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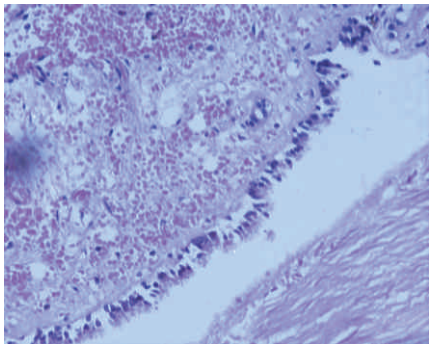

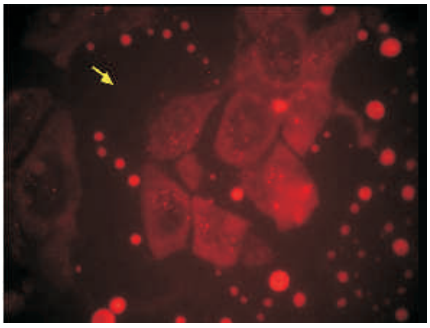

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SRI RAMACHANDRA JOUNRAL OF MEDICINE
JAN-JUNE 2012
CONTENT

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From the Editor's Desk

There has been an increasing interest shown for the publication of articles. We would like this practice to continue and I would like to place on record my sincere thanks to all the editorial board members as well as internal and external reviewers for providing timely and high quality reviews.

I am happy to announce that the Editoral Board has planned to bring out future issues with special themes. The next issue would be on **"Oncology".**

This issue has two Original Articles, eight Case Reports, two Brief Communications and one article on Best Practice.

I take this opportunity to wish you all a VERY HAPPY AND PROSPEROUS NEW YEAR.

P.V. VIJAYARAGHAVAN
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MIDTERM ANALYSIS OF TOTAL HIP ARTHROPLASTY USING HARRIS HIP SCORE

Ganesan G Ram[a], S. Sundar[a], P.V. Vijayaraghavan[a]

ABSTRACT

Background: *In today's clinical practice, total hip replacement (THR) for non-traumatic conditions is just not an option for elderly alone but for younger patients as well. With immense advancement in this technique with respect to operative technique, technology and biomaterial a better outcome can be generated. An assessment of the long-term outcomes of this procedure would help in determining its usefulness and effectiveness. In addition, a focused study can evaluate its drawbacks, limitations and complications.*

Aim: *To analyse the mid term follow up of total hip arthroplasty done for non traumatic indications using Harris hip score.*

Material & Methods*: Thirty one patients with 38 cemented (or)uncemented hip prosthesis were followed retrospectively for 5-13 years. Harris hip score[1] (Modified) for clinical and functional evaluation and plain x-ray pelvis with both hips and proximal femur - AP view and x-ray of the operated hip - lateral view for radiological evaluation. The Andrew Whaley and Daniel et al criteria for uncemented cups and the De Lee and Charnley criteria for cemented cups were used to assess cup loosening. The Gruen zones for cemented stems and the Enghs criteria for uncemented stems were used to assess femoral stem loosening.The Brookers Classification[1] was used to assess Heterotropic Ossification.*

A cemented prosthesis was used in men older than 60 years and women older than 55 years and in younger patients in whom adequate initial fixation could not be obtained without cement. Uncemented implants were used in all other patients.

Results : *The overall clinical results were similar for both groups. The mean Harris hip score at latest follow up of both cemented and uncemented total hip replacement were 88 and 89 respectively. On analyzing the difference in pre-operative and latest Harris hip score for various nontraumatic indications, our study showed that the results were better in patients with avascular necrosis followed by osteoarthritis and rheumatoid arthritis. In our series of uncemented total hip replacement we have 95% excellent/good results while in case of cemented THR's we have 82% excellent/good results.*

We had 1 case (2.6%) of aseptic loosening of the acetabular component which was revised. Pain in the thigh, usually slight and not disabling, occurred at 6 month-1 year in 16% of our patient in whom uncemented components were used. We had 1 case (2.6%) of dislocation following posterior approach. In our series we have 13% (5 cases) of class II heterotrophic ossification seen only in uncemented hips. In bilateral hip diseases there is considerable pain relief and improvement after the first total hip replacement, but the optimal improvement is not seen until after the second replacement.

Conclusion : *The Harris hip score is a useful scoring system in assessing total hip replacement done for non traumatic indications and showed high validity and reliability.*

Key words: *HARRIS hip score, osteoarthritis, THR.*

SRJM 2012;5:1-5

INTRODUCTION

The hip joint functions as one of most important joint in the human body, designed for both mobility and stability. The hip allows the entire lower extremity to move in three planes of motion, while providing an important shock absorption function to the torso and lower body.

Pain in the hip joint is one of the important causes that disable locomotion. There are many ways and methods by which this crippling pain in the hip can be treated. This includes analgesics, using a walking stick or bilateral axillary crutches, arthrodesis, excision arthroplasty and hip arthroplasty. Hip arthroplasty is a surgical procedure in which the diseased part of the hip joint are removed and replaced with artificial implant.

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AIM

To analyse the mid term follow up of total hip arthroplasty done for non traumatic indications using Harris hip score.

MATERIAL AND METHODS

This was a retrospective study of thirty one patients who underwent total hip replacement (THR) in Sri Ramachandra medical center. The study period was from March 2008 to Dec 2011. The youngest patient was 23 years and the oldest was 75 years. Twenty four patients underwent unilateral total hip replacement while 7 underwent bilateral total hip

Table 1: Outcome of cemented and uncemented Total Hip Replacement using Harris Hip Score

Outcome	Number of patients	Percentage
Excellent	22	58%
Good	12	31%
Fair	0	0%
Poor	4	11%

Table 2: Outcome in uncemented Total Hip Replacement using Harris Hip Score

Outcome	Number of patients	Percentage
Excellent	13	59%
Good	8	36%
Fair	0	0%
Poor	1	5%

Table 3: Outcome in cemented Total Hip Replacement using Harris Hip Score

Outcome	Number of patients	Percentage
Excellent	9	57%
Good	4	25%
Fair	0	0%
Poor	3	18%

replacement. Of the 38 hips, 16 were cemented and 22 uncemented. We did the uncemented hip replacement in males below 60 years and females below 55 years of age.[1,2] However, the cemented hip replacement was used in patients for whom economy was a constraint. The follow up was from 5 years to13 years. The indications were rheumatoid arthritis(9), ankylosing spondylitis(2), avascular necrosis(14) and osteoarthritis(13). The posterior approach was used in 19 cases and the lateral approach in 19 cases. The approach was selected randomly by choice of the surgeon for both unilateral and bilateral patients. Informed consent was obtained from patients after discussion of the advantages and risk of each approach.

We used the Harris hip score[3] (Modified) for clinical and functional evaluation and plain x-ray pelvis with both hips and proximal femur - AP view and x-ray of the operated hip -lateral view for radiological evaluation. All the patients were followed up at immediate postop, 6 weeks, 3 months, 6 months and then yearly upto 5-13 years. The Andrew Whaley and Daniel et al criteria[4] for uncemented cups and the De Lee and Charnley criteria for cemented cups were used to assess cup loosening. The Gruen zones[5] for cemented stems and the Enghs criteria for uncemented stems were used to assess femoral stem loosening. Other radiological components that were taken into consideration were cup inclination, femoral stem position, vertical subsidence of femoral component, vertical migration of acetabular component and heterotrophic ossification. The Brookers Classification[6] was used to assess Heterotropic Ossification.

Table 4: Mean Harris Hip Score

Condition	Pre-Op	Latest	Difference
Osteoarthritis	49	92	43
Rheumatoid Arthritis	35	74	39
Avascular Necrosis	46	90	44
Ankylosing Spondylitis	46	89	43

RESULTS

In our series as per Harris hip score criteria of excellent score (100-90) we had 58% excellent, good score(89-80) we had 31% good, fair score (79-10) we had 0% fair and poor score (below 69) we had 11% poor results (Table 1). The uncemented total hip replacement's had 95% excellent (59%) / good (36%) results and 5% poor/fair results (Table 2) and in cemented total hip replacement's we had 82% excellent (57%) / good (25%) results and 18% poor results (Table 3). The mean pre and latest Harris hip score were 44 and 88 respectively. The mean Harris hip score in 1st, 3rd and 5th years were 86, 87 and 87 respectively. The mean pre and latest Harris hip score in osteoarthritis was 49 and 92, in rheumatoid arthritis it was 35 and 74, in avascular necrosis it was 46 and 90 and in ankylosing spondylitis it was 46 and 89 respectively (Table 4).

In our series we had a normal cup inclination (30-45 degrees) in 21 patients (55%), a vertical cup inclination (> 45 degrees) in 15 patients (39%) and a horizontal cup inclination (<30 degrees) in 2 patients (6%). We had a Central Femoral stem position in 26 patients (69%), a Valgus position in 9patients (23%) and a Varus position in 3patients (8%). There was no incidence of vertical femoral subsidence in our study. There was no incidence of migration of the acetabular component in our study. We had 1 case (2.6%) of acetabular loosening and no cases of femoral loosening.

COMPLICATIONS

In our series we had a dislocation rate of 2.6% (1 hip), 2.6% incidence of aseptic acetabular loosening (1 hip), 16% incidence of anterior thigh pain (6 patients) and a 13% incidence of heterotrophic ossification (5 hips). All the heterotrophic ossifications were Brookers Type II. Five patients (13%) had a limb length discrepancy of 1-1.5 cms. No infection was documented in our series.

DISCUSSION - CLINICAL OUTCOME

HARRIS HIP SCORE

The mean pre-operative and latest Harris hip score in our study were 44 and 88. This was comparable to the study by Wixson et al[7] whose mean pre and post op Harris hip score was 44 and 93 respectively and Siwach et al[8] whose

Table 5: Unilateral Vs. Bilateral Harris Hip Score

	Our		Anders et al	
	Pre	Latest	Pre	Latest
Unilateral	48	88	43	96
Bilateral				
After one hip	40	65	41	73
After both hips	40	87	41	93

mean pre and post op Harris hip score were 44 and 83.5. Mean pre op and latest Harris hip score in cemented hips was 40 and 85 which was comparable to that of Wixson et al 42 and 90. Mean pre and post op Harris hip score in uncemented hips was 48 and 89 which was comparable to that of Wixson et al who had 47 and 95.

Our mean 1st, 3rd, 5th year Harris hip scores of 86, 87 and 87 were comparable to that of C.Y.Ng et al[9] and Goran et al[10] who both had 88, 89 and 89 respectively. The greatest change occurred between pre op assessment and review at 6 months. The patients had the potential to improve further until 18 months. Further the scores plateaued. Our series had a ceiling effect [11] of 15% which was considered as the acceptable limit in literatures.

Our study of unilateral vs bilateral total hip replacement was comparable with the study of Anders Wykman et al.[12]. Although patients with bilateral disease had considerable pain relief and improvement after the first total hip replacement, the optimal improvement was not seen until after the second replacement (Table 5).

Difference in Harris Hip Score between Pre-operative and Latest Followup:

On analysing the difference in pre-operative and latest Harris hip score for various indications, our study showed no difference in gain of Harris hips for various indication like avascular necrosis, osteoarthritis and Rheumatoid arthritis. Our series of patient with osteoarthritis have pre and latest Harris hip score as 49 and 92 The Harris hip score in rheumatoid arthritis patients in our series was 35 and 74 which is comparable with Johnson et al's scores of 41 and 78[13].

In our series we had one patient (2.6%) for whom bilateral total hip replacement and total knee replacement was done for rheumatoid arthritis. Her Harris hip score of 20 and 68 were comparable with the results of Kenneth et al[13] score of 25 and 75.The preferred method of arthroplasty in these case is to operate on the hips before the knees, and on the more diseased of each pair of joint. The relief of pain was the single factor that accounted for the increase in hip rating[13].

In our series of uncemented total hip replacements we have 95% excellent/good results and 5% poor/fair results which can be compared with Wixson et al who has 89% excellent/good and 11% poor/fair results. In case of cemented total hip replacement's we had 82% and 18% results which can be compared with Wixson et al's 89% excellent/good and 11% poor/fair results respectively. In our series the poorer results (4 hips) occurred in cases of rheumatoid arthritis however the gain in Harris hip score was same as in other indications. All these patients had involvement of other joints and one patient had bilateral total hip replacement and total knee replacement done .

RADIOLOGICAL OUTCOME

1. **Implant loosening**: In our study we had one case (2.6%) of acetabular cup loosening (aseptic loosening). The loosening occurred in the case of an uncemented acetabular component (Fig 1). The patient was a case of rheumatoid arthritis operated through the posterior approach with a St.Nabor cup and Wagner stem.

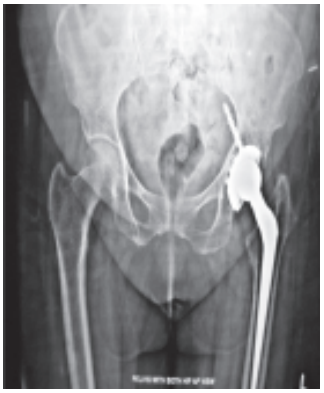

Fig 1: Loosening of uncemented acetabular component

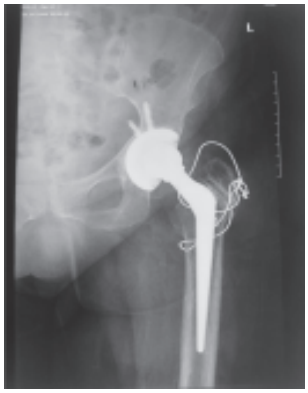

Fig.2 : 1 yr 6 month following acetabularr cup revision

The hip showed radiographic evidence of cup loosening at 8 years following the primary surgery. Since the patient did not have any symptoms of cup loosening, revision of the cup was not advised to the patient. 12 years following the primary surgery, the patient presented with symptomatic loosening of the acetabular component (St.Nabor Cup). Acetabular cup revision (Fig 2) was done retaining the intact femoral component (Wagner stem). The mean interval between primary surgery and diagnosis of loosening was 7.8 years in the study by Engh C.A et al[14] which is comparable with our study.

The age of this patient at the time of primary total hip arthroplasty was 43 years. This corresponded to the results of Engh C.A who revealed a higher incidence of osteolysis and reoperation in younger patients. In the study by John C and W.H. Harris[15], 4% of the acetabular components were revised. In our study 2.6%, i.e. 1 acetabular component was revised.

2. **Cup inclination**: The ideal cup position is neutral. In our study we had 21 cups in neutral position, 15 vertical and 2 horizontal. One of the vertical cup had aseptic loosening and an other vertical cup had dislocation .
3. **Femoral stem position**: The ideal femoral stem position is central. In our study we had 26 stems in central, 9 in valgus and 3 in varus. As of now we do not have any complications.
4. **Subsidence and migration**: In our study we had no subsidence or migration.
5. **Dislocation**: In our series we have 1 case of dislocation (2.6%) (Fig.3).The dislocation occurred during the first month of the surgery at home and was treated with open reduction and trochantric osteotomy (Fig 4). For this patient the cup was placed vertically and the posterior approach was used. Our study can be compared to that

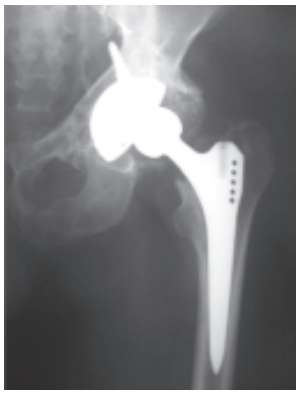

Fig.3: Dislocation of prosthesis

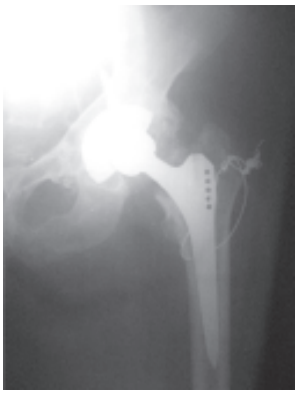

Fig.4: Post-operative reduction of dislocation

of M.A. Ali Khan et al whose study shows 2.1% dislocation.[16] The study of Wayne M.Goldstein et al [17] shows an increased rate of dislocation following the posterior approach. His study shows a dislocation rate of 2.8% following posterior approach which correlates with our study (5.2%).

6. **Heterotopic ossification:** Heterotopic ossification usually first becomes visible on radiographs three to four weeks after surgery and matures by three to six

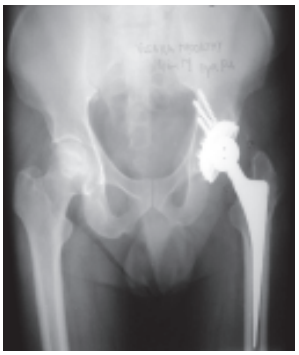

Fig.5 : First year follow up showing heterotopic ossification.

months.[18] The incidence ranges from 5% to 90% in various literatures.[19] In our series we have 5 cases (13%) of class 2 heterotophic ossification (Fig. 5). This corresponded to the results of Micheal A. Mont et al (9.6%). All the cases were uncemented hips. Three cases were through the lateral approach and 2 through the posterior approach. The particulate bone debris and the escape of femoral bone marrow elements, which are normally sealed off by bone cement in a cemented femoral component may be increased when an uncemented implant is used.

In our study we had 7.8% heterotophic ossification when the indication for surgery was osteonecrosis and 5.2% in osteoarthritis which can be comparable to the study of Michael A. Mont et al[20] who had 9.6 % in both groups. All the cases in our study had Brooker Class II heterotopic osssification. This is comparable with the Michael A. Mont et al study which had 9.6% Class II heterotopic ossification.

CONCLUSION

We have attempted to do a mid term follow up of total hip arthroplasty done for non traumatic indications using Harris hip score. From our study we have arrived at the following inferences.

- The Harris hip score is a useful scoring system in assessing total hip replacement done for non traumatic indications and showed high validity and reliability. We have 89% excellent/good results in our series.
- The results in patients with avascular necrosis and osteoarthritis were better than those with rheumatoid arthritis, however the gain in Harris hip score is same.
- Both uncemented and cemented total hip replacement gives good results in non traumatic indications.
- In bilateral hip diseases there is considerable pain relief and improvement after the first THR, but the optimal improvement is not seen until after the second replacement.

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Trichophyton raubitschekii: CHARACTERIZATION AND CLINICAL CORRELATES ISOLATES FROM DERMATOPHYTOSIS

Elangovan Elavarashi[a], Anupma Jyoti Kindo[a], Jagannathan Kalyani[c], Rangarajan Sudha[b]

ABSTRACT

First report of ten cases of raubitschekii, a variant currently considered as a synonym of Trichophyton rubrum was identified in South India, based on the colony morphology, urease test, growth on bromocresol purple casein glucose agar, in-vitro hair perforation test, PCR based restriction fragment length polymorphism (RFLP) and PCR based DNA sequencing targeting the internal transcribed spacer (ITS) region of rRNA.

Keywords*: Dermatophytosis, Trichophyton rubrum var. raubitschekii, PCR, RFLP, DNA sequencing.*

SRJM 2012;5:6-9

INTRODUCTION

Dermatophytes are a wide variety of keratinophilic fungi that cause skin, hair and nail infections. *Trichophyton rubrum* is the most common causative agent of dermatophytosis. *Trichophyton rubrum* var. *raubitschekii* is included in the *T. rubrum* complex. It was first described as a new species[1,2] and now it is currently considered as a synonym of *T. rubrum*.[3] Classically, the conventional methods require 3-4 weeks for identification of dermatophytes to the genus level. Later on sub-culture, these fungi produce different morphological features, which would make the identification of species or varieties difficult by the routine laboratory methods. Therefore, the objective of this study was to add an effective method – genotyping for rapid identification of dermatophytes, which would be helpful in epidemiological survey.

MATERIAL & METHODS

A total of 12,189 patients who attended the Dermatology outpatient Department of a tertiary care centre were screened between January–December 2010, of which 138 patients had lesions resembling dermatophytosis were randomly selected for the present study.

The clinical specimens were subjected to 10% KOH mount, cultured in Sabouraud's dextrose agar (SDA) containing gentamycin and cycloheximide and were also inoculated on Dermatophyte test medium (DTM), in duplicates and incubated at 25^{0}C and 37^{0}C. The culture of dermatophytes were identified based on colony morphology, slide culture, growth on bromocresol purple casein glucose agar, urease test and *in-vitro* hair perforation test.

The fungal DNA was extracted from isolates following the *OmniPrep*™ kit for fungus (G Biosciences, USA). The PCR targeting the internal transcribed spacer (ITS) region was performed using universal fungal primers ITS 1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS 4 (5′-TCCTCCGCTTATTGATATGC-3′). Amplification was performed using Eppendorf Master Cycler Gradient (Model 5331). The primers and the PCR reagents were purchased from Bangalore Genei Pvt. Ltd, India. The reaction mixture constituted of 200 µM concentration of each dNTP, 25 pmol of each primer, 1 U of Taq polymerase, 5 µl of Taq buffer, 10 μl of template DNA and sterile double distilled water was added to make up the final volume to 50 µl. PCR amplification conditions - an initial denaturation at 95^{0}C for 5 min, followed by 35 cycles of 95^{0}C for 30 s, 55^{0}C for 1 min, 72^{0}C for 1 min and a final extension at 72^{0}C for 5 min. The amplified products were electrophoresed in a 2% agarose gel in 1X Tris acetate – EDTA buffer and visualized using ethidium bromide under UV trans-illuminator (Bio-Rad Laboratories India Pvt. Ltd).

Subsequently, restriction fragment length polymorphism (RFLP) assay was performed using Mva I restriction enzyme (Fermentas Inc. USA) and incubated at 37^{0}C for 2 hours. The digested products were electro-phoretically separated in a 2% agarose gel stained with ethidium bromide and observed under UV trans-illuminator.

For further confirmation of isolates, PCR based DNA sequencing targeting the ITS region was performed[4] at the Vision Research Foundation (VRF) Referral laboratory (A unit of Sankara Nethralaya), Chennai. Due to financial constraints, only two of the representative isolates were sent for confirmation. The PCR amplified products were sequenced by the dideoxy chain termination method using an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, USA).

RESULTS

Among 138 clinical specimens, 76 patients were positive for dermatophytes by culture. The dermatophytes isolated were *T. rubrum* (37) the most common followed by *Trichophyton mentagrophytes* (17), *T. rubrum* var. *raubitschekii* (10) *Epidermophyton flocossum* (9) and *Trichophyton interdigitale* (3). The patients from whom *T. rubrum* var. *raubitschekii* were isolated had similar scaly lesions as other isolated dermatophyte species. Infections caused by *T. rubrum* var. *raubitschekii* were isolated from tinea corporis and tinea cruris. Three were males and seven were females, their age ranging from 19-72 years. The patients

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had papulo erythematous lesions on the buttocks, shoulder, armpit, groin, thighs and lower abdomen. Among them, eight patients were positive by direct microscopy. After two weeks of incubation, all 10 specimens showed growth in culture. The isolates showed granular and fluffy colonies (Fig.1a) the color on the reverse ranged from reddish brown, yellow orange (Fig.1b), whitish with yellow margin to reddish purple resembling *T. violaceum*. In two isolates, the red pigment was fully diffused in the medium. Growth of the isolates on bromocresol purple casein glucose agar showed restricted growth and did not show any change in color. Urease test on

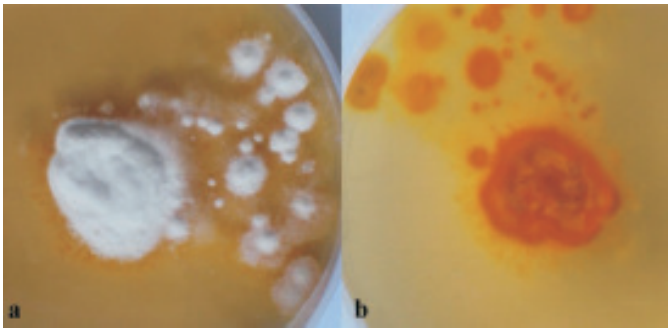

Fig. 1: Colony morphology of *T. rubrum* var. *raubitschekii*.
(a) Colony on SDA at 25^{0}C after 14 days of incubation
(b) reverse of the colony.

Christensen'urea agar was positive within five days. *In-vitro* hair perforation test was negative. Microscopically, numerous clavate or pyriform and club shaped microconidia (Fig. 2a), abundant production of cylindrical or cigar shaped macroconidia (Fig.2b) and in few cases, intercalary and terminal chlamydospores were seen (Fig.2c). One of the isolate was deposited in the National Culture Collection of Pathogenic Fungi (NCCPF) at PGIMER, Chandigarh, India (Accession number: NCCPF-900025).

The dermatophyte DNA was extracted from isolates and was identified by amplifying ITS region in all clinical specimens, resulting in amplified product of approximately

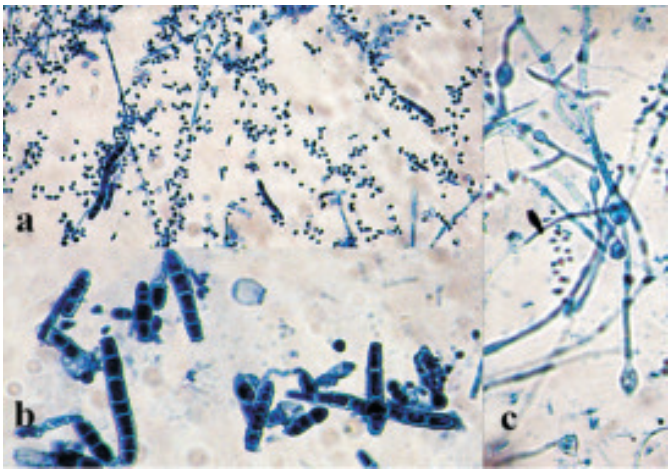

Fig. 2: Microscopic morphological features of *T. rubrum* var. *raubitschekii*.
On slide culture (a) microconidia (b) macroconidia (c) intercalary and terminal chlamydospores.

690 bp length. The band pattern obtained using Mva I restriction enzyme for *T. rubrum* var. *raubitschekii* were 400, 180, 120 bp (Fig. 3) which showed consistent identical band profile of *T. rubrum* ATCC 28188. The DNA sequences were aligned with those in GenBank database by BLAST analysis. The two queried strains displayed 99% and 100% identical to both *T. raubitschekii* ATCC 42631 and *T. rubrum* ATCC 28188. The two DNA sequences reported in this paper had been deposited in the genetic sequence database at the National Center for Biotechnology Information (NCBI) (GenBank ID: JQ844452, JQ886109). Therefore, both the strains were confirmed as *T. rubrum* var. *raubitschekii* by standard morphological characteristics, physiological test and by genotyping. Moreover, the other eight clinical isolates were also confirmed as *T. rubrum* var. *raubitschekii* based on the above-mentioned phenotypic characteristics.

The patients were treated systemically with fluconazole 150mg/week for four weeks in combination with topical application of terbinafine. The patient's symptoms improved and were followed up there after for any recurrence.

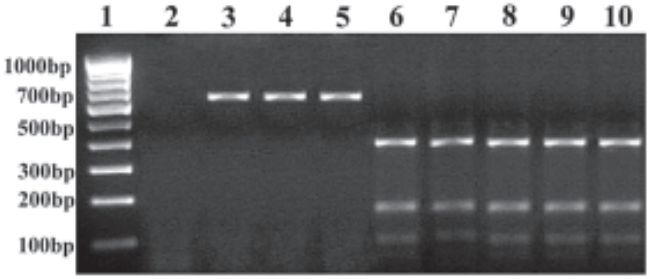

Fig.3 : Amplification and RFLP profile using Mva I restriction enzyme.
Lane 1: Molecular weight marker – 100 bp DNA ladder;
Lane 2: Negative control (no template DNA);
Lane 3, 4: Undigested product of *T. rubrum* var. *raubitschekii*;
Lane 5: Undigested product of *T. rubrum* ATCC 28188 (690 bp);
Lane 6, 7, 8, 9: Digested product of *T. rubrum* var. *raubitschekii*; Lane 10: Digested product of *T. rubrum* ATCC 28188 (400, 180, 120 bp).

DISCUSSION

T. rubrum complex is the most common causative agent of dermatophytosis. The most predominant species of the complex is *T. rubrum* worldwide. In the past few years, molecular methods have proven to be more useful in rapid diagnostics. Gräser *et al* (2000) compared the conidial morphology and physiologic features of 15 species and varieties (*T.circonvolutum,T.fischeri, T. fluviomuniense, T. glabrum, T. gourvilii, T. kanei, T. kuryangei, T. megninii, T. pedis, T. raubitschekii, T. rodhaini, T. rubrum* var. *nigricans, T. soundanense, T. violaceum* var. *indicum* and *T. yaoundei*) with the DNA sequence targeting the ITS region of rRNA, PCR fingerprinting and amplified fragment length polymorphism (AFLP) assay.[3] Eventually they were reclassified or reported as a synonym of *T. rubrum* or *T. violaceum*.[3]

Table 1: Review of clinical literature.

Identified by	No. of cases	Described in	Origin
Ilkit *et al* (2011) [6]	2	Turkey	Turkey
Kano *et al* (2010) [7]	1	Japan *	Japan
Ma *et al* (2009) [8]	1	China	China
Gómez Moyano *et al* (2008) [9]	2	Spain	Nigeria
Brasch and Jensen (2008) [10]	1	Germany	Germany
Brasch (2007) [11]	7	Germany	Africa
Brasch and Gräser (2005) [12]	6	Greece	Greek (5), Bulgarian (1)
Arabatzis *et al* (2005) [13]	1	Germany	Cameroon
Papini *et al* (2004) [14]	1	Italy	Cameroon
Costa *et al* (2003) [15]	1	Brazil	Brazil
Tietz *et al* (2002) [16]	4	Germany	Ghana (1), Cameroon(3)
van Gelderen de Komaid and Borges de Kestelman (2001) [17]	2	Argentina	Argentina
Taplin (2001) [18]	1	Vietnam	Vietnam
Lacaz *et al* (1999) [19]	1	Brazil	Brazil
Caiuby *et al* (1996) [20]	4	Brazil	Brazil
Kane et al (1990) [2]	38	Canada	Asia (23), Southern Europe (7), Northeast Europe & North America (8)

*Animal dermatophytosis, isolated from a dog.

T. rubrum var. *raubitschekii* was initially found in indigenous parts of Africa, Asia, and South America. First report of ten cases of *T. rubrum* var. *raubitschekii* was identified from South India. Even though the organism is now synonymized as *T. rubrum*, isolation of *T. rubrum* var. *raubitschekii* from clinical specimens was not reported from India. A brief clinical review of isolation of *T. rubrum* var. *raubitschekii* reported until date is listed in Table 1. In the past, *T. raubitschekii* was identified as a distinct species from the more common *T. rubrum* and *T. mentagrophytes* and the fungus is significantly associated with tinea corporis.[2] In our study, we isolated the fungus from patients having tinea corporis and tinea cruris. The reddish pigment on the reverse of the colony and negative for *in-vitro* hair perforation test are highly suggestive of *T. rubrum*, while granular appearance, rounded microconidia, urease positive are suggestive of *T. mentagrophytes*. In the present study *T. rubrum* var. *raubitschekii* possessed minor differences in laboratory features like urease positive, produced rounded microconidia a feature uncommon in *T. rubrum*. As described by Ishizaki et al (1993) using restriction fragment length polymorphism (RFLP), *T. rubrum* var. *raubitschekii* produced the same pattern as *T. rubrum* suggesting conspecificity with *T. rubrum*.[5] Our study data correlated with these earlier findings. Our study results correlated with Gräser *et al* (2000) showing that the gene sequence of the clinical strain of *T. rubrum* var. *raubitschekii* shared sequence similarities of ITS region of rRNA with the ATCC strain of *T. rubrum*.[3] Since they produce minor morphologic and physiologic variations, *T. rubrum* var. *raubitschekii* is currently synonymized as *T. rubrum*.

CONCLUSION

We identified ten cases of *T. rubrum* var. *raubitschekii* for the first time from South India, which is phenotypically closely related to the typical cottony *T. rubrum*, but can be differentiated by positive urease test and genotypically shared similar ribosomal ITS sequence of *T. rubrum*, suggesting conspecificity with *T.rubrum*. In addition, DNA sequencing confirmed the isolates as *T. rubrum* var. *raubitschekii*. Since misidentification of *T. rubrum* var. *raubitschekii* is possible with other closely related dermatophytes, typical morphological and physiological characteristics of *T. rubrum* var. *raubitschekii* should be considered for future identifications.

ACKNOWLEDGMENTS

We thank the Vision Research Foundation (VRF) Referral laboratory (A unit of Sankara Nethralaya, Chennai) Dr. R. Bagyalakshmi, for confirming the isolates by DNA sequencing and Dr. Arunaloke Chakrabarti and his team for depositing our culture at the National Culture Collection of Pathogenic Fungi (NCCPF) at PGIMER, Chandigarh, India.

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AN INTERESTING CASE OF ACUTE PULMONARY EDEMA WITH SEVERE PRE-ECLAMPSIA AND MITRAL REGURGITATION IN LATE PREGNANCY

V. Arun Muthuvel[a], UshaVishwanath[a], T.V. Ramakrishnan[b], Preet Agarwal[a], Lavanya[a]

ABSTRACT

Alow risk late booked primi gravida without any co-morbid conditions developed sudden severe pulmonary edema at rest on a routine antenatal visit. She was admitted in Intensive care unit, intubated and started on frusemide. She was diagnosed as having acute pulmonary edema with severe pre-eclampsia and moderate mitral regurgitation. Emergency cesarean section was planned after starting on magnesium sulphate therapy and labetalol infusion. She improved after delivery.

Key words: *Pulmonary edema, pre-eclampsia, heart disease.*

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INTRODUCTION

Unexpected acute pulmonary edema in late pregnancy is uncommon and often generates suspicions of undiagnosed valvular heart disease, acute pulmonary embolism, peripartum cardiomyopathy, severe preeclampsia and thyrotoxic heart failure (Table-1).[1] Pathophysiology of pulmonary edema resulting from pregnancy induced hypertension is not understood. Severe preeclampsia is associated with medical, surgical and obstetric complications. The occurrence of pulmonary edema in such patients is associated with high maternal and perinatal mortality and morbidity.[3]

CASE REPORT

A twenty three year old primigravida having regular antenatal checks outside was booked in this hospital from 32 weeks of gestation. Her blood pressure was normal through out pregnancy and she had no family history of hypertension and did not reveal any history of heart disease.

During her present visit at 38 weeks for an ultrasound growth profile, she was uncomfortable and complained of sudden breathlessness, had bouts of haemoptysis and also complained of reduced urine output for 1 week. On examination she also had bilateral pedal edema. She was immediately shifted to emergency room. Oxygen saturation was around 75% with severe bilateral basal crepitations and her blood pressure was 220/120 mmHg (Fig.1). Anti-cardiac failure drugs were started and she was put on non-invasive-ventilatory support.

Patient's attenders narrated a history of heart disease at young age for which cardiology opinion was sought. Echocardiogram showed a moderate mitral regurgitation, tip of anterior mitral leaflet (AML) prolapsing into left anterior (LA) with a normal chambers and left ventricular (LV) function. Cardiac markers (CK-MB, Myoglobin, Troponin) were within normal limits. (T.Protein – 5.2 and Albumin – 1.8). Her oxygen saturation started falling to 65%, hence patient was intubated and intravenous morphine administered. Patient was shifted to Cardiac Intensive Care Unit (ICU) for further management where anti-hypertensives and prophylactic magnesium sulphate were started and maintained. Blood pressure settled at around 150/100 mmHg and spot urine albumin was 3+. This reiterated the presence of severe preeclampsia.

Termination of pregnancy was decided upon and patient was shifted to operating room. Under general anaesthesia, emergency cesarean section proceeded, baby delivered and shifted to Neonatal ICU. She developed atonic post-partum hemorrhage with a blood loss of 1000 ml. Uterus well contracted after administration of oxytocics and uterine compression sutures. With adequate antibiotic coverage and anti hypertensives (Metoprolol, Labetolol) blood pressure was brought under control. Patient was extubated on postoperative day 2. She was shifted to ward on day 4. She was discharged on eighth post operative day.

DISCUSSION

Acute pulmonary edema may develop secondary to major cardiovascular insult, such as critically high blood pressure in preeclampsia, congenital or rheumatic valvular heart disease, peripartum cardiomyopathy, thyrotoxic heart failure, beta agonist tocolytic therapy or cardiogenic shock associated with myocardial ischaemia. Undiagnosed heart disease is a major cause.

Undiagnosed mitral regurgitation and severe preeclampsia were the causes for severe pulmonary edema in this patient. Supine position during ultrasonography could

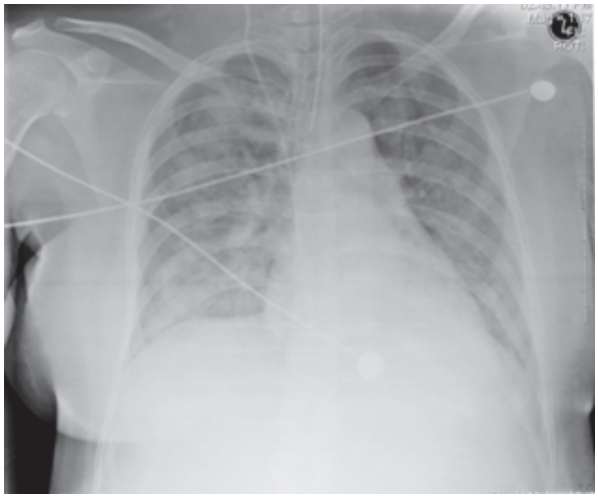

Fig.1: X-ray showing acute pulmonary oedema

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Table 1: Risk factors for the development of acute pulmonary edema in pregnancy[14]

Category	Specific risk factors
Pre-existing pre-pregnancy conditions	Cardiovascular disease (hypertension, ischaemic heart disease, congenital heart disease, valvular heart disease, arrhythmias, cardiomyopathy) Obesity Increased maternal age Endocrine disorders (phaeochromocytoma and hyperthyroidism)
Specific diseases in pregnancy	Pre-eclampsia, Cardiomyopathy, Sepsis, Preterm labour, Amniotic fluid embolism, Pulmonary embolism
Pharmacological agents	ß-Adrenergic tocolytic agents, Corticosteroids, Magnesium sulphate, Illicit drugs including cocaine
Iatrogenic intravenous fluid therapy	Positive fluid balance > 2000 ml
Fetal conditions	Multiple gestation

have provoked this episode. Estimated rates of acute pulmonary edema in pregnancy vary from as low as 0.08% to as high as 0.5%.[2,7,8,10]

Acute pulmonary edema is a significant cause of morbidity and mortality in pregnant and recently pregnant women.[11,12] It is characterized by sudden-onset breathlessness, tachypnea, rales or rhonchi upon auscultation, evidence of hypoxia by pulse-oximetry or arterial blood gas. The Scottish Confidential Audit of Severe Maternal Morbidity, one of the largest maternal morbidity audits, reported that acute pulmonary edema was the fourth most common form of maternal morbidity.[11] It is also frequently the reason for intensive care admission[13] and may occur during the antenatal, intrapartum or postpartum periods.[14]

Echocardiography in healthy pregnant women enables the detection of subclinical reduced systolic and/or diastolic function, and may thereby identify women at risk of acute pulmonary edema. It also assists in determining underlying cardiac function at the time of acute pulmonary edema.[2] However we do not routinely do echocardiography in low risk patients.

Normal cardiovascular and respiratory changes in pregnancy can predispose women to the development of pulmonary edema. Potential complications to mother and child can be decreased if risk factors and signs and symptoms of pulmonary edema are recognized early.[5] Structural or functional cardiac defects predispose a patient to developing pulmonary edema. However, cardiac disease in most likely underdiagnosed and underreported because of underutilization of diagnostic utilities such as echocardiography.[2] A cardiac cause of edema is difficult to predict based on history and examination.[6] Echocardiography is the key diagnostic and management tool.[15] A prospective study done in normal pregnant women revealed a progressive and significant increase of multi-valvular regurgitation which was maximal in full term compared to early pregnancy and mitral regurgitation resolved in puerperium.[9]

Preeclampsia is a major cause of pulmonary edema due to underlying endothelial damage and decreased colloid osmotic pressure, which causes leakage into the pulmonary interstitium or alveolar space.[2]

CONCLUSION

Treating obstetrician should be aware that preeclampsia and undetected valvular diseases in pregnancy may result in pulmonary edema in order to deliver a prompt and specific treatment.

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A RARE CASE OF ACARDIAC TWIN

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ABSTRACT

Acardiac twins are a rare complication of monozygotic twins. It is characterized by a structurally normal twin pump perfusing an anomalous recipient twin via an artery to artery anastamosis in a reverse direction. Morbidity and mortality of the pump twin is high. We present an interesting case of an acardiac twin, with a favorable outcome of the pump twin.

***Key words**: Acephalic acardiac twin, Monochorionic diamniotic twins, out come of pump twin, TRAP (Twin Reversed Arterial Perfusion) sequence.*

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INTRODUCTION

Monochorionic twins share a common placenta. This shared placenta provides an opportunity for vascular anastamosis. Twin reversed arterial perfusion is caused by a sizeable artery to artery placental shunt, often accompanied by a vein to vein shunt. The vascular pressure of one twin overpowers the other, who then has reverse blood flow from the co twin. The recipient twin develops without a normal heart (acardiac) and various other missing structures. The donor twin is normally formed and may show features of heart failure, growth restriction and an increased risk for preterm labour.

CASE REPORT

A twenty seven year old gravida with two abortions was suspected to have an acardiac twin at 9 weeks gestational age (GA) by ultrasound which showed monochorionic diamniotic twin pregnancy with fetal movements in both fetuses but fetal heart in only one fetus.

At 12—13 weeks, twin A was diagnosed by ultrasonogram (USG) to be acardiac acephalic perfused twin with single umbilical artery with retrograde flow in the single umbilical artery (Fig. 3b). Twin B was the pump twin corresponding to the gestational age. (Fig 1(a) & Fig.1 (b).

Patient had regular antenatal checks with appropriate weight gain, normal BP, and had no complaints. At 20 weeks, fetus A, the perfused twin measured 10x7x9 cm volume 332 cc with subcutaneous edema and normal liquor. Fetus B, the pump twin had no anomalies and polyhydramnios. Subsequent follow up USG at 24 weeks, 27weeks, 30 weeks showed the pump twin to be normal with normal Doppler in umbilical and middle cerebral arteries. The perfused twin reached a volume of 1445 cc.

At 34 weeks the perfused twin showed an increase in volume to 2890 cc with gross subcutaneous edema with increased diastolic flow in its aorta. The pump twin reached a weight of 1664 grams and was in breech presentation. It showed a lag in interval growth of head, abdomen and femur indicating growth restriction. It showed high resistance flow in umbilical artery and raised diastolic flow in the middle cerebral artery. Cerebro Placental (CP) ratio was 0.84. Biophysical score of the pump twin was normal. Steroid coverage with betamethasone 12 mg, 2 doses was given 12 hrs apart. Lower segment Cesarian section (LSCS) was done at 34 weeks in April 2005. There was difficulty in delivering the acardiac twin which weighed 2.9 Kgs. The normal twin was delivered as breech and a live female baby weighing 1.6 Kgs APGAR (Appearance, Pulse, Grimace, Activity, Respiration)

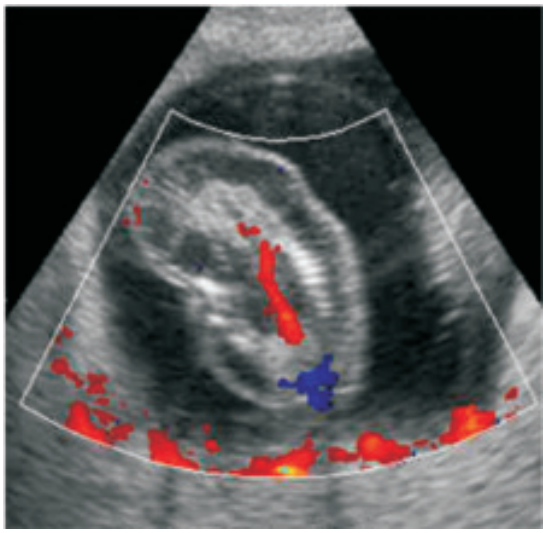

Fig. 1 (a) : Pump twin

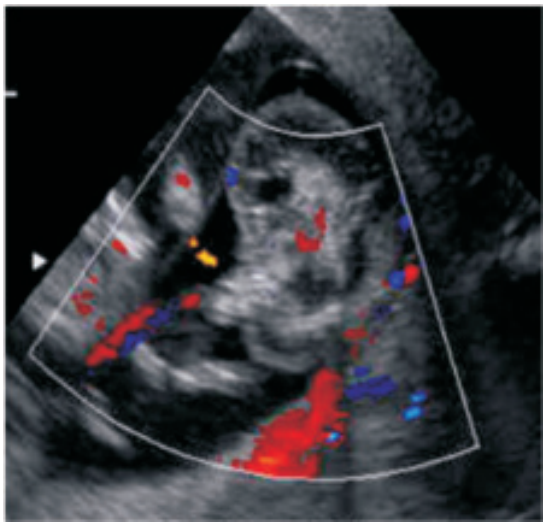

Fig.1 (b): Pefused twin

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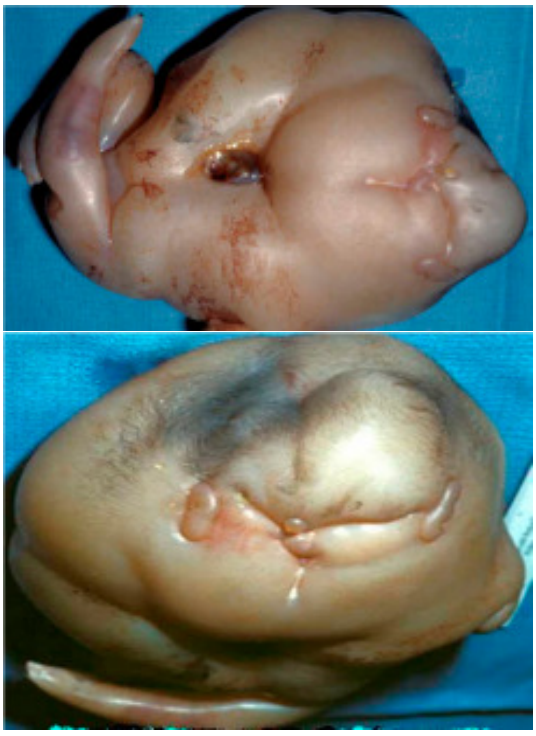

Fig. 2: Acardiac, acephalic twin with two stalks resembling the lower limbs

6/10 and 9/10 was born. Baby was in neonatal intensive care unit (NICU) for preterm care. Baby was discharged after 10 days. The child was followed up for five years (2005 to 2010) after birth and was normal and doing well.

AUTOPSY OF THE PERFUSED TWIN

***External features*:** (Fig.2) The face was not formed. and a cleft was seen with one partially developed ear. There were no upper limbs. Both lower limbs were edematous, anus was imperforate, genatalia was ambiguous and there was a cystic swelling on the back.

Cut section: There was no demarcation between thorax and the abdomen. Thoracic organs were absent. All abdominal organs were absent except the intestines.

Placenta was monochorionic and diamniotic type (Fig. 3a).

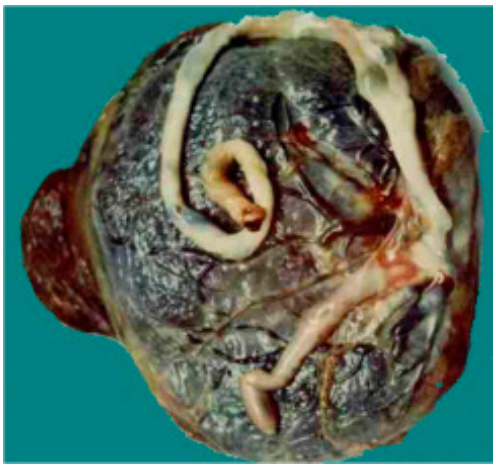

Fig 3a: Monochorionic diamniotic placenta

Fig. 3 b: Doppler showing reverse blood flow from pump twin to perfused twin

DISCUSSION

Twin reversed–arterial-perfusion(TRAP) sequence occurs 1 in 35,000 births. It occurs in mono chorionic twins. Trap sequence is known as Acardius or Chorioangiopagus parasiticus. It occurs in 1% of monozygotic twins with a recurrence rate of 1 in 10,000.[1]

Artery to artery anastomosis between the monochorionic twins in the first trimester is the fundamental event in the TRAP sequence. The structurally normal twin pumps arterial blood (deoxygenated blood of the fetus) into the other twin. The recipient twin is perfused with the de oxygenated blood, which circulates in a reverse direction through the umbilical artery. The lower part of the recipient fetus receives more of the de oxygenated blood, compared to the upper half of the fetus. This abnormal circulation results in early tissue hypoxia and the absence of development of the upper parts of the body.

The artery to artery placental shunt with reversed blood flow from pump twin to perfused twin during embryogenesis results in inadequate perfusion leading to lethal anomalies including acardia and acephalus in the perfused twin.[9]

There are three ways in which the acardiac twin can threaten the well being of the normal pump twin. The artery to artery shunt increases the cardiovascular demand and leads to congestive cardiac failure of the pump twin. The acardiac twin can grow to extraordinarily large size and cause mass effects like preterm labour. Thirdly the deoxygenated blood from the pump twin is further deoxygenated in the acardiac

twin. If this "double used blood" is circulated back through a vein to vein anastamosis into the pump twin it can lead to chronic hypoxia and growth restriction.[2,6]

Based on the morphology of the acardiac twin, four types of acardiac twins have been described. Acardius acephalus is the most common type (60-75%) which shows an absence of the head and heart .The rarest is the Acardus acornus, in which there is a head without a body. In Acardius anceps head and face are partially developed. In Acardius amorphous a formless blob of tissue is found which can be differentiated from a teratoma only by the presence of the umbilical cord.[3]

Successful use of transabdominal fetoscopy to ligate the umbilical cord of 11 fetuses at 21 weeks has been described by Quintero et al.[5] Intra fetal ablation techniques use different energy sources like monopolar current, Nd:YAG Laser, radio frequency generator, which can be performed through a per cutaneous needle under ultrasound guidance or fetoscopy, to coagulate the intra abdominal vessels of the acardiac twin.[4,8] Expectant management with close monitoring of the pregnancy is the main stay of the treatment. It is recommended to use intra fetal ablation only when deterioration sets in before the age of viability and survival of the pump twin.[7]

To conclude, in the reported case the pump twin remained unaffected till 34 weeks and hence was salvaged. Close monitoring for effects in the pump twin facilitates prompt rescue either in the form of delivery or intra uterine intervention like intrafetal ablation of the perfused twin.

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RARE ASSOCIATION OF IDIOPATHIC, CENTRAL DIABETES INSIPIDUS WITH HYPOGONADOTROPHIC HYPOGONADISM : A CASE REPORT

Muthamil Selvan S[a], Vinoth PN[a], Venkataraman P[a], Krithika P[a]

ABSTRACT

Central diabetes insipidus (CDI) is characterized by decreased release of antidiuretic hormone (ADH) which can be caused by disorders that act at one or more of the sites in brain involved in ADH secretion. Patients with untreated CDI typically present with polyuria, nocturia and polydipsia. The causes of CDI are multiple including idiopathic disease. We present a 17 years old adolescent boy with idiopathic CDI and hypogonadotrophic hypogonadism, a rare association.

Keywords: *Antidiuretic hormone (ADH), central diabetes insipidus, hypogonadotrophic hypogonadism.*

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INTRODUCTION

Decreased release of antidiuretic hormone (ADH) also called arginine vasopressin or AVP, results in a variable degree of polyuria in central diabetes insipidus (CDI). It is due to disorders that act at one or more of the sites involved in ADH secretion: the hypothalamic osmoreceptors; the supraoptic or paraventricular nuclei; or the superior portion of the supraopticohypophysial tract.[1] Central diabetes insipidus can result from multiple etiologies, including genetic mutations in the vasopressin gene, trauma to vasopressin neurons, congenital malformations of the hypothalamus or pituitary, neoplasms, infiltrative, autoimmune, and infectious diseases affecting vasopressin neurons. In approximately 10% of children with CDI, the etiology is idiopathic.

CASE REPORT

A seventeen years old adolescent boy, third born to non-consanguineous parents, with normal developmental milestones, presented with history of loose stools of 3 days duration, signs of severe dehydration, tachycardia and hypotension. Fluid resuscitation was done and baseline workup revealed normal cell counts, sodium of 171 mEq/L and normal serum potassium. He continued to have hypernatremia even after the correction of dehydration.

Further evaluation revealed an apparently normal child till some six months ago, had gradual onset of polyuria, polydipsia associated with increased weight gain. He was also concerned about the yet to develop secondary sexual characters and small sized phallus. His anthropometry revealed a Body Mass Index of 22.4, height of 148 cms which was < 3rd centile consistent with short stature, he was weighing 48 kgm, his Stretched Penile Length was 7cms (microphallus) with testicular volume of 3ml (prepubertal). He was found to have Stage1 sexual maturity rating (SMR).

In view of polyuria, polydipsia, hypernatremia and clinical evidence of hypogonadism, provisional diagnosis of central diabetes insipidus was made. Biochemical evaluation revealed elevated serum osmolality of 333mOsm/L, reduced urine osmolality of 91mOsm /L, and ratio of urine /serum osmolality less than 1 which were in conformity with a diagnostics of CDI. Endocrine evaluation revealed a low serum FSH of < 0.3mIU/L (Normal,2.6-11 mIU/L), low LH of < 0.3mIU/L (Normal,0.4-7.0mIU/L) and low testosterone levels of 39.8ng/dl (Normal,350-970 ng/dl) consistent with hypogonadotrophic hypogonadism. He was clinically and biochemically euthyroid. Serum cortisol and growth hormone levels after clonidine stimulation were within normal limits. A X-ray elbow for bone age revealed bone age of 14-18 years. Skeletal survey was done with no evidence of Langerhan's cell histiocytosis. A routine ultrasonography (USG) of abdomen was found to be normal. Magnetic resonance imaging (MRI) brain of sella and supra sella regions revealed absence of hyperintensity in posterior pituitary region.

With the above cited clinical, auxological and biochemical parameters a working diagnosis of central idiopathic diabetes insipidus with hypogonadotrophic hypogonadism was considered. He was started on oral desmopressin 0.1mg once daily and monthly parenteral testosterone enanthate 100mg. The patient was monitored clinically and biochemically with measurement of anthropometry, Tanner staging, serum sodium, osmolality and urine osmolality levels monthly (Table -1). With above treatment regimen serum sodium levels were normalized and SMR staging improved to stage3. Currently, he is on oral desmopressin 0.1mg twice daily and monthly parenteral testosterone enanthate of 125 mg.

DISCUSSION

The causes of CDI, are idiopathic diabetes insipidus (DI) primary or secondary tumors or infiltrative diseases (such as Langerhans cell histiocytosis).[2] The etiology of central DI is usually organic in 64% of children, trauma in 5.9% and idiopathic in 29.4%.[3] Anterior pituitary hormone deficiencies have been documented in 53% of children. Organic central DI group has a greater prevalence of anterior pituitary hormone deficiency when compared with the idiopathic type.[3] In one study of 16 patients first diagnosed with idiopathic CDI, the detection of evolving gonadotropin deficiency in three individuals resulted in the diagnosis of pituitary or suprasellar germinomas 20 cases about 6, and 3 years after the initial presentation.[4] Our case had evidence of gonadotropin

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Table - 1: Clinical and Biochemical markers during therapy and follow up

Duration since therapy	Weight (KGs)	Height (Cms)	Body Mass Index	Sexual Maturity Rating	Serum Osmolality mOsm/L	Urine Osmolality mOsm/L	Serum Sodium mEq/L
At Diagnosis	48	148	22.4	Stage I	333	91	171
1st month	48	148	22.4	Stage I	307	98	155
2nd month	49	149	22	Stage I	300	110	145
3rd month	49.1	150	21.8	Stage I	303	120	142
4th month	49	150.5	21.8	Stage II	309	122	140
5th month	48	151	21.8	Stage II	299	126	146
9th month	50.9	153	21.1	Stage II	305	133	149
1 year	50	155	20.8	Stage II	300	184	146
1year 3months	50	156	20.6	Stage III	290	210	141

deficiency but did not have pituitary or suprasellar germinomas in neuro imaging.

Since the primary problem in central DI is deficient secretion of ADH, control of the polyuria can be achieved by hormone replacement. In the past, this was achieved by intramuscular injections of vasopressin (Pitressin) tannate in oil, which is no longer available.[6] This has been replaced by desmopressin (dDAVP), a two-amino acid substitute of ADH that has potent antidiuretic but no vasopressor activity.[7] An oral preparation of desmopressin is also available[8] which is being used for our patient.The initial dose of the tablet form is 0.05 mg (one-half a 0.1 mg tablet) at bedtime The usual daily maintenance dose ranges from 0.1 mg to 0.8 mg in divided doses but may be as high as 1.2 mg/day.

There are few reports on the long-term use of the tablet form of desmopressin. In one study, eight children with central DI were treated and followed for up to 3.5 years.[9] There was no attenuation of the antidiuretic effect and no side effects or antibody formation were noted. In another report, ten adults had satisfactory maintenance of the antidiuretic effect over one year with doses of 0.3 to 0.6 mg/day given in two to three doses per day; doses larger than 0.2 mg had no greater effect.[10]

The goal of therapy is to control nocturia and partial control of polyuria during the day since more aggressive therapy can promote the development of hyponatremia. The serum sodium concentration should be checked at 24 hours after the initiation of desmopressin therapy, there after monthly. Patients are educated about warning symptoms of hyponatremia .The duration of central DI varies with the cause. DI is permanent in idiopathic disease, most often transient following neurosurgery. Therapy should be continued as long as the patient has symptomatic central DI.

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A MOSAIC KARYOTYPE AND Y CHROMOSOME MICRODELETION IN AN INFERTILE MALE

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ABSTRACT

The advances in assisted reproduction techniques and genetic diagnoses have increased the percentage of couples who are able to conceive. We report on an infertile male patient with azoospermia carrying one cell line (45,X) lacking the entire Y chromosome and a second cell line(46,Xdel(Y) (q12)) with a large terminal deletion of the Y chromosome. Fluorescence in situ hybridization (FISH) performed on blood lymphocytes confirmed the presence of the two cell lines in a proportion of 80% and 20% respectively and a Y chromosome (Yq) microdeletion in the azoospermia factor (AZF) regions, AZFb and AZFc. Histological examination of the testicular biopsy revealed very few seminiferous tubules, which were hyalinized and composed only of Sertoli cells. This case highlights the importance of a combined molecular and cytogenetic approach as well as thorough histological analysis for proper evaluation of genotype-phenotype correlation in patients with spermatogenic failure carrying AZF deletions.

***Key words**: Male infertility; sex chromosome mosaicism; Y microdeletion (Yq)*

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INTRODUCTION

Infertility affects 10-15% of all couples and the male factor is responsible in approximately half of them.[1,2] Genetic evaluation of couples prior to assisted reproductive techniques (ART) shows that the genetic contribution could either involve numerical and structural chromosomal abnormalities or deletions of genes necessary for male germ cell development.[2,3] In those males who have spermatogenetic failure, abnormalities of azoospermia factor genes in the euchromatic region on the long arm of the Y chromosome (Yq) have been reported.[2] The Yq microdeletions of azoospermia factor (AZF) regions are major causes of infertility associated with severe oligospermia and azoospermia.[2,4] and may also be associated with somatic and germinal gonosomal mosaics.[5-7] Among the structural abnormalities of the Y chromosome, large cytogenetically visible deletions of the long arm are commonly seen and are often associated with a percentage of cells showing a 45,X chromosomal complement.[3]

While it is possible to use polymerase chain reaction (PCR) methods to determine the presence or absence of specific Y chromosome sequences such as AZF, the obtained results do not provide enough information to analyse the structure of abnormal Y chromosomes.[1] Therefore many investigators chose to use complementary conventional cytogenetic and molecular cytogenetic techniques such as FISH in order to identify the causes of infertility. In the present case, we characterized an abnormal Y-chromosome, detected as a mosaic in an azoospermic male investigated for infertility.

CASE REPORT

A 30-year-old infertile male with non obstructive azoospermia was referred to our genetics laboratory for diagnostic studies before ART treatment. In sperm analysis, the seminal volume was 2.7 mL and no motile or immotile sperm was seen. Endocrinological studies showed raised FSH, LH, and prolactin with low testosterone level. Histological examination of the testicular biopsy revealed hyalinization in the few seminiferous tubules present and they were composed only of sertoli cells. No germinal epithelium or spermatogenesis was detected. The family history indicated that the parents had no fertility issues and the two offspring were conceived naturally. However, sperm analysis of the patient's brother revealed that he was also azoospermic. Genetic investigations on the patient, his father and brother were undertaken after obtaining informed consent.

Cytogenetic and Fluorescence In Situ Hybridization Analysis- Chromosomes were prepared from phyto-hemagglutinin-stimulated lymphocytes, G-band was done according to standard technique and fifty metaphases of G-band were analyzed. Two hundred interphase cells from the lymphocytes were analyzed by fluorescence in situ hybridization (FISH) with a probe specific for chromosomes X and Y (Vysis, CEP X alpha satellite Spectrum Red and CEP Y satellite III Spectrum Green, respectively). Signals were observed under Olympus BX61 microscope equipped with Cyto Vision 2.7 image analysis software (Applied Imaging).

Analysis of the Azoospermia Factor Regions by Sequence-Tagged Site Polymerase Chain Reaction— Genomic DNA was extracted from peripheral blood of the patient, his father and brother. Multiplex polymerase chain reaction (PCR) was performed for the 6 sequence-tagged site (STS) loci along the Y chromosome (sY84, sY86, sY127, sY134, sY254, and sY255 from azoospermia factor region AZFa, AZFb, and AZFc). Primers and the amplification protocol were according to the European Academy of Andrology/ European Molecular Genetics Quality Network best practice guidelines for molecular diagnosis of Y chromosomal microdeletions. PCR products were separated on 2% agarose gel. A normal woman and a normal fertile man were used as negative and positive controls.

Cytogenetic analysis by conventional G banding of cultured blood lymphocytes showed a mosaic karyotype 45,X(80%)/ 46,Xdel(Y)(q12) (20%) and the karyotype

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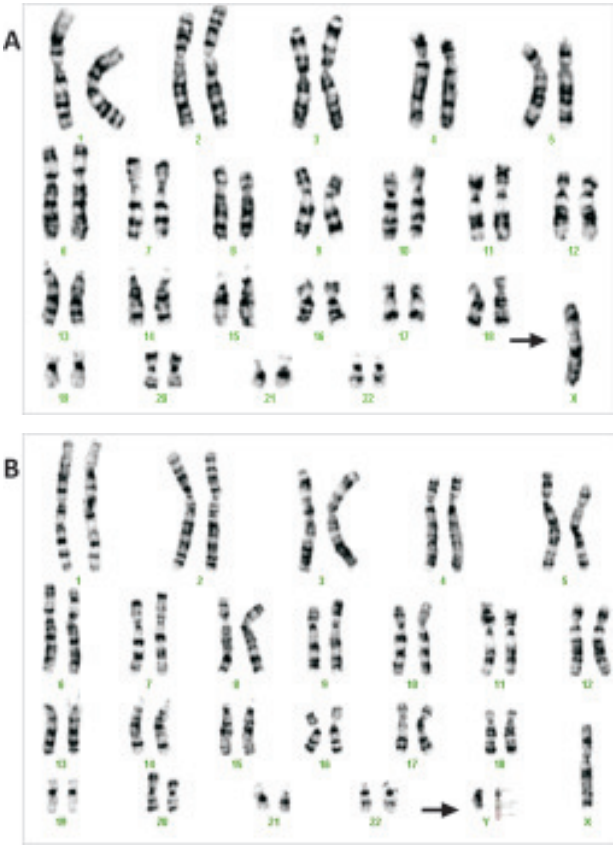

Fig.1: Photos of 45,X and 46,Xdel(Y)(q12) analyzed by G-band staining. (A) Karyotype 45,X analyzed by G-band staining. (B) Karyotype 46,Xdel(Y)(q12) by G-band staining. The arrow indicates the monosomy X chromosome in panel A. The arrow indicates del(Y) in panel B.

interpretation was made according to ISCN 2009 [8] (Fig.1). To further investigate the cell lines in blood lymphocytes and to evaluate larger number of cells, two hundred cells were analyzed by FISH and the XY signal was detected in 20% of the lymphocytes (Fig.2). In the Yq microdeletion analysis performed, the sY254 and sY255 regions in AZFc and sY127 and sY134 regions in AZFb, were found to be deleted (Fig.3). The father's and brother's results for the chromosomal analysis as well as the Yq microdeletion analysis were normal.

DISCUSSION

Chromosomal and Y-chromosomal microdeletion analysis has been done in cases of idiopathic infertility with the objective of evaluating the frequency of chromosomal and molecular anomaly as the causal factor of infertility. The relationship between infertility and chromosomal abnormalities has been well documented over the past 25 years.[2] Since an increase in chromosomal abnormalities correlates with a decrease in sperm count, abnormalities in sperm count are the most important indications for chromosome analysis in infertile males.[2] Numerical sex chromosome aberrations constitute a small percentage of the anomalies; since only 3.32% were reported in a study of 2,196 infertile men; and they tend to have a broad spectrum of phenotypic effects.[2].

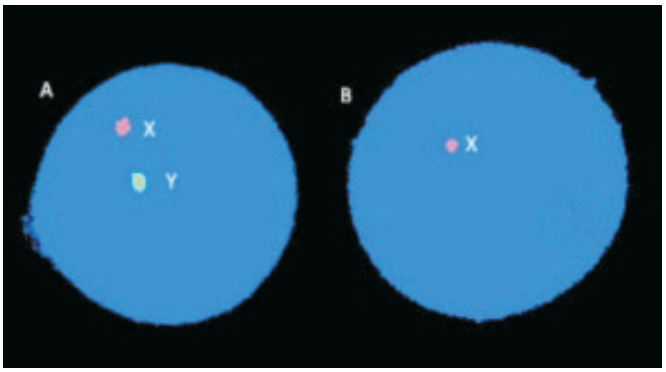

Fig.2: Flourescence in situ hybridization (FISH) probes for centromeric X alpha satellite (red) and Y satellite III (green) sequence were used. (A) A green and red signal indicates presence of both a Y and X chromosome in the cell. (B) A red signal indicates monosomy X.

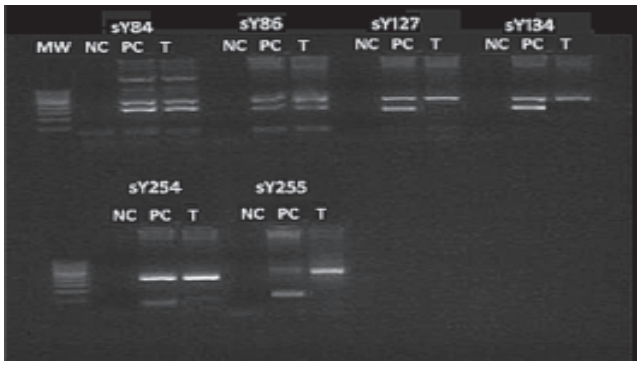

Fig.3: Result of multiplex polymerase chain reaction (PCR). M indicates molecular market, T, patient; NC, DNA sample from a woman as a negative control; PC, a DNA sample from a normal fertile man as a positive control. The arrows indicate the deletion of sY127 and sY134 regions in AZFb and sY254 and sY255 regions in AZFc.

Our patient revealed a 45,X/46,Xdel(Y)(q12) mosaicism during routine blood analysis. Our patient was infertile because of azoospermia, but he also had an Yq microdeletion covering the AZFb and AZFc regions. It has been reported that numerical Y chromosomal defects may accompany Yq microdeletions, as deleted regions tend to be lost during cell division as a result of mitotic instability.[6,7] There is a close association between large Yq deletions and gonosomal mosaicism in both somatic and germinal cells.[5,7] As in our case, mosaicism in germinal cells may remain undetected unless a specific analysis is performed.

Patients with a karyotype 45,X/46,Xdel(Y)(q11) can present a wide spectrum of sex phenotypes, including complete masculinization, ambiguous genitalia, or Turner syndrome These phenotypic differences are related to the proportion of 45,X line in gonadal tissue. If the 45,X line was predominant in gonadal tissue, the phenotype of Turner syndrome would appear. Our patient showed a higher percentage of 45,X cell line (80%) in the peripheral blood, but we speculate that the proportion in gonadal tissue is lower than that in the blood. This is supported by the patient's normal male phenotype.

The formation of 45,X/46,XY mosaicism may be caused by at least two mechanisms. The first is paternal non disjunction at meiosis II followed by loss of the chromosome in subsequent mitoses and the second is a post-zygotic mitotic error.[3] Either mechanism could be the cause of mosaicism in our patient, while the presence of Yq microdeletion makes him especially interesting. When sex chromosomal aneuploidy is detected in infertile males, it should be remembered that Yq micro deletions may be present and induce mitotic instability of the Y chromosome, and cause mosaicism. This may be undetected in blood cells, but may be significant in the germinal tissue. Evaluation of infertile males with spermatogenetic defects for gonosomal mosaicism could help in a better assessment of the outcome of assisted reproduction techniques.

In conclusion, our results highlight the importance of a combined molecular and karyotypic approach and thorough histological analysis for proper evaluation of genotype-phenotype correlation in patients with spermatogenic failure carrying AZF deletions.

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OPHTHALMIA NODOSA - A CASE REPORT

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ABSTRACT

Hairs of certain insects and plants, if exposed to the eye can penetrate the conjunctiva and the cornea and produce an inflammation called Ophthalmia Nodosa. Hairs of caterpillar and tarantula (a species of spider) are usually implicated. Patients present with severe congestion, chemosis, photophobia, blepharospasam, lacrimation and pricking sensation. Protruding hair, lodged in the palpebral conjunctiva will lead to linear corneal abrasions and intolerable irritation. Here we describe such an inflammation (acute uveitis) following an insect fall inside the eye of a 31 yr old bike rider who was treated with thorough washing of the eye and removal of numerous hair on the palpebral conjunctiva and corneal surface. He was subsequently treated with topical antibiotic steroid combination drops to control the inflammation and to prevent secondary bacterial infection. This case report is being presented to highlight the importance of early diagnosis and the urgency of treatment. Removal of caterpillar hair should be attempted without causing further damage to the eye.

Key words: *Caterpillar hair, ophthalmia nodosa,tarantula hair.*

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INTRODUCTION

Ophthalmia nodosa is known to be caused by caterpillar and tarantula hair. These black and brown hairy caterpillars belong to the order Lepidoptera.[1]

These hairs are highly irritant in nature due the toxins at its base.[2] Hairs can be barbed or smooth. It was earlier described as nursemaid's disease due to the frequency with which it was seen among the nursemaids who watched over their charges in Hyde Park. Saemirsch in 1904 first coined the term Ophthalmia Nodosa used to describe the nodular granulomatous inflammation of the conjunctiva and iris with insect hair inside it.[3,4] In a case report in England a 6 year old boy in whose eyes a caterpillar had been thrown by his friend, developed such severe iridocyclitis that eventually the eye had to be enucleated.[5]

CASE REPORT

A thirty one year old male patient presented with complaints of pain, redness and photophobia in the left eye for 3 weeks. He gave history of an insect falling into the eye 3 weeks ago while riding a bike and what he felt was like "acid thrown into the eye". Patient was on treatment with topical antibiotics without any relief. His systemic condition was normal. On slit lamp examination, multiple fine hairs were seen on the surface (Fig1:- black arrow), intra stromal layers of the cornea, anterior chamber (AC), iris (Fig.1: white arrow) and on the lens (Fig.1: double head arrow). Mild anterior uveitis was present as indicated by the cells and flare in AC. Fundus appeared normal. Vision was 6/6 in both eyes. The patient was diagnosed to have Ophthalmia Nodosa due to caterpillar hair. A thorough saline wash was given. Removal of hair on the surface of the cornea and palpebral conjunctiva was attempted with a fine needle and forceps. Most of them were very brittle and friable and difficult to remove. The patient was started on antibiotic steroid drops and atropine drops with a subsequent decrease in the inflammatory response. Reactivation of anterior uveitis occurred 2 weeks later and subsided on increasing the frequency of drops. On follow up visit eye was quiet with no evidence of uveitis and nonsteroidal anti inflammatory drops were substituted for steroid drops in view of the complications of long term steroid use (cataract, glaucoma, secondary infection). As the hair could not be completely removed, the potential for chronic inflammation in the form of recurrent pain, photophobia, watering and defective vision was explained to the patient. The importance of follow up was also explained in order to prevent inflammation related complications.

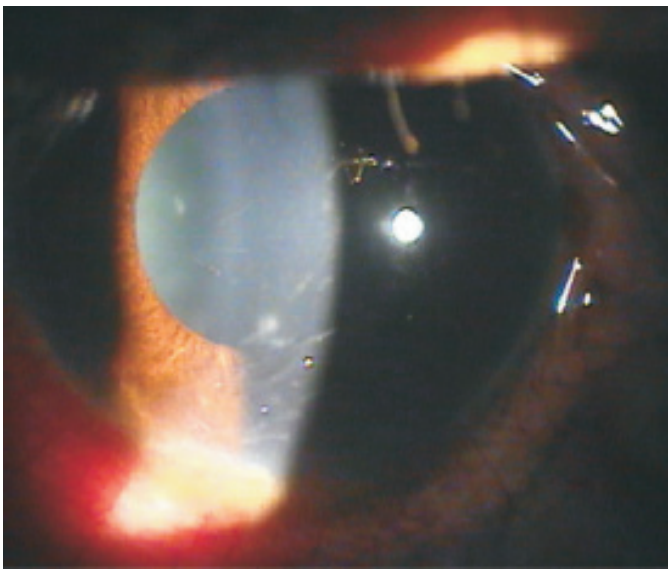

Fig1: Slit lamp photograph showing caterpillar hairs on the cornea (black arrow), on the iris (white arrow) and on the lens (double head arrow).

DISCUSSION

Ophthalmia nodosa has been known since the last hundred years. It is very common in Australia where entire sections of forests would be closed off during autumn due to these outbreaks. In India the seasonal incidence is from September to January when the caterpillars are in plenty.[6] Nodular conjunctivitis, keratitis, hypopyon uveitis, nodular iritis,

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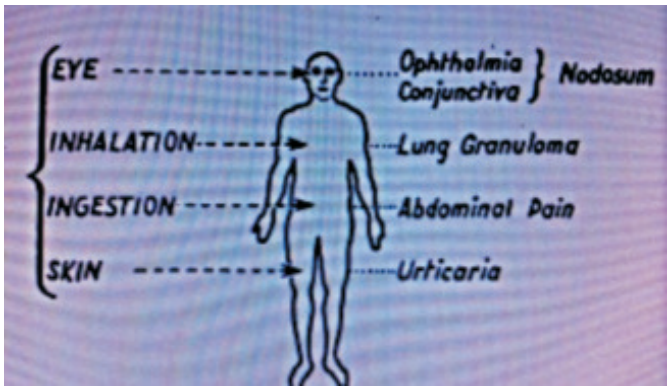

Fig 2: Diagram showing systemic problems that can be caused by caterpillar hair (Courtesy - Peter GW, David S Brit J Ophthalmol 1966;50:213)

cataract, vitritis, retinal detachment, endophthalmitis and finally phthisis bulbi has been known to occur requiring treatment like lensectomy, vitrectomy and systemic immunosuppressants. Hair can also cause systemic problems like urticaria, lung granuloma on inhalation, abdominal pain on ingestion (Fig .2).

The hairs of the insect are propelled like a missile in the eye due to the wind and enter the tissues by rubbing the eye or by lid movement. Removal of superficial hair should be attempted with a fine needle on initial visit, as they become friable and would break off easily later. Copious irrigation is advised. They have to be followed up on a regular basis to treat any recurrent inflammation in the form of uveitis for at least 6 months. Topical steroids provide more symptomatic relief by reducing the toxin mediated inflammation, edema and discharge. Despite the grave range of possibilities in manifestations, the outcome in most of the cases is satisfactory if diagnosed early and treated effectively. When nodular lesions develop in the conjunctiva, they harbor caterpillar hair and should be coaxed out from the nodule. Endophthalmitis has been reported even years later despite adequate treatment of the anterior segment manifestations. Enucleation is restricted to those cases in which the eye is phthisical and painful.[6,7]

CONCLUSION

In this report we wish to highlight the importance of recognizing the disease and treating it early. Long term follow up is important and should be explained to the patient. Protective eye wear like goggles is advised for those people who work in gardens, bike riders and other people at risk.

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SCIMITAR SACRUM- INFECTED ANTERIOR SACRAL MENINGOCELE

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ABSTRACT

Anterior sacral meningocele is a rare anomaly most frequently presenting as a presacral mass. It is associated with a number of congenital abnormalities of the pelvis. Plain radiograph of the pelvis may demonstrate a concave defect in the side of the sacrum (scimitar shaped sacrum) which is often diagnostic while CT scan and MRI scan would confirm the diagnosis.

Key Words: *Anterior sacral meningocele [ASM], currarinotriad, scimitar sacrum*

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INTRODUCTION

Anterior sacral meningocele (ASM) is a herniation of the cerebrospinal fluid (CSF) filled dura mater through either an enlarged sacral foramen or a defect in the sacral bone. It's posterior counterpart-the posterior meningocele is a much more common condition presenting as an external protuberance and hence diagnosed earlier. In case of anterior sacral meningocele, the bony defect doesn't enlarge much, while the hernial sac driven by the CSF pulsations grows in size, due to less resistance offered by the pelvic structures. Symptomatic cases present with non specific symptoms like constipation and urinary disturbances. These lesions rarely cause neurological complications, but meningitis, sepsis, obstetric problems, and bowel and bladder difficulties are common secondary conditions. The lesions can even be fatal.[1] Hence, a high degree of clinical suspicion and imaging are indispensable in the diagnosis of this potentially fatal condition. The identification of sickle shaped defect of sacrum-scimitar sacrum in radiograph is highly suggestive of this condition.

Anterior Sacral Meningocele (ASM) is a rare congenital anomaly first described in 1837 by Bryant.[2] Anterior sacral meningocele is defined as a unilocular or multilocular protrusion of the dura and arachnoid out of the sacral canal into the retroperitoneal and infraperitoneal space. Anterior sacral meningocele may protrude either anteriorly through the body of the sacrum or anterolaterally through an enlarged intervertebral foramen. Its wall has outer dural and inner arachnoid elements which are filled with cerebrospinal fluid (CSF) and may contain neural tissue.[2]

CASE REPORT

A 22 year old male patient presented to emergency room (ER) with complaints of low back ache, weakness of bilateral lower limbs and difficulty in micturition. He also had fever and headache of one week duration. On clinical examination, patient was febrile with weakness of power in bilateral lower limbs [grade 3/5]. Neck rigidity was present. All baseline investigations were found to be normal except for a mildly elevated leukocyte count.

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Radiology findings:

Plain X-ray of sacrum [Fig.1a] showed partial agenesis of the sacrum on the left. A Computed tomography (CT) [Fig 1b,c]: revealed a soft tissue mass in presacral region protruding through the sacral defect displacing the rectum anteriorly. Volume rendering [Fig.1d]: (VR) images showed butterfly vertebra at L5 and scimitar-shaped sacrum.

A magnetic resonance imaging (MRI) T2 & T1 post contrastaxial images [Fig 2a,b,c] demonstrated a well-defined cystic lesion in the presacral region with an intraspinal component. The lesion shows peripheral enhancement with air pockets within it. Diffuse spinal meningeal enhancement is seen in contrast-enhanced MR. In sagittal images T1 & T1C MRI [Fig.2d], spinal cord is seen to end at L2 level and is tethered by thin cauda equina lipoma ending at S2–S3. Reduction in size of lesion was seen in follow up MRI [Fig 2e] after conservative treatment.

DISCUSSION

Anterior sacral meningocele (ASM) a rare anomaly is a form of caudal dysgenesis secondary to failure of sclerotome development. It is present at birth and becomes symptomatic at a later age, usually manifesting as a presacral mass. It is

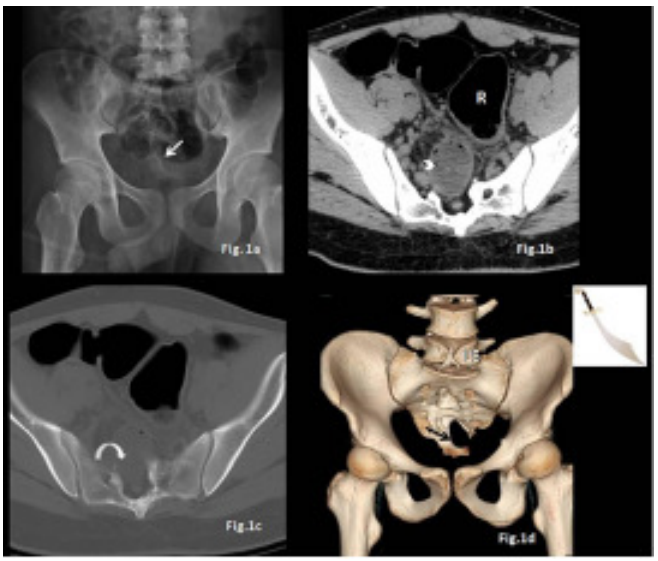

Fig.1: Investigations of an infected anterior sacral meningocele showed partial agenesis (white arrow) in x-ray (a); a presacral soft tissue mass (double arrow) protruding through the sacral defect (curved arrow) seen in CT (b,c); L5 butterfly vertebra (L5), scimitar-shaped sacrum and scimitar sword (inset) were observed involume rendering (VR) images (d).

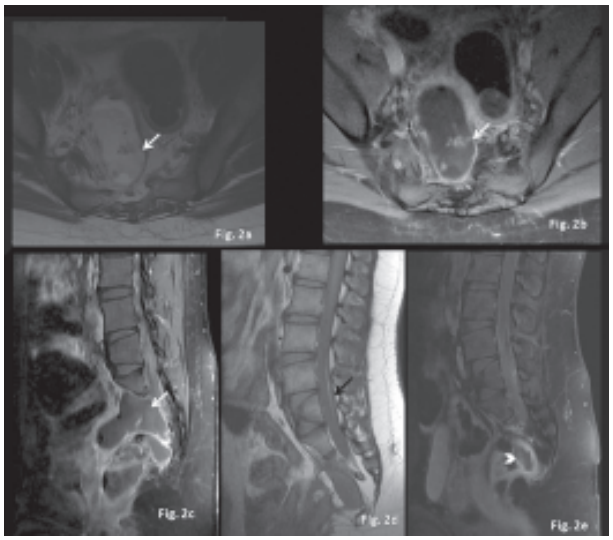

Fig.2a,b,c: MRI T2 & T1 post contrast axial images showed a peripherally enhancing presacral cystic lesion with an intraspinal component (white arrow). Few air pockets noted within it. Diffuse spinal meningeal enhancement is also seen on contrast administration.

Fig.2d: Spinal cord ends at L2 level and is tethered by thin caudaequinalipoma (black arrow) ending at S2–S3.

Fig.2e: After conservative treatment a reduced size of the lesion was seen in follow up MRI.

more common in women with a male to female ratio of 1:4 to 31:116.[2,3] It is part of the Currarino triad which includes anorectal malformations, scimitar sacrum, and a presacral mass.[4,5,6] It is also associated with Marfan syndrome and neurofibromatosis. Although anterior sacral meningocele occurs sporadically, autosomal dominant or x-linked dominant inheritance has been attributed.[5,6]

Anterior sacral meningocele can manifest at any age, but usually occurs in the second or third decades of life, or during childbearing years in women. In men, it is diagnosed most often in the first decade. The symptoms are related to mass effect causing compression on the rectum, bladder, and sacral nervous plexus. Patients may also present with increased pressure headache due to compression of anterior sacral meningocele by increased abdominal pressure. This can result in emptying the contents of the anterior sacral meningocele into the CSF spaces. Low-pressure headaches due to refilling of anterior sacral meningocele and decrease in CSF volume can occur when the patient is in erect posture. Rarely, anterior sacral meningocele may present with bacterial meningitis.[7,8, 9] The cord and cauda equina are usually normal, but there may be tethering, as in this patient.

Radiography of the pelvis may show the scimitar sign of the sacral bone. The bone defect may range from an enlarged foramen to complete sacral agenesis. In this case CT and MRI helped us to arrive at a diagnosis of ASM and also in subsequent treatment planning. As the patient presented with infected meningocele, conservative treatment with antibiotics was given and patient was advised to come at a later date for a definitive management.

Usually the treatment is surgical as ASM does not regress spontaneously and generally enlarges with an increase in the risk of complications. In the surgical management, care is essential to avoid precipitating bacterial meningitis.[2,9] The posterior approach is preferred by some where a sacral laminectomy is done and the neck of the anterior sacral meningocele is tied off. Nevertheless, trans-abdominal ('anterior abdominal approach' with over-sewing of the neck of the meningocele) and perineal approaches have been reported.[2] Transvaginal or transrectal aspiration is not advisable, as this can lead to a fatal outcome due to sepsis.

CONCLUSION

Anterior sacral meningocele is a rare form of spinal dysraphism and often occult disorder of clinical importance. The radiological manifestation is quite typical. CT and MRI play an important role in confirming the diagnosis as in our case. It is essential to be aware of the imaging features of ASM in order to arrive at the right diagnosis.

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SEX REVERSAL-SIBLING CASE REPORTS

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ABSTRACT

Intersexuality or sex reversal is defined as a condition of imperfect sexual differentiation, into either male or female. Sexual and physical development of an individual depends on genetic, gonadal, hormonal factors, response to the end organs and psychological factors. This study was aimed to characterize the genetic abnormalities associated with XY sex reversals by chromosomal and DNA testing. Blood samples were collected from siblings of two families with clinical features of sex reversal. The karyotype results showed the presence of Y chromosome in all 4 samples. The male genotype was further confirmed by invitro amplification of the SRY gene showed the presence of SRY gene in four samples. Genetic counselling was provided to the patients and was advised to consult endocrinologist for further management.

Key words: *Gonadal dysgenesis, sex reversal, SRY gene.*

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INTRODUCTION

Intersex is a general term used for a variety of conditions in which a person is born with a reproductive or sexual anatomy that doesn't seem to fit the typical definitions of female or male. For example, a person might be born appearing to be female on the outside, but having mostly male-typical anatomy on the inside. Or a person may be born with genitals that seem to be in-between the usual male and female types-for example, a girl may be born with a noticeably large clitoris, or lacking a vaginal opening, or a boy may be born with a notably small penis, or with a scrotum that is divided so that it has formed more like labia. This could be congenital, involving chromosomes, morphologic, genital and/or gonadal anomalies. Sex reversal is defined as a sexual condition in which gonadal sex and chromosomal sex are dissimilar, such as diversion from typical XX-female or XY-male presentations (XY-female, XX-male).[1] The gonadal dysgenesis phenotype features may be due to incomplete or defective formation of the gonads, resulting from a disturbance in germ cell migration or organization, caused by structural or numerical chromosomal abnormalities or mutation in the genes involved in the formation of the urogenital ridge and sexual differentiation of the bipotential gonad. The estimated incidence of intersex is 0.018% in general population of which 1% live births exhibit some degree of sexual ambiguity, and approximately 0.2% of live birth are ambiguous enough to become the subject of specialist medical attention, including surgery to disguise their sexual ambiguity.[1]

Sex is determined by the presence or absence of a testis determining factor (TDF) encoded by a sex determining region (SRY gene) on the short arm of the Y chromosome.[2] During gestation, the cells of the primordial gonad that lie along the urogenital ridge are in a bipotential state, meaning they possess the ability to become either male cells (Sertoli and Leydig cells) or female cells (follicle cells and Theca cells). SRY initiates testis differentiation by activating male-specific transcription factors that allow these bipotential cells to differentiate and proliferate. SRY accomplishes this by upregulating SOX9, a transcription factor with a DNA-binding site very similar to SRY's. SOX9 in turn upregulates fibroblast growth factor 9 (Fgf9), which is necessary for proper Sertoli cell differentiation. Fgf9 then feeds back and upregulates SOX9. Once proper SOX9 levels are reached, the bipotential cells of the gonad begin to differentiate into Sertoli cells. Additionally, cells expressing SRY will continue to proliferate to form the primordial testis.[3] Action of the SRY gene about six weeks after conception triggers the formation of the testes. The testes subsequently make testosterone which floods through the body, making it male. Without a Y chromosome and hence an SRY gene, a fetus would develop the default gender, which is female. Even if people have an X and a Y chromosome, they can develop into females if they have a mutation in the SRY gene, or in one of the other sex-determining genes downstream of SRY in the pathway of male sex determination. But if the SRY gene is transferred to the X chromosome during the production of sperm, an XX male can result.[4]

In the present study, we examined the chromosomes and DNA in four individual, who were sibling with the phenotypic features of intersex. The study was approved by the Institutional medical ethics committee and informed consent was obtained from the patients before collecting blood sample.

CASE DESCRIPTION

Case 1: Two sisters, 22 years and 24 years old, were referred for genetic testing for primary amenorrhoea. The external genitalia and mullerian structures were typically female. Ultrasound showed streak gonads and hypoplastic uterus.

Case 2: Two sisters, 11 years and 14 years old, suspected to have Testicular feminization syndrome were referred for genetic testing. Pelvic sonography and CT scan pelvis showed fibrous band in retroperitoneal area.

In both the cases while the phenotype appears female, normal feminine looks, the secondary sexual characters like axillary and pubic hairs absent, and breasts were under developed (Tanner-II). Hair line was low occipital. No thyroid swelling. However, smell and vision were normal. Serum

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gonadotropin levels (serum FSH, LH) and testosterone level were within normal limits for male.

CYTOGENETIC ANALYSIS

Peripheral blood culture was followed using standard protocol.[5] Peripheral blood lymphocyte cultures for all the four samples were set up in 10-ml culture flasks using RPMI media supplemented with 20% fetal bovine serum. Phytohemagglutinin was added to stimulate cell division. Dividing cells were arrested at metaphase stage with colchicine and fixed in methanol/acetic acid (3:1). Fixed cells were dropped onto glass slides and allowed to air-dry. Chromosomes were G-banded by treating the preparations with trypsin followed by staining with giemsa. All the samples about 25 metaphases were analyzed from duplicate cultures. Three metaphases were documented using image analysis system (Fig 1).

SRY GENE ANALYSIS

High molecular weight genomic DNA was isolated from these samples and Invitro amplification of the SRY gene was performed using Polymerase chain reaction (PCR)[6] to identify the presence or absence of SRY gene in the Y chromosome, similar to karyotypes the four samples showed the presence of SRY gene (Fig 2).

The chromosome analysis and SRY gene by PCR results obtained from subjects are given in Table 1

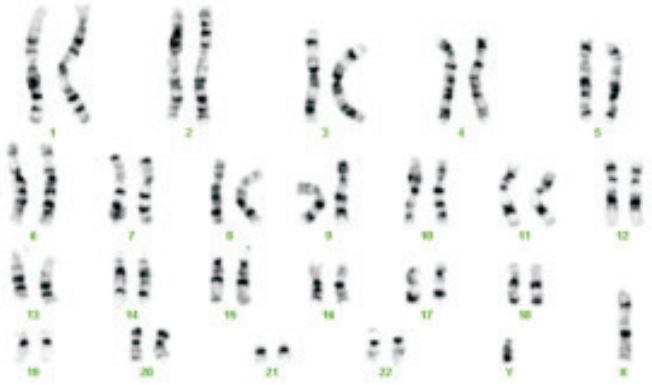

Fig. 1: Karyotype obtained from case

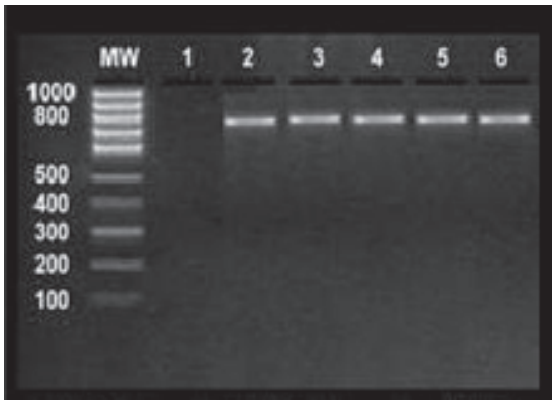

Fig. 2: PCR amplicons of SRY gene

Table -1:

Case	Age	Karyotype	SRY gene
1	22	46,XY	Positive
2	24	46,XY	Positive
3	11	46,XY	Positive
4	14	46,XY	Positive

DISCUSSION

The sex-determination cascade constitutes a model of the exquisite mechanisms of gene regulation that lead to the development of mammalian embryos. Abnormal sexual development may result from multiple etiologies; however, the clinical presentation is rarely pathognomonic of the molecular defect responsible for the intersex condition. Phenotypic females with an XY chromosome constitution, complete gonadal dysgenesis, occur with a frequency of 1/ 20,000 births.[7] At puberty these individuals are typically deficient in secondary sex characteristics, have primary amenorrhea, and tend to be taller than XX females, yet, despite the presence of only a single X chromosome, they lack the stigmata of a Turner syndrome phenotype.

Earlier studies have shown that gonadal dysgenesis may present with a wide spectrum of findings, ranging from individuals with either testicular tissue on one side or a streak gonad on the other or bilateral streak gonads, although internal sex organs are most often female.[8] In the presence of the Y chromosome, these individuals are at a significant (30%) risk for the development of malignant gonadoblastoma, and early gonadectomy is recommended. Hormonal replacement therapy can induce puberty and menstruation.[9]

XY sex reversal occasionally has been reported to occur in families, however we report the presence of XY sex reversal in two families and in both the families SRY gene was also present. As reported earlier this could be recessive mutation that is inherited. However, further family studies involving mutation analysis of the genes involved in the sex determining pathway, linkage analysis of these family samples will enable us to get a lucid picture on the role of different genes in the aetiology and pathogenesis of sex reversal. This will help in better management and counselling of patients with sex reversal.

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GRYNFELT'S HERNIA - A RARE ENTITY

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ABSTRACT

Lumbar hernia is a rare hernia which accounts for less than 1.5% of total hernia incidence. Only 200-300 cases have been reported in the literature. Lumbar hernia can occur either through superior (Grynfelt) or the inferior (Petit) lumbar triangle. Twenty percent of the reported hernias are congenital. We encountered a 65 year old male who was diagnosed to have a superior lumbar hernia which was surgically managed by hernioplasty.

***Key words** : Grynfelt's hernia, lumbar hernia, Petit, hernioplasty*

SRJM 2012;5:28-29

INTRODUCTION

Lumbar hernias are rare lesions[1,2] arising through posterolateral abdominal wall defects, named superior triangle (Grynfelt) [3,4] and inferior triangle (Petit). They occur most often in the superior lumbar triangle (Grynfelt's hernia).[4,5] The most common presenting symptom of lumbar hernia is a dragging sensation or discomfort in the flank.[6] It can mimic a soft tissue mass above the iliac crest, that increases with coughing and strenuous activity, usually reducible and tending to disappear with the patient in the decubitus supine position.[7] Otherwise it can be revealed by complications like incarceration or strangulation[8].

CASE REPORT

A sixty five year old male was admitted with complaints of swelling in left lumbar region for the past 5 years associated with dull aching pain. The swelling increased in size on coughing and reduced on lying down. There was no history indicative of irreducibility. No history of trauma or surgeries in the past. He was a known smoker with history of chronic cough for the past 2 years. He is a known hypertensive on regular treatment.

On general examination, the patient was hemodynamically stable. Cardiac and respiratory systems were normal. On local examination there was a single ovoid swelling of size 8cm x 5 cm, in the region of left superior lumbar triangle.

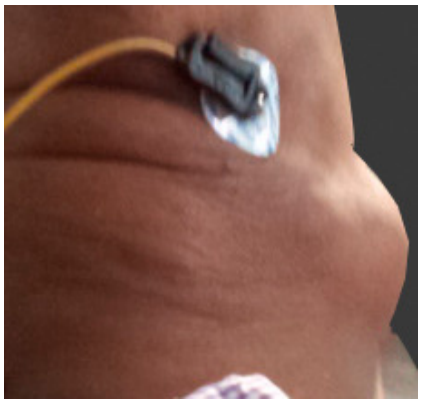

Fig.1: Left Lumbar Hernia (Anterior view)

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On coughing, expansile impulse was elicited. On palpation there was no warmth or tenderness; there was a gurgling sound on reducing the swelling [Fig. 1]. Hernial orifices and the opposite lumbar triangle were normal.

Patient was investigated .Ultrasound abdomen revealed a 3.5cm x 3.3 cm defect in the left lateral abdominal wall with bowel as content. The hernia was partially reducible

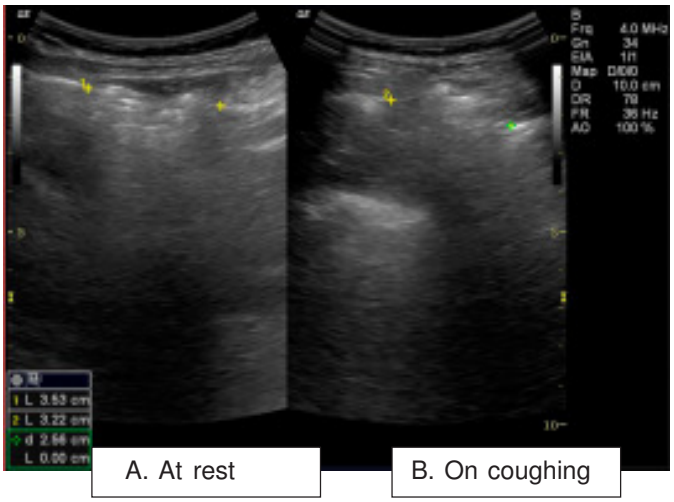

Fig. 2: Ultrasound Abdomen

[Fig.2]. Chest X ray revealed features of chronic obstructive pulmonary disease (bilateral hyper inflated lung fields).

Treatment:

Under epidural anaesthesia with patient in right lateral position, a transverse incision of 10 cm length was made over the swelling. The incision was deepened in layers, thereby exposing the left superior lumbar triangle with the herniated content. The bowel was viable [Fig.3]. The sac was plicated with non absorbable suture after reducing the content. Polypropylene mesh was placed pre-peritoneally and fixed with interrupted non absorbable sutures [Fig.4] Postoperative period was uneventful .The patient was well on follow up after 3 months.

DISCUSSION

The lumbar triangle can refer to either the superior lumbar (Grynfelt) triangle or the inferior lumbar (Petit) triangle. Of the two, the superior triangle is the most common site for herniation. The superior lumbar triangle is bounded medially by the quadratus lumborum muscle, laterally by the internal abdominal oblique muscle, and superiorly by the 12th rib. The floor is formed by the transversalis fascia and its roof by

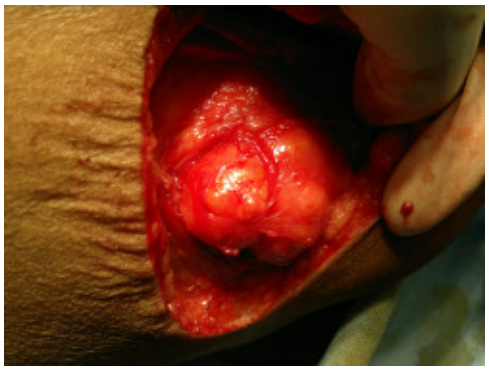

Fig.3: Intra operative - Hernial sac with the content

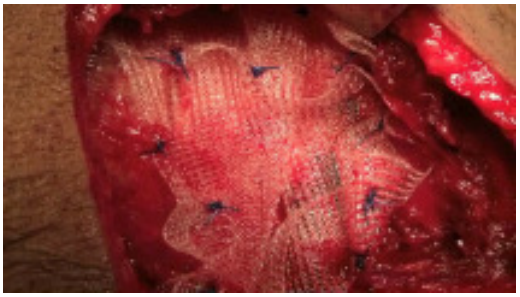

Fig. 4: Preperitoneal mesh placement

the external abdominal oblique muscle. The inferior lumbar triangle is bounded medially by the latissimus dorsi muscle, laterally by the external abdominal oblique muscle and inferiorly by the iliac crest. The floor is formed by the internal abdominal oblique muscle [Fig. 5].

Lumbar hernia is a rare hernia which accounts for less than 1.5% of total hernia incidence. Only 200-300 cases have been reported in the literature. All lumbar hernias must be treated with surgery[1]. Simple suture of the defect may lead to recurrence[1].There are two possible surgical approaches: the anterior approach with lumbar incision and the laparoscopic approach.[4] The objectives of operation for hernia are to reduce the hernia, to remove or reduce the sac and to repair the defect. Many techniques have been described, including primary repair, local tissue flaps and conventional mesh repair. Historically, Dowd repair[9] was practised, which involved the closure of defect by a pedical flap of tensor fascia lata and gluteus maximus from below the iliac crest with side to side opposition of external oblique and latissimus dorsi for Petit

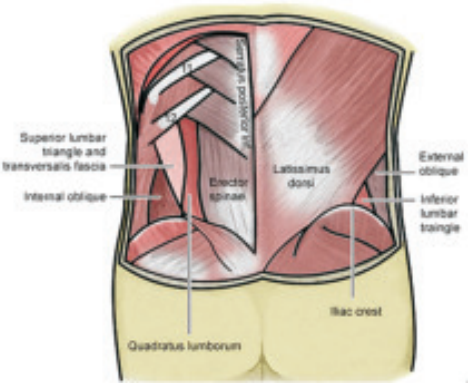

Fig.5 : Anatomy of lumbar triangle

triangle hernia. For superior triangle, flaps from adjacent structures were developed.

As far as minimal access surgeries are concerned, very few cases have been reported till date with the first successful lumbar hernia repair done in 1996 by Burick and Parascandola.[10] The basic principles to be followed for an intraperitoneal laparoscopic approach are as follows. Patient to be placed in modified flank position, to create a wide peritoneal flap around the hernial defect after reducing the contents, to fix the lower margin of the mesh to the lumbodorsal fascia and finally to reperitonealize with peritoneal flaps to place the mesh in the retroperitoneum. However in our case, laparoscopic technique could not be adopted in view of the comorbid COPD in him.

Strangulation of a lumbar hernia is relatively uncommon, reported in approximately 10% of patients.[7] Computed tomography is a very useful tool for the diagnosis of lumbar hernia. It can delineate the neck of the hernia and hernial contents.[7]

CONCLUSION

Lumbar hernias are rare with scattered evidences of both superior and inferior lumbar hernias. Hernias of the superior triangle are most commonly associated with either straining or direct trauma in the lumbar region. The diagnosis is relatively easy if there is a reducible mass beneath the 12th rib which transmits a cough impulse. Our patient had a chronic history of cough which was the contributing factor for the hernia. All lumbar hernias must be treated surgically.

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SAFETY MEASURES AND PRUDENT PRACTICES IN HISTOPATHOLOGICAL LABORATORY IN DENTAL SCIENCES

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ABSTRACT

Implementation of safety measures is essential for a proper functioning of any laboratory. Laboratory accidents can occur due to incomplete knowledge of the various chemicals. This article details some of the common chemicals used in the histopathology laboratory and their hazards. The design of a laboratory along with the safety equipment that needs to be installed during the designing are also elaborated. Some common accidents that can occur in the work area of a laboratory, their protocol and ways to circumvent such errors including the laboratory design to risk assessment and implementation of personnel protection have been elaborated.

Key words: *Chemical hazards, lab safety, lab accidents, risk assessment, personnel protection.*

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INTRODUCTION

The term "*Laboratory*" refers not only to a space within four walls but also to a "*field*" such as chemical, biological, genetics etc. The term "*safety*" refers to protection against all hazardous biological, chemical and physical materials including radiation.[1] Accidents happen in the laboratory as in other work places and a greater understanding of the biological or medical consequences offers no greater protection as familiarity often leads to a more casual approach in dealing with the chemical or biological hazards. Determining the degree and nature of hazard of a particular chemical or biological is only part of the process; the way in which the material is used is equally important in determining the risk.[2]

Since the time Moses directed the children of Israel to construct a parapet for their roofs, "that thou bring not blood upon thine house, if any man fall from thence", the matter of workplace safety and health has been on the agenda, sometimes high on the agenda of civilized societies.[3] The Occupational Safety And Health Act was signed unto law by President Richard Nixon on December 29, 1970 and became effective on April 28, 1971. This law, considered a landmark in the history of labor and public legislation, has as its purpose "to assure so far as possible every working man and woman in the Nation safe and healthful working conditions."

The Occupational Safety and Health Administration was established in the Department of Labor with major responsibility for the development and enforcement of occupational safety and health standards. The National Institute for Occupational Safety and Health (NIOSH) was established in the Department of Health and Human Services with the responsibility for research and training activity in the area of occupational safety and health. The Centre for Disease Control and Prevention (CDC), along with its federal agencies such as Agency for Toxic Substances and Drug Registry (ATSDR), Environmental Protection Agency (EPA) has compiled the various toxicological profiles of various hazardous chemical substances along with their adverse effects which are of immense use in various fields.[4,5]

As a developing country India is making advances in the field of technology, research, infrastructure and skilled manpower. However, much of these advances are limited to urban centres. In healthcare too, such disparity exists .[6] The Kolkata hospital fire accident on the 9th of December 2011 portrayed the discrepancies that still existed in the hospital safety sector. The Indian Council of Medical Research has laid down the mandate to foster personnel protection in laboratories.[6] National Accreditation Board for Laboratories specifies guide lines for safe functioning of laboratory facilities.

Histopathology plays a vital role in diagnosis. A well-equipped and maintained laboratory forms the backbone of histopathology. Certain stains routinely employed in immunohistochemistry such as amino-ethylcarbazole, diaminobenzidine, fast red are potential carcinogens and must be used with utmost precaution. Certain chemicals are not compatible with each other. Thus it becomes mandatory to be familiar with the various chemical agents employed in the laboratory, their properties, disposal ways and their hazards.

Common accidents in the laboratory and their safety protocols:

Chemical spills:

A chemical spill is the most common accident in the laboratory. In most cases it can be cleaned up by the laboratory personnel with minimal effort or risk. The response to the spill depends on nature of the hazard, the volume of the hazard and the qualifications of the staff.[7] A gallon of alcohol spilled onto the floor presents a risk of fire but little health hazard but the same quantity of formalin is life- threatening.[8]

The minor spills can be wiped off with towels or paper towels and properly disposed off in an impermeable plastic bag or container.[9] In case of a larger spill, all personnel should evacuate room quickly and emergency response teams should be summoned. Appropriate first-aid must be provided to the involved persons. In case of chemical splash, the eyes must be flushed with copious amounts of water from an eyewash station or a deluge shower. The eyes must be open while being washed. In case of exposure to dry chemicals then it

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should be brushed off the material. A good basic kit including cleanup items such as dustpans, brush for powders, sponges, towels and mops for liquids, adsorbent material, bleach for biohazards, baking soda for acids, vinegar for alkalis and a commercial formalin neutralizing product should always be present in the vicinity of the work area.[7, 8]

Fire:

Laboratory fire stems from many sources- the Bunsen burner, runaway chemical reactions, electrical heating units, failure of temperature controls in the equipment etc.[7,10]

Various types of fire extinguishers are commercially available for the fire due to various reasons and must be available in the vicinity of the work area. In the event of a person's clothing catches fire, it is important not to run as this provides additional air to support the flames.[9,10] It is also recommended that a person aflame should roll on the floor to attempt to smother the flames. In the laboratory a deluge shower is the best way to put off the fire. In a crowded laboratory there is often a risk of involving solvents and other materials in the fire. However a deluge shower is an effective way to put out the fire if it is in the immediate area, or, if a fire blanket is available, the fire can be smothered by the person quickly wrapping himself in it. If others are present, they can help smother the flames or they might employ a fire extinguisher to put the fire out. [7,11,12]

Explosions:

An explosion may result from a runaway chemical reaction, a ruptured high- pressure vessel, reactive metals coming in contact with moisture etc.[7,11]

During explosions, shock waves and extreme air pressures are generated that can result in chemical spills that can exacerbate the existing situation. During explosions apart from physical injuries often toxic fumes are released which pose a more serious hazard leading to respiratory emergencies and sometimes even death.[12,13] Fortunately explosions are less common in a histopathology laboratory but can occur and the technical staffs must be well prepared to handle such emergencies.[1] The victims must be immediately taken out of the room and the fire department and other professionals must be summoned immediately. Usage of personal protective equipment such as masks, goggles, heavy gloves and gauntlets should be made mandatory.[8,13,14]

Other accidents that can be expected to occur in laboratories for which every personnel should be prepared to combat are [1, 7]:

* Inhalation, ingestion, or absorption of toxic materials
* Release of compressed toxic, explosive, asphyxiating, and corrosive gases
* Release of radioactive materials
* Release of pathogens and restricted biological materials, though uncommon in a histopathological laboratory setup
* Power failure, involving loss of lights or ventilation
* Electrical shocks
* Explosions, or runaway reactions
* Failure of a facility exhaust system

Certain accidents may result due to incomplete knowledge of the reactivity of the chemical substances. Reactivity data are available in the Material safety data sheet which should be perused while arranging chemicals in the racks in order to prevent undesirable chemical reactions. Selected incompatible chemicals are listed out in Table 1.

Table 1: List of incompatible chemicals

Chemical	Incompatible with
Formaldehyde	Metals, alkalis, acids
Gluteraldehyde	Alkalis, oxidizing agents
Acetone	Acids, hydrogen peroxide
Mercury, Iodine	Ammonia
Hydrogen peroxide	Metals & metal salts, alcohol
Sulphuric acid	Potassium permanganate
Nitric acid	Acetic acid, flammable liquids

DISCUSSION

"Better safe than sorry" is true in the sense that it is always better to be preventive. This begins with designing of a laboratory to training the personnel to combat such mishaps.

Laboratory design:

The primary objective in laboratory design is to provide a safe environment for laboratory personnel to conduct their work. A secondary objective is to allow for the maximum flexibility for safe research use. [13] Therefore all health and safety hazards must be anticipated and carefully evaluated so that protective measures can be incorporated into the design.

The laboratory should be completely separated from outside areas restricting the entry of visitors. Such enclosed laboratories help in containing spills while keeping unauthorized persons from entering the areas where hazardous chemicals are stored.[7] The floor should be preferably made of a single piece impervious to liquid spills. Tiles and wooden flooring is not preferred as liquids can seep through the gaps.

Good quality modular furniture in a variety of materials that is available in today's market can be installed in configurations to fit almost any need.[9] Units can be obtained pre-cut to accommodate connections to utilities. Base units can be obtained in steel, wood or plastic laminates. The steel should be of a heavy gauge with a pre-treatment to reduce the corrosive effects of chemicals.[7] Usage of a chemically resistant paint will also minimize the effects of corrosive chemicals. Bench- tops with incorporated lips to prevent run- off onto the floor can also be obtained of several materials such as laminate veneers, plastics etc. Although the cost of wooden furniture is high, its durability outweighs the cost.

Laboratory shelves must not be placed at a height above 30 centimetres form the work table. This not only reduces the ergonomic issues but also prevents the accidental slipping of containers while accessing them from such high levels.[9,13] Firmly attached shelves are a must for the laboratory in order to prevent the collapse of the shelves due to the weight of the materials kept inside. [7,11] Shatter resistant containers are preferred for storage of substances in order to minimize spillage due to accidents or earthquakes. Corrosive substances such as acids must be grounded to the floor in order to help prevent splashes. Light–sensitive substances should be stored in amber coloured bottles in order to prevent oxidation[9].

The laboratory room should have mechanically generated supply air and exhaust air. There should be no return of fume hood and laboratory exhaust back into the building. Fume hoods should be located away from any site of activity which produces turbulence[10]. An emergency eye wash station should be present from within ten seconds of each fume hood installed in the laboratory.[7,13] Apart from fume hoods general room exhausts should also be provided to maintain the air circulation in the room. Local exhaust ventilation such as 'snorkels' or 'elephant trunks' can also be designed to adequately control exposure to hazardous chemicals.[9]

Laboratory area should be provided with adequate natural and artificial illumination that ensures visibility during working.[13,14] This also contributes to safe working environment. The laboratory should be designed in such a way that it can be cleaned easily. Adequate spacing in between the work tables must be provided to keep the area clean. Adequate place for additional facilities such as food storage/ consumption, personal hygiene etc., must be incorporated while designing the laboratory.[7,10]

Personnel protection:

Trained personnel and the practice of personal hygiene also contribute to the safety of the working environment. Trained people work more safely, more efficiently and more economically. The training must include the various first-aid techniques for the emergencies, handling of the various toxic/ carcinogenic substances and their exposure protocol.

There is no alternative to the practice of personnel protective equipment.[7,14] A simple plastic apron, a vented splash proof goggle and nitrile gloves are the best options for the histological work.[8] Serious exposures can be safely tolerated with 8mm thickness of Nitrile gloves.[8,15,16] While designing the laboratory these should be borne in mind and adequate cabinets that are labeled should be installed appropriately. Plumbed eyewash station should be provided for all work areas where during normal operations or foreseeable emergencies the eyes of an employee may come into contact with a substance which can cause corrosion, severe irritation, or is toxic by skin absorption. An emergency shower (Fig.1) should also be installed to handle emergencies involving body contact of hazardous chemical substances.[7,11] In histopathology laboratories which handle formaldehyde higher than 1% must have a deluge shower installed in work area in order to provide a quick drench facility to personnel who have had exposures to corrosive acids and other chemicals. Emergency eyewash facilities and deluge showers shall be in unobstructed and accessible locations that require no more than 10 seconds for the injured person to reach along an unobstructed pathway. The floor beneath the facility could be properly delineated by using a distinctive pattern in order to facilitate clear viewing of the wash area.[7,10]

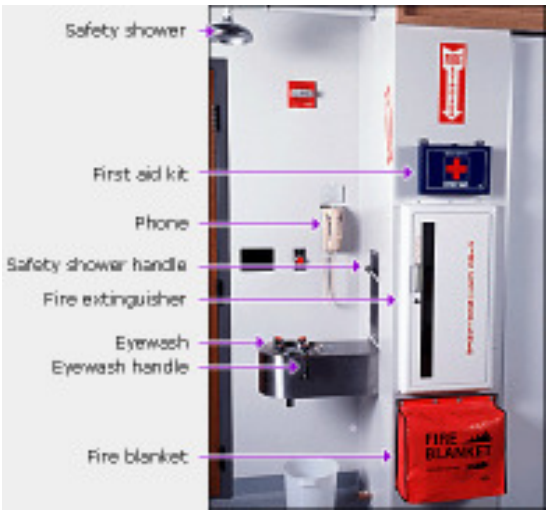

Fig 1: Components of a safety shower

Different classes of fire extinguishers are available in the market. They should be mounted on the walls of the laboratory in ideal locations and the personnel should be given adequate training to use these appropriately.[1] Class A extinguishers containing water are intended to be used on fires involving solid fuels such as paper, wood and plastics. Class B extinguishers intended for use on petroleum and solvent fires usually contain carbon dioxide or a dry chemical such as potassium or sodium bicarbonate. Class C extinguishers are to be used for electrical fires and Class D extinguishers are primarily used for reactive metal fires.[1,7,10] For an effective use the extinguisher must always be full and should be aimed at the base of the fire.[11,12]

All these facilities must be properly maintained and checked on a yearly basis. Appropriate training, mock fire drills etc., should be should be made as a protocol for the employees in order to familiarise the Standard operating procedures (SOPs) to the personnel.

Risk assessment and management:

Risk assessment and management are valuables that have become incorporated into modern safety legislation. The various steps in risk management are[8]:

* Identification & evaluation of the hazards.
* Plan to minimize the risk.
* Implementation of the plan.

All possible hazards should be identified and listed out. The severity of each hazard should also be evaluated.[7,8,10] Knowledge regarding the technical aspects, their hazards, storage, handling and disposal details of the various chemicals used in the histopathological lab can be obtained from the Material Safety Data Sheet (MSDS) available in the internet.

Fig 2: National Fire Prevention Association (NFPA) universal hazard diamond

Few chemicals used in the histopathology lab along with their potential hazards are listed in Table 2.

Every institution should have an emergency committee that should develop an appropriate emergency response plan which should include the telephone numbers of the emergency assistance cell, the location of the MSDS sheets for the various chemicals used in the laboratory, a simplified list of emergency actions to be taken for most likely emergencies, evacuation instructions etc.[7,8] Additional information such as placing a National Fire Prevention Association (NFPA) universal hazard diamond (Fig 2) that indicates the type of hazard of the chemicals available in the lab is also highly desirable. This contains four smaller diamonds which are together assembled into one larger diamond. The four smaller diamonds are: Blue for health/ toxicity, Red for flammability, Yellow for reactivity and White for special warnings such as radiation or carcinogenicity. Printed in each segment is a number representing the degree of hazard of the chemical ranging from[1, 7]:

Table 2: Potential hazards of some chemicals used in the histopathological lab

Chemical	Laboratory use	Potential hazard
Formalin	Most common tissue fixative	Suspected human carcinogen
Gluteraldehyde	Fixative for electron microscopy	Mutagenic for mammalian somatic cells. Risk of explosion in presence of static discharge
Paraformaldehyde	Vapour fixative for frozen tissues	Flammable when heated (forms formaldehyde gas)
Glyoxal	Tissue fixative	Extremely flammable in presence of heat and flames
Osmium tetroxide	Secondary fixative in electron microscopy	Skin irritant. Extremely hazardous when ingested
Isopropyl alcohol	Tissue dehydrating agent	Produces narcotic effects at increased concentrations
Xylene	Tissue clearing agent	Respiratory and skin irritant
Acetone	Tissue clearing agent	Toxic to cardiovascular system, respiratory system and the kidney. May contain traces of benzene and formaldehyde which may cause cancer. Highly flammable in presence of heat or sparks.
Benzene	Tissue clearing agent	Potential carcinogenic and clastogenic (can cause chromosomal aberrations)
Picric acid*		Highly explosive. Should always be kept wet
		(water concentration above 20%)
Acetic acid*		Mutagenic for mammalian somatic cells
Sodium hydroxide*		Mutagenic for somatic mammalian cells. Explosive in presence heat, water and ammonia
Hydrogen peroxide*		Mutagenic for somatic cells. Explosive in the presence of combustibles such as wood, cotton etc.
Mercuric chloride*		Developmental toxicity leading to birth defects
Iodine*		Improper handling can cause allergic responses such as urticaria, angioedema etc.

** Part component of the various stains employed in the histopathological laboratory*

0- No known hazard
1- Slight hazard
2- Moderate hazard
3- Severe hazard
4- Extreme hazard

Although this appears simple it is difficult to implement as there are literally hundreds of chemicals present in a typical lab. A simple alternative to this would be posting of the possible risks and the precautions needed to be undertaken in the form of symbols/ photographs in the area containing such chemicals. On a yearly basis all standard operating procedures (SOPs), risk assessments and training programs must be reviewed and updated. It is also a prudent practice to record the regulator compliance, risk assessments and exposure monitoring.

CONCLUSION

Emergencies are, by definition, not planned. However, planning for emergencies can not only be done but is an essential component of laboratory safety. This is especially true for the laboratory environment where the *potential* for accidents is much higher than in many other working situations. There are many regulatory standards that now require that organizations using chemicals in laboratories and elsewhere or that produce chemical waste have formal emergency plans. These plans must cover emergency evacuation and response procedures, emergency equipment to be kept on hand, security, training of personnel handling hazardous chemicals, reference materials, identification of emergency personnel and access to external resources including aid agreements with local emergency organizations. Safe disposal of both used and unused chemicals must be practised in order to prevent pollution of our resources.

Nearly all laboratories operate under a set of written standardized procedures (SOPs) mandated by a variety of accrediting or regulatory agencies. Meticulous application of these stringent protocols involved in handling of chemicals could pave way for a "*Hazard – free laboratory*".

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ANALYSIS OF IMPACT OF PSYCHOMOTOR SKILL OF THE EXAMINER ON THE RESULTS OF "PASSIVE REPOSITIONING TEST" USED FOR PROPRIOCEPTION EVALUATION

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ABSTRACT

Background *: Evaluation of Proprioception is an important part of neurological examination. Passive repositioning test is a commonly used tool to evaluate the integrity of Proprioception. But this tool can be influenced by the psychomotor skill of the examiner. This component has not been analysed in the studies done previously. Hence, this study is done to analyse the influence of psychometric skill of examiner on the results of the passive repositioning test.*

Methodology*: Thirty physiotherapists with varied level of experience in administering the test were included in the study after their oral informed consent to participate. The proprioception was measured with electronic goniometer attached to Phenix USB 8 system. The knee joint was taken for measurement. One base line test and three trials were given. The examiners skill to identify the same degree of position of knee every time is noted using the goniometer. The angles measured were used for analysis.*

Results*: Pearson's correlation coefficient was calculated for base line test angle and average of three trial angles, and found r = 0.9.*

Conclusion: *The study suggests that psychomotor skill of the examiner did not affect the test result. Hence this test is a easy to use test in clinical set up for evaluating proprioceptive system.*

Key words: *Proprioception, evaluation, psychomotor ability*

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INTRODUCTION

Proprioception is defined as the specialized variation of sensory modality of touch that encompasses the sensation of movement and joint position. It provides the immediate experience of our body.[1] Proprioception is essential for normal coordination and grading of muscle contraction and maintenance of equilibrium.[2] Proprioception is the process occurring along the afferent pathways of sensory motor system. The afferent Proprioceptive feedback results from impulses transmitted by mechanoreceptors to the central nervous system, relaying information about joint position and movement sense.[3,4]

Minimal angle of movement an individual can detect or ability to judge the position of the part in space are used to test Proprioception.[2] The threshold to detect passive movement is considered as an objective way to measure Proprioception. Passive repositioning test is a commonly used test in clinical practice to test Proprioception. It involves repositioning of the limb to the same angle as moved earlier[5-8](Skinner HB 1994, L Remedios et al 1998, Michael V Hayley et al 1998, Ander Jo et al 2007).

Psychomotor skills includes varying levels of well coordinated physical activity and precise manipulative procedures. It can affect the skill of a physiotherapist in various testing and treatment procedures.

In passive repositioning test the subjects will be made to note or recognize the test position of the limb which will be positioned by the examiner. The subject will be asked to inform the examiner when the test angle is reached during subsequent repetition of movement. It will be the examiner's skill to identify whether repositioning of the limb to the same angle, as placed during the test position was achieved. If there is a difference noted then it can be inferred as a deficit in proprioception. Interpreting whether the subject identifies the test position during repositioning is the psychomotor skill of the examiner. Hence, it forms an important aspect in physiotherapy evaluation and can influence test procedure. The present study was carried out as it is thought that examiners' psychomotor skill is important in interpreting the result of the test in clinical set up without sophisticated instrumentation.

During proprioception examination, therapist will interpret the results as proprioception normal or abnormal. In research purposes interpretation of deficit or normalcy will be made with goniometer, by identifying the presence or absence of difference between test angle and angle identified during repositioning maneuvers. Electronic goniometer is instrumentation, which is capable of measuring the angle more accurately. It is equipped with a goniometer attached with electronic device which can measure the degrees of movement of the arms of the goniometer or with more advanced technology where the information regarding the movement and angle can be recorded in the computer. In this study we have used advanced version of electronic goniometer recording the movement in the computer for analysis of the results.

METHODOLOGY

Permission to conduct the study was obtained with the Principal, Faculty of Physiotherapy as it was not involving the patient population and as there is no specific issues identified.

Participants were recruited from the Faculty of physiotherapy, Sri Ramachandra University. Participants

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included faculty, staff and postgraduates. A total of 30 participants were recruited. The study was done with 12 faculty members, 8 clinical therapist and 10 post graduate students in physiotherapy. Clinical experience of the participant ranged from 6 months to 12 years. Their level of clinical experience and experience in doing the passive repositioning test were noted. It was confirmed that all those who have been included had used this test in their clinical practice. They did not require any assistance or training prior to the study.

The passive repositioning test was performed by the participants on an individual (model) who had no musculoskeletal impairment of the knee joint. The same individual was used all through the study.

The model was positioned in side lying with a sliding board supporting the knee joint to be tested. Efforts were made to exclude any visual clues that the participants might get from the sliding board while performing the passive repositioning test. An electronic goniometer was used for the purpose of measuring the angles. This electronic goniometer was incorporated with phenix USB, version-8, Biofeedback. The electronic goniometer was secured to the lateral aspect of the knee joint (Fig.1). The participants were given a demonstration of the testing procedures, following which they were given a trial of the procedure. The testing procedure required the participants to position the knee joint into flexion which was taken as the test angle and following this they had to reposition the knee joint to the same test angle in 3 trials. The initial test angle was not made constant for the individual participants, but rather it was chosen by individual participant. But they were instructed not to use extremes of knee joint ranges, as tissue end feel can give feedback for the position. Following each trial the knee joint was brought to resting position of zero degrees with the guidance of the researcher. The researcher will guide the participant to get to the zero degree by using the readings from electronic goniometer. But during the whole testing procedure none of the participant will be allowed to see the recording by electronic goinometer in the computer screen.

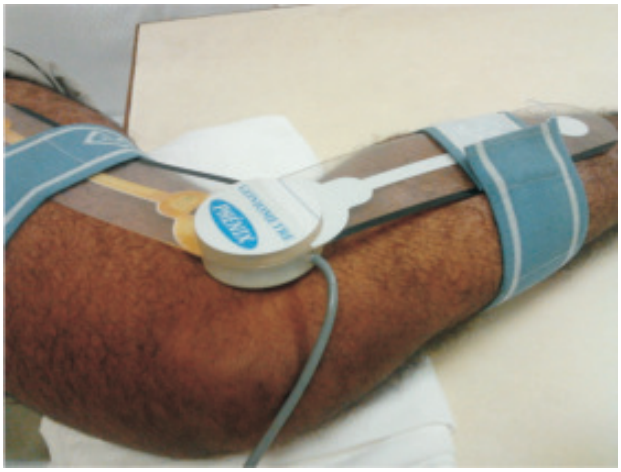


Fig.1: Electronic goniometer on the knee.

Table 1: Data of test and trial angles for 30 participants tested.

Participant No	TA	T1	T 2	T 3	Aver T	T Diff TA& Aver T
1.	29.8	29.4	31.1	30.7	30.4	0.6
2.	24.6	27.1	22.6	25.9	25.2	0.6
3.	19.6	20.9	18.4	17.5	18.9	0.7
4.	64.3	64.5	67.7	55.6	62.6	1.7
5.	38.8	33.4	28.4	25.5	29.1	9.7
6.	35.7	32	33.2	33.6	32.9	2.8
7.	21.5	23.5	19.5	21.1	21.4	0.1
8.	22.6	10.9	12.2	7.7	10.3	12.3
9.	10.1	10.5	8.1	9.3	9.3	0.8
10.	43	46.4	55.2	39.7	47.1	4.1
11.	39.7	45.1	41.4	42.2	42.9	3.2
12.	46	48.5	46.8	45.1	46.8	0.8
13.	30.9	28.4	27.6	29.3	28.4	2.5
14.	38	39.3	35.5	31.3	35.4	2.6
15.	38.5	35.5	37.6	36.8	36.6	1.9
16.	22.2	21.3	16.7	17.1	18.4	3.8
17.	30.1	28	30.9	28	29.0	1.1
18.	32.7	27.1	31.8	27.1	28.7	4.0
19.	29.7	28.4	27.1	20.2	25.2	4.5
20.	26.7	24.1	22.4	19.8	22.1	4.6
21.	18.1	21.9	23.6	23.2	22.9	4.8
22.	35.7	35.7	27.1	25.4	29.4	6.3
23.	45.6	43.9	37.4	37.4	39.6	6.0
24.	55.5	57.2	53	56.8	55.7	0.2
25.	25.4	22.8	24.5	26.7	24.7	0.7
26.	35.3	41.3	38.7	46.9	42.3	7.0
27.	15.5	21,9	20.2	15.5	17.9	2.4
28.	30.5	28.8	42.6	27.1	32.8	2.3
29.	62.7	67.2	61.4	46.4	58.3	4.4
30.	18	19	21	23	21.0	3.0

TA – Test Angle

T1- T3 – Trial 1 to 3

Aver T – Average Trial angle

Diff TA& Aver T – Difference between Test angle and Average Trial angle

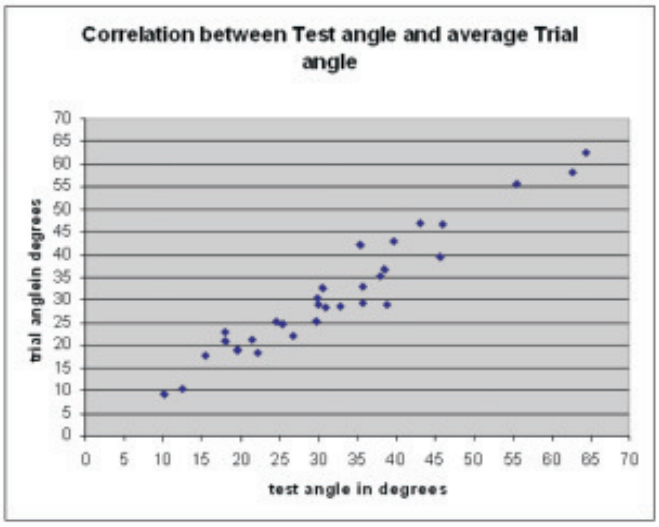
RESULTS

The average of three trial angles was calculated (Table 1). Pearson's Correlation coefficient was calculated for test angles and average of trial angles. Correlation coefficient was $r = 0.9$, which was strongest correlation. p value was calculated for correlation coefficient and found significant at $p < 0.5$ level. Inter observer difference was not calculated as the angle used by each tester was different, hence there will be a difference between the individual tester. If the angle used

was constant then, inter observer difference could have been calculated, but it is not the objective of this study. As this study was aimed to identify whether a tester/examiner doing passive repositioning test will be able to identify the test position used in the following trials, correlation coefficient was used to calculate the relationship between the test angle and trial angle. A scatter plot graph for correlatin of test angle in degrees and trial angle in degrees showed an association (Graph 1). As the test and trial angles were correlated strongly it can be assumed that the test and trials angles or mean of trail angles were similar. This can be inferred that psychomotor skill had minimal influence on the procedure.

DISCUSSION

In our clinical set up proprioception forms an important aspect of the evaluation process. Normal Proprioception is important for well coordinated motor activity and an improved functional performance. The passive repositioning test is commonly used to evaluate Proprioception. This study analyzed the impact of psychomotor skills of the examiner on the results of passive repositioning test. This study found that psychomotor skills of the examiners irrespective of their experience in doing the test did not influence the test. There was a strong correlation between the test value and trial value,



Graph 1: Scatter plot for correlation

inferring the values were similar. Study showed a mean difference of three degrees between test and trial angles. But this difference may not be clinically significant. This is because the electronic goniometer is very sensitive, and this degree of accuracy may not have an impact in the clinical set up. This study also emphasizes the need of using accurate means of measuring changes in angles of repositioning test when used for research purpose. In this study though the experience of the examiners/ participants were documented, they were not correlated with the results, as it is difficult to state that more the experience does mean that their experience in doing the test maneuvers will also be more.

Literature search made with respect to the influence of psychomotor skill of the examiner on passive repositioning test did not retrieve any results. There are studies which deals with consistency of positioning, ie skill of doing the test in terms of handling, recording with instrumentation and their reliability in different joints. A study by Lönn J et al[9] on the inter day reliability of the passive repositioning test revealed that even though with ANOVA no difference was identified in intraday accuracy of measurement, ICC was moderate. Hence, this study suggests the usage of passive repositioning test with caution. However, in this study influence of psychomotor skill of the examiner was not considered.

Future studies may be aimed at different clinical experience level in more structured format. The studies may also be done with electronic goniometer and non electronic goniometer for their accuracy.

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DETECTION OF R127H MUTATION IN CONNEXIN 26 GENE IN CHILDREN WITH HEARING LOSS USING PCR-RFLP METHOD – AN INITIAL REPORT

R. Selvi[a], Rosa Raj[a]

ABSTRACT

Background and Objective: *Congenital hearing loss is estimated to occur in about 1 in 1000 births. Approximately 50% of cases are thought to be due to environmental factors and the reminder due to genetic causes. Mutations in the GJB2 gene encoding connexin 26 protein are a major cause of autosomal recessive non-syndromic hearing loss. R127H is a common GJB2 mutation that involves a G to A substitution at 380bp. The present study is aimed to detect the occurrence of R127H mutation in children with hearing impairment by the PCR-RFLP method.*

Materials and Methods: *PCR-RFLP was carried out in 20 hearing impaired subjects and 6 age matched controls. Genomic DNA was isolated using salting out method and was subsequently screened for the R127H mutation using PCR- RFLP technique and the products were run on 2% agarose gel and the band pattern were analysed.*

Results and conclusion: *The present study assessed the prevalence of R127H mutation in subjects with hearing loss and all the 20 cases were found to be negative and this was established by the presence of two bands corresponding to the 242bp and 104bp regions as seen in the control samples. Although the test results of the present study were found to be negative, hearing loss could be due to the presence of some other mutation in the GJB2 gene. The present study brings out the need to focus in future on the screening of newborn for various mutations in Cx26 gene to identify the carriers and counsel them appropriately to avoid the occurrence of the NSHI in their progeny and such an approach helps in early intervention by way of speech therapy and language development.*

Key Words*: GJB2, R127H, PCR-RFLP, Connexin 26.*

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INTRODUCTION

Hearing impairment is a full or partial decrease in the ability to detect or understand sounds. It is caused by a wide range of biological and environmental factors. Several hundred genes are known to cause hereditary hearing loss and deafness. The human ear can hear frequencies ranging from 20 to 20,000 Hz.[1] Hearing loss can be unilateral or bilateral and it can be categorised as conductive hearing loss and sensorineural hearing loss. Hearing loss is said to be mixed when it is both sensorineural as well as conductive.[2]

The incidence of congenital hearing loss is estimated to be 1 in1000 births, of which approximately equal numbers of cases are attributed to environmental and genetic factors.[3] Of the hearing disorders attributable to genetic causes, ~70% are classified as nonsyndromic and the remaining 30% as syndromic.[4] Nonsyndromic hearing impairment (NSHI) can be associated with abnormalities of the middle ear and/or inner ear. Over 400 genetic syndromes have been described in syndromic hearing impairment.[5]

GJB2 gene is encoding for Gap junction protein, beta 2. It is located on chromosome 13q11 spanning 5.5 kilobases.[6] The GJB2 gene has simple organization of 5427 bases with two exons. Exon 1 is a non – coding region of 160 bases and exon 2 is a coding region of 681 bases. Mutations in the GJB2 gene are pathogenic in both autosomal dominant and autosomal recessive forms of hearing loss.[7] More than 50 GJB2 mutations have been identified and account for as many as 50% of all congenital cases of non – syndromic hearing impairment.[8]

Connexin or gap junction proteins are a family of structurally-related transmembrane proteins that assemble to form vertebrate gap junctions. A remarkable aspect of connexin is that they have a relatively short half life of only few hours and the result is the presence of a dynamic cycle by which connexin are synthesized and replaced.[9,10] It is believed that mutations in the GJB2 gene would lead to complete or partial loss of function of the Cx26 protein, interfering with recycling of potassium ions and thus hampering the normal process of hearing.[11] Hearing loss is one of the frequent sensory defects and the genetic basis of hearing impairment is well established with about 60% of the cases with definite genetic aetiology. Mutations in the GJB2 gene (13q11-q12) is implicated as a major cause for the development of congenital hearing impairment specially the recessive types.[12,13]

In a study conducted by Ramshankar et al., (2003)[14], R127H was found to be the most common polymorphism among the normal subjects and in subjects with hearing loss. They suggested that the substitution of G to A at 380bp could be a polymorphism and not causative for NSHI.[14] Minarik et al., (2003)[15] reported a high frequency of R127H mutation in Slovak Romanies (Gypsies)which may be attributed to their shared origin with the groups of Indian subcontinent.[15] In a study by Padma G et al., R127H was found in homozygous state in four probands (1.3%) and in heterozygous state in 77 probands (25.4%).[16] In the study of Ramchander et al., Cx26 mutations were found in one of the 13 families with inheritance of R127H (G to A at 380bp) and concluded that R127H could be a polymorphism in Indian population.[17]

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From previous literatures high incidence of heterozygosity with R127H is found in both NSHI patients and in controls, indicating that the mutation could be a polymorphism and may not be the cause of NSHI.Thus, it is apparent that the role of R127H mutation in hearing impairment is not clear. Whether R127H allele has any mild effect in the aetiology of hearing impairment or not is still a debatable issue and needs to be investigated. Hence, a study was conducted to detect the occurrence of R127H mutation in the connexin 26 gene in hearing impaired subjects and to identify whether R127H could be a causative mutation for hearing impairment or could be an associated polymorphism.

AIM

(1) To study the role of R127H mutation in the exon 2 of connexin 26 gene in subjects with hearing impairment

(2) To detect the occurrence of R127H mutation in the connexin 26 gene using PCR-RFLP technique.

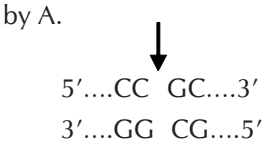
MATERIAL & METHODS

Ethical clearance was obtained from the Committee for Students Proposals at Sri Ramachandra University, in order to proceed with the sample collection. A formalized informed consent form was used to obtain approval from the subject's guardian(s) to proceed with the sampling. Demographic and family information were obtained from the subject's respective guardian(s) and the study enrolled 20 hearing impaired subjects and 6 age matched controls.

The inclusion criteria were subjects with non- syndromic, bilateral, and sensorineural or mixed type of hearing loss. Subjects of both sexes and all age groups were taken into consideration for the study. All subjects included for the study showed mild to profound type of hearing loss. All those patients who were diagnosed with conductive hearing loss and those subjects with a known non – genetic cause underlying the hearing impairment were excluded from the study.

The study enrolled 20 hearing impaired subjects and 6 age matched controls. DNA was isolated from peripheral blood samples according to a standardized salting out procedure.[18] The leucocytes were separated from the red blood cells by the addition of red cell lysis buffer followed by centrifugation to pellet down the intact leucocytes. DNA containing leucocytes were lysed using nucleated lysis buffer and 10% SDS and incubated at 55°C for 20min -2hours to degrade integral proteins and thereby releasing the DNA. The lysate was transferred to an eppendorf tube and 400μl of 5M NaCl was added to the lysate and centrifuged at 10000rpm for 15min at 4°C. To the supernatant double the volume of ethanol was added and the tube was gently inverted until the DNA precipitates out. The DNA was transferred to an eppendorf and 500μl of 70% ethanol was added, centrifuged at 2000g for 5 min at 4°C. The supernatant was discarded and the pellet was air dried, to the pellet 150μl of TE buffer was added and stored in refrigerator at 4°C. Quality and quantity of DNA was checked using agarose gel electrophoresis and nanodrop.

The isolated DNA was then subjected to PCR followed by restriction digestion.[19] A gradient PCR was first performed in order to determine the optimum annealing temperature. After the optimum annealing temperature was identified, a standard PCR was performed. All the reagents were briefly centrifuged in a Spinwin microcentrifuge after thawing. A master mix was prepared using nuclease free water, 10X PCR buffer, dNTPs, Primers, Taq DNA Polymerase and template DNA and it was then aliquoted into individual PCR tubes. The tubes were placed in the thermocycler and on completion, the PCR products were run on a 2% agarose gel and the gel was then visualized using a UV transilluminator in order to check for the amplification of the gene of interest. The PCR products were further subject to restriction digestion using AciI. R127H mutation analysis was done by following PCR/ AciI restriction digestion. The presence of R127H mutation abolishes AciI restriction site at 380bp where G is replaced by A.



A 20 μl enzyme reaction mixture was prepared containing AciI restriction enzyme, 10X assay buffer, Sterile distilled water, Purified PCR product in a microfuge tubes which were placed in a water bath set to 60ºC for one hour for digestion. The digested products were run on a 2% agarose gel in order to observe the band patterns.

RESULT

The present study assessed the prevalence of R127H mutation in subjects with hearing loss by PCR RFLP technique. The DNA was isolated by salting out method; the isolated DNA was run on 0.8% agarose gel to check the quality and nanodrop was used to check the quality and quantity of the DNA. The quantity and quality of the DNA on an average was found to be around 1.8 and the quality of the DNA in 0.8% agarose was found to be satisfactory. The isolated DNA was then subjected to gradient PCR to identify the annealing temperatue and it was found to be 56ºC. The same annealing temperature was adapted to run the standard PCR. Following PCR, the products were subjected to restriction digestion using AciI restriction enzyme. The products of the PCR-RFLP were run on a 2% agarose gel and the band patterns were visualized using the UV transilluminator. All the 20 cases were found to be negative for the R127H mutation. This was established by the presence of two bands corresponding to the 242bp and 104bp regions as seen in the control samples. The presence of R127H abolishes AciI restriction site at 380bp, where G is replaced by A.Normal individuals will have AciI restriction site and hence restriction digestion will generate 242bp and 104bp fragments. Individuals with R127H mutation lack this restriction site and therefore the 346 bp fragment will remain intact. The results of the PCR-RFLP are represented in the Figures: 1, 2 and 3. Figure 1 and 2 illustrate the band patterns

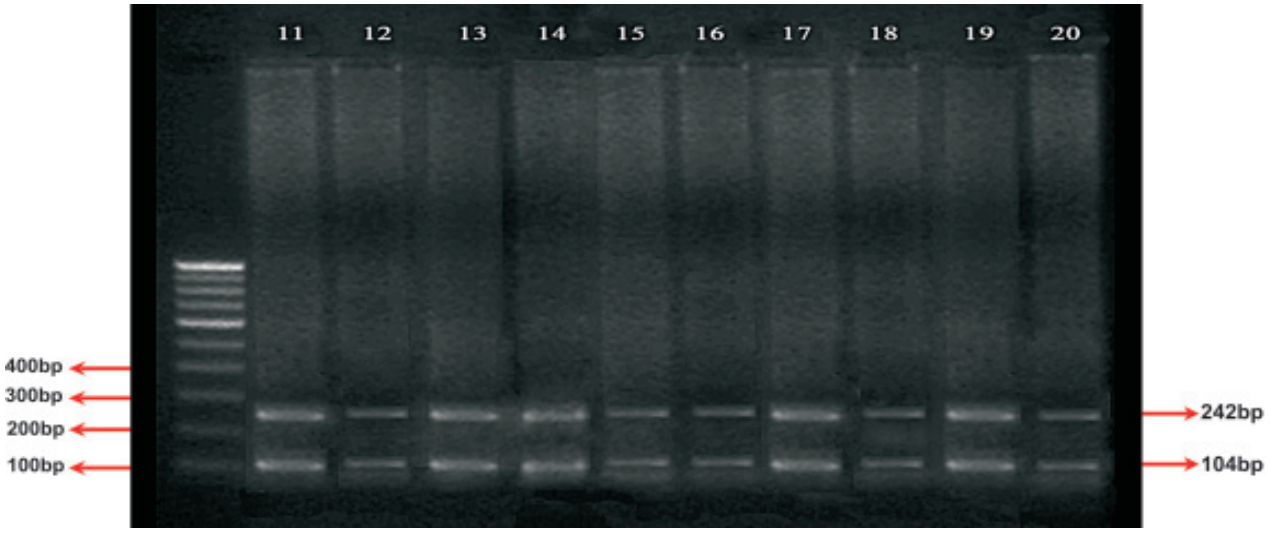


Fig. 1: PCR-RFLP band patterns: Case samples 1 to 10

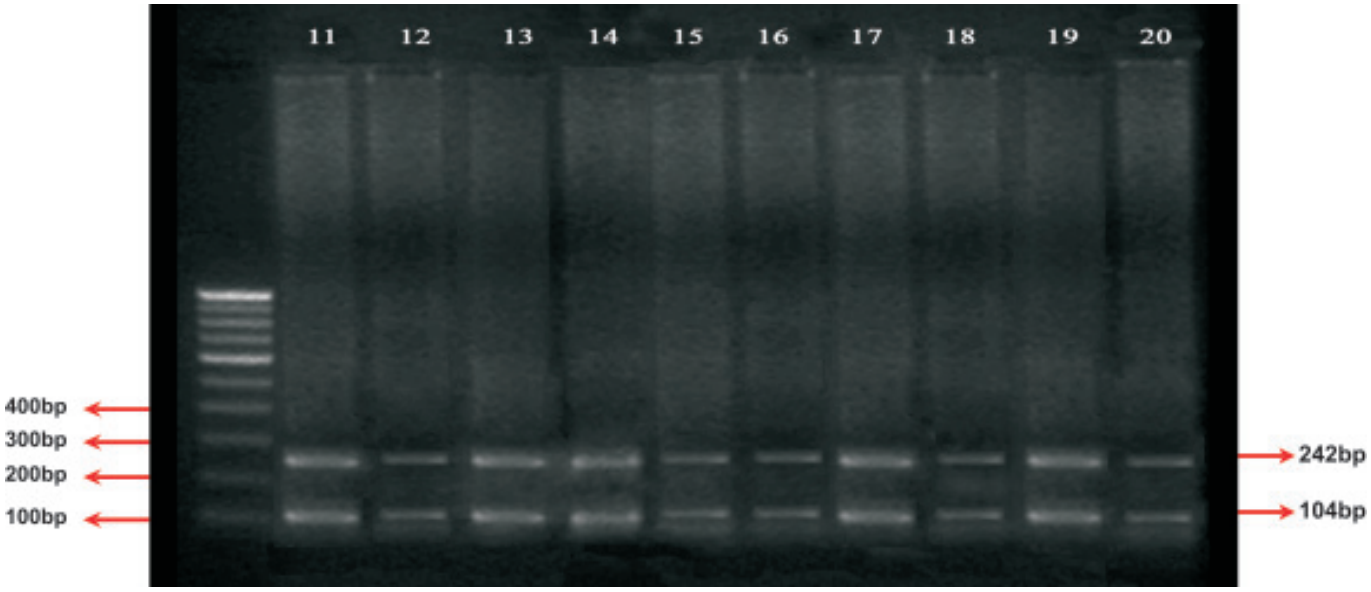


Fig. 2: PCR-RFLP band patterns: case samples 11 to 20

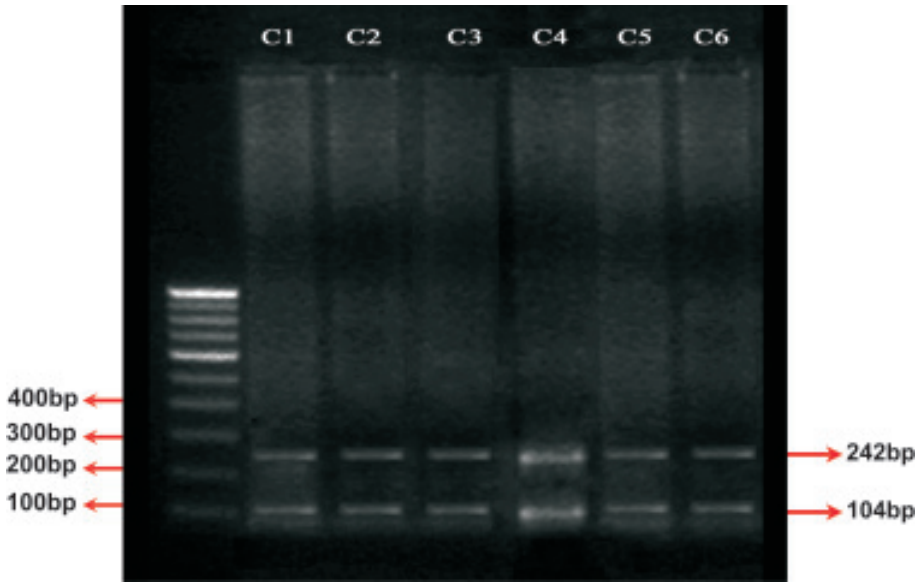


Fig. 3: PCR-RFLP band patterns: control samples C1 to C6

of PCR-RFLP for cases 1-20. Figure 3 represents the PCR- RFLP band patterns of control samples.

DISCUSSION

GJB2 (connexin 26) analysis should be the first step in mutation analysis for non-syndromic sensorineural hearing loss, as it is the most common cause in its category. Hearing loss is one of the frequent sensory defects and about the mechanism of hearing loss, connexin have a relatively short half life of only few hours and it has been suggested that this short life span allows for more finely regulated physiological processes to take place. In the inner ear, connexin 26 is expressed in the supporting cells, stria vascularis, basement membrane, limbus, and spiral prominence of the cochlea. The sensory hair cells of cochlea allow potassium ions to pass through during the mechanosensory transduction process of normal hearing. These potassium ions are recycled across the supporting cells and fibrocytes at the base of hair cells through the gap junctions of the stria vascularis and back to the K+ rich endolymph.

Mutations in the GJB2 gene would lead to complete or partial loss of function of the Cx26 protein, interfering with recycling of potassium ions. Unlike many other genes, GJB2 is small, so screening for mutations is fast and relatively simple. GJB2 mutation analysis has therefore secured a place as a useful tool in clinical practice. So far, many different mutations in the Cx26 gene causing DFNB1 have been identified. Nevertheless, connexin 26 mutation analysis provides a good starting point in the molecular diagnosis of patients with non-syndromic congenital deafness. In the present study, all the 20 cases analyzed were found to be negative for the R127H mutation. However, the results of the present were inconclusive as the study subjects were not from high risk families and sample size were only 20 and ethnic background should also be considered. In a study conducted by Ramshankar et al., R127H was found to be a common polymorphism among the normal subjects and subjects with hearing loss.[14] The reason for this could be attributed to the fact that a very large sample size of 215 hearing impaired subjects was utilized in the study. Another study by Ramchander et al., showed the prevalence of heterozygotes for R127H in probands (28.0% heterozygotes) and as well as in controls (36.5% heterozygotes).[17] However, this study also involved a very large sample size of 200 hearing impaired subjects.

In a study by Ramchander et al., (2004),[20] only probands from high risk families were included for analysis. The same was the case in the study conducted by Ramshankar et al., (2003).[14] Hence, the high frequency of mutation in the previous studies may perhaps be due to the fact that those studies considered extremely high – risk families that included multiple affected individuals in each family. However, in the present study, the number of affected individuals in the family of the patients was much lower, which may perhaps explain the absence of the mutation in the samples. In view of the diversity present in Indian population and reports on the ethnic association of mutations causing NSHI worldwide, it is also important to consider the ethnic background of the affected as it would help in identifying founder population. Cx26 mutations causing hearing impairment are found to be specific to certain populations exhibiting ethnic diversity. Perhaps, it may have some role in instances where it occurs in compound heterozygous state atleast with causative mutations or under special environmental conditions or modifier genes in heterozygous state. The phenotypes of the set of subjects included in the present study varied from those with mild to those with profound type of hearing loss. It remains unclear why mutations in connexin 26 gene lead to different degrees of hearing loss. It has been proven that connexin 26 gene mutations exhibit intrafamilial and interfamilial variability. Different genotypes of connexin 26 mutations have been found in the families of multiple affected members. There are many reports finding no correlation between genotype and phenotype. It has been hypothesized that additional mutations in other interacting genes can influence the extent of hearing impairment. Positive test results are typically highly accurate, although ambiguities may exist in the interpretation of specific or newly recognized mutations. Negative results may not always rule out the diagnosis of a particular disorder or other genetic causes of deafness. Even if the test procedure involves DNA sequencing of coding regions of a deafness gene, regulatory mutations in non – coding regions cannot be ruled out as a potential cause or risk factor.

CONCLUSION

The present study assessed the prevalence of R127H mutation in subjects with hearing loss and all the 20 cases were found to be negative. Although the test results of the present study were found to be negative, hearing loss could be due to the presence of some other mutation in the GJB2 gene. The present study brings out the need to focus in future on the screening of newborn for various mutations in Cx26 gene to identify the carriers and counsel them appropriately to avoid the occurrence of the NSHI in their progeny and such an approach helps in early intervention by way of speech therapy and language development.

By keeping this study as an initial report similar or advanced molecular diagnostic techniques can be applied in screening large samples in subjects from different ethnic background and subjects from high risk families for different mutation in GJB2 gene. There are many benefits of moving from the mere detection of hearing loss to the identification of its cause; such genetic tests would provide a powerful strategy for identifying infants at risk for the development of hearing loss. Once the aetiology of the hearing loss has been identified, patients can benefit from accurate genetic counseling, and in some cases the treatment may be affected, with a substantial possible benefit for development.

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THE MANUSCRIPT REVIEW PROCESS

The manuscript review process is an important aspect of journal publications. Once a manuscript is received at a journal office, it goes through distinct stages of the review process. Broadly, the review process comprises of two main steps, the initial editorial review and then the peer review steps.

The initial editorial review looks for the following in the submitted manuscripts:

* If the content is within the journal scope and areas of interest
* The right manuscript category
* Comprises of all manuscript components-includes cover letter, complete author affiliations, contact details of the corresponding author, etc.
* Adherence to the format requirements
* Author declarations and related clearances if any

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If the manuscript is rejected, the decision is communicated to the author along with a list of reasons for the non-acceptance. Although, for the new authors this decision can be a trifle disappointing, it actually helps in improving the manuscript quality if the authors understand the peer review comments and try to satisfy them before submitting it to any journal in the next instance.

If the peer review process requires authors to revise the manuscript, the decision is communicated along with a list of clarifications/comments/suggestions. Authors are expected to satisfy ALL the peer review clarifications/comments/suggestions and submit a revised manuscript accordingly. A list of corrections and rebuttals with reference to the peer review clarifications/comments/suggestions should be submitted along with the revised manuscript. The manuscript is accepted at this stage provided it has satisfied all the peer review clarifications/comments/suggestions.

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The two-staged manuscript review process ultimately benefits the authors the most, as their published manuscript will be of high standards due to the fine polishing received by the review process. Authors therefore should consider the review process as an important one and accept the review decisions, whatever they might be. Well, the aim of any journal is to publish quality manuscripts for knowledge enhancement and sharing; for them, authors are the most valuable assets.

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Presentation of manuscripts should conform with the uniform requirements for manuscripts submitted to biomedical journals [http://www.icmje.org]. Manuscripts should be submitted as per the instructions given below. Failure to follow these instructions may result in the manuscript being returned to the author(s) for revision. The manuscripts submitted to the Journal is considered not submitted elsewhere nor under consideration for publication in other Journals.

General :

- All contents related to manuscript submission should be in English on a White paper of A4 size with margins of 25mm (1 inch) width on all the four sides.
- Print should be on one-side only with double spacing throughout.
- Pages should be numbered consecutively, beginning with title page.
- Lettering should in Times New Roman with a font size of 12.
- Three paper copies should be submitted to the editor.
- In addition to the paper copies, a digital copy should also be submitted through e-mail or on a compact disc.
- Studies involving human subjects or animals should have received the approval of the institutional ethics committee. A statement about this should be mentioned in the methods of the manuscript.
- Pictures having visible identity of patients should be accompanied by a duly signed patients consent form.

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Authorship credit should be based only on substantial contributions

1) to conception and design or acquisition of data or analysis and interpretation of data;
2) drafting the article or revising it critically for important intellectual content; and
3) final approval of the version to be published.

Conditions 1, 2, and 3 must all be met

GUIDELINES FOR ORIGINAL ARTICLE:

Articles of original research are welcome in this category. Articles should not exceed 4000 words. It must include an abstract of 250 words. Minimum of three MesH words to be mentioned at the bottom of the abstract. Upto 50 references may be included in these articles. The manuscript should be prepared as title page, abstract and keywords, introduction, materials and methods, statistical analysis, results, discussion, acknowledgement , references, tables and figures . Each of the above mentioned should begin in a fresh pag .

I. TITLE PAGE: List

(i) title of the manuscript

(ii) the initials followed by the name of each author and highest academic qualification;

(iii) the name of the department(s) and institution(s) to which authors are affiliated;

(iv) the name, address, contact number and email of the corresponding author to whom queries and proofs should be sent.

The authors are strictly advised not to mention their name and affiliation details in any of the subsequent pages other than the Title page since it may interfere with the review process.

II. ABSTRACT AND KEY WORDS: The second page should carry a structured abstract of not more than 250 words with subheadings of

(i) Background and objectives,

(ii) Methods,

(iii) Results and

(iv) Conclusions .

It should be written for the readership of both clinicians and basic investigators and should state the hypothesis or central question of the study or investigation, the study subjects or experimental animals, observational and analytical methods, the main findings, and a final statement of the principal conclusions. Three to six key words using, where possible terms of medical subjects headings list from Index Medicus [MeSH].

III. INTRODUCTION. It should commence on separate page and should briefly review the current state of knowledge about the topic of the paper. It should also explain clearly the reasons for undertaking the study being reported and what it hoped to achieve. Any mention about the results obtained or conclusions observed should be strictly avoided.

IV. MATERIAL AND METHODS. The material (patients, laboratory tests, experimental animals, etc.) used for making observations must be described along with all other relevant information. The methods used in the study should be described, giving sufficient

information to permit the work to be repeated. If a generally accepted technique has been used, only a reference to that is enough. If, however, such a technique has been modified by the workers, the manner in which this has been done should be clearly stated.

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The relevant statistical methods used for analysis should be briefly explained mentioning the objective of each statistical test in relation to the variables in the reported study that is meaningful. When 'p' value is mentioned the exact number should be mentioned [exception is a highly significant value which may be mentioned as <0.001]. Mention should be made about the predetermined level of 'p' value which will be considered significant. Details of the statistical software used and its version needs mention.

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VI. DISCUSSION. This should be limited to significance of results obtained and what can and what cannot be concluded and why. It should not be a repetition of the findings already given under 'Results'. Results should be discussed in the light of others' work in the field. Speculative and purely theoretical discussion to which results presented are not related will not be accepted.

VII. ACKNOWLEDGEMENTS: Acknowledgement should be brief and made specific for scientific/technical assistance and financial supports in the form of grants/drugs/ equipment only .

VIII. REFERENCES: References should be typed on a separate page after the text and these should be numbered consecutively in the order in which they are first mentioned in the text. In accordance with best practices in scientific writing, latest articles published in relevant area must be referenced. Identify references in text, tables, and legends by Arabic numerals in parentheses.

The titles of journals should be abbreviated according to the style used in Index Medicus. Consult the List of Journals Indexed in Index Medicus. The list can also be obtained through the library's web site (http://www.nlm.nih.gov).. List all the authors when there are six or fewer; but when there are seven or more, list the first six, then 'et al'. Examples of correct form of references are given here:

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Tian D, Araki H, Stahl E, Bergelson J, Kreitman M. Signatureof balancing selection in Arabidopsis. Proc Natl Acad Sci USA. In press 2002.

BOOKS AND OTHER MONOGRAPHS

3. CHAPTER IN A BOOK

Meltzer PS, Kallioniemi A, Trent JM. Chromosome alterations in human solid tumours. In: Vogelstein B, Kinzler KW, editors. The Genetic Basis of Human Cancer. New York: McGraw-Hill. 2002; pp 93-113.

4. CONFERENCE PAPER

Christensen S, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. EuroGP 2002: 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale Ireland.

5. DISSERTATION

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6. ELECTRONIC MATERIAL:

Dengue haemorrhagic fever: diagnosis, treatment, prevention and control. 2nd edition. Geneva : World Health Organization. available at: http://www.who.int/csr/resources/publications/dengue/Denguepublication/en/

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Correctness of the reference list is the entire responsibility of the author (s).

IX. FIGURES AND TABLES

FIGURES.:

(i) Glossy print photographs (in triplicate) are required (usually 10 cm × 8 cm); good contrast is essential for good reproduction.

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(iv) All Figures must be numbered and cited in the text.

(v) Legends should be provided for each figure , listed on a separate page.

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(i) Each table should be typed double-spaced on a separate sheet.

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(iv) Explanatory matter should be in a footnote, not in the title. The symbols *,†,‡,§,||,¶,**,††,‡‡ can be used in the table or its foot note..

GUIDELINES FOR REVIEW ARTICLE:

Articles addressing an theme of current interest is welcome in this category. Articles should not exceed 4000 words. The manuscript should be prepared as title page, abstract and keywords,

introduction followed by discussion, acknowledgement, reference , tables and figures . Each of the above mentioned should begin in a fresh page

I. ABSTRACT AND KEYWORDS:

(i) In an unstructured format not more than 250 words.

(iii) It should describe the background and summary of the discussion related to the topic of interest.

(iii) Minimum of three MesH words to be mentioned at the bottom of the abstract. Upto 50 references may be included in these articles.

II. INTRODUCTION: It should commence on separate page and should briefly explain the reason for the review. This should be a brief overview about what is already known on the topic of the article. This should be followed by a statement on the method of review of literature. A systematic explanation of the methods followed to search the literature on the topic of interest is desirable.

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Title page, acknowledgement, references , tables and figures should be prepared as per instructions already mentioned under guidelines for original article.

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Properly analyzed cases reflecting important clinical problems that contribute to the understanding of pathogenesis, diagnosis and management of a condition are welcome for this section. Manuscripts discussing more than one case will be given preference. The manuscript should not exceed 750 words with no more than 2 tables/ 3 figures and 10 references. The manuscript should be arranged as title page, abstract, Introduction, description of the case and discussion, acknowledgements, references , tables and figures.

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INTRODUCTION: A brief mention about the background literature related to the case discussed. This should focus on epidemiology and clinical relevance of the case.

DESCRIPTION OF THE CASE AND DISCUSSION: The case should be narrated in a simple and logical manner with important observations shown as tables and figures[the latter two should be kept at the end of the manuscript as described earlier]. Discussion should focus on similar or related case reports published in the global literature and important or unusual features in the case described.

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Thoughtful discussions of current topics are welcome in this category . Should be no more than 500-1000 words, no tables or figures and references to a maximum of 10. The manuscript should be prepared as title page, abstract of 150 words with 3 Mesh terms, text of the manuscript which may be self styled followed by references

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GUIDELINES FOR IMAGES IN MEDICINE:

Interesting images which is of practical importance are welcome for this section. It should not exceed 300-500 words.Should be prepared as title page, text which briefly explains the details of the image with a short relevant discussion, images which are appropriately labeled and accompanied by legend and references no more than 5. Images should be as per our instructions mentioned under original article. Abstract is not required.

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Correspondence with comments on a recently published journal article or scientific content not related to a journal article [case report, research protocol, original research,etc] is welcome. Word limit is 300 words and 5 references. Abstract is not required. Title page should be as already described.

The submission should be accompanied by

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Each Manuscript received by the journal is assigned a manuscript number. The author is expected to mention the respective manuscript number in all relevant communications. Editorial decision will be conveyed to the corresponding author within 6 weeks from the date of submission.

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Three Paper copies of the manuscript in the prescribed format should be submitted to Dr.K.V.Somasundaram, Chief Editor – SRJM & Dean of Faculties, Sri Ramachandra University, Porur, Chennai – 600 116

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If hard copies of images are not available then a soft copy is sufficient.

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