate infiltrative vesical malignancy.

A 58-year old male presented with difficulty in voiding and slow stream of urine for 3 years and dysuria for 2 years. He also had increased frequency with twenty times during the day and nocturnal enuresis for last 6 months. Patient was a known hypertensive on irregular treatment with no other significant past history.

General physical examination was essentially unremarkable except for pallor.

Per abdomen examination showed no organomegaly or ascites. Bladder was palpable. Other systemic examination was normal. On per rectal examination, prostate was enlarged (approximately 40 gms), smooth, firm and, non-tender.

Cystoscopy showed grade I trabeculations in bladder. Both the uretric orifices were normal. Baseline and other relevant investigations were done, findings of which are available in table 1.

Table 1: Laboratory findings

<table>
<thead>
<tr>
<th>Test</th>
<th>Patient’s value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin</td>
<td>9.2 gm/dl</td>
</tr>
<tr>
<td>PCV</td>
<td>28.9 %</td>
</tr>
<tr>
<td>Total leucocyte count</td>
<td>8670 cells/cumm</td>
</tr>
<tr>
<td>Differential leucocyte count</td>
<td>Polymorphs: 80%; Eosinophils: 1%; Lymphocyte: 14%; Monocyte: 5%</td>
</tr>
<tr>
<td>PSA</td>
<td>1.44 ng/ml</td>
</tr>
<tr>
<td>Serum iron</td>
<td>29 µg/dl</td>
</tr>
<tr>
<td>Serum creatinine</td>
<td>1.7 mg/dl</td>
</tr>
<tr>
<td>BUN</td>
<td>15 mg/dl</td>
</tr>
<tr>
<td>Urine protein</td>
<td>++ +</td>
</tr>
<tr>
<td>Urine sugar</td>
<td>Nil</td>
</tr>
<tr>
<td>Urine microscopy</td>
<td>Pu cells 10-15; RBC: 25-30; occasional granular cast +</td>
</tr>
<tr>
<td>Urine culture</td>
<td>No growth</td>
</tr>
</tbody>
</table>

Perurethral catheterization done drained one litre of urine. A diagnosis of voiding dysfunction with chronic urinary retention, anaemia and hypertension was made. A transurethral resection of prostate and bladder biopsy was done and submitted for histopathological examination.

Histopathological examination of bladder biopsy received showed focal ulceration of the mucosa, edema, congestion, few lymphocytes and dense eosinophilic infiltrates in the lamina propria (Figure 1, 2, 3 and 4). Focal areas of hemorrhage was also noted (Figure 3). Muscular propria showed eosinophilic infiltrates (Figure 5) and focal areas of fibrosis (Figure 6). Histopathological diagnosis of eosinophilic cystitis was made. Prostatic tissue received showed features of benign prostatic hyperplasia.

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Eosinophilic cystitis, an unusual disease process presents clinically with episodes of marked dysuria, hematuria, suprapubic pain, diurnal and nocturnal urinary frequency. Patient in the study case had several of these symptoms. Although mostly encountered in children and women, can also occur in men.

The disease has been associated with vesical injury, chronic vesical irritation, surgery, parasitosis, food / drug allergy, tuberculous cystitis and malignancies. Acute flare of eosinophilic cystitis has also been noticed after bladder instillation with dimethyl sulfoxide for presumed interstitial cystitis. Pathogenesis of eosinophilic cystitis still remains unclear. It could possibly be caused by antigen-antibody reaction leading to production of various immunoglobulins which in turn cause activation of eosinophils that initiates inflammatory process. Another reason suggested is dysregulation of cytokines predominantly involving interleukin 4 and 5.

Cystoscopy findings may vary considerably with edema, erythema, ulceration to papillary lesions in bladder mucosa. Sometimes an infiltrative mass-like lesion may also be visualized. Imaging studies of patients with eosinophilic cystitis are not very specific. It can show thickening of the wall and sometimes mimic tumoral mass. Other laboratory findings include proteinuria, microscopic hematuria and at times peripheral eosinophilia. The present case had microscopic hematuria and proteinuria without blood eosinophilia.

The gold standard for diagnosing this lesion is histopathological examination of bladder biopsy. It is mandatory to obtain a deep biopsy otherwise the diagnosis can be missed.

Histological changes can be acute, chronic or both. The acute changes are characterized by edema, congestion...
with inflammatory infiltrate composed of eosinophils and lymphocytes. Eosinophils are more prominent in cases with muscle necrosis. The chronic stage shows few / absent eosinophils, few lymphocytes with fibrosis in the lamina propria and interspersed among superficial muscle layers. The overlying epithelium can show proliferative changes or squamous metaplasia.

There is no specific curative treatment available for this condition[6]. However, patients have responded to treatment modalities such as transurethral resection of the lesion in the bladder and combination of corticosteroids and antihistaminic. A regular follow up is essential as this lesion though self limiting, at times can recur. The knowledge of this distinct clinicopathological entity is essential for urologist since the symptoms are not very specific for the clinician to suspect this lesion and can often mimic malignancy.

REFERENCES:
INTRA-ABDOMINAL ROUND CELL TUMOUR OF CHILDHOOD - A DILEMMA OF SITE AND ORIGIN

Shanmugapriya S*, Harsha Chadaga⁴, Santosh Joseph ⁴, V K Panicker⁺, S Rajendiran⁺

ABSTRACT:

Neuroblastoma and Wilms tumor are the common intra-abdominal round cell tumours of early childhood. The treatment modalities of these malignancies are very different. Neuroblastoma arising from the kidney or an aggressive adrenal neuroblastoma invading the kidney may easily be misdiagnosed as a case of Wilms tumor preoperatively. We report a case of small round cell tumour entirely replacing right kidney and adrenal gland. Although, the exact site of the tumour remained undetermined, immunohistochemistry confirmed the diagnosis of neuroblastoma resolving the dilemma of origin.

Key words: Adrenal gland, kidney, neuroblastoma, Wilms tumour.

INTRODUCTION:

Pediatric abdominal tumors often present in an advanced stage at first clinical examination. The primary differential diagnosis of a large palpable right upper quadrant mass in a young child includes neuroblastoma, Wilms tumor and hepatoblastoma. The location of the mass may prove useful in preoperatively identifying the origin of these lesions. A mass localized to kidney suggest Wilms tumor. The possibility of neuroblastoma needs to be considered for a mass arising within adrenal gland while a tumor localized to liver suggest a hepatoblastoma. Intrarenal neuroblastoma is very rare and originates from either adrenal rests found within the renal tissue or from intrarenal sympathetic ganglia.

CLINICAL SUMMARY:

An eight year old girl presented with distension of upper abdomen which was noticed by her mother incidentally. She also complained of dragging sensation in the abdomen. There was no history of vomiting, loose stools, constipation, fever, weight loss, joint pain, hematuria or frequency of micturition. On examination, there was a palpable mass in the right upper abdomen measuring 15x10 cm, which was vaguely nodular, firm in consistency and did not cross the midline. Hematological and biochemical parameters were normal except for an increased level of Vanillyl mandelic acid (VMA) 41.8g/mg/creatinine (Normal value of 24 hours VMA urinary excretion-<8mg/day). Ultrasonography of abdomen showed a large well circumscribed lobulated lesion with areas of calcification and necrosis involving the right kidney, suggesting the possibility of Wilms tumor. Computed tomography (CT) of whole abdomen showed a large heterogeneously enhancing right renal mass with necrosis and amorphous calcification displacing the adjacling structures. Adrenal gland was not identified on CT abdomen (Fig. 1). Intraoperatively, a bosselated vascular tumor was seen occupying the upper pole and involving 2/3rd of right kidney extending up to the inferior surface of the liver and involving the inferior vena cava. Adrenal gland was not identified intraoperatively also. Right radical nephrectomy was performed and the resected specimen was sent for histopathological examination.

HISTOPATHOLOGICAL FINDINGS:

The nephrectomy specimen received weighed 4.5 kg and measured 19x12x7cm. Cut surface showed a grey white tumour measuring 15x11cm involving whole kidney. Adrenal gland could not be identified grossly (Fig. 2).

Fig. 1: CT whole abdomen showing right renal mass displacing the adjoining structures with normal left kidney.

Fig. 2: Large solid, nodular, grey white tumour. Grossly no normal renal or adrenal tissue could be made out.

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Neuroblastoma is an aggressive malignancy as compared to Wilms' tumor and usually presents with secondaries at the time of initial presentation in approximately 75% of the patients. Renal invasion by neuroblastoma occurs by direct penetration through the renal capsule and/or lymphatic perivascular spread. Generally, the renoinfiltrative neuroblastoma (a stage III or IV disease) are extensive and have unfavourable histological features with lymph node involvement.

Bone metastasis with renal tumors is uncommon and seen mostly with clear cell sarcoma of the kidney. In our case, there was evidence of secondary metastasis to the bones at the time of presentation. The ratio of adrenal to extra-adrenal primary site is approximately 1.5 to 2:1. In about 10% of cases it is not possible to establish the primary site of origin with certainty[2]. Six of such cases were identified in the National Wilms Tumor Study Pathology Centre in 1993[3]. Adrenal neuroblastomas infiltrate the superior pole of adjacent kidney in 5% of cases. Ultrasonogram and computed tomography determines its position in relation to adrenal, kidney, and other intraabdominal and retroperitoneal organs. MRI and bone scans are helpful to detect metastases. In the index case, the adrenal gland was neither identified radiologically, intraoperatively nor microscopically. The tumor was seen extensively involving and destroying the entire right kidney. The possibilities were neuroblastoma of adrenal gland involving the kidney or an intrarenal neuroblastoma with adrenal invasion and destruction. Because of the extensive renal involvement, a renal origin was favoured in our case.

Urinary catecholamines may be negative and the imaging modalities may at times be unable to differentiate between neuroblastoma and Wilms tumor. The features of vascular (aorta) encasement, non-visualization of the kidney or its displacement, bony or muscular invasion and suprarenal location favours the diagnosis of neuroblastoma as seen in our case. However, there is always a risk of 5-10% of misdiagnosis on these imaging modalities.

Review of literature suggested that intrarenal origin or extension of neuroblastoma from adrenal gland may be confused with Wilms tumor[4,5,6,7]. In such situations radiological correlation, urinary catecholamines levels, immunohistochemistry, cytogenetics studies and bone marrow biopsy would be helpful in establishing the diagnosis[4,5,6,7]. In our case, though genetics was not performed, WT-1 negativity and synaptophysin positivity on immunohistochemistry and elevated serum catecholamine level with hot spots in bone scan clinched the diagnosis of neuroblastoma. Lall et al studied in three children between two months and four years diagnosed as intrarenal neuroblastoma and found that differentiation between Wilms tumor and intrarenal neuroblastoma is imperative as the prognosis and treatment are different for these tumors and a
correct preoperative diagnosis would be important in the management of these cases[8].

With the advent of CT and MRI, the differentiation of neuroblastoma from Wilms tumor has improved dramatically. Application of immunohistochemistry and detection of N-myc amplification and 1p deletion has greatly facilitated in differentiating neuroblastoma from other small round cell tumor of childhood. Intrarenal neuroblastoma is usually associated with a poor prognosis as the tumor presents with secondary metastasis at the time of the initial presentation.

Dactinomycin and Vincristine are the most effective drugs for Wilms tumor with favourable histology. These drugs are used in conjunction with surgery, without radiotherapy, for all patients with stage I and II lesions with favourable histology. Radiotherapy and more toxic chemotherapeutic agents are reserved for patients with stage III and IV disease. The treatment of Wilms tumor is successful with remission in 80% of children, with less than 20% experiencing serious morbidity at twenty years from diagnosis[9]. Outcomes remain poor in neuroblastoma despite intensive treatment. The bortezomib-doxorubicin combination is effective for neuroblastoma[10,11]. Neuroblastoma, because of its unique biology, continues to be a challenging tumor to treat, and many times these tumors are refractory to standard chemotherapeutic regimens. Trichostatin A (TSA) and Interferon-beta has a significant antitumor activity against neuroblastoma[12].

A high proportion of intrarenal neuroblastoma are of unfavourable histology as defined by the International Neuroblastoma Pathology Classification and have a higher incidence of anaplasia(32%) when compared to both their adrenal counterparts and to Wilms tumor. The factors that affect survival are age and health of child, extent of the disease, size, type and location of the tumor, metastasis, tumors response to therapy and overall child's tolerance to medications. Clinical, radiological and pathological correlation is very essential for diagnosis and appropriate management of this type of unusual cases.

To conclude, even though the exact site remained undetermined in this case of small round cell tumour completely replacing the right kidney and adrenal gland, immunohistochemistry played a pivotal role in confirming the diagnosis of neuroblastoma thereby, resolving the dilemma of origin.

REFERENCES:
ATYPICAL VASCULAR LESION OF LIVER- A CASE REPORT

S.Sankar *, M. Subramanian*, Santhosh Joseph b, Harsha Chadaga b, T. Arunkumar a

ABSTRACT

Computerized axial tomography has revolutionized the diagnosis of space occupying lesions of the liver. Hemangioma of the liver is the most common solid tumor of the liver that can be diagnosed with great degree of accuracy, as it produce characteristic enhancement pattern in Contrast enhanced CT scan. But nevertheless, atypical radiological findings could cause diagnostic problems. Herein we report an interesting and atypical vascular lesion of the Liver, possibly an atypical hemangioma that was managed successfully with interventional radiological method.

KEY WORDS: Hemangioma, Liver, Embolization

INTRODUCTION:

Hemangioma of the liver is the most common solid tumor of the liver. With the advent of Contrast enhanced CT scan the diagnosis of hemangioma of liver is easy as it produces typical enhancement pattern. However atypical radiological features create diagnostic problems. The case reported here is interesting as it was atypical radiologically and treated with embolization successfully.

CASE SUMMARY:

A 14 year old boy presented with complaints of right upper quadrant pain of three months duration. The pain was dull aching in nature that was constant and not radiating. His bowel habits were normal. There were no constitutional symptoms or jaundice. Physical examination revealed a well nourished boy with a palpable liver. No other abnormalities were made out in physical examination. Hemogram and biochemical parameters were normal. Serum Alpha fetoprotein was normal. Ultrasound abdomen picked up a well encapsulated mixed echoic lesion of size 10 cm in the posterior sector of right lobe of liver. CT scan was done to characterize the lesion better. Plain CT showed a hypo dense lesion in the right lobe of the liver in the posterior sector (Fig. 1). In the arterial phase the lesion was found to be very vascular as evidenced by the brisk and homogenous contrast enhancement (Fig 2, 3 & 4). This pattern was very unusual as none of the space occupying lesions of the liver described in the literature has this pattern of enhancement. Ironically this atypical vascular pattern had favorable therapeutic implications. Hepatic artery angiogram was done and selective cannulation of right hepatic arterial branch supplying the lesion was done with Simmonds
catheter (Fig. 5). Embolization was done using polyvinyl alcohol particles and gel foam. Postembolization pictures revealed complete interruption of the blood supply to the lesion (Fig.6). Repeat CT imaging done after six weeks showed complete necrosis of the lesion (Fig. 7 & 8). The necrotic lesion was drained completely with Laparoscopic guidance and the fluid sent for culture was found to be sterile. Patient was relieved of his symptoms. He is doing well on follow up. The possible diagnosis in this case could be an atypical hemangioma due to the radiological appearance and as it was producing symptoms in the form of pain it was treated with angio-embolization.

DISCUSSION:

The most common solid tumor of the liver is hemangioma with an incidence of about 0.4% to 20% in the population (1). Hemangiomas are usually solitary lesion, but in 50% of the cases it may be multiple (2). These lesions are usually detected as an incidental finding on imaging. Though congenital in origin hemangioma usually present in adults. Hemangioma consists of cavernous vascular spaces lined by endothelium and interspersed with thin stroma. Giant hemangiomas are lesions that are more than 4-5 cm (3). Though growth of hemangiomas is reported, the most accepted cause of enlargement of hemangioma is by the mechanism of “progressive ectasia” of the vascular spaces (4). Surgical series report a distinct female preponderance whereas autopsy series report an equal incidence. Hemangiomas are usually clinically silent. Symptoms are produced due to enlargement and compression of adjacent structures, secondary changes and rupture. Large hemangioma may sequestrate platelets to produce consumption coagulopathy, an entity labeled as Kasabach Merrits syndrome. Symptomatic hemangiomas warrant treatment. The ideal treatment is liver resection or enucleation. Hence it is imperative to rule out other causes of pain before labeling it as a “symptomatic hemangioma”. In children large hemangiomas can produce cardiac failure. Interestingly hemangiomas are uncommon in cirrhotics as the fibrotic process impede the development of hemangioma (5).

Hemangiomas are characteristically hyper echoic lesions on ultrasound examination. The typical radiological finding on a CT scan is a hypo dense lesion that has a very characteristic enhancement pattern. In the arterial phase there is “peripheral and nodular” enhancement which fills towards the center (centripetal) in the delayed phase (6, 7). This pattern of enhancement is attributed to the arrangement of vascular spaces tightly in the periphery of the lesion. In the delayed phase the entire lesion becomes uniformly enhanced. The “Light bulb sign” is the typical MRI finding of hemangioma, wherein the hypo intense lesion on T1 weighted images turns into hyper intense on T2 weighted sequences. Angiogram and Single Photon Emission Computerized Tomogram (SPECT) scans are other investigations that are described for the diagnosis of hemangioma. Extremely small and very large hemangiomas may not produce the typical radiological finding. The other causes of atypical radiological finding include degeneration, hemorrhage, thrombosis, hyalinization and calcification. Atypical contrast enhancement may occur when there are arterial portal shunts (8). The lesion described in this case report is atypical and fails to fit into any of the classical space occupying lesions of the Liver. But nevertheless the whole lesion is very vascular as evidenced by the brisk and uniform enhancement of the lesion in the arterial phase of CT. This atypical vascular pattern had favorable therapeutic implications, as embolization of the feeding vessels caused complete necrosis of the tumor and the patient was rendered asymptomatic. Arterial embolization is one of the well accepted modality of treatment of symptomatic hemangiomas of the Liver (9).

REFERENCES:


CONVERSION DISORDER: A CASE REPORT

G. Aarzoo\textsuperscript{a}, S. Bhasi\textsuperscript{b}

ABSTRACT:
Conversion/dissociative disorders are more prevalent in childhood and adolescence, generally affecting females more than males. They are associated with stressors which are perceived as unmanageable, and the symptoms generally reflect a means to avoid the stressor. The case report presents a successful intervention involving five sessions.

Mesh words: Conversion disorder, case report

INTRODUCTION:
Conversion disorder refers to mild and temporary symptoms which can be motor or sensory in nature involving anaesthesia or paresthesia, especially of extremities, abnormalities of movement, gait disturbance, weakness and paralysis, gross rhythmic tremors, choreiform movements, tics and jerks\cite{1}. Any sense modality may be involved. Reflexes remain normal. There might be associated primary and secondary gains which act as maintaining factors\cite{2}. The disorder is more common in adolescence than in childhood\cite{3,4}.

CASE REPORT
A 10 year old boy, Standard V student, presented complaints of stiffness in body and inability to flex knee joints.

History revealed occasional complaints of body pain for the last 2 months which was relieved by body massage. One week back the boy complained of body pain and vomited after having breakfast. He was not sent to school. He slept for about 2 hours and woke with stiffness of body and inability to flex upper and lower limbs. He was admitted in a hospital, where he regained mobility of the upper limbs but was not able to bend his knees and walked with a stiff gait. His mother noticed that when the child was asleep his limbs were not rigid and would be flexed. The following morning he was able to walk and run. When discharge was planned there was a relapse. He was then referred to the Department of Clinical Psychology, SRU.

There was no significant past history of psychiatric or neurological disturbances. Developmental history was reported to be unremarkable. Family relationships were reported to be cordial. Problems in the school were reported. A gradual decline in performance was reported He feels discriminated and victimised by his class teacher and expressed strong resentment for not getting required attention and reinforcement from his class teacher.

Psychological evaluation using Children’s Apperception Test (CAT) and Malhotra’s Temperament Scale (MTS) did not give more insight. However, good prognostic indicators were elicited in terms of resolution of problems and favourable outcomes. The clinical picture is indicative of a diagnosis of Dissociative Motor Disorder, F 44.4, according to ICD 10\cite{5}.

The child was seen for five therapy sessions. On the first visit the child was seen to be sitting in the chair with his legs held parallel to the ground since he was not able to flex his knees. He was dragging his feet while walking. The child was provided reassurance regarding the management of symptoms. Possible consequences of persistence of symptoms were also discussed. He was made to do movement exercises by slightly moving his feet preceded by deep breathing. As he was moving his feet suggestions of increased flexibility were given. With continued effort of 10 – 15 minutes he could bend his knees and sit in a normal position for a brief period. His effort to move his lower limbs were encouraged and appreciated. The child was asked to continue the movement exercises at home and given a suggestion that he would flex his knees at right angles. In the second session held the next day child walked less stiffly and was able to bend his knees to right angles as suggested. His parents were educated about the psychosomatic nature of his symptoms and advised to encourage him for developing a symptom free lifestyle. They were also told not to pay attention to his complaints of physical symptoms.

By the third session held the next day, his gait was normal. He reported to have pain in his lower limbs but was able to flex his knees. He was still unable to bend his knees fully. He was reinforced for the improvement and asked to continue the movement exercises at home and resume all usual activities.

Addressing the school related issues he was allowed to talk about alternatives available to deal with the current situation. His parents were advised to allow him to communicate his difficulties freely, look at issues objectively and help him develop an adaptive coping style.

The child was asymptomatic and had resumed his earlier routine by the fourth session which was held the next day. He was seen once more after a period of one week during which improvement was maintained. Follow up was maintained for 2 more sessions with the parents with a week’s interval in between during which also improvement was maintained. Telephonic contact was maintained upto 3 months during which he continued to be symptom free.

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Conversion disorder, somatoform disorder, and malingering remain diagnostic challenges for the clinicians. The prompt identification of these patients, use of appropriate and validated physical examination manoeuvres, and coordination of care and information exchange between all members of the care team may facilitate the expeditious care of these patients in a cost effective manner[6]. Early themes including stress and conflict are linked to conversion symptoms[7]. Psychogenic symptoms should be treated using suggestions, patience and reassurance[8]. Early recognition of a conversion disorder will limit unnecessary tests and medications. The quality of doctor-patient relationship can influence outcome. The existing literature supports a multidisciplinary treatment approach, with specific interventions, such as cognitive behaviour therapy for cognitive restructuring and psychodynamic therapy for addressing symptom connections to trauma and dissociation[9].

REFERENCES:
INTRODUCTION

In the era of interdisciplinary esthetic therapy, discoloured, fractured, malformed and malposed teeth can be restored to a highly esthetic and desirable form due to development of wide range of materials and techniques. Discolouration of enamel and dentin occurs due to administration of tetracycline for prolonged periods of time during tooth formation. Discolouration is usually bilateral, affecting multiple teeth in both arches. The discolouration can range from yellow-brown, brown, dark grey or blue depending on the type of tetracycline, dosage, duration of intake and patients age at the time of administration.

Tetracycline discoloration has been classified into three groups according to severity. First degree discoloration is light yellow, light brown or light gray and occurs uniformly throughout the crown, without banding. Second degree discoloration is more intense and also without banding. Third degree discoloration is very intense, and the clinical crown exhibits horizontal color banding. This type of discoloration usually predominates in the cervical region of the teeth.¹

Laminate veneering is an ultra conservative method of restoring the appearance of discoloured, pitted teeth and diastemas. It provides extremely good esthetic results and longevity of laminates have been reported to be approximately over 8 years [2] and a suitable alternative to more extensive restorative procedures.

Case Report

A 21-year old female patient reported to the Department of Conservative Dentistry and Esthetic Dentistry with the chief complaint of discolored teeth (fig. 1). All upper and lower teeth had uniform reddish brown discoloration prominently on the incisal/occlusal third without any pitting or grooves. This case report falls into the third degree of discoloration with intense continuous reddish brown discoloration throughout out the incisal and middle third and a horizontal grayish band at the cervical region.

History and clinical examination revealed that the discoloration was due to tetracycline staining. Esthetic correction with ceramic laminates for upper anterior teeth was planned after clearly explaining to the patient, the various other treatment modalities along with their advantages and limitations highlighting the reasons for choosing the laminate veneering as the treatment modality.

Shade selection:

It is desirable to select a shade that is slightly lighter than desired by the patient. Therefore it is advisable to choose a shade with higher value and lower chroma. Keeping in mind that the the final colour of the restoration depends on the original tooth colour, B2 was selected as the shade for laminate and flowable luting cement.

The teeth were prepared for laminates as follows:

1. Labial reduction of 0.5 mm was achieved by depth cutting diamond instruments from mesioproximal line angle to distoproximal line angle.

2. Finish line was established using double grit tapered diamond point. A definitive chamfer margin of 0.3mm was prepared beginning at the height of the free gingival margin and extended towards distal papilla tip and then towards mesial papilla tip. The chamfer margin was continued from distal papilla tip to beginning of contact point, far enough linguually to hide veneer margin when viewed from lateral oblique view. Without breaking the contact from labial side, the finish line was carried from this point to the incisal embrasure, cutting just labial to entire contact area. Similarly the tooth was prepared on mesial side.

3. The procedure was repeated from left upper canine to right upper canine (fig 2).

4. Gingival retraction was achieved by retraction cord.

ABSTRACT:

Laminate veneering is a conservative method of restoring the appearance of discolored, malformed anterior teeth. We present a case of a 21 year old female patient with discolored tetracycline stained anterior teeth managed successfully by ceramic laminates.

Key words: Esthetic, laminate Veneers
5. Elastomeric impression was made and sent to laboratory for fabrication of ceramic laminates (fig 3).

**Try in of ceramic laminates**
1. The intimate adaptation of each laminate to the prepared tooth was checked
2. The teeth surfaces were first cleaned with slurry of fine flour of pumice.
3. Each laminate was individually tried starting from distal tooth.
4. Fit, margins and shade of all laminates were checked

**Cementation of ceramic laminates**

The post operative instructions were to avoid hard foods and extreme temperatures, avoid alcohol based mouth rinses in the first 72 hours. The patient was also instructed to maintain oral prophylaxis once in every four months and to avoid excessive biting forces and habit patterns (nail biting).

**DISCUSSION:**

Discolouration of either deciduous or permanent teeth may occur as a result of tetracycline deposition during prophylactic or therapeutic regimens instituted either in the pregnant fetus or postpartum in the infant. Moffitt et al have observed that the critical period for tetracycline related discolouration in the primary dentition is 4 months in utero to 3 months postpartum for maxillary and mandibular incisors and 5 months in utero to 9 months postpartum for maxillary and mandibular canines. According to Grossman and his associates, the use of oxytetracycline or possibly doxycycline may diminish tooth discolouration. Above all, effective alternatives to tetracycline are available and hence should be avoided from approximately the fourth month to the 12th year of childhood.

According to the literature esthetic correction of discolored, pitted, malformed teeth with laminates is a better choice over full coverage restorations. Ceramic laminates appear more polychromatic and natural tooth like because of its closer shade matching. Laminate veneers are technique sensitive and also requires art and skill to create natural appearance. Proper teeth preparations with proper definitive finish line and utmost care at try in and cementation of laminates can bring esthetically acceptable results. In addition advancements in shade matching, bonding and cementing media makes it the most accepted treatment for esthetic correction of the anterior teeth. An excellent esthetic rehabilitation of the patient’s discoloured teeth, with minimal sacrifice of natural tooth structure has been achieved. This has resulted in an immense boost in the patient’s morale and self confidence.

**REFERENCES:**
Dear Editor,

Bioaerosols are particulate matter of biological origin, comprising of micro-organisms and fragments, and their metabolites (toxins and particulate waste products)[1]. Reports indicate the presence of pathogenic bacterial and fungal species in healthcare settings, especially air,[2,3] and a possible link to cause nosocomial infections and occupational health hazards is described[4]. Though several methods are available for airborne microbial measurements, petri-plate gravitational settling (passive) method of sampling is widely used due to simpler methodology and technical feasibility[5]. Since varying sampling durations ranging from 10 min to 30 min are being used as sampling protocol,[2,3] this study was designed to compare the extent of recovery of microorganisms from the indoor air at varying durations of time exposures.

This study was undertaken during February – April 2007 to determine the microbial loads in indoor air of areas in hospital and non-hospital settings, using petri-plate gravitational settling (passive) method using two different exposure periods, 15 minutes and 30 minutes and to characterise the organisms isolated. A preliminary walk-through was conducted to determine the level of activity in the sampling locations. Sampling was done in locations with minimal activity and in places where routine activities are known to generate aerosols. Nutrient agar, blood agar, MacConkey agar and Sabouraud Dextrose agar were the media used for sampling. The plates were incubated at 37°C for 24-48 h and processed for the identification of predominant Gram-positive and Gram-negative bacteria and fungi grown using standard methods of microbiological analysis[6].

The locations sampled were coded in order to maintain confidentiality of areas sampled. The microbial loads obtained at 15 min and 30 min is shown in Fig. 1. The extent of isolation of microorganisms was uniformly high when exposed at 30 min than at 15 min irrespective of the sampling locations. It was observed that the recovery of microorganisms increased with increased duration of exposure. For example, only Micrococci were isolated in location VII when exposed for 15 min, whereas exposure for 30 min enabled the recovery of Staphylococcus aureus along with Micrococci, and the microbial loads obtained also increased. Similarly, Aspergillus niger was isolated from location VII only when exposed for 30 min. This increased recovery of microorganisms at 30 min may be attributed to the size of the airborne microbial particles; larger particles once airborne take time to settle. Gram-positive cocci (GPC) were more frequently isolated than Gram-negative bacilli (GNB). Staphylococci and Micrococci were the predominant GPC. GNB and moulds recovered included Klebsiella oxytoca, Pseudomonas sp. and Enterobacter sp., and Aspergillus niger, Aspergillus fumigatus, Aspergillus flavus, Penicillium sp. and Trichophyton sp. GNB and moulds were isolated from areas with high activity.

In the context of a developing country, where resources are limited, use of petri-plate gravitational settling (passive) method of sampling will be beneficial, as it is simple, cost-effective and facilitates the use of media available in the laboratory for routine diagnosis. Increased durations of sampling exposures at 30 min can be used to determine the indoor air quality in hospitals. This may be of use in locations with minimal activity where expected loads are thought to be less or in sensitive patient care areas to obtain a more accurate estimate of level of contamination.

REFERENCES: