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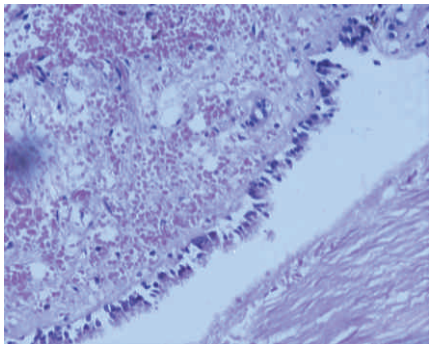

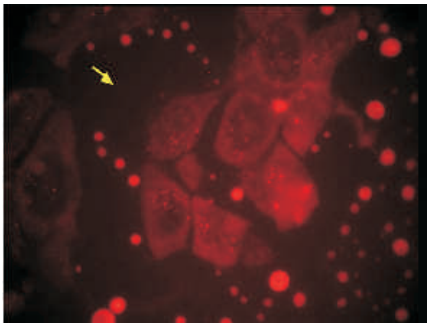

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SRI RAMACHANDRA JOUNRAL OF MEDICINE

JULY - DEC 2011

CONTENT

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MANUSCRIPT FORMAT – A PRIMARY REQUIREMENT

Manuscript preparation towards journal submission requires considerable skill and effort. Manuscript preparation encompasses many components and adherence to the required/accepted format is of primary importance.

This column highlighting the importance of manuscript format although directed towards the budding authors who would like to increase the acceptance rates of their manuscripts, we hope will also get the approving nod of the experts too.

The sense of achievement and good feeling one gets when his/her scientific manuscript is published in a Journal is well known. One reason for this is in getting the recognition and acceptance of the hard work done coupled with the satisfaction of having contributed to knowledge enhancement in an important area of science. Considerable effort would have gone into conducting the study, collection of data and its subsequent analysis by a team, which makes them realize the importance of their achievement as well as the need to share the same to others with similar interests. Of course, the best way of communicating and sharing of such new findings is to publish in scientific peer reviewed journals. This leads us to the art of manuscript preparation, the skillful technical and scientific writing entities which can make a big difference on the acceptance and rejection of a submitted manuscript by a journal. Manuscript adherence to the accepted and required format as directed by the journal guidelines is a primary requirement and the first one to be checked by the editorial team of any journal.

All components of a manuscript have specific format requirements, with every journal following a specific style as given in their Author Guidelines. For example, the various formats for the Title are the 'Sentence case', the 'Title Case' and the 'FULL CAPITALS' or 'UPPER CASE'.

Author names formats have variations that differ in the sequence of the first, last names and the initials. The inclusion of author highest academic qualifications are also insisted in a few format styles. While the affiliations and contact details of the corresponding authors along with the Key words/Mesh words generally follow a common style, the Abstract has at least 2 format style variants. One style requires that the Abstract is presented as a single continuous running text paragraph; the other 'structured' style requires that the Abstract be presented with separate Introduction, Material and Methods, Results and Discussion and Conclusion components.

The main manuscript text also has a few format variants as accepted by specific journals. For example, subsections within the main Introduction, Material & Methods, Results & Discussion and Conclusion sections are not accepted in a few format styles. Similarly, the requirement of numbering the sections/subsections is not universally common.

Of particular importance is the reference citation and listing format that is probably the most complex and has the maximum number of variants. This warrants a complete understanding of the format requirements and considerable amount of attention to fine detail to ensure absolute adherence to the required format. It is too exhaustive to present all the reference citation and listing formatting variants here, but it is worth mentioning that all authors should go through the manuscript preparation guidelines thoroughly. It is a good idea to refer to recently published articles in the journal of choice to obtain a clear idea regarding the accepted format requirements.

It is also important to note that the manuscript format requirements of a few manuscript parts can vary according to the manuscript category within a same journal. This variation might only be to certain parts of the manuscript while the a few such as the reference citation and listing formats will remain constant for manuscripts in all categories. For example, while a 'structured' Abstract is the requirement for Research Articles, a single paragraph Abstract as a running text might be the required format for 'Case studies' and 'Clinical reports' for a same journal.

To conclude, it is reiterated that complete adherence of a manuscript to ALL the format guidelines is a primary requirement and will no doubt play an important role in the initial editorial decisions regarding the suitability of a manuscript to the chosen journal.

Note: The SRJM format requirements and Guidelines for authors are provided in all print issues for easy reference.

From the Editor's Desk

On behalf of the Editorial Board, I am glad to bring out the July-Dec 2011 issue of the Sri Ramachandra Journal of Medicine. It is a welcome sign that there is a better awareness amongst the University faculty and the scientific contribution to the journal are gradually improving.

I would like to reinforce that the publication process evolved over the years has improved our quality of publication and the authors are requested to initially ratify their Publication through the Publication oversight Committee.

This issue includes three (3) original articles, one (1) Review article and five (5) case reports.

I must place on record my sincere thanks to all the Peer Reviewers, the Members of POC and the Members of the Editorial Board and Secretarial Staff for fast tracking the release of this issue.

We hope to see the Jan - July 2012 issue before 30th October 2012.

P.V. VIJAYARAGHAVAN
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The Editorial Board gratefully acknowledges their contribution

PREVALENCE OF EXCLUSIVE BREAST FEEDING PRACTICES AMONG RURAL WOMEN IN TAMIL NADU

S. Sangeetha Balamurugan[a], R Shankar[a]

ABSTRACT

Background: *Poor infant feeding practices and their consequences are one of the world's major problems and a serious obstacle to social and economic development. Various studies have shown that infant feeding could be influenced by socio-economic status, maternal education, place of living and many other factors. Hence a prevalence study on exclusive breast feeding was conducted in rural Tamil Nadu.*

Aim & Objective: *To assess the prevalence of exclusive breast feeding practices and the socio-demographic factors influencing them, among women in a rural area in Tamil Nadu.*

Material & Methods: *A Cross-sectional study was conducted in Attyampatti Panchayat Union, Salem district, Tamil Nadu, from March 2011-June 2011. All the children in the age group of 6 months to 2 yrs, constituting 291 children, and their mother's were included in the study, irrespective of any sample. The data was analysed using SPSS package.*

Results: *Among the study population of 291 children, 52.6% were male children and 47.4% were females. Only 99 (34%) children were exclusively breast fed for 6 months. Majority of women 60.5% initiated breast feeding within half an hour after delivery. Factors like education of the mother, type of delivery, type of family, number of children, had direct influence on exclusive breast feeding. Most of the mothers (44.7%) inferred that main reason for giving bottle feed is because of inadequate breast milk secretion.*

Conclusion: *The prevalence of exclusive breast feeding is low in this area. Hence to focus on the factors affecting them is highly warranted in this area.*

Key words: *Exclusive Breast feeding, Bottle feed, Rural women.*

SRJM 2011;4:1-4

INTRODUCTION

Breast feeding has been conclusively demonstrated as one of the important determinants for comprehensive growth and development of infants, more so, among low birth weight infants. For neonate, 'Breast is best' is now a universal concept. In spite of world wide campaign for promotion of breast feeding, achievements are not to the desired target. There are many factors, which may affect feeding practices in our country. Various studies have shown that infant feeding could be influenced by socio-economic status, maternal education, place of living and many other factors.[1]

Poor infant feeding practices and their consequences are one of the world's major problems and a serious obstacle to social and economic development. It is not only a problem of the developing world, it occurs in many parts of the developed world as well.[2]

For the first time in its 10th plan, the Government of India has included state specific goals, to improve infant feeding practices to reduce Infant Mortality Rate (IMR), malnutrition and promote integrated early child development. The 10th plan goals aim to increase the rate of initiation of breast feeding within 1 hour to 50% from current level of 15.8%. To increase the exclusive breast feeding rate to 80% during the first six months from the current level of around 41%.[3]

Though there has been global movement towards protecting, promoting and supporting breast milk as a part of optimal feeding practices among newborn babies, there exist many discrepancies between what has been recommended and what has been practiced in reality.[4] In general, the move is towards addressing all the newborn babies.

In this background, the present study was undertaken to identify the prevalence of breast feeding and various factors influencing them and the prevailing infant feeding practices in a rural area in Tamil Nadu.

AIM & OBJECTIVE:

1. To assess the prevalence of exclusive breast feeding practices among women in a rural area in Tamil Nadu.
2. To assess the socio-demographic factors influencing exclusive breast feeding among those study population.

MATERIAL AND METHODS

The study was conducted at Attyampatti Panchayat Union of 10,000 population. It is a cross-sectional study done during March 2011-June 2011. All the children in the age group of 6 months to 2 yrs in Attyampatti Panchayat Union, constituting 291 children, and their mother's were included in the study, irrespective of any sample.

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Using pre-tested questionnaire, mother's having children in the age group of 6 months to 2 yrs, were interviewed and asked questions related to breast feeding practices, complementary feeds, socio-demographic factors and illness episodes among children.

Statistical Analysis-Statistical tests like Chi-square, Multiple Logistic Regression and Proportions were used by using SPSS software.

RESULTS

Among the study population of 291 children,153 (52.6%) were male children and 138 (47.4%) were female children. (Table 1).

Table 1 : Age and sex wise distribution of the study population

Age Group	Male (%)	Female (%)	Total
6-9 months	22(45.8)	26(54.2)	48
10-13 months	41(56.9)	31(43.1)	72
14-17 months	36(58.1)	26(41.9)	62
18-21 months	32(53.3)	28(46.7)	60
22-24 months	22(44.9)	27(55.1)	49
Total	**153**	**138**	**291**

Table 2 : Duration of exclusive breast feeding among the study population

Duration	Frequency	Percent
< 2 months	27	9.3
2-3months	40	13.7
3-4months	24	8.2
4-5months	101	34.7
=/> 6 months	99	34.0
Total	**291**	**100.0**

Table 2 shows that only 99 (34%) children were exclusively breast fed for 6 months. Among male children, it was 51(51.5%) and among females it was 48 (48.5%), who were exclusively breast fed and this difference was not statistically significant.($p > 0.05$). Hence sex of the child, is not a factor, which influences exclusive breast feeding. Table 3 shows that majority of women 176 (60.5%) initiated breast feeding within half an hour after delivery. Table 4 shows various socio-demographic factors like education of the mother(literate/not literate), type of delivery(normal/caesarean), type of family (nuclear/non-nuclear), occupation (occupied/nonoccupied), number of children(< 2/> 2), monthly income (< 5000/> 5000), family size(< 4/> 4), age at marriage (< 18/> 18) and religion (Hindu/non-Hindu) having influence on exclusive breast feeding by using multiple-logistic regression analyses.($p < 0.0001$). It was found that, type of delivery, type of family, family size and number of children had direct influence on exclusive

Table 3 : Duration of Initiation of breast feeding

Initiation of breast feeding	Frequency	Percent
< 1/2 hr	176	60.5
1/2 - 4 hrs after birth	51	17.5
4-12 hrs after birth	14	4.8
> 12 hrs	50	17.2
Total	**291**	**100.0**

Table 4 : Multiple Logistic regression for various socio-demographic factors influencing on exclusive breast feeding

Factors	Chi-Square	P value	Final intercept (p value)
Family size	15.360	.002	
Type of delivery	6.865	.032	
Type of family	26.829	.000	
No. of children	48.981	.000	
Education of mother	8.196	0.146	
Monthly income	5.902	0.316	.0001
Occupation	9.792	0.280	
Age at marriage	9.596	.088	
Religion	1.948	.378	

breast feeding,which was found to be statistically significant ($p < 0.002$). Fig 1 shows the reasons for bottle feeding, where most of the mothers inferred that, main reason for giving bottle feed is because of inadequate breast milk secretion 130 (44.7%). Reasons like, bottle feeding more nutritious than breast milk 74(25.4%), unable to breast feed due to soreness of the nipple, inverted nipple, pain in the nipple etc. constituted 52 (17.8%), poor weight gain of the baby 27(9.3%) and others, such as lack of knowledge regarding breast feeding practices and its importance by 8 (2.8%) women. Table 5 showed that, among children, who were exclusively breast fed (n = 99), 62 (62.6%),did not have any episode of illness. Only 26 (26.2%) had 1-3 episodes of illness and 11(11.1%) had more than 3 episodes. Contrary to those who had not breast fed (n = 192),

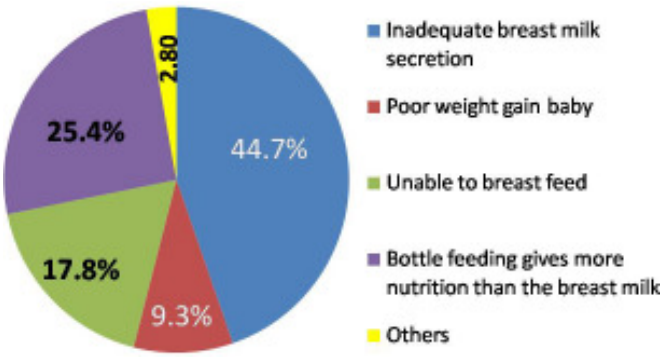

Fig. 1: Reason for bottle feeding in the study population

Table 5 : Breast feeding practices and episodes of illness among the study population

Exclusive breast feeding	Episode of illness between 6months to 1 year		Total	Pearson Chi-square	P value
	Nil	1-3 episodes			
Yes	62(70.4)	26(29.5)	88		
No	63(44.7)	78(55.3)	141	13.693	<0.001
Total	125	104	229		

Exclusive breast feeding	Episode of illness between 6months to 1 year		Total	Pearson Chi-square	P value
	Nil	>3 episodes			
Yes	62(84.9)	11(15.1)	73		
No	63(55.3)	51(44.7)	114	18.637	<0.001
Total	125	62	187		

78 (40.6%) of them had 1-3 episodes of illness, and 51 (26.6%) of them had more than 3 episodes of illness. This was found to be statistically significant. ($p < 0.001$). This shows exclusive breast feeding is protective to the baby, as it helps to combat the diseases by increasing the immune status of the child.

DISCUSSION

In a study done by Medhi.G.K and Mahanta.J in Assam tea garden workers, exclusive breast feeding rate was 69.35% upto 6 months of age, which was higher than their counterparts in Assam[5] and a similar study done by C.R.Banapurmath and Renu Sobti in Davanagere district, 73 percent of infants in the age group of 0-3 months were exclusively breastfed and 60 percent for the 4-6 month age group.[6]

In a study undertaken by Arun Gupta. and Y. P. Gupta, more than half of the children (54%) in the age group of 0-3 months were exclusively breastfed whereas this percentage was much lower (26%) for children in the age group of 4-6 months.[7]

According to NFHS-3 survey, the report showed that 35.6 % of mothers in Karnataka had initiated breast feeding within 1 hour; especially for urban population, it was 36.9 percent. The national average of mothers who had initiated breast feeding within one hour after the birth was 23.4 percent.[8] Similarly a study done by Patrica A. Haggerty and Shea O. Rutstein, 11.6% of infants in urban and 9.4% of infants in rural area were put to breast within first hour. 33.3% of infants in urban and 25.6% of infants in rural area of infants were breastfed within first day[9] and a similar study done by Ranjana Fotedar etal revealed that 20% of women initiated breast feeding their newborns within 1 hour, 50% of women initiated breast feeding their newborn baby within 6 hours, while 30% women initiated breast feeding their newborn baby after 24 hours.[10]

A study by Rajmahal P et al. noted that mothers belonging to the higher socio-economic status had a greater chance of feeding colostrum than the poor income groups[11] and a similar study by P.V.Gopujkar et al. inferred that, the higher the educational status of the family, of the father, mother or both, the lower the percentage of infants exclusively breastfed for even three months. The infants of 68% of educated mothers in Bombay, 32% in Calcutta and 44% in Madras were being exclusively breastfed till they were three months old as against 80%, 57% and 61% in the corresponding poorly educated groups[12] where as in our study neither the education nor the income had an impact over exclusive breast feeding.

A study by R.Parmar et al stated the various reasons for starting bottle feeding before 6 months, which were, like insufficient milk (59.7%), working mother (13%), to habituate the baby to bottle (12%) maternal illness and child illness (6.5%)[1] and a study by Aggarwal A etal concluded that the most common reason for early food supplementation was insufficient breast milk (49.4%).[13] Similarly a study by Chintan Parekh etal stated the predominant reasons for practice of bottle feeding were inadequate breast milk secretion, unable to breast-feed and inadequate breast milk secretion and poor weight gain of baby.[14]

A study by Onayade et al in Nigeria, found that babies reported fewer symptoms and had fewer illness episodes (0.l episodes per child) compared to those who started

complementary feeding before six months.[15]. A similar study by Deepikasur etal on incidence of diarrhea among low birth weight infants of urban slum of Calcutta, showed that early weaned infants had, significantly higher risk of occurrence of diarrhea than the exclusive breast feeding infants.[16] Another study by Seema Mihrshahi etal in Chittagong, Bangladesh, had found that, partially breast fed infants had a higher incidence of acute respiratory infection and diarrhoeal infection when compared to exclusive breast fed infants and that difference found to be statistically significant[17],which is in par with our study.

The prevalence of exclusive breast feeding in our study was 34% for the duration of six months, which is lesser than the national average of 41%.Though many National health Programmes were working for the improvement of mother and child health, still the prevalence of exclusive breast feeding has not reached 50%, so it is a right time to think for initiating a separate National health programme concentrating on improvement of exclusive breast feeding practices.

ACKNOWLEDGEMENT:

The authors are grateful to the Head of the Institution and Head of Community Medicine department of VMKV medical college, Salem, Tamil Nadu, for their permission and encouragement to conduct the study and they also thank the mother's for their co-operation and support.

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ROLE OF PCA3 GENE POLYMORPHISM IN PROSTATE CANCER

Karthika Pillai[b], Maalavika Ragunathan[b], Sunil Shroff[c], Venkatachalam P[a], Vettriselvi V[a]

ABSTRACT:

Background & Objective: *Prostate cancer is the most commonly diagnosed malignancy and the second leading cause of cancer-related deaths and its incidence is still increasing. PCa can be cured by radical surgery or radiation therapy if the disease is localized within the prostate. However, when this carcinoma has spread locally or distantly, no curative therapy can be offered, and these patients will suffer from a poor prognosis. Therefore, there is an urgent need for early diagnosis of the disease to increase the cure rate for PCa. The present study was aimed to analyse the role PCA3 gene polymorphisms in prostate cancer.*

Methodology: *Blood samples were collected from 10 histologically confirmed prostate cancer patients and 10 healthy controls from South India. DNA was isolated and in vitro amplification of the PCA3 gene by PCR was performed. PCR amplification was confirmed using a 2% gel after which the genotypes were analysed by DNA sequencing. Calculation of odds ratio at 95% confidence interval facilitated the comparison of genotype frequency distribution between the controls and cases.*

Results: *Three polymorphisms were identified: 4, 5, 6 (number represents the repeat times of TAAA in the promoter of PCA3 gene). Our results revealed a significant association between the (TAAA)6 repeats and PCa indicating a close relation between the number of repeats and incidence of prostate cancer.*

Conclusion: *PCA3 TAAA repeat polymorphism may be associated with the risk of PCa, the results are confined to a small group of samples; additional larger studies are needed to provide further insight into these preliminary findings.*

Key words : *STR, PCA3, polymorphism.*

SRJM 2011;4:5-8

INTRODUCTION

Prostate cancer (PCa) is the most common cancer in men and the fifth most common cancer in the world.[1] It is a complex, heterogeneous disease, exhibiting a spectrum of clinico-pathologic presentations. It is the second most common cancer among men with substantial difference in prevalence in different ethnic groups.[1] Inspite of the high incidence and mortality rates its etiology is poorly understood. Clinically detected prostate cancer displays a variety of phenotypic features and malignant potential.[2] Prostate cancer may be an indolent, latent disease without clinical symptoms during the lifetime of an elderly patient, or it may take an aggressive clinical course metastasizing into seminal vesicles, bladder, rectum, lymph nodes, bone and other organs. Each year in the UK about 36,000 men are diagnosed with prostate cancer. Most prostate cancers (about 75%) are considered sporadic. Familial prostate cancer is less common (about 20%) and occurs because of a combination of shared genes and environmental or lifestyle factors. Hereditary (inherited) prostate cancer is rare (about 5%) and occurs when gene mutations are passed within a family from one generation to the next.

In India, Mumbai and Delhi have reported highest incidence rates for prostate cancer. In Chennai, prostate cancer is the fourth most common cancer among men.[3] The age adjusted incidence per 100,000 reported in important centres from North India were 7.9 in Mumbai, 5.7 in Delhi and 2.6 in Kolkata. In South India, the incidence rates were 6.1 in Trivandrum, 4.7 in Bangalore and 3.6 in Chennai.[4] While the incidence rates may be low in India, studies have reported that 84% of patients in India present with advanced stages and high-grade prostatic intraepithelial neoplasia,[5,6] which suggest that early screening for prostate cancer is limited in the Indian population.

The cancer registry of our institute, Sri Ramachandra Medical College & Research Institute while reporting the relative proportion of cancers by specific site reported the highest incidence for prostate cancer (14.67%) in males, followed by the Brain (11.3%), stomach (9%) and others.[7] Although serum prostate-specific antigen (PSA) measurement is regarded as the best conventional serum tumour marker available there is not enough specificity and sensitivity for PSA in detecting prostate cancer early.[8] More prostate cancer specific and sensitive biomarkers are urgently needed.[8] The high incidence of prostate cancer recorded from our institute further stressed the need to identify the genetic variations associated with prostate cancer risk.

Prostate cancer antigen 3 (also referred as DD3) is a gene which encodes a prostate specific non-coding mRNA, which was found to be 10–100 fold over-expressed in 53 of 56 human prostate cancer samples in a Northern blot analysis whereas it was not expressed in adjacent non-malignant prostatic tissues. The PCA3 gene is located on chromosome 9 at location 9q21.2. The current understand-ing is that the PCA3 gene contains a high density of stop-codons and expresses a non-coding messenger RNA in epithelial prostate cells; it functions as a polyadenylated RNA transcript, but no cytoplasmic

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protein results from its transcription. The prostate-specific expression and the sharp up regulation of PCA3 mRNA in prostate cancer suggest a unique transcriptional regulation.[9] Since PCA3 gene expression is increased in high grade prostate cancer, study of genetic alterations in PCA3 gene may be helpful in elucidating the incidence and pathogenesis of prostate cancer. Hence the present study was aimed to analyse the role PCA3 gene polymorphisms in prostate cancer samples.

MATERIALS AND METHODS

Subjects

Blood samples were collected from 10 histologically confirmed prostate cancer patients and 10 control, healthy unrelated individuals with normal PSA levels from South India. The age of prostate cancer patients ranged from 61 to 75 years with a mean age of 67 years and the age of the controls ranged from 62 to 69 years with a mean age of 65 years. The PSA levels of the patients at diagnosis were recorded. The PSA ranged from 100 to120 ng/ml. Pathological grading of the tumors represented as Gleason scores (GS) was obtained by the histopathological examination. The pathologic grades were classified into three groups: well differentiated (GS 2-4), moderately differentiated (GS 5-6) and poorly differentiated (GS 7-10)[10]. The well and moderately differentiated groups were considered as low grade and thus patients were stratified as low grade if their Gleason scores were less than 7 and high grade if their Gleason scores were greater than or equal to 7. The GS was less than 7 in 4 patients and greater than or equal to 7 in 6 patients. Informed written consent was obtained from all the participants and the study was approved by the Institutional Ethics Committee.

DNA EXTRACTION AND GENOTYPING

5 ml of venous blood was collected from the patients and controls, genomic DNA was isolated using phenol chloroform method and ethanol precipitation, dissolved in TE buffer (pH 7.4) and stored at -20ºC. Short tandem repeat (TAAA)n polymorphisms in the promoter region of PCA3 gene was amplified using specific primers. Standard PCR reaction was performed in a total 20µl reaction volume containing 50-100ng of genomic DNA, 1X PCR buffer (1.5mM MgCl2, 10 mM Tris (pH 9.0), 50 mM KCl and 0.1% Triton X-100), 200µM dNTPs, 50pM of each primer and 1 U of TaqDNA polymerase. The cycling condition was 95º C for 5min of one cycle; 95º C for 1min, 57º C for 45 sec and 72º C for 1min 30sec for 30 cycles and final elongation cycle of 72ºC for 5min. The PCR products were visualized by 2% agarose electrophoresis and the genotype was determined by DNA sequencing. Amplified fragments were purified and subjected to cycle sequencing reaction followed by DNA sequencing.

STATISTICAL ANALYSIS

The expected genotype and allele frequencies were calculated for cases and controls. These frequencies were used to test if the population followed Hardy-Weinberg equilibrium. Calculation of odds ratio at 95% confidence interval (CI) facilitated the comparison of genotype frequency distribution between the controls and cases. All the analysis was done using SPSS (Statistical Package for Social Sciences (Version 16.0).

RESULTS

A total of 20 subjects, including 10 patients with PCa and 10 healthy individuals as control group, were analyzed for polymorphism in promoter of the PCA3 gene. DNA samples from individual patients exhibited a singleband after PCR amplification. The sequencing analysis revealed a STR in the promoter region of the PCA3 gene.In the present study, three polymorphisms were identified: 4, 5, 6. (number represents the repeat times of TAAA in the promoter of PCA3 gene, Fig 1,2,3) Number of TAAA repeats in cases and controls are given in Table 1. Six genotypes 4/4, 4/5, 4/6, 5/5, 5/6, 6/6 were observed in the present study. The frequencies of (TAAA) repeats were represented in Fig 4. The (TAAA)6 was 70 % in cases and only 10 % in the controls. The frequency of (TAAA)4 and (TAAA)5 repeats are high in controls than the cases. Our results revealed a significant association between the (TAAA)6 repeats and PCa (OR -16.33; 95% CI - 1.34 to 197.7) indicating a close relation between the number of repeats and incidence of prostate cancer. Of the 10 samples analysed 6 samples were high grade and 4 samples were low grade. TAAA repeat distribution among patients with low grade and high grade has been presented in Table 2. The frequency of (TAAA)6 were more in high grade tumor.

Table 1: TAAA repeat polymorphism in Cases and controls

No.of (TAAA)n repeats	Cases (N = 10)	Controls (N = 10)
4	0	2
5	3	7
6	7	1

Table 2: Distribution of TAAA repeats in low and high grade cancer

	Low grade (N = 4)	High grade (N = 6)
5 repeats	2 (67%)	1(33%)
6 repeats	2 (29%)	5 (71%)

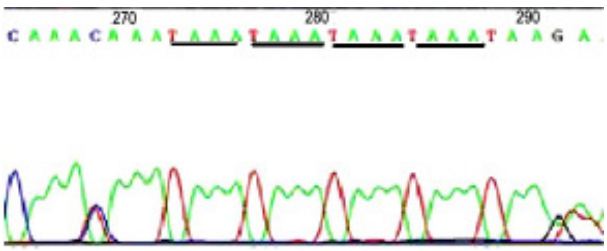

Fig 1: PCA3 gene promoter polymorphism - (TAAA)4 repeats

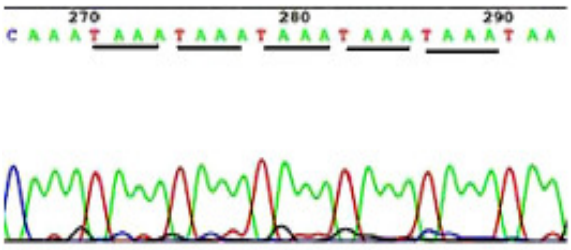

Fig 2: PCA3 gene promoter polymorphism - (TAAA)5 repeats

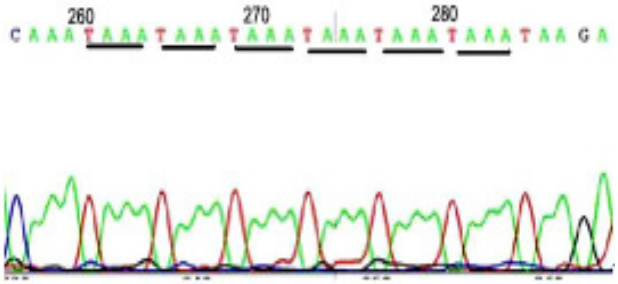

Fig 3: PCA3 gene promoter polymorphism - (TAAA)6 repeats

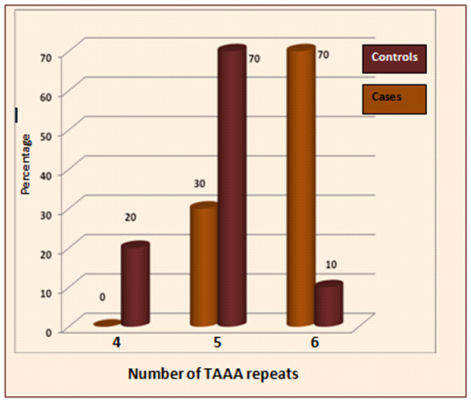

Fig. 4: Frequencies of TAAA polymorphism in cases and control

DISCUSSION

The present study was aimed at analysing the role of PCA3 gene promoter STR polymorphism in prostate cancer. STRs are repeating DNA sequences that contain 2 to 6 base-pair units and widespread throughout the human genome and polymorphic in nature. STRs are important genetic markers for mapping studies, disease diagnosis and forensic studies. Our results suggested that PCa was closely related to the TAAA repeat numbers in the promoter of PCA3, more TAAA repeats are associated with increased risk and aggressiveness of prostate cancer. Similar study in the Chinese population also reported that more TAAA repeats are associated with increased risk for prostate cancer. In the Chinese population, individuals carrying 11 repeat numbers of TAAA had a 1.76 higher risk of PCa (95% CI = 1.07 – 2.89) than those carrying 10 repeat numbers.[8] It is reported that the expression of PCA3 is very high in the PCa patients than that in healthy individuals .[9] Owing to the fact PCA3 promoter has no TATA-box, earlier studies have hypothesized that the transcription would begin from other positions. Instead of TATA-box, the (TAAA)n polymorphic region may be one of the transcriptional initiation positions. Therefore, the increase of (TAAA)n repeats in the promoter of PCA3 would increase the transcriptional initiation sites of PCA3 and up-regulated the expression of PCA3 mRNA in PCa patients.

In summary, the present findings indicate that PCA3 TAAA repeat polymorphism may be associated with the risk of PCa. However, the results are confined to a small group of samples; additional molecular and epidemiological studies analyzing the functional significance and the genes in linkage with this polymorphism are needed to clearly elucidate the role of PCA3 in the risk and pathogenesis of prostate cancer.

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STUDY OF GFP GENE INHERITANCE IN *Drosophila melanogaster*

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ABSTRACT

Back ground & Objective: *The green fluorescent protein (GFP) is a mobile artificial exon carried by a transposable P-element, is tagged on chromosome 2 of Drosophila melanogaster. This study aims at finding the pattern of inheritance of the GFP gene by using the same as a marker or a reporter.*

Method: *A simple cross was performed using the virgin females of Canton S and males of CyO GFP II.*

Results: *Analysis of the results revealed the dominant nature of the GFP gene.*

Conclusion*: This protein reveals in real time the dynamics of gene distribution in the whole, live organism and proves to be a useful marker for a variety of experiments.*

Key words*: Green Fluorescence Protein (GFP), Drosophila melanogaster, Biological marker, GFP gene*

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INTRODUCTION :

Green fluorescent protein (GFP) is a fluorescent molecule found in the jellyfish *Aequorea victoria*. It was first isolated in 1961 and cloned in 1992. GFP has become a popular reporter molecule for gene expression in both mammalian and non mammalian systems. GFP have been used as reporter molecules that provide superior monitoring capabilities to those of other reporters such as luciferase or alkaline phosphatase. GFP is also used as a nontoxic reporter in whole organisms.

The present study aims at identifying the pattern of inheritance followed by GFP gene in Drosophila by crossing virgin females of the wild type (non GFP) with males of Cyo GFP II(a double mutant of Drosophila, which has curly wings and GFP gene cloned on chromosome 2).

GFP is part of a large class of evolutionarily unrelated molecules that facilitate bioluminescence, found in organisms from bacteria to fishes. It exists as a 238-amino-acid protein; its crystal structure illustrates its conformation as a rigid β-can enclosing a β helical region that contains the chromophore. Because of its compact structure, and because the chromophore is protected from the external environment inside the β can, GFP is extremely stable: it is resistant to bases, mild denaturing and reducing agents and high temperatures, and it is capable of renaturing after exposure to harsh conditions. GFP reassembly can be used to monitor protein– protein interactions and protein expression patterns in cells and whole organisms.[1,2]

The wild-type fluorescence absorbance/excitation peak is at 395nm, with a minor peak at 475nm.

The normal emission peak is at 508 nm, continued excitation leads to a decrease over time of the 395nm excitation peak and a reciprocal increase in the 475nm excitation peak. This interconversion effect are especially evident with irradiation of GFP with UV light.[2,3]

The currently known GFP variants may be divided into seven classes based on the distinctive component of their chromophores: class 1, wild-type mixture of neutral phenol and anionic phenolate; class 2, phenolate anion; class 3, neutral phenol; class 4, phenolate anion with stacked¼-electron system; class 5, indole; class 6, imidazole; and class 7, phenyl. Each class has a distinct set of excitation and emission wavelengths. Classes 1–4 are derived from polypeptides with Tyr at position 66, whereas classes 5–7 result from Trp, His, and Phe at that position.[2,4,5,6,7]

Transposon-mediated germ-line transformations have been the approach most extensively used to obtain transgenic insects and other animals. In this approach a DNA Transposon is used in a binary vector/ helper system that allows the precise and stable insertion of any desirable DNA sequence into the target genome.[7,8,9]

Balancer chromosomes are valuable research tools. GFP acts as a genetic tag for the presence of the balancer allowing identification of homozygotes by virtue of their lack of GFP signal.[8]

Cell biological applications of GFP may be divided into uses as a tag or as an indicator. In tagging applications, the great majority to date, GFP fluorescence merely reflects levels of gene expression or subcellular localizations caused by targeting domains or host proteins to which GFP is fused. As an indicator, GFP fluorescence can also be modulated post translationaly by its chemical environment and protein-protein interactions.[9,10,11]

The rigid shell in GFP surrounding the chromophore enables it to be fluorescent and protects it from photo bleaching but also hinders environmental sensitivity. GFPs that act as indicators of their environment have been created by combinations of random and directed mutagenesis.[11,12]

MATERIALS AND METHODS:

Establishment of the Cross and F1 Generation analysis:

Stocks (Canton S and Cyo GFP II) were procured and maintained in aseptic conditions at 22°C in an incubator.

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Stocks were bred on corn meal agar(medium containing corn flour, sugar, dextrose, yeast extract and antifungal agents) for two generations and healthy flies were collected for the cross. Virgin females of the Canton S strain (wild type) were collected by the removal method. The CyO GFP II flies were etherized and the males were separated. The virgin females of Canton S and males of CyO GFP II were seeded in a bottle containing fresh corn meal agar. When eggs are observed on the surface of the food, the parent flies are transferred to fresh food. The bottles were maintained at 22°C under sterile conditions to avoid fungal attack. After about 2 weeks the F1 emerges. The maintenance of the stocks and crosses were performed employing standard procedures.[13] The flies of the F1 generation were etherized and observed under the fluorescent microscope to check the pattern of expression of the GFP gene (by looking for the flies that glowed green under the GFP filter). The results of the cross were documented manually by scoring all the flies that emerged in the F1 generation. The scoring criteria was simple, since the study was about evaluating the inheritance of GFP gene, only flies which inherited the GFP gene would glow green under the fluorescence microscope. Since the GFP parent also had curly wings, the wing structure was also observed in the F1 generation to look for the dominant/ recessive expression of GFP and the curly wing expression.

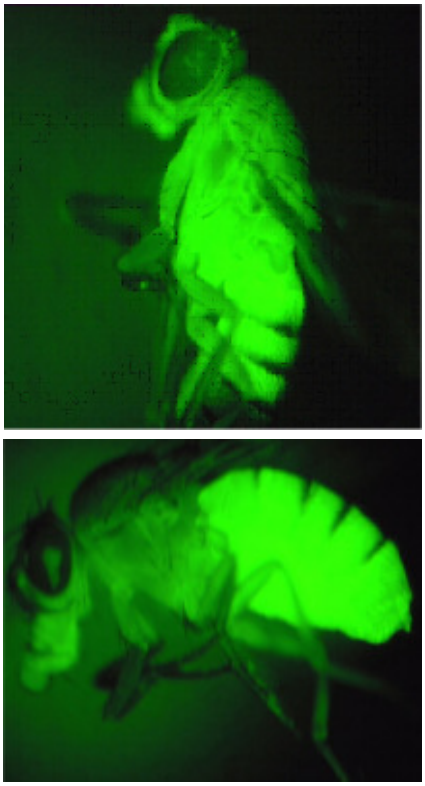

Fig. 1 & 2: Analysis of the cross : Cyo GFP(II)

RESULTS:

Around 200 flies (males and females) obtained from the F1 generation were etherized and observed under the fluorescent microscope to analyze the pattern of GFP gene expression. The Experimental cross was set up in triplicates and the F1 generation obtained from all the three bottles demonstrated similar patterns of inheritance which is indicative of consistence and reproducibility minimizing the chances of errors in observation and analysis (Fig.1& 2)

ANALYSIS REVEALED:

- Dominant expression of the GFP gene was visualized in both males and females - all the males and females of the F1 generation glowed green under the GFP filter indicating that all the flies had inherited the GFP gene from the parent which indicates the dominant nature of the GFP gene.
- Wild type wing structure was observed in both males and females – since the GFP parent had curly wings, the wing structure of the F1 was analyzed to look for the inheritance of the curly wing phenotype. Since, all the flies in F1 showed wild type wing structure, it was concluded that curly wing was recessive to the wild type wing phenotype.
- Wild type color of the eye (red) was prominent in both males and females – there was no *in vitro* mutation in the eye color or other body parts as a result of the cross.

DISCUSSION:

The ability to study the pattern of inheritance of a gene within an organism relies on the availability of techniques that can detect changes in gene expression within specific cells or tissues during cell movements and migrations. The green fluorescent protein from the jellyfish, *A. victoria*, acts as a viable marker in Drosophila to observe certain characteristic phenotypes of interest, in this study- the inheritance pattern or the expression pattern of the GFP gene itself.[1,2]

The GFP gene in *Drosophila melanogaster* is tagged on chromosome 2. CyO is a balancer of Chromosome 2. It serves as a suppressor of crossing over and helps maintain lethal and sterile mutations to be maintained in the heterozygote.[1,2]

Analysis made on the F1 generation in both the sexes of the cross, suggests that the inheritance pattern of the GFP gene was dominant and not sex linked. By performing a test cross it is possible to determine the genotype and confirm the homozygous or heterozygous status of the GFP gene. The wings of the flies and the eye color in both males and females took after the wild type, proving the dominant nature of the wild type genes involved in wing formation and eye pigmentation. There has been no study conducted 'hitherto' to record the inheritance of GFP in the cross with wild type flies. However other studies have employed GFP tagged flies to track and record genes specific for the study since GFP serves as a tagging/indicator molecule with stability, reproducibility and non toxic properties.

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Review Article

MOLECULAR PATHOLOGY- GENTLY STEPPING OUT FROM RESEARCH AREA TO DIAGNOSTIC ARENA

Sandhya Sundaram

ABSTRACT:

Molecular pathology is a discipline within pathology which focuses on the study of diseases through the examination of molecules within organs, tissues or bodily fluids.

It encompasses the development of molecular and genetic approaches to the diagnosis and classification of human tumors.Molecular pathology which was once a specialized component of the research laboratory is slowly emerging as a significant player in the field of cancer biology. Molecular testing is expanding as archival paraffin embedded tissue, fresh and frozen tissues have become good sources of DNA and RNA. This review addresses the basic molecular pathology concepts, its increasing applications in practical diagnosis, prognosis and therapeutic interventions

Key words: *Molecular techniques, Cancer diagnosis, Pathology.*

SRJM 2011;4:12-21

INTRODUCTION:

In this new era of personalized medicine, advances in the biological sciences and technology are providing molecular targets for diagnosing and treating cancer. The present classifications in surgical pathology for staging malignancies are based primarily on histopathological appearance (e.g., grade) and anatomic parameters (e.g., tumor-node metastasis). However, newer molecular techniques are revealing a molecular diversity among cancers that promises to form the basis for classification of tumors according to their prognostic and, more importantly therapeutic significance. Molecular pathology was once a specialized component of the research laboratory is now slowly emerging and assuming an important role in the realm of cancer biology. Just as we entered the era of immunohistochemistry a quarter of a century ago, we are now at the threshold of "molecular cellular pathology".

The present review addresses the basic aspects of molecular pathology, the benefits, the challenges and its possible clinical applications.

So, what is molecular pathology?

Cancer develops when the functions of the genes controlling cell division, cell repair, apoptosis and angiogenesis are impaired. A unique signature can be found within the genetic programming of each tumor revealing its molecular history. For example mutations of p53 correlate with aggressive histologic features,[1] early invasive potential and resistance to therapy.[2] In breast cancer, detection of p53 mutation by molecular analysis is an independent predictor of poor response to tamoxifen.[2, 3]

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Molecular pathology is an emerging discipline within pathology which is focused in the study and diagnosis of disease through the examination of molecules within organs, tissues or bodily fluids. It is a scientific discipline that encompasses the development of molecular and genetic approaches to the diagnosis and classification of human tumors. It recognizes the disease at the fundamental level in relation to nucleic acid abnormalities or by means of techniques involving DNA or RNA analysis.[4]

Goals of Molecular Pathology

Foremost goal of molecular pathology is to establish a definitive diagnosis, based on recognition of "fingerprints" of unique molecular alterations in specific tumor types. It also provides the ability of early detection of tumor cells using sensitive molecular techniques, thus anticipating therapeutic intervention. It further helps to render prognostic information of clinical relevance, through the assessment of molecular predictors of outcome. In addition it also contributes towards selection of individualized treatment regimens, thus saving unnecessary drug toxicity.

Protocols based on molecular markers are expected to increase the chances for cure by opting for the right management approach, and improve the quality of life of patients with cancer.

Background Basics

Genes are made up of nucleic acids and contain the information necessary for the construction of proteins from amino acids within a cell. DNA makes up genes, and RNA transcribes the genetic code held within the DNA into proteins.

So, the DNA has the blue print or the master plan!!

The genetic code within the genes is composed of nucleic acids, for which nucleotides are the building blocks. Nucleotides, made up of a sugar-phosphate backbone with a nitrogenous base, are either purines- adenine (A) and guanine (G) in DNA and RNA- or pyrimidines-thymine (T) and cytosine (C) in DNA while uracil (U) replaces T in RNA. The nucleotides that makeup the genes are arranged in a

double- stranded right handed helix. Nucleotides in DNA are arranged sequentially so that a gene will code for a matching protein. Within a double helix pattern, A, a purine, always binds with T, a pyrimidine, and G always binds with C, giving a nucleotide sequence for which one strand is a "mirror image" of the other strand.[5-10] There are 46 chromosomes (23 pairs) in a human diploid cell, on which all genes are located. Chromosomes are paired, and as such a gene is found on a locus on each of the 2 paired chromosomes, giving 2 copies, or alleles, of genes. Transcription, the synthesis of messenger RNA (mRNA) from a DNA strand, is a key step in the formation of protein coded by DNA. Base pairs are matched with the DNA template to produce a mirror image of the DNA template, except with the substitution of U for T, forming a strand of mRNA.[11]

A series of 3 base pairs in a gene, called a codon, code for a specific amino acid resulting in the synthesis of a specific protein. Transfer RNA assists in translation.[5-10] After translation, modifications to the newly formed protein occur in order for it to function, to move within the cell, or to fold properly. Methylation, acetylation, phosphorylation, glycosylation, posttranslational cleavage, and the addition of lipid groups are examples of posttranslational modifications. The newly formed protein is inert until made functional by a posttranslational modification such as phosphorylation or proteolytic cleavage. Phosphorylation, the addition to the protein of a phosphate group catalyzed by enzymes called kinases, may cause, for example, translocation of the protein from the cytosol into the nucleus. Dephosphorylation is the removal of a phosphate group catalyzed by enzymes called phosphatases. Phosphorylation and dephosphorylation of proteins are important in the activation and deactivation of cell cycle proteins, signaling pathway proteins, and transcription factor proteins.[5-10]

Molecular pathology techniques

Before the analysis of nucleic acids can be undertaken they must, of course, be extracted from the cells or tissues of interest! Sources of tissue could be peripheral blood, body fluids, fresh tissue, frozen tissue or even paraffin embedded tissue.

Tissue preparation

All fixatives damage nucleic acids to some degree through chemical interactions. Some types of fixatives, such as picric acid, mercury-containing solutions, and acid decalcifiers, may not be compatible with molecular testing.[12-15] Formalin- and alcohol-based fixatives preserve DNA reasonably well, but there is high variability introduced by several different factors. Optimal time of fixation is between 12 and 24 hours, after which time, the DNA that is obtained is of much lower quality.[16] The average fragment length of DNA obtained from formalin-fixed, paraffin-embedded tissues is often only between 300 and 400 base pairs.[17] In contrast, DNA from fresh tissue or blood will often range up to thousands of base pairs in length.[18]

RNA is substantially degraded during formalin fixation and paraffin processing as compared with parallel fresh tissue.[19,20] RNA fragments obtained from paraffin-embedded tissue samples are often small, ranging from 100 to 500 base pairs in length. Optimization includes avoiding over fixation and under fixation (from 12 to 24 hours is ideal).[21] Fixation at 4°C is preferred to room temperature, although this may not be feasible in pathology laboratories.[22] Because DNA is fairly robust and does not degrade rapidly, fresh tissue or fluid can be stored in the refrigerator.[18]

RNA, on the other hand, is more labile because of RNase in tissue. Therefore, tissue or fluid samples intended for RNA testing should be handled carefully and quickly. Stored tissues will yield the best DNA or RNA if they are snap frozen in liquid nitrogen.[23] Degradation will occur if the frozen tissue is subjected to multiple freeze-thaw cycles. It is usually recommended to pulverize frozen tissue before extraction. This can be done mechanically using a variety of mortar and pestle implements. Tissue can be cored out of paraffin blocks or tissue sections can be cut from the blocks at 10 to 20μm thick and made into tissue scrolls (rolled sections), which are stored in tubes. A number of scrolls can be placed into one tube for digestion; however, if too many tissue scrolls are digested together, the sample may paradoxically yield less DNA. The most commonly used micro dissection procedures include manual micro dissection and laser capture micro dissection.

Nucleic acid extraction

The main function of an extraction technique is to purify DNA and to eliminate contaminants. Nucleic acid extraction historically has used organic techniques using chloroform and phenol; however, automated nucleic acid extraction exists today and is frequently used to purify nucleic acids for their use with other molecular methods. Other techniques include salting out, guanidium thiocyanate method, extraction via metal chelating agents, or purification using commercially available extraction.

DNA is remarkably physicochemically stable in relation to RNA. RNA has an almost "mercurial" function in the cellular environment and might be expected to be less stable.[4] The potential enemies to the extraction of the two types of nucleic acid lie in their catalytic enzymes (DNase & RNase). It is easy to extract DNA because of the easy removal of DNase by heat treatment or adding chemicals. RNA extraction is difficult due to removal of stable RNase and contamination.

Nucleic acid analysis

Nucleic acid based testing is becoming a crucial tool not only in the setting of research or inherited genetic disease but in a wide variety of neoplastic and infectious processes.

Analysis of nucleic acids is commonly performed to determine the average concentrations of DNA or RNA present in a mixture, as well as their purity. Reactions that use nucleic acids often require particular amounts and purity for optimum performance. There are several methods to establish the concentration of a solution of nucleic acids. Two common methods of analyzing purity and concentration of nucleic acid used are: Spectro-photometry and fluorometry.[24] Spectrophotometers measure absorbance of light in the UV spectrum. Nucleic acids absorb light maximally at 260 nm, whereas protein contaminants absorb at 280 nm.[25] The ratio of absorbance at 260 to 280 nm is a measure of its purity. The ideal ratio is generally greater than 1.8. Fluorometers use a fluorescent dye that incorporates into double-stranded DNA.[26] Other methods include column chromatography, selective precipitation, selective staining of RNA and DNA, and ultracentrifugation on cesium chloride layers. Nanodrop 1000 spectrophotometer and 3300 fluorospectro-photometer provide nucleic acid quantification using only 1 μL of sample, which may be useful when isolating nucleic acids from small micro dissected samples.[18]

Extracted and purified DNA can be stored for prolonged periods, especially when stored optimally at -80°C.[27,28] Storage at typical freezer or refrigerator temperatures (-20°C or -4°C) will cause degradation of the DNA over time. RNA should be stored frozen, either as an aqueous solution at -70°C or as an ethanol precipitate at -20°C.[29] To use RNA as a template for PCR, it must first be converted into a DNA-like sequence called complementary DNA (cDNA). The process of conversion of RNA to cDNA is called reverse transcription (RT), and it is accomplished using the enzyme reverse transcriptase.

Gel electrophoresis

Nucleic acids or their fragments are separated on a layer of gel (usually agarose). The gel is usually buffered at neutral pH, at which phosphate groups in nucleic acids are negatively charged and the molecules migrate towards the positive electrode. The speed that the molecules move depends on their sizes, and they separate into bands along the gel because the gel has "pores" that variably limit the rate of migration. The nucleic acid bands are then demonstrated by means of ultraviolet light or by reaction with certain chemical reagents (such as ethidium bromide). It is used for the detection of neoplastic genes, especially when there is a very small mutation. It is a rapid technique and depends on the fact that small changes in single stranded DNA molecules can be detected by their changes in speed in gel preparations when compared with control DNA. The rationale of this method is that such alterations lead to a new molecular structure at the three dimensional level. This method can detect even single base changes. DNA samples are amplified by the polymerase chain reaction then denatured into single strands and separated on gels. This method allows screening for gene changes, followed by sequencing.

Tools of molecular Pathology

A variety of techniques exist for molecular pathology diagnosis, which include Southern blotting, restriction fragment length polymorphism, sequencing, liquid bead microarrays, mass spectrometry, and comparative genomic hybridization, among others. For practical pathology laboratory based molecular diagnosis, polymerase chain reaction (PCR), fluorescence in situ hybridization (FISH) and microarray are the important techniques commonly practiced.

FLORESCENT IN SITU HYBRIDIZATION (FISH)

In situ hybridization uses DNA or RNA probes to evaluate intact cells for genetic changes. Probes visualized with a chromogen that produces a coloured chemical at the reaction site is called Chromogenic in situ Hybridization (CISH) and probes using fluorescent labels are called Florescent in Situ Hybridization (FISH).

FISH is a technique used to detect and localize the presence or absence of specific DNA sequences on chromosomes.(Fig.1) FISH uses fluorescent probes that bind to only those parts of the chromosome with which they show a high degree of sequence similarity. Probes that are complementary to nucleic acid sequences in both directions along the molecule can be produced, so that both "sense" and "antisense" probes are available.

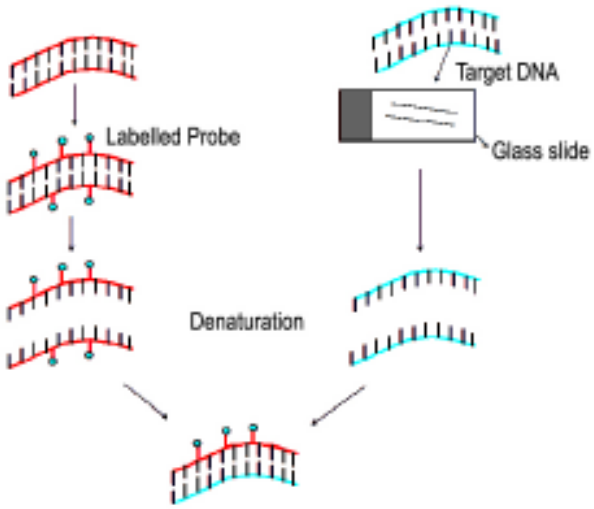

Fig. 1: Principle of FISH technology

FISH is based on the use of fluorescence-labeled oligonucleotide probes that specifically attach to their complementary DNA sequence target on the genome and label that region with fluorescence color (e.g., Texas red, FITCI green, acridine orange). The labeled region can then be easily visualized under a fluorescence microscope.[30] Peripheral blood, urine, sputum, endoscopic brushings and washings, frozen tissue and paraffin-embedded, formalin-fixed tissue are all suitable for FISH. However, fixation of tissue with formalin for longer than 48 hours may yield poorer FISH results.

Applications

FISH is often used for finding specific features in DNA for use in genetic counseling, medicine, and species identification. It can also be used to detect and localize specific mRNAs within tissue samples. It offers great advantages over conventional cytogenetics in the study of chromosomal deletions and translocations and gene amplifications.[31,32] FISH is commonly performed for the identification of abnormal chromosome numbers in cancer.[33] Common examples include the identification of trisomy 8 in many hematologic tumors and sarcomas and trisomy 12 in chronic lymphocytic leukemia (CLL).[34-36] These abnormalities are detected by using centromere-specific probes and may provide important prognostic information.[37,38] Studies suggest that detection of numeric chromosomal abnormalities; gain of chromosomes 3, 7 and 17; and loss of 9p21 help increase the sensitivity of urine cytology for the presence of transitional cell carcinoma.[39-41]

Identification of chromosomal translocations by FISH has been used frequently for tumor diagnosis. The best known example is the detection of the t(9;22) (q34;22q11) in chronic myelogenous leukemia (CML). This cytogenetic abnormality occurs in > 90–95% of CML and in a small subset of acute lymphoblastic leukemias and results in the fusion of the BCR to the ABL gene.[42,43] Gene amplification is an oncogenic mechanism observed in most types of cancer.[44-46] FISH has been used to detect gene amplification because the results may possess prognostic and therapeutic implications. Role of FISH in Her 2 neu is a major area where it has therapeutic applications.

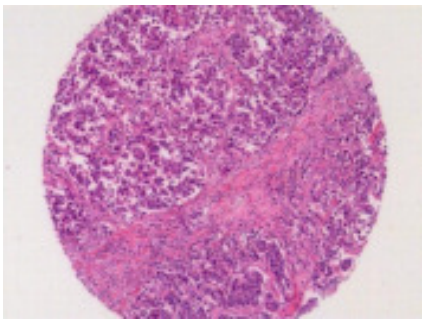

Fig. 2: Tissue micro array of breast carcinoma

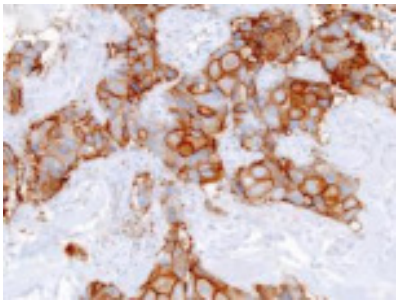

Fig. 3: Immunohistochemical staining of Grade II Her 2 Neu staining

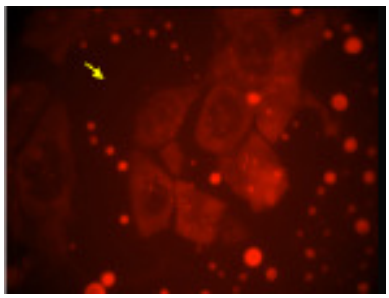

Fig. 4 : Ner 2 Neu over amplification in FISH

In breast carcinoma (Fig.2) Her2 Neu is a prognostic and therapeutic marker. The most commonly used methods for the detection of Her-2/Neu status is immunohistochemical staining. (Fig.3) However, this is not an optimal quantitative assay as there is significant variation particularly in 2+ score/reactions. The Her 2 Neu FISH amplification testing (Fig. 4) is highly accurate and of great value in resolving such cases.

Inactivation of tumor suppressor genes, such as p53 and RB, is another rather commonly observed genetic event in cancer.[47-50] Several mechanisms of tumor suppressor gene inactivation have been reported, including loss of a chromosome segment or locus containing the normal allele of a tumor suppressor gene that has already been inactivated in the homologous chromosome, usually by a point mutation.[51] This is referred to as loss of heterozygosity and can be detected with FISH probes specific to the commonly deleted regions. FISH deletional analysis is particularly useful for cytologic specimens in which cyto-morphologic overlap between reactive and neoplastic cells makes the diagnosis challenging.

The Polymerase chain reaction (PCR)

Since its introduction in 1985, PCR has been refined to be an efficient and sensitive method of studying the molecular pathology of primary and metastatic neoplasms, inflammatory mechanisms and infectious diseases. The discovery of PCR marked the beginning of an explosion of molecular applications to samples that could never have been analyzed before. In PCR, the nucleic acid area of interest is selectively amplified up to 1 million-fold, resulting in production of abundant and specific amplicons. It relies on the presence of DNA polymerase to catalyse the reproduction of a specific DNA sequence; however, to enable DNA to undergo this process, it must be heated to a high temperature to split it into single strands. This temperature is between 94°C and 95°C, a temperature at which proteins such as the polymerases would be denatured.

Using a pair of priming complementary sequences (oligonucleotide primers) flanking a location of interest, multiple copies of a targeted chimeric gene can be obtained. Each PCR cycle involves 3 basic steps: denaturing, annealing, and polymerization. During denaturing, the 2 strands of the helix of the target genetic material are unwound and separated by heating at 90° to 95°C. During annealing, or hybridization, oligonucleotide primers bind to their complementary bases on the single-stranded DNA. This step requires a much cooler temperature, 55°C. Finally, during

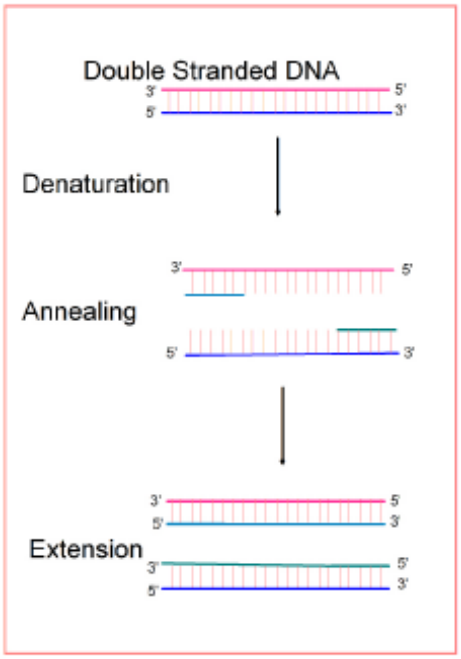

Fig. 5: Schematic drawing showing the steps of PCR

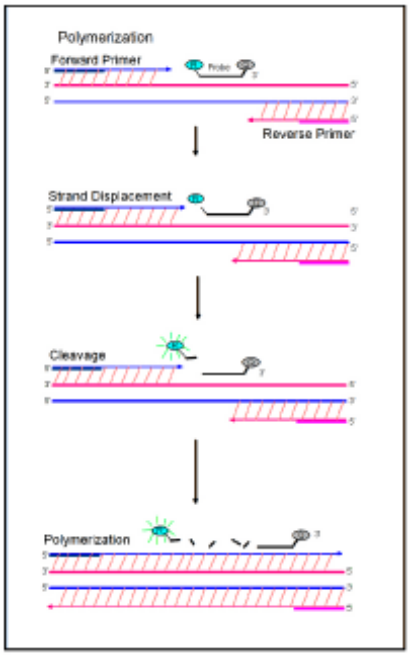

Fig. 6: Schematic drawing showing the steps of PCR

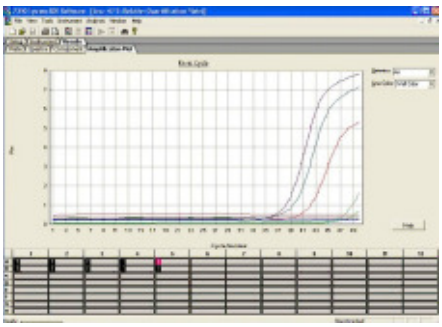

Fig. 7: Realtime PCR graph showing expression of diverse genes

polymerization (at 75°C), the polymerase reads the template strand and quickly matches it with the appropriate nucleotides, resulting in 2 new helixes consisting of part of the original strand and the complementary strand that was just assembled. The process is repeated 30 to 40 times, each cycle doubling the amount of the targeted genetic material. At the end of the PCR procedure, millions of identical copies of the original specific DNA sequence are generated.(Fig.5) Since these copies are identical in electrical charge as well as molecular weight, they are expected to migrate simultaneously, forming a single band, when applied to an electrophoretic gel.

PCR can also be used to amplify an RNA target sequence. This procedure is termed reverse transcriptase PCR (RT-PCR). The RNA sequence is first converted to a double-stranded nucleic acid sequence (cDNA) by using a reverse transcriptase enzyme. The cDNA sequence can then be amplified by using the regular PCR cycles process.[30]

The recent development of "real-time" PCR added great advantages to traditional PCR. Real-time PCR improves upon quantitative endpoint PCR by measuring target amplification early in the reaction when amplification is proceeding most efficiently. Real-time PCR measures the amount of amplicon produced during each cycle of amplification using fluorescence-based technology.(Fig. 6) Real-time PCR can quantify amplicon production at the exponential phase of the PCR reaction in contrast to measuring the amount of product at the end-point of the reaction. The amplicon is monitored in "real-time", or as it is being produced, by labeling and detecting the accumulating product with a fluorescently tagged substrate during the amplification procedure.(Fig. 7) This method has many advantages over conventional PCR including increased speed due to reduced cycle number, lack of post-PCR gel electrophoresis detection of products, and higher sensitivity of the fluorescent dyes used for the detection of the amplicon. It is, moreover, less prone to contamination since the entire process of amplification and quantitation of the original target DNA for each sample is done in a single sealed tube. However, real-time PCR requires sophisticated equipment in comparison to conventional.[52] Non-specific fluorescent dyes/ Sequence-specific DNA probes labeled with a fluorescent reporter can quantify messenger RNA by using reverse transcriptase.

Applications of PCR in molecular pathology

The Polymerase Chain Reaction (PCR) has widespread use in several areas particularly in genetic analysis, medical applications; infectious diseases, forensic studies, research areas. Many forms of cancer involve alterations of oncogenes. By using PCR-based tests these mutations can be studied and therapy regimens can sometimes be individually customized to a patient as represented below. Few key areas of its importance in relation to neoplastic lesions are depicted in table 1.

Table 1 : PCR APPLICATIONS IN PATHOLOGY

S.No.	Uses	Description
1.	Lung Cancer	Epidermal Growth Factor Receptor (EGFR) mutational analysis in lung cancer. Amplification of myc in lung cancer, leads to overproduction of the p62 protein, which has been observed in small cell and non-small cell carcinomas. This is associated with tumor progression and a poor response to chemotherapy.[4]
2	Cervical & Testicular tumors	The over expression of myc in cervical cancers has been associated with poor prognosis and increased relapse while in testicular teratoma myc associated protein p62 is related to better differentiation of the tumors.[4]
3	Breast Cancer	HER2/neu over expression is increasingly being used in breast cancer for targeted therapy with monoclonal antibodies. Increased expression of ras seen in 60% of breast carcinoma is linked to poor prognosis.
4	Colorectal and Head & Neck Cancers	K- ras mutations analysis in colorectal and head & neck cancers targeted treatment. Mutated ras gene may be found in stool sample in case of colorectal cancers.
5	Bladder and Prostate Cancer	Presence of alterations of p53 and p27/Kip1 in bladder and prostate cancer suggest a poor prognosis. Primary prostatic carcinomas displaying the p27 low or negative phenotypes were found to be biologically more aggressive.
6	Pancreatic Cancers	ras mutation studies. Up to 85% of pancreatic cancers have mutations of ras which can be found in stool samples.[4]
7	Soft tissue tumors	Detect the most common types of *EWS-FLI1* translocations and also to differentiate between the *EWS-FLI1* type 1 and type 2 fusions SSX 1 & SSX 2 in synovial sarcomas
8	Endocrine pathology	RET mutation in medullary carcinoma thyroid .
9	Hematologic malignancies	Gene Rearrangement assays and clonality studies for different categories of lymphomas.
10	Gene testing in established cancers	Breast and ovarian cancers (BRCA1 and BRCA2), breast carcinoma and childhood sarcomas (p53), colonic carcinoma and polyps (MSH2 and MLH1), and retinoblastoma (Rb1).[4]
11	Single Nucleotide Polymorphism (SNP) studies	Predictive genetic testing and identification of relevant SNP's in neoplastic processes.
12	Microsatellite Instability Analysis (MSI) studies	Identification of Microsatellite Instability Analysis (MSI) in several tumors.
13	Identification of micro-metastases	Identification of micro-metastases or minimal residual disease in some cancers particularly in colorectal cancer, neuroblastoma and prostate cancer.
14	Others	Construction of cancer predisposition tests Thymidine phosphorylase and other nucleotide excision repair genes expression in assessing response to therapy. Methylation analysis for MGMT, FUS1, MDM2 promoter in brain tumors.

DNA Microarray Technology

DNA micro array offers the ability to look at the expression of thousands of gene in a single experiment called the gene expression profiling which determines the phenotype of any particular cell. It consists of an arrayed series of thousands of microscopic spots of DNA oligonucleotides containing picomoles (10^{-12} moles) of a specific DNA sequence. Probe-target hybridization is usually detected and quantified by detection of fluorophore, silver, or chemiluminescence-labeled targets to determine relative abundance of nucleic acid sequences in the target. The 2 types of DNA microarrays that are widely used are cDNA microarrays and oligonucleotide / DNA chips.

In cDNA microarrays, DNA sequences complementary to a library of mRNA from thousands of genes are mechanically placed on a single glass slide. The immobilized cDNA sequences serve as anchoring probes to which mRNA extracted from the tested sample will specifically attach during hybridization. If the tested mRNA is first tagged with a fluorescent dye, the intensity of fluorescence at each anchoring probe location would be proportional to the amount of mRNA (degree of expression) of the gene at that location. A microarray reader displays the intensity of fluorescence at each cDNA location as a colored dot per gene location on a grid. (Fig. 8)[30]

Oligonucleotide/DNA chips are silicon chips on which the "anchoring" oligonucleotide sequences are directly synthesized and serve as the immobilized probes to which the complementary specific mRNA will hybridize.[30] The applications of these technologies are limitless. By analyzing and comparing hundreds of tumor samples, databases of gene expression "fingerprints" can be built with specific patterns of expression linked to both prognosis and outcome of therapy.[53-58] The usefulness of the microarray technology is in the fact that the several genes can be studied at the same time in a rapid way. It can compare the activity of many genes in diseased and healthy cells and categorize diseases into subgroups.[59]

Applications of Microarray

Some of the important applications of microarrays in pathology are listed in table 2.

Microarray technology holds great promise for the future of molecular diagnostics of cancer biology and with cautious monitoring may be used to provide better diagnostic methods and therapeutic strategies.

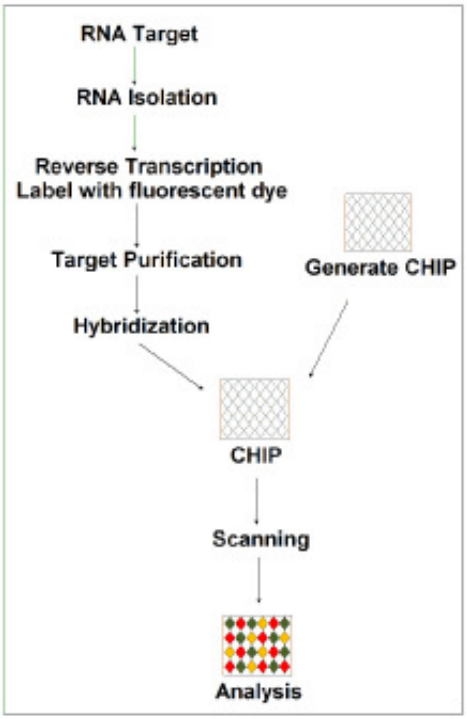

Fig. 8: Schematic drawing showing the steps of cDNA microarray reaction

Table 2 : APPLICATIONS OF MICROARRAY IN PATHOLOGY

S.No.	Uses	Description
1.	Gene expression profiling	Microarray reveals the level of expression of tens of thousands of genes in the nucleic acids extracted from complex tumor samples.[60]
2.	Genotyping of tumors	Genomic DNA from blood or saliva can be used for genotyping thousands of genetic markers in a single hybridization.[60]
3.	DNA sequencing	Thousands of base pairs of DNA can be screened on a single microarray for mutations in specific genes whose normal sequence is already known thus providing a precise molecular diagnosis in single gene and genetically complex diseases.[60]
4.	Identification of Single-Nucleotide Polymorphism (SNPs)	"SNP chips", capable of genotyping up to 2000 polymorphisms in a single hybridization, helps in construction of an individual's "genetic fingerprint", that can be related to his or her risk of developing single gene disorders or other complex diseases.
5.	Mutation analysis	Microarray-based mutation detection may become a fast and reliable method for mutational analysis such as K-ras, BRCA1, BRCA2 and p53 genes.
6.	Discovery of novel genes and pathways associated with transformation	Target genes for several gene products, which regulate transcription either directly or indirectly, have been identified using microarray-based expression profiling.
7.	Discovery of new diagnostic and prognostic indicators and biomarkers of therapeutic response	DNA microarray-based expression profiling is also used to identify markers of diagnostic and prognostic value.

8.	Improved tumor classification	Several groups have used DNA microarrays for classifying tumors into different grades or groups that may have therapeutic implications. Microarray analysis has proven capable of distinguishing subtypes of leukemia and lymphoma[61, 62] and in molecular classification of breast carcinoma.
9.	Identification of the gene(s) involved in metastatic process	DNA microarrays have proved useful in identifying gene(s) specifically involved in the transition of non-metastatic to metastatic tumors.
10.	Simplifying steps in drug discovery and validation	Traditional methods of drug discovery are time-consuming and not cost-effective. With the use of microarray-based gene expression profiling, it may be possible to do several steps of drug design and validation in a much simpler way.[63]
11.	Identification of genes associated with drug sensitivity and chemo resistance	The NCI60 microarray data by Scherf et al unveiled a very strong negative correlation between the level of dihydropyrimidine dehydrogenase (DPYD) mRNA and the potency of 5-FU (5-fluorouracil), with most of the cell lines deficient in DPYD showing high levels of sensitivity to 5-FU.[64]

CONCLUSION

Molecular testing can help guide appropriate treatment by identifying specific targets of several newly tailored drugs, thus playing an integral role in reshaping our approach to diagnosis and therapy. Molecular Pathology is a rapidly evolving field with tremendous implications in the newly emerging array of individualized therapies. The most critical factor however, is the proper standardization of procedures in the laboratory to ensure a high quality diagnostic, theranostic and genomic applications.

Just as a surgeon can never be separated from his knife in spite of a levy of modern methods, a pathologist can never do away with the old faithful armamentarium that is formalin fixed paraffin embedded hematoxylin –eosin stained sections.

To conclude the molecular techniques can be used to confirm, complement and refine the information obtained from routine histological slides. These powerful tools have to be judiciously used and cannot replace the traditional histomorphology in providing basic diagnosis.

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MEDIASTINAL FOREGUT DUPLICATION CYST

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ABSTRACT

We report an uncommon case of mediastinal foregut duplication cyst in an infant. Our case is a 2 months old infant (female) who presented to the outpatient clinic at Sri Ramachandra Medical College and Research Institute with a history of cough and breathing difficulty. CT scan showed a non enhancing cystic lesion in the superior mediastinum, which was completely excised and proved to be a Bronchogenic cyst.

Key words: *Bronchogenic cyst, Foregut duplication cyst, Mediastinal mass*

SRJM 2011;4:22-24

INTRODUCTION

Embryological cysts of the mediastinum are uncommon lesions that constitute 15% of all mediastinal masses[1]. Three types of foregut cysts are described: Bronchogenic, intramural esophageal and enteric cysts.[1] They represent an abnormal budding from either dorsal or ventral portion of the primitive foregut.[1] The imaging features of these different types are almost identical and a definite diagnosis is only by histology. The Bronchogenic cysts are extremely uncommon and hence we are reporting this case of bronchogenic cyst with its distinct characteristics.

CASE HISTORY

A 2 month old female infant presented to the outpatient clinic at Sri Ramachandra Medical College and Research Institute with history of cough for 3 days and breathing difficulty for a day. The infant was born to non consanguineous parents, had no significant medical illnesses in the past, was on exclusive breast feeds and thriving well. On examination, the child was tachypnoeic with respiratory rate of 54/min, with sub costal and intercostal retractions. The mediastinum was shifted to the right side and left lung field was hyper resonant.

On auscultation of the chest, air entry was decreased on left side and bronchial breath sounds were heard over the right interscapular and infrascapular regions. The clinical diagnosis that was considered initially was right sided collapse consolidation with obstructive emphysema on the left side. A chest x-ray was performed to confirm the diagnosis. The chest x-ray showed right sided collapse consolidation with left sided emphysema. Complete blood count, liver function and renal function test were all normal. CT scan showed a non enhancing cystic lesion in the superior mediastinum displacing the esophagus posteriorly with no communication with the trachea or bronchi (Fig 1 & 2).

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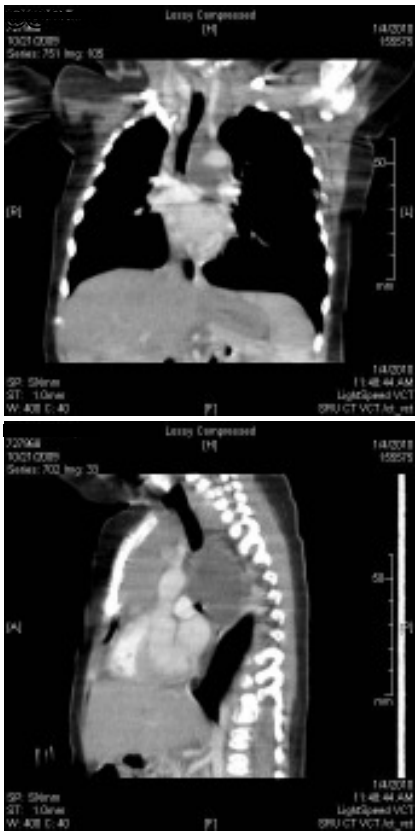

Fig 1 & 2 : CT Thorax with Contrast

A well defined cystic lesion in the middle mediastinum splaying the trachea anteriorly and the esophagus posteriorly, with no communication with the trachea or bronchi causing hyperinflation of left lung due to extraneous compression of left bronchus.

RESULT

Thoracoscopic excision of mediastinal cyst was performed with complete dissection of the mass. The cystic lesion was situated between the trachea and esophagus measuring 3x2.5x2.5cm approximately. The Azygos vein was lying just above the swelling. The cyst wall was thin, about 1mm thickness with mucinous material and was adherent to bronchial wall. There was minimal adhesion between the cyst and the esophagus allowing a clear plane of dissection. The specimen was sent for histopathology.

Histopathology of the cyst showed pseudo stratified columnar epithelial lining with bundles of smooth muscle,

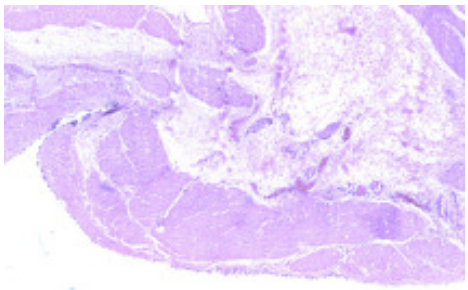

Fig. 3: Histopathology
Cyst wall showing bundles of smooth muscle & mucinous glands.

mucinous glands and fibro collagenous tissue (Fig 3 & 4). The findings were suggestive of a bronchogenic cyst. Post operatively, the infant needed supportive care and was discharged on day 8 of hospitalization. On follow up, the baby is doing well with normal growth and development.

DISCUSSION

Congenital cystic disease of the lung and mediastinum are rare in infants and children. It includes congenital lobar emphysema (CLE), cystic adenomatoid malformation (CAM), bronchogenic cyst, and pulmonary sequestration (PS).[2,3] These lesions show close relationship in terms of embryology and clinical presentation. [4] Some cause life threatening

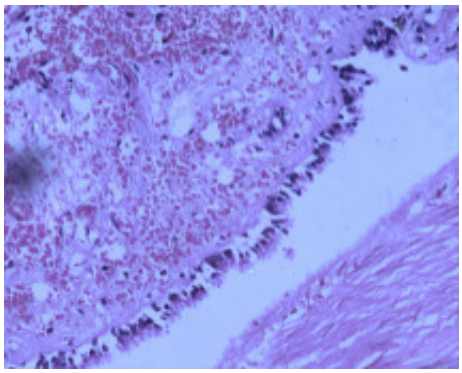

Fig 4: Histopathology
Pseudo-stratified squamous epithelium lining the cyst wall.

respiratory distress at birth, while others appear late in life as an unexpected findings on an accidental roentgenogram. Bronchogenic cyst was first described in 1948 by Maier [5]. Gerle first proposed the term "broncho-pulmonary foregut malformation" to encompass these pulmonary congenital abnormalities in 1968.[6]

There are three main hypotheses for the occurrence of these malformations. First, as a result of ectoendodermal adhesion in the early stages of embryonic life with abnormal separation of germ cell layers. Secondly, an ectodermal origin as the ectoderm of the primitive streak is capable of forming both endoderm and paraxial mesoderm. Thirdly, there is partial duplication and separation of the notochord through which the ventrally placed yolk sac or gut anlage endoderm may herniate and rupture with a resultant fistula between

Table1: Bronchogenic cyst - Characteristics

Mediastinal (86%)	**Intrapulmonary (14%)**
Usually asymptomatic; stridor, Dysphagia	Infection (75%), Dyspnoea, Hemoptysis
Male/female = 1:1	Male > female; LL/UL = 2:1, usually medial third
Associated with spinal Abnormalities	36% may contain air
Communication with tracheal lumen possible	
Location: usually on the right; posterior (50%), superior (14%) media-stinal pericarinal (35%)	

the yolk sac and the amniotic cavity that passes through and divides the future cord and spine. Subsequent differentiated growth of the embryo tends to close the fistula and the site and size of the resultant lesion depends on the degree of obliteration attained by this process. The latter process is called the split notochord syndrome.[7]

Of all the primary mediastinal tumors, bronchogenic cysts make up 6% to 15%.[8] Bronchogenic cysts are the most common type of intrathoracic foregut cysts (71%). The most common primary cysts of the mediastinum are bronchogenic cysts. They usually demonstrate no patent communication to the airway and are often adherent to major airways or oesophagus. Most frequently they are unilocular, round or oval in shape and contain clear fluid. Bronchogenic cysts typically have cartilaginous structures and sub mucosal glands in their walls. Bronchogenic cysts can be classified as mediastinal bronchogenic cysts (86%) or bronchogenic cysts surrounded by lung parenchyma (14%) (Table 1). The developmental stage of the budding is responsible for the location of the cysts.

The first successful surgical resection of bronchogenic cysts was reported in 1948 by Maier. [5] Maier classified mediastinal cysts into 5 groups on the basis of their topography: paratracheal, carinal, hilar, paraesophageal, and miscellaneous (e.g., in the diaphragm, chin, parasternal soft tissue, lower cervical areas, or scapular region.[9,10]

Surgical therapy is effective as a treatment of bronchogenic cysts and should be performed as early as possible for postnatal patients because of the risk of pulmonary compression, infection, or malignant degeneration.[11] Ideal surgical procedure is a parenchyma-saving operation and should be selected whenever possible. However, it is of utmost importance that bronchogenic cysts should be completely excised along with the tissue around the parenchyma if the dividing line is unclear, since recurrence has been reported following incomplete excision and multiple attempts of percutaneous aspiration.[12,13]

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WERNICKE'S ENCEPHALOPATHY- AN ELUSIVE COMPLICATION OF HYPEREMESIS GRAVIDARUM - A CASE REPORT

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ABSTRACT :

Hyperemesis gravidarum is a condition of severe nausea and vomiting during pregnancy leading to fluid, electrolyte and acid-base imbalance, nutritional deficiency and weight loss. We present a case of a Gravida 2 Para1 Live1 admitted at 34 weeks of gestation with hyperemesis gravidarum and Wernicke's encephalopathy along with truncal ataxia. Patient responded well to parenteral thiamine supplementation and showed symptomatic improvement over a course of time with subsequent improvement in her gait and cerebellar functions.

Key words: *Ataxia, hyperemesis, thiamine, Wernicke's encephalopathy*

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INTRODUCTION:

Hyperemesis gravidarum in pregnancy is a state with excessive vomiting resulting in electrolyte/ acid-base imbalance, nutritional deficiency and weight loss. Hyperemesis gravidarum occurs in 0.3 – 2 % of all pregnancies. [1] Symptoms usually begin at 4-5weeks of gestation and improve by 14-16 weeks. However, in upto 20% of patients, symptoms persist throughout pregnancy. [2]

In addition to severe nausea and vomiting, patients may report ptyalism, spitting, retching and gastro-esophageal reflux symptoms such as retrosternal discomfort and heartburn. Patient may present with evidence of volume depletion with dry mucus membranes, tachycardia and postural hypotension. Severely affected patients may have muscle wasting and weakness. Common maternal complications include weight loss, dehydration, micronutrient deficiency, muscle weakness, Mallory Weiss tears. If left untreated may lead on to retinal hemorrhage, spontaneous pneumomediastinum with or without subcutaneous emphysema, central pontine myelinolysis, severe depression and Wernicke's encephalopathy with or without Korsakoff's psychosis.[3,4]

CASE REPORT:

A 27 yr old Gravida 2 Para 1 Live 1 at 34 weeks period of gestation was admitted at a tertiary care centre in Chennai, with repetitive episodes of vomiting and loss of more than 10 kg of her body weight over 2 months. She had repetitive episodes of vomiting from the time of conception and was on anti-emetics for the same. On admission, patient was in a state of dehydration, appeared cachexic with a body mass index (BMI) of 18.5, sunken eyes and coated tongue. She had a pulse rate of 110/minute with blood pressure of 90/60 mmHg. There was no icterus and generalized lymphadenopathy.

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On examination of central nervous system, she was found to be conscious and in a mildly confused state. Her recent memory was intact. Muscle tone was reduced in left upper and lower limbs with truncal ataxia. Deep tendon reflexes were exaggerated with diminished plantar reflexes. All cranial nerves were normal. On cerebellar examination, finger-nose test and finger-finger test were positive. Pendular knee jerk and knee heel test were positive. Rebound phenomenon was positive. There were no nystagmus and ocular fundus examination was normal. Per abdomen examination revealed uterus corresponding to 28 weeks of gestation with features of fetal growth retardation.

Her laboratory parameters revealed hypokalemia (Potassium level was 2.5 mEq/l) with normal renal and liver function tests. Urine ketones were present in large amount. Magnetic Resonance Imaging (MRI) of brain was done which showed a thin rim of peri-aqueductal hyper-intensity on T2 weighted images, features suggesting Wernicke's encephalopathy.

Patient was conservatively managed with intravenous (I.V) fluids, parenteral antibiotics and metoclopramide 10 mg I.V. 8th hourly. Potassium correction was done by giving I.V. Potassium chloride infusion. Thiamine infusion was given at a dosage of 500 mg I.V 3 times / day for 3 days followed by 250 mg I.V. once daily for the next 5 days with which patient showed symptomatic improvement. Patient was managed based on the guidelines formulated by Thomas et al. [5] Patient went into spontaneous preterm labor and delivered a preterm baby weighing 1.5kg. Her gait improved and patient was started on oral thiamine thereafter. Patient was discharged on her 18th postnatal day.

DISCUSSION

Wernicke's encephalopathy is seen in cases of hyperemesis gravidarum with untreated thiamine deficiency, especially if these patients are given glucose or fed before thiamine has been replaced. A review of 45 reported cases established that the most common presenting symptoms are ocular signs (diplopia, sixth nerve palsy or nystagmus- 82%), confusion (71%) and ataxia (69%).[7] If left untreated, Wernicke's encephalopathy may lead to Korsakoff's psychosis (amnesia, impaired ability to learn) or death.

The three components of the classic triad of Wernicke encephalopathy are encephalopathy, ataxic gait and oculomotor dysfunction. All three features of the triad are recognized in only about one third of the cases.

Caine et al have proposed criteria for the diagnosis of Wernicke's encephalopathy and Korsakoff's psychosis with minimum of two of these features: dietary deficiency, oculomotor abnormalities, cerebellar dysfunction, either altered mental status or mild memory impairment.[8]

Magnetic resonance imaging is more sensitive than Computerized Tomography (CT) in detecting acute diencephalic and peri-ventricular lesions. Typical findings include areas of increased T2 and decreased T1 signal surrounding the aqueduct and third ventricle and within the medial thalamus and mamillary bodies.[9,10]

Pharmacologic therapies with anti-emetics and anti-reflux medications constitute the first line treatment for outpatients who fail dietary changes. Phenothiazines, dopamine antagonists or pyridoxine are often used as the first line of therapy. In patients who fail to respond with these drugs, 5 – hydroxytryptamine receptor antagonist is preferred. Oral and intravenous corticosteroids are used in refractory cases.[6] Patients who are ketotic and not able to maintain hydration by oral intake require intravenous hydration. Normal saline, lactated Ringer's solution or Hartmann's solutions are preferred since fluids containing dextrose may precipitate Wernicke's encephalopathy, if administered before thiamine replacement.[7] Hypertonic saline is contraindicated as it may precipitate central pontine myelinosis by the overly rapid correction of serum sodium levels. Potassium supplements should be added to the intravenous fluid replacement therapy as required.

An immediate and imperative treatment is to administer thiamine than confirm the diagnosis whenever Wernicke's encephalopathy is suspected. The disorder may be precipitated by the administration of intravenous glucose. Hence in susceptible patients, glucose administration should be accompanied by thiamine 100 mg I.V.

To conclude early identification of the patient with hyperemesis and administration of right choice of intravenous fluids may help in overcoming the neurological sequale of hyperemesis.

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GIANT ODONTOME REMOVAL BY UNILATERAL SAGITTAL SPLIT OSTEOTOMY -A CONSERVATIVE TECHNIQUE AND A CASE REPORT

C.Ravindran[a], Santhosh Kumar[a], Emmanuel D Azariah[a]

ABSTRACT:

Odontomes are considered to be hamartomatous malformations rather than true neoplasms. They are generally asymptomatic and are an incidental finding during a routine radiological examination. They are usually small in size and are enucleated surgically without much morbidity. An alternative method of managing a Giant odontome when conventional managements can cause great surgical morbidity has been discussed in this article.

Key words: *Large odontome, Complex-compound odontome, Sagittal split osteotomy.*

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INTRODUCTION:

Odontomes are the most commonly occurring benign odontogenic tumor and it accounts about 22% of all odontogenic tumours of jaws [9]. They are slow growing, non aggressive and usually asymptomatic, found accidentally by a routine radiographic examination.[1,9,11] The accepted treatment for smaller odontome not involving the vital structure is enucleation. Routine enucleation is usually accomplished by a buccal approach. Large odontome of the mandible are rare and their removal may endanger vital structures like the inferior alveolar nerve, and may result in conditions like an unfavorable fracture.

Sagittal split osteotomy is a proven surgical technique routinely used for orthognathic correction of mandible and had undergone various modification for ease of surgery and to reduce the operative complication. Rittersma and Van Gool[2] first introduced the technique of sagittal split osteotomy in 1972 to gain access in the angle of the mandible for the removal of large odontogenic keratocyst, and later this technique was applied to the management of large odontome by other authors. This article is about a case of large odontome in the molar area removed by unilateral sagittal osteotomy (SSO) technique and highlights the advantage of SSO compared to other surgical approaches.

CASE REPORT:

A 20 year old male reported to the Department of Oral and Maxillofacial Surgery with complaint of gradually increasing swelling in the right angle of mandible for past five years and occasional discomfort and pain in the last year. No significant underlying medical condition was present. On extra-oral examination, a swelling is evident extending antero-posteriorly 5 cm from the commisure of mouth to right angle of mandible and supero-inferioly up to the lower border of mandible. Intraoral examination reveals missing 47 & 48 with soft tissue inflammation distal to 46 regions. On palpation, intraorally expansion of buccal and lingual cortex is present and was hard in consistency.

Radiographic examination of orthopantomogram showed a large, irregular, calcified mass extending from the mesial root of 46 to the right mandibular angle region and to the lower border of the mandible. The second and third molars were embedded in the distal part of the calcified mass which depressed the inferior alveolar nerve to the lower border of mandible (Fig 1A). The axial computed tomography scan showed expansion of buccal and lingual cortical plates of about 2cm x 2.3cm with a thin plane of cleavage between the mass and the cortex and the cortical thickness was maintained in most of the regions (Fig 1B).

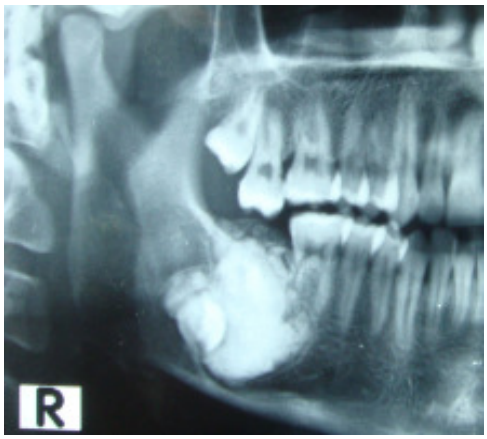

Fig1A: Orthopantamogram X-ray shows irregular calcified mass extends from the mesial root of 46 to the lower border of mandible till the ramus on right side.

Based on clinical and radiographic findings, a diagnosis of odontome was made. The surgical management considered is removal of odontome by sagittal split osteotomy under general anesthesia. Convential sagittal split osteotomy carried out with the little modification of extending the mesial osteotomy cut till the 45 region as the odontome starts from the mesial root of 46 (Fig 2). The miniplates were pre-adapted and applied on the bone fragments to ensure precise reapproximation of the osteotomy ends. Care was taken while doing the sagittal split of the mandible to avoid a bad split. After splitting, the odontome was removed in multiple segments along with the 47 and 48 teeth (Fig 3 & 4). The osteotomised segments were stabilized by use of rigid miniplates that were pre-adapted and the occlusion

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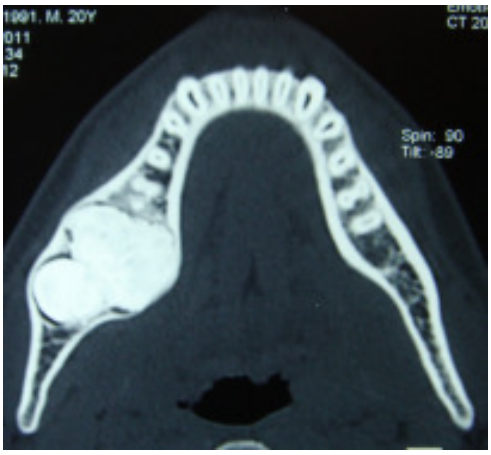

Fig. 1B: Axial section of CT Shows expansions and thinning of buccal and lingual cortex.

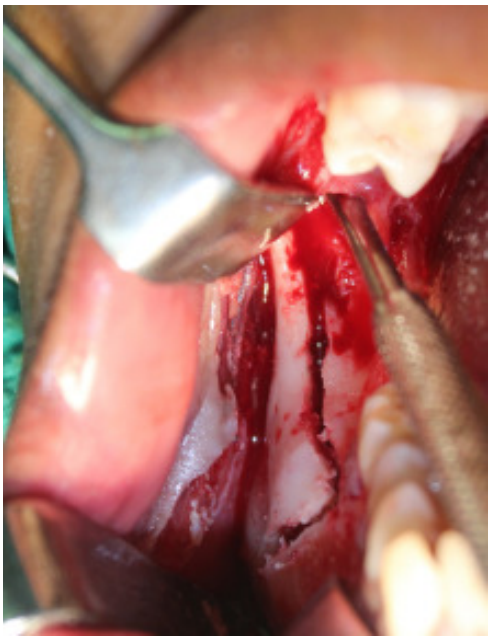

Fig. 2 : Intraoperative view-osteotomy cut for sagittal split of mandible on right side

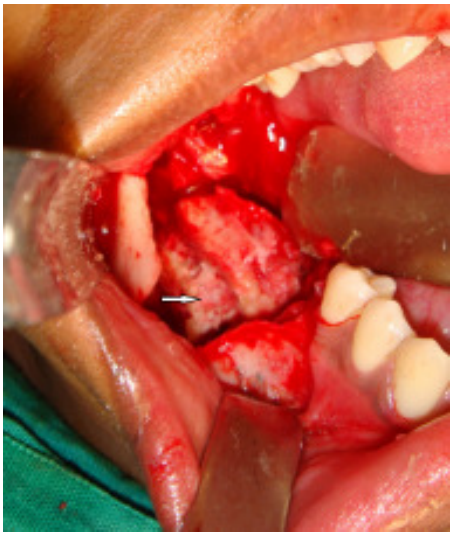

Fig. 3: Exposure of odontome following the sagittal split of mandible

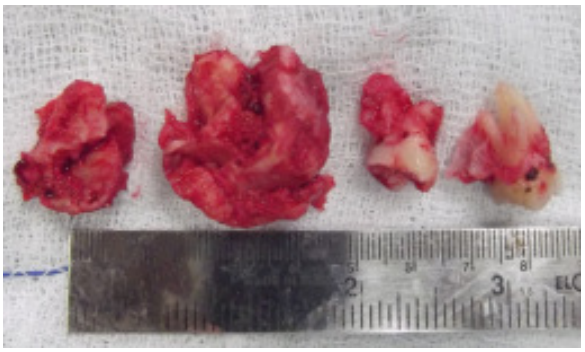

Fig. 4 : Excised multiple specimens of odontome with 47 and 48 teeth.

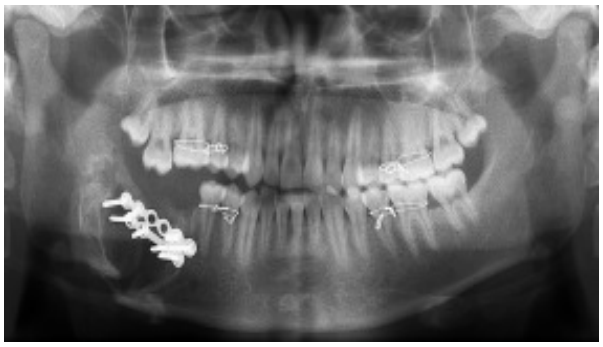

Fig. 5 : Immediate post-operative orthopantogram X-ray shows rigid miniplates fixation and MMF in situ

was achieved by 3 weeks of IMF post operatively. The immediate postoperative orthopantamogram shows the mandible free of tumor mass and miniplates fixation and MMF in situ (Fig 5).

Histopathogloical examination of the mass reveals decalcified section of agglomerate masses of tooth like structures resembling dentin and cementum which suggestive of complex compound odontome.

DISCUSSION:

Odontome is one of the common odontogenic tumours. They are classified based on their ability to resemble tooth like structure as simple, compound and complex compound odontome. The lesion being a hard calcified mass; it necessitates cortical bone removal almost to the size of the lesion to allow complete enucleation. Small to medium sized lesions can allow such bone removal but in large lesions such aggressive cortical bone removal may endanger the surrounding vital structures like inferior alveolar nerve and may result in weakening the jaw and leads to pathological fracture.[3,4,5,7,10] Blinder et al [8] indicated the risk of the intraoral removal of odontome by buccal and lingual approach results in unfavorable fracture of mandible and lingual nerve injury.

Lakshmi N et al [11] suggests that the use of CT in addition with orthopantamogram is a valuable tool in diagnosis of odontome and visualizing vital structures like inferior alveolar nerve and position of the unerupted tooth in relation to the odontome, thus aiding in the treatment plan. In our case,

the CT slices shows the odontome displacing the inferior alveolar nerve inferiorly to the lower border and the axial sections shows a thin radiolucent space present around the lesion on either side with expansion of buccal and lingual cortex.

Based on these radiographic and CT findings, that the odontome was a free structure enclosed within cortical bones it was decided to choose a procedure that can give access to the odontome without losing the cortex and thereby retaining the continuity of the mandible.

Several authors like Rittersma and Van Gool (1972) [2] have used sagittal spilt technique for removal of large odontogenic kerotocyst, similarly Petti et al [6] used the same technique for mandibular myxoma removal. Nardy casap (2006) [10] et al used the sagittal spilt technique for the removal of large odontome and concludes that it's a versatile technique as it avoids the sacrifice of a large amount of bone removal and damage to inferior alveolar nerve.

The thick expanded cortex on the buccal side made the sagittal split possible. By this adequate access, the odontome removal can be achieved by maintaining the continuity of the mandible. The pre-bending or adapting of the miniplates prior to completing the sagittal split helps in anatomically repositioning the osteotomised segments. The miniplates is planned in an area which has sound bone and will not be affected by the enucleation of the lesion. Application of intermaxillary fixation prior to completion of plating helps in achieving good occlusion. Post operative IMF is an optional procedure considering the extent of medullary bone loss and may be avoided in most cases. The only side effect may be the inferior alveolar nerve paresthesia which is usually temporary and many a times unavoidable even with the lateral approaches.

In conclusion, sagittal split osteotomy technique is an excellent, safe and conservative method as it provides three dimensional access to the tumor in the angle and body of mandible region for complete removal without damaging the inferior alveolar nerve and also reduces the risk of mandibular weakness or fracture, when compared to the other conventional surgical approach. It also helps in maintaining the continuity of the bone thus preventing a contour defect and enabling early restoration of function.

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TOTAL LARYNGO – PHARYNGO - OESOPHAGECTOMY WITH GASTRIC TRANSPOSITION FOR ADVANCED POST CRICOID MALIGNANCY – A SURGICAL CHALLENGE

Sanjeev Mohanty[a], S Sankar[b], John Samuel[a], M Gopinath[a]

ABSTRACT:

Malignant tumours of the hypopharynx usually present very late and have an overall poor prognosis. The management of these malignancies is complex with various modalities of treatment like surgery, chemotherapy and radiation being available. The surgical option is rarely exercised since the tumour is locally advanced at presentation. Surgery involves a multidisciplinary team approach. The challenges involved in the performance of total laryngo-pharyngo-oesophagectomy with gastric pull up for a case of locally advanced malignancy of the hypopharynx along with the various treatment options available are presented here.

Key words: *Postcricoid malignancy, total laryngo-pharyngo-oesophagectomy, modified radical neck dissection*

SRJM 2011;4:30-32

INTRODUCTION

Malignant tumours of the hypopharynx constitute 7% of head and neck tumours. These tumours generally present very late with regional lymph node metastasis.[1] The symptoms and subtle signs are often missed in the early stages. Due to the late presentation, most of the patients are managed by chemo-radiation and surgical interventions are rarely done. Amongst the hypopharyngeal cancers, post cricoid region is known to have poor prognosis, due to late presentations. Here we profile one such patient of advanced malignancy for whom successful surgical intervention was done for the first time in our institution. A multi disciplinary team approach ensured gratifying results in the post operative period.

CASE REPORT

A 50 year old female presented to the ENT outpatient department with complaints of progressive difficulty in swallowing and throat pain of 6 months duration. Examination revealed a proliferative growth of about 4x2 cm in the left pyriform fossa extending to the postcricoid region with impaired left vocal cord mobility. The findings were confirmed by videolaryngoscopy. Neck examination showed level II lymphadenopathy significant enough to be coined as advanced malignancy.

A direct laryngoscopy biopsy of the growth confirmed as well differentiated squamous cell carcinoma. At this stage the treatment options of chemoradiation and surgery were discussed with the patient and family. The advantages and disadvantages of both the modalities were explained. Chemoradiation involved lifelong gastrostomy feeds which add to the morbidity. Although surgery resulted in loss of voice, a decent swallow without PEG was an advantage and voice could be restored with electrolarynx. The 5 year survival rate following chemoradiation and surgical intervention are comparable.[1] Surgery plus concomitant chemoradiotherapy results in a better survival rate than concomitant chemoradiotherapy plus salvage surgery in patients with stage III-IV hypopharyngeal cancer.[2] Bearing all these factors in mind and the clinical stage (T3N1M0 Stage III), a favorable tumour grade and a relatively fit patient, with no co morbid conditions, the family members and the patient were counseled to undergo surgical excision of the tumour.

After thorough counseling, a multi-disciplinary evaluation of the patient was done. Along with the ENT Surgeon, this included evaluation by Surgical Gastroenterologist, Plastic Surgeon, Pulmonologist, Cardiologist, Psychologist and Speech therapist. The results of the UGI endoscopy did not suggest extension of the tumour beyond the confines of the oesophagus. A contrast enhanced CT scan of the neck indicated presence of lymph nodes in levels 2, 3 and 4 bilaterally. The pulmonary function, cardiac function and biochemical parameters were within normal limits.

After complete workup, the patient underwent Total Laryngo-pharyngo-oesophagectomy with bilateral modified radical neck dissection, total thyroidectomy and gastric pull up (Ong's procedure) under general anaesthesia. Although the procedure is recommended for hypopharyngeal malignancies, it is rarely done because of the advanced stage of disease and the high levels of morbidity involved. This procedure was performed for the first time in our institution by a team of ENT surgeons and Surgical Gastroenterologist.

The two teams worked simultaneously to mobilize the tumour and excise it in toto with adequate tumour free margins and to mobilize the stomach for pull up after oesophagectomy (Fig.1). After pharyngo-gastric anastomosis, an end tracheostomy (Fig.2) was fashioned and a temporary feeding jejunostomy was done. This patient also had temporary ICD bilaterally as per protocol. After 24 hour monitoring in the ICU, she was shifted back to the ward and early ambulation was encouraged. All through the stay in hospital, meticulous monitoring of the serum electrolytes and nutritional status was done to prevent any negative nitrogen balance.

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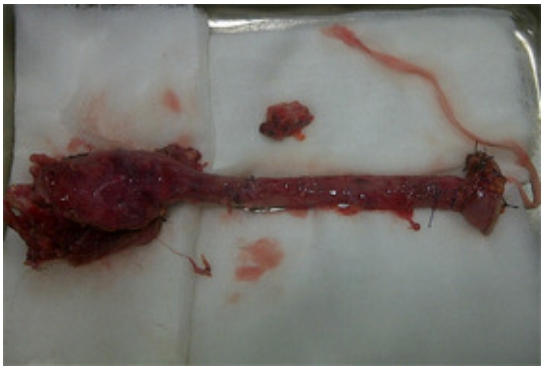

Fig 1: Excised specimen of total Laryngo-pharyngo-oesophagectomy

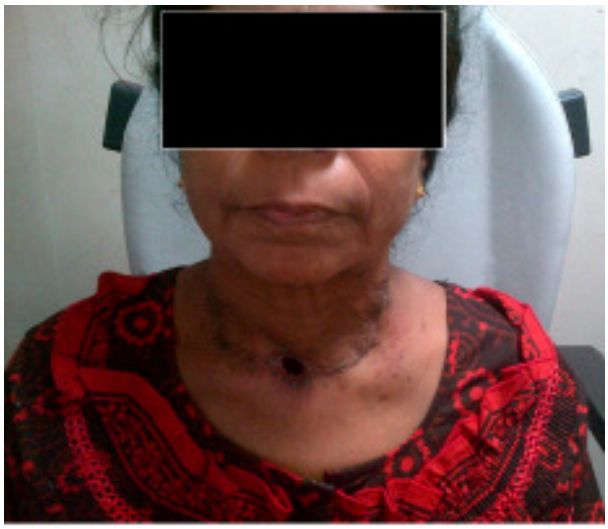

Fig 2: Post-op status with end tracheostomy.

Post-op recovery was uneventful and patient was started on oral feeds on the 12th post operative day, after confirming the integrity of the pharyngo-gastric anastomosis by a Gastrograffin swallow. The patient was discharged on the 14th post operative day and is on continued follow up. Oncologist suggested irradiation of the tumour bed 6 weeks after surgery.

DISCUSSION

Management of hypopharyngeal malignancy poses a challenge to the otorhinolaryngologist because of the morbidity associated with it. Both the surgical and non surgical modalities have their own merits and demerits. Management depends on the stage of disease, operability, co-morbid conditions and metastasis. The major problems associated with surgery are the reconstruction after pharyngolaryngectomy, permanent tracheostomy and voice restoration. The reconstruction options available are gastric pull up, free jejunal transfer, colonic transposition and augmentation with myocutaneous flap if required. The procedure of choice is total laryngopharyngo-esophagec-tomy without thoracotomy, with gastric pull-up for reconstruction.[3,4] Meticulous surgery without compro-mising oncological principles and post operative care with an aggressive post operative follow up is vital to prevent problems like anastomotic leak and pulmonary complications during the prolonged hospital stay. The morbidity associated with trans-hiatal oesophagectomy is considerably less than the thoracotomy approach. The advantage of surgical procedure over radiotherapy is the good swallow [5] obviating need for gastrostomy or enterostomy feeding adding to the morbidity. The 5-year survival is significantly related to the stage of the disease [6] (stage I, 74%; stage II, 63%; stage III, 32%; and stage IV, 14%). Electrolarynx is the recommended method of choice for voice restoration.

In our patient, a team approach coupled with the confidence of the family members and the patient ensured a smooth recovery post operatively and entailed a good quality of life for the patient. The patient is on oral feeds and phonating satisfactorily with an electro-larynx (Fig 3). She is counseled for a regular follow up with the surgical team and to comply with the recommendations of the radiation oncologist.

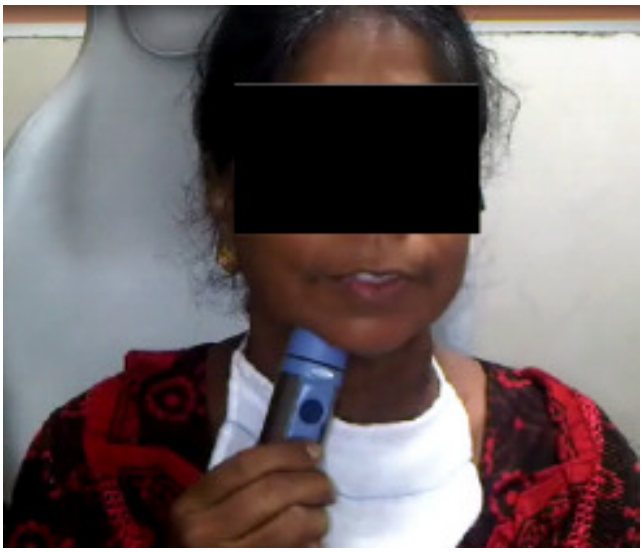

Fig 3: Patient phonating with electrolarynx

CONCLUSION

Surgical rehabilitation is still a viable option for advanced hypopharyngeal malignancies, with stomach interposition the preferred method of reconstruction.[4]. Although the long term prognosis is poor, satisfactory short-term palliation can be achieved. The significant risk factors affecting survival should be taken into account prior to selecting the candidates for surgery. Counseling of the patient and family, multidisciplinary workup of the patient and meticulous post operative care is essential for a optimal surgical outcome in patients with hypopharyngeal malignancies.

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POSTERIOR URETHRAL VALVE WITH URINARY ASCITES

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INTRODUCTION:

Urinary ascites is a rare in utero complication of posterior urethral valves (PUV). We report two cases of PUV and urinary ascites and have described a management algorithm.

Case 1: *A one-month old male baby was admitted with gross abdominal distension and respiratory distress. Clinical examination revealed ascites and distended bladder. Ultrasonogram (USG) revealed bilateral hydrouretero-nephrosis, and gross ascites. Voiding cysto urethrogram (VCUG) confirmed PUV, bilateral vesico ureteric reflux (VUR) and right side urine extravasation. Catheter drainage, nephrostomy, valve fulguration and bilateral Double J (DJ) stenting were performed in the order. However, hydronephrosis and urinary ascites did not improve. Bilateral ureterostomies eventually resulted in control of ascites.*

Case 2: *In a Gravida 2, Para 1 mother, foetal urinary ascites and PUV was detected in the third trimester antenatal scan and baby was delivered at 36 week by Lower Segment Cesarean Section (LSCS). Gross ascites and right sided perinephric collection was found on postnatal USG. Catheter drainage, nephrostomy and valve fulguration were performed in the order. In view of persistent right sided urinoma, right ureterostomy was performed. Repeat VCUG at 6 months revealed no residual valve and the ureterostomy was closed.*

Conclusions: *Management of urinary ascites involves initial percutaneous drainage and stabilization. If drainage controls urine leak, valve fulgration alone is sufficient. Otherwise diversion in the form of ureterostomy is required.*

Key words: *Urinary ascites, posterior urethral valves.*

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INTRODUCTION:

Ascites in the newborn is a rare condition. Urinary ascites accounts for one third of the cases. In male neonates posterior urethral valves (PUV) is the most common cause of urinary ascites.[1] Incidence of PUV ranges from 1:8000 to 1:25000. Clinical features are secondary to the obstructive effect of the valves. In utero clinical presentations include: oligohydramnios, bilateral hydroureteronephrosis and 'key hole' sign detected on antenatal ultrasonograms. Post natal features include recurrent urinary tract infection and failure to thrive and renal failure.[2]

Urinary ascites is an uncommon manifestation of a PUV. It is often due to the leakage of fluids through the renal fornices with transudation of this fluid into the peritoneal cavity. The systemic absorption of this fluid causes the renal function test abnormalities.[3] It can be diagnosed antenatally and is considered as a favourable prognostic marker as it vents the pressure effect secondary to the obstructive uropathy.[4] The treatment of neonatal ascites caused by PUV can be a simple valve fulguration or may require several staged procedures.[1-5] We report management of two such cases and compare our experience with the literature[1-5] and describe an algorithm for management.

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CASE SUMMARY

CASE 1:

A one- month old boy presented with gross ascites, anuria and breathing difficulty. Clinical examination revealed ascites and distended bladder. Investigations revealed elevated Blood Urea Nitrogen (BUN) and creatinine. X ray abdomen showed ascites with splinting of the diaphragm (fig. 1a) Ultrasonogram (USG) of abdomen showed ascites and a right sided urinoma. Nephrostomy was done under USG guidance. Subsequent voiding cystourethrogram (VCUG) revealed leak of contrast from the right kidney and confirmed presence of PUV (fig. 1b). Cystoscopic valve fulguration and bilateral Double J (DJ) stenting was performed. Bilateral stenting was performed to prevent the urinoma on the other side which was also grossly enlarged. The urinoma resolved in 5 days after bladder catheter was inserted but recurred when the catheter was removed. Re-catheterisation of the bladder failed to resolve the urinoma and therefore the baby was taken up for open surgery. Right kidney was explored and upper forniceal leak was repaired. Bilateral ureterostomies were done to ensure a pressure free vent. Post operatively ascites resolved and renal function improved. At one year follow up the child was stable with good renal function and VCUG showed no residual valves. Ureterostomies were closed and the patient is doing well at 2 year follow up.

CASE 2:

Antenatal scan at 32 weeks of gestation revealed bilateral hydroureteronephrosis, bladder wall thickening and classical 'keyhole sign' suggestive of PUV (fig. 2a). Follow up USG revealed foetal urinary ascites and the baby was delivered by Lower Segment Cesarean Sections (LSCS) at

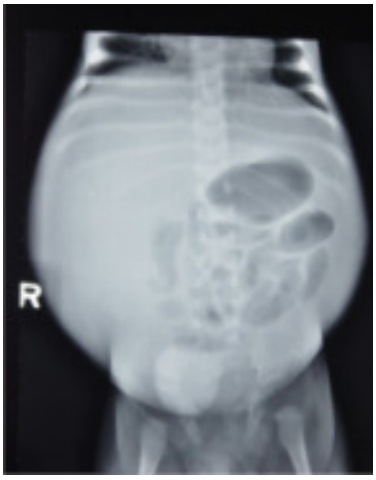

Fig 1a: X Ray abdomen showing gross distension due to ascites and splinting of diaphragm

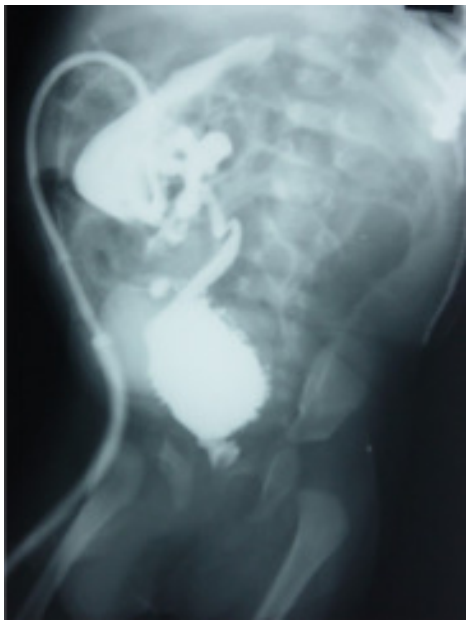

Fig 1b: VCUG showing PUV and right sided reflux. Contrast is seen extravasating from the right renal parenchyma in perinephric space and peritoneum (urinoma & urinary ascites)

36 weeks. Gross distension of abdomen and mild tachypnoea were noticed at birth. Ultrasonogram showed ascites, thickening of the bladder wall, bilateral hydroureteronephrosis and a right perinephric collection (fig. 2b). As an emergency management the ascites was tapped, bilateral nephrostomies were placed under USG guidance (fig. 2c) and the bladder was catheterised. Voiding cystourethrogram (VCUG) revealed a trabeculated bladder with bladder neck hypertrophy, dilated posterior urethra with calibre change and narrow anterior urethra confirming the diagnosis of PUV (fig. 2d). Once stable the patient was taken up for primary valve fulguration. Both the nephrostomies were removed on day 5 and per urethral catheter on day 7. Although the urinary ascites started

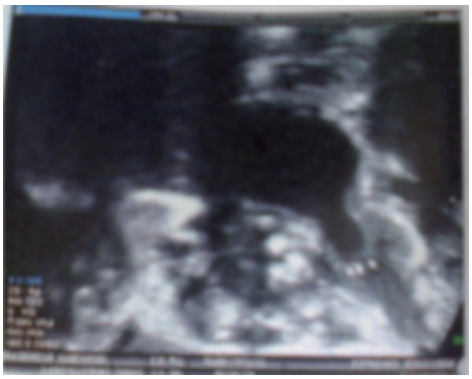

Fig 2a: Antenatal ultrasound showing 'key hole sign' a classical appearance in those with PUV. Distended bladder and dilated posterior urethra together constitute the key hole

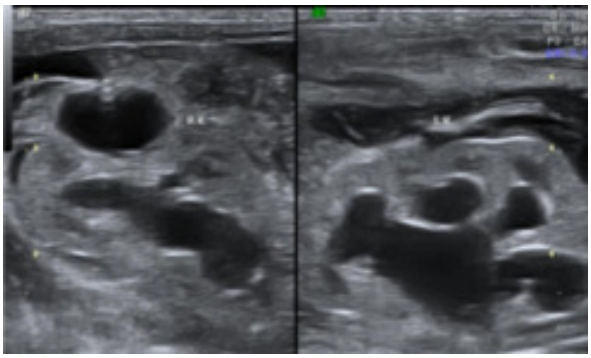

Fig 2b: Ultrasonogram showing bilateral hydronephrosis and right sided urinoma

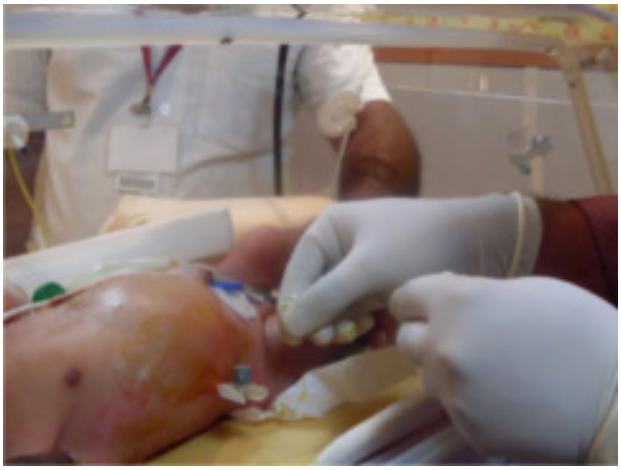

Fig 2c: Urinary ascites being tapped with the help of IV cannula

resolving, the right sided urinoma persisted. In view of this a right ureterostomy was performed. The urinoma resolved promptly following this and the baby was discharged; Ureterostomy was closed at the age of 6 months; and the baby has recovered well with normal renal function at 1 year follow up.

DISCUSSION:

With widespread availability of ultrasound hydronephrosis is being frequently detected in the antenatal

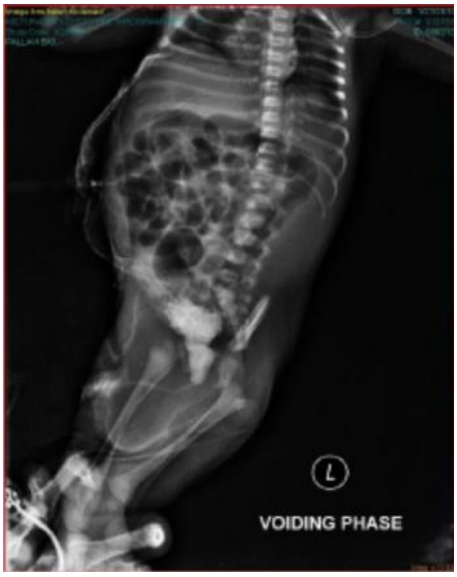

Fig 2d: VCUG showing dilated posterior urethra and a calibre change confirming diagnosis of PUV. Contrast is seen extravasating on the right side in to the peritoneum.

period. PUV is the commonest cause of bilateral hydro-ureteronephrosis in a male foetus. Ultrasound findings include oligohydramnios, bilateral hydronephrosis, thickening of the bladder wall and a classical 'key hole' sign due to a dilated posterior urethra. The incidence of urinomas and urinary ascites in neonates with PUV varies between 1 to 8% according to various studies.[1-5] The postulated mechanisms are forniceal rupture, trans-peritoneal transudation and intraperitoneal leakage following bladder rupture.[1] Often it is not possible to delineate the exact site of extravasation.[1]

Although urinomas and urinary ascites have been documented as protective mechanisms, this concept has not been widely accepted.[1-3] Patil et al[1] felt that bilateral urinomas are associated with good renal function but unilateral urinomas led to impairment of ipsilateral renal function. They also felt that PUVs with urinary ascites had a poorer prognosis. The postnatal effects of urinary ascites are profound on the newborn ranging from splinting of diaphragm, respiratory distress, absorption urine, electrolyte imbalance and sepsis. It is prudent to deal with urinomas urgently after delivery.

There is insufficient literature on the management of urinomas associated with PUV.[2-5] Patil et al [1] have suggested that needle aspiration or drainage as initial management for urinary ascities associated with massive distension, respiratory distress, rising plasma creatinine, increasing urinoma, parenchymal compression, infection and hypertension. Percutaneous nephrostomy, ureteric stenting or ureterostomies were advised for those with deteriorating clinical features. In cases with urinoma, a drainage has to be done as an initial step to prevent absorption of urine and sepsis settling in. In our first case stepwise management of aspiration, nephrostomy, valve fulgration and stenting alone did not help and ureterostomy had to be done. In the second case although the urinary ascites settled, urinoma persisted and a ureterostomy had to be performed despite a successful primary valve fulgration.

With widespread availability of neonatal resecto-scope, primary PUV fulgration has become a standard and ureterostomies have become a thing of past. However, in the presence of urinoma or urinary ascites, primary fulgration and bladder drainage for a prolonged period alone many not be sufficient. High pressure with in the system, especially when the bladder wall is thickened, promotes continued extravasation of urine in to the low pressure peritoneal cavity. In such situations temporary ureterostomy diversion will help in quick resolution of urinary leak, early renal recovery and enable the neonate to be discharged early before sepsis develops. We have proposed an algorithm (fig .3) in the management of urinary ascites based on our experience and existing literature.[1]

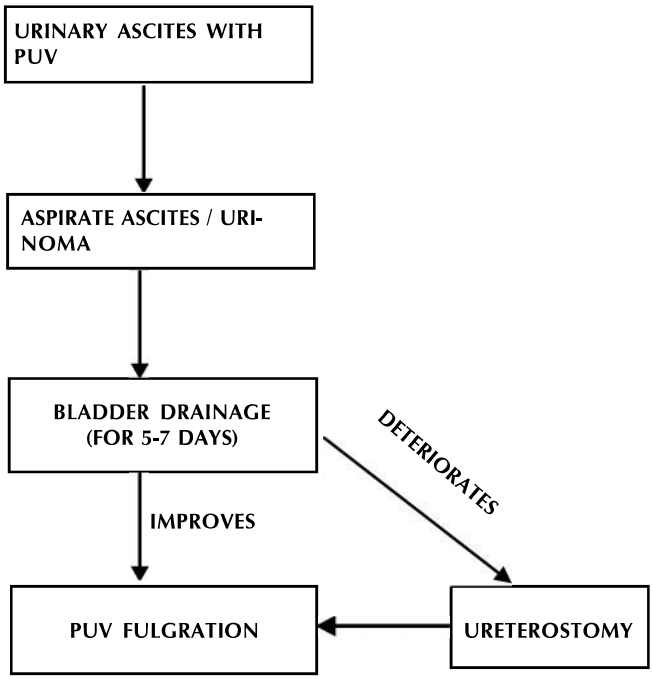

Fig 3: An Algorithm in the management of PUV with urinary ascites

CONCLUSION:

The combination of urinary ascites and PUV is rare. Hence a multi centre study will be of help in arriving at a consensus in its management.

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