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Kamothe, Navi Mumbai, Maharashtra, India

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MGM Institute of Health Sciences (Deemed University u/s 3 of UGC Act 1956) is committed to promote excellence in medical education and research finding better, safer and affordable ways of diagnosing, treating and preventing diseases. With this view in mind, the university has launched a quarterly peer-reviewed scientific journal "MGM Journal of Medical Sciences" in 2014. It publishes original research papers, review articles, interesting case reports, pictorial reviews, letters to editor, book reviews and notices of important professional meetings and conferences. All submitted articles are scrutinized by peer-reviewers and the editorial team, before being published. From time to time, this journal may bring out special issues focusing on specific themes. A section may also be devoted exclusively to young researchers and students to stimulate their innovative and creative ideas.

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From the Editor's Desk

We are pleased to announce the release of third issue, volume 4 of MGM Journal of Medical Sciences for our esteemed readers. Among the 10 papers published in this issue, 3 pertain to the discipline of anesthesiology. World Anesthesia Day is celebrated every year on 16th October, to commemorate all those brilliant physicians and innovators of the past who worked hard to introduce and develop the art and science of anesthesiology. It was on 16 October, 1846 when Dr William TG Morton, a young dentist of Boston administered Ether Anesthesia to a 17-year-old boy who was operated successfully for a vascular tumor in his neck by Dr John C Warren, a reputed surgeon of Massachusetts General Hospital. The surgery was performed first time on this day safely without pain. Over last 171 years, numerous advances and developments have taken place making anesthesia a safe, state-of-art technique that has enabled surgeons to develop their skills and boldly carry out all types of advanced surgeries on every organ of the body safely. Of the 3 anesthesiology articles published in this issue, two are interesting case reports and one original article.

It is our sincere endeavor to maintain highest standards of quality of this publication. We again solicit our esteemed readers, clinicians and medical scientists to submit their papers. Let us disseminate our knowledge and research work for the wider benefit of practitioners and students of health sciences.

Shibban K Kaul MS MCh FIACS
Editor-in-Chief
MGM Journal of Medical Sciences
Pro-Vice Chancellor
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MGM Journal of Medical Sciences

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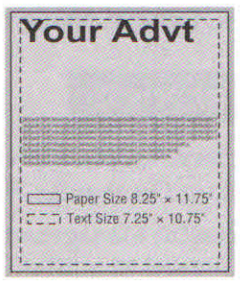

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ORIGINAL ARTICLE

Studies on Single-nucleotide Polymorphisms in the *FUT2* Gene and Their Association with Host Susceptibility to Rotavirus Infection of P[4] and P[8] Genotypes

[1]Kshitija S Rane-Yadav, [2]Divashree Jhurani, [3]Dattatraya S Joshi, [4]Niman C Mohanty, [5]Nitin N Kadam

ABSTRACT

Rotavirus is a segmented double stranded ribonucleic acid virus with typical surface antigens, viral protein 7 and viral protein 4. Almost 5 million children all over world are reported to be infected with this virus. In vitro studies have shown binding of rhesus rotavirus surface protein with 'Sia' groups of a histo blood group antigens (HBGAs). In case of humans, rotavirus type A is more prevalent. The precise genomic diversity of ABO (H) and Lewis Blood Group System in various ethnic populations may provide plausible explanation for prevalence of specific P genotypes. Present study aims to identify role of Single Nucleotide Polymorphisms in the host *FUT-2* gene in the host susceptibility to risk of rotavirus infection of P genotypes.The study indicates that null allele at certain loci of fucosyltransferase-2 (*FUT2*) gene, also known as secretor (Se) gene leads to lack of functionally active enzyme. This results in absence of α-1/2 fucosylated glycan and may protect the child against rotavirus infection of specific strain. *FUT2* gene alleles at loci 428 (AA) and 302 (TT) are found to be associated with group A rotaviruses. Both these alleles were frequent in population under study. Presence of any of these allele in children of Indian origin leads to non-secretor phenotype and hence if exposed to P[4] and P[8] genotypes of rotavirus, can resist the infection.

Keywords: Fucosyltransferase-2, Genetic predisposition, Histo-blood group antigens, Rotavirus, Single-nucleotide polymorphism.

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INTRODUCTION

Genetics of secretor status has attracted considerable attention in recent times as it appears to have significant association with predisposition to many infectious diseases, such as acquired immunodeficiency syndrome, gastric cancer, etc.[1] Identification of secretor status is carried out based on Lewis Blood Group System. In this system, individuals are classified based on expression of Lewis glycoproteins on the surface of RBCs, endothelial tissues, kidney cells, genitourinary and gastrointestinal epithelial cells as well as in body secretions.[2-4] There are two main Lewis HBGAs, Le^a and Le^b, expressing three common phenotypes: Le(a+b–), Le(a–b+), and Le(a–b–).[5] The HBGA can serve as a ligands for pathogen initiating infection via cell attachment.

The major alloantigen system in blood tissue is ABO, but even Lewis antigens have acquired attention in the past few years because of their expression on histological tissue cells and in mucous secretions. The expression of Lewis paratopes on the erythrocytes is dependent on adsorption of secreted Lewis antigen present in serum by RBC membranes. The Lewis blood group antigen system is associated with the ABO system. It depends on two different types of fucosyltransferases. Genes for enzymes are mapped to chromosome 19p13.3 (*FUT3* or Lewis gene) and 19q13.3 (*FUT2* or secretor gene).

The *FUT2* gene encodes for an enzyme α-1,2 fucosyltransferase, which produces type I and 3 H antigens. Another enzyme encoded by gene *FUT3*, α-1,4 fucosyltransferase, regulates fucosylation of H type I antigen substrate to produce Le^a or Le^b antigens[6,7] (Fig. 1). The gene *FUT2* is conserved through evolution and is composed of two exons and one intron. Exon 2 is an ORF for transcription and protein expression.[8] Approximately 20% of Europeans and Africans are found to present with null allele of *FUT2* gene.[9] Any mutation or polymorphism causing disruption of the active site may compromise the expression of fucosyltransferase-2 enzyme. In this case, as there will not be α-1,2 fucosylation, subsequently there will be lack of expression of Lewis antigen.[10] Heterozygous

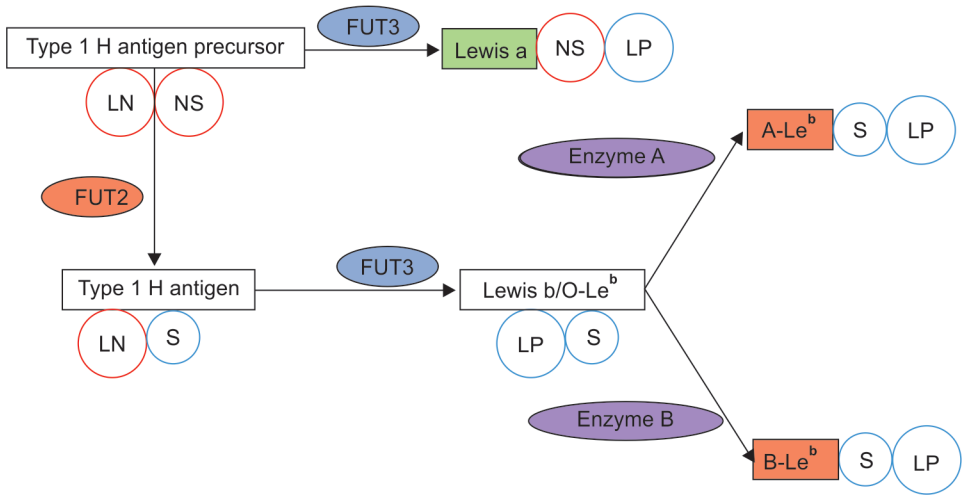


Fig. 1: Synthesis of type I ABO, H, and Lewis blood group antigens. Histo blood group antigens are synthesized by simultaneous addition of monosaccharides (fucose, galactose, *N*-acetyl galactosamine) by three major gene families that code for α-1,2 fucosyltransferase (*FUT2*), α-1,3 or α-1,4 fucosyltransferase (*FUT3*) and A and B glycosyltransferases. When *FUT2* and *FUT3* genes are active, type I precursor prefers accepting fucose by enzyme FUT-2 at α-1,2 position of galactose and later by enzyme *FUT3* at position α-1,3 or α-1,4 (LN: Lewis negative; LP: Lewis positive; NS: Nonsecretor; S: Secretor)

mutation at the allele leads to less expression of active enzyme; hence, partially Le^a is converted to Le^b. About 30 to 40% of Asians typically are found to show such a phenotype. The *FUT3* gene regulates expression of Lewis antigens, but *FUT2* gene regulates secretion of these antigens. Subcellular localization of enzyme is in the Golgi apparatus.[11] In the absence of *FUT3* gene product, the individual is not able to produce any of the Lewis antigens.

In recent times, HBGAs in its various forms are found to be associated with various diseases including viral diarrhea. Diarrhea has consistently been described as the second leading cause of mortality among children under 5 years of age worldwide. The RV, followed by norovirus, is the most lethal agent of acute diarrhea associated with mortality in this age group. Worldwide, nearly 453,000 children die each year due to RV infection, of which about 98,621 die in India each year.[12] These are segmented RNA viruses undergoing genetic reassortment. There are 27 G and 35 P genotypes known so far.[13] The P[4], P[6], and P[8] are major P-genotypes globally. The RV causes acute or sometimes severe diarrhea.

In 2012, two different groups in the Baylor College of Medicine, located in the Texas Medical Center in Houston, Texas, USA, and University of Cincinnati, Ohio, USA, showed that the distal portion of RV VP4 spike protein (VP8*) is implicated in binding to cellular receptors, thereby facilitating viral attachment and entry. A-type HBGA binds to the HR VP8* at the same location as the Sia in the VP8* of animal RV, and suggests how subtle changes within the same structural framework allow for such receptor switching.[14,15] A few studies have been conducted on European, American, African, as well as Asian population.[16-20] Scientists reported that binding to these host ligands is shown to be strain-specific, but no reports have been recorded so far to support involvement of host genetics in this receptor recognition and pathogenesis. The ABO(H) and Lewis blood group system is synthesized by the fucosyltransferase family of enzymes, the gene pool for which is highly polymorphic and ethnicity specific.

It has been reported worldwide that genetic polymorphisms of *FUT-2(Se)* and *FUT-3(Le)* gene determine presence of HBGAs on tissues other than RBCs and body secretions. Particularly secretor and Lewis phenotypes exhibit differences in diverse human populations. Expression of Lewis b (Le^b) antigens which are reported to interact with RV is regulated by *FUT-2* gene. The risk of RV infection thus depends on successful recognition of host ligand by the viral surface protein (VP8). Any change in the ligand could lead to fulfilling of such interaction and subsequent infection. Such change in host ligand could result from the functional deficiency of fucosyltransferase-2 enzyme. This has been recognized in a few recent reports. The polymorphisms in the *FUT2* gene seem to be responsible for induction of these changes and, hence, the present study is focused on finding such SNPs in the population under study. Single-nucleotide polymorphisms data available for Europeans, Caucasians, Americans, or Japanese population cannot be extrapolated for the Indian population as ethnic diversity is larger. To the best of our knowledge, no report has appeared which comments on polymorphisms of the *FUT2* gene and susceptibility to RV in Indian population. The current study is possibly the first of its kind carried out in India.

MATERIALS AND METHODS

This study has been performed at the MGM Institute of Health Sciences, Navi Mumbai, India, from December 2014 to November 2015. It has a well-equipped, state-of-the-art Pediatric Department for diagnosis and treatment of RV infection. Stool sample in sterile container and ethylenediaminetetraacetic acid (EDTA) blood was obtained from the patient on the very first day of admission. The project and the protocol were followed by the MGM Institutional Ethics Review Committee.

Inclusion Criteria

The study included children below 60 months (5 years) of age, having acute diarrhea as defined by the World Health Organization.[21] Informed consent was taken from parents who were willing to participate in the study.

Exclusion Criteria

Any child having bacterial diarrhea, mucus, or blood after clinical and lab evaluation was excluded. Also, the child with small and frequent stools >15 days with or without tenesmus was not considered.

The day of initiation of loose stool was considered. Patients negative for RV in order to show similar symptoms were asked for another fresh sample daily until the symptoms subsided or discharged. The blood sample of the same patient has been used for Lewis typing and extraction of host DNA. Informed consent was duly signed by the guardian or parent of the child patient.

Electropherotyping and RT-PCR from Stool Samples

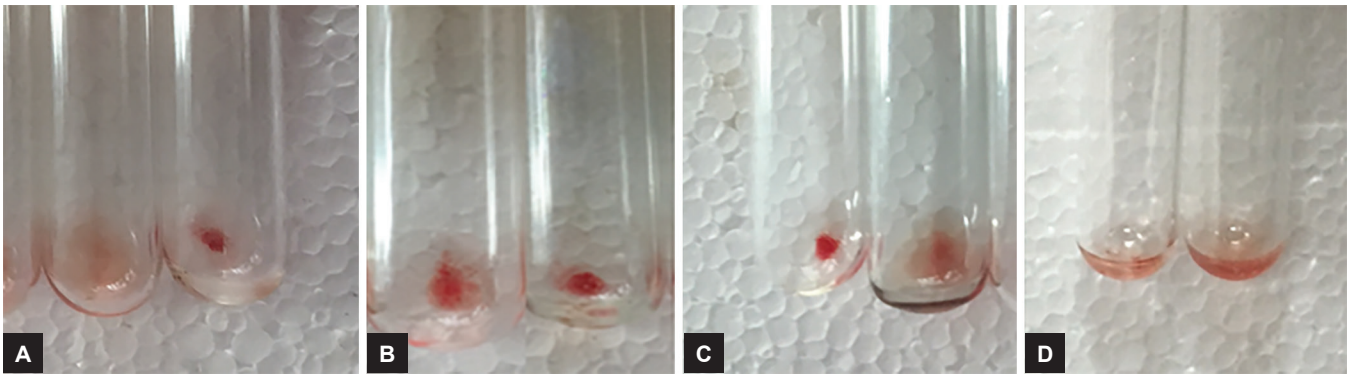
Generally, for screening purposes, the enzyme-linked immunosorbent assay is in use, but as this technique lacks sensitivity, electropherotyping[22] was chosen. About 10% of stool suspension was prepared and viral nucleic acid extracted using QIAamp viral RNA mini kit (Qiagen). Sample was maintained at 4°C during centrifugation and all other steps. Pellets were resuspended in 25 µL diethyl pyrocarbonate water. Sample was stored at –20°C until use. A 7% RNA polyacrylamide gel electrophoresis (PAGE) was used for RNA electrophoresis. A 30% acrylamide (Merck), 10% ammonium per sulfate (HiMedia), and tetramethylethylenediamine (HiMedia) were used as standard protocol.[22] A 1.5M Tris base (HiMedia) at pH 8.8 was used as resolving gel buffer and Tris glycine as tank buffer. The gel was stained with standard protocol of silver staining (Sigma) after methanol fixation and developed by chilled 3% sodium hydroxide (HiMedia) and immediate addition of formaldehyde (Merck) to catalyze reaction.[22] P-genotyping was performed by RT-PCR mentioned elsewhere (Qiagen).[22]

Le^b Phenotyping of RBC by Hemagglutination Test

All acute gastroenteritis cases were typed for Lewis antigens to check for the presence of Le^b on RBCs. This confirms exocrine secretion of ABO antigens as RBCs adsorb this molecule from plasma. Lewis antisera by Immucore Pvt. Ltd. were used for agglutination test. Agglutination with any of the tubes confirms presence of the corresponding antigen; only Le^a positive sample is supposed to be a nonsecretor, Le^b is a secretor, whereas agglutination in both the tubes confirms weak secretor status. Lewis antigen-negative patients were not considered in this study (Fig. 2).

Scoring Diarrhea Symptoms on Vesikari System

In 1990, Ruuska and Vesikari[23] described a numerical scale to assess severity of gastroenteritis based on duration and frequency of diarrhea, vomiting, fever, dehydration, and type of treatment required (Table 1). These have been used



Figs 2A to D: Hemagglutination tube test. Lewis antisera from goat is used to analyze presence of antigen on RBC. Clotting of RBCs can be seen clearly in left tube, whereas tube at right side is negative for hemagglutination. Lewis phenotyping. Antisera for Le^a and Le^b antigens were mixed with phosphate-buffered saline (PBS) washed with 5% suspension of blood cells. Presence of antigen on RBC leads to visible hemagglutination reaction. (A) Le^a − Le^b + secretor positive, (B) Le^a + Le^b + weak secretor, (C) Le^a + Le^b − secretor negative, (D) Le^a − Le^b − Lewis negative

Table 1: Vesikari scoring[23] to determine severity of RV diarrhea

	Observations and scoring		
Parameter	*Score 1*	*Score 2*	*Score 3*
Diarrhea			
Maximum no stools/day	1–3	4–5	≥6
Diarrhea duration (days)	1–4	5	≥6
Maximum no vomiting/day	1	2–14	≥5
Vomiting duration (days)	1	2	≥3
Temperature	37.1–38.4	38.5–38.9	≥39.0
Dehydration	N/A	1–5 %	≥6%
Treatment	Rehydration	Hospitalization	Hospitalization

Table 2: Severity category[23] on basis of Vesikari scoring

Mild	*Moderate*	*Severe*	*Maximum score*
<7	7–10	≥11	20

to grade the severity of rotaviral diarrhea in epidemiological studies in the form of Vesikari score from 0 to 20 and interpreted as mild, moderate, and severe depending on the score (Table 2).

Direct Sequencing of *FUT2* Gene to Monitor SNPs

The partial sequencing of exon 2 of *FUT2* gene was carried out by Sanger's method to identify polymorphisms or mutations, which can be correlated to expression of fucosyltransferase-2 enzyme. The ORF of the gene spanning 1272 bp of exon 2 is amplified with flanking sense and antisense primers given below. Products were checked on agarose gel with DNA marker. Primers[24] (Table 3) used for the reaction were confirmed using the National Centre for Biotechnology Information Primer Blast and were supplied by Sigma.

The PCR products were purified by slicing the band in gel using SV wizard gel purification kit (Promega). Nanodrop reading of purified product was taken. Next protocol was performed at Scigenome labs, Kochi. Around 50 to 70 ng DNA was used with primer 1.5 pmol/µL. About 7 µL of sequencing reaction was set up in thermal cycler containing 5× sequencing buffer (ABI[tm]), purified PCR product, primers, and milliQ water. Postcycling cleanup of extension products was done by BigDyeX terminator kit. Primary data analysis software processes raw sequence data in an *.ab1 file using algorithms. Then KB™ basecaller is used that processes the fluorescence signals followed by Mobility Shift Correction. Electropherograms obtained were checked for signal-to-noise ratio. Good quality results were accepted. Secondary data analysis was performed using FASTA format sequence by multiple sequence alignment with wild-type sequence using basic local alignment search tool. Every electropherogram was assessed manually for overlapping peaks at any base call, conclusive of heterozygous allele.

Table 3: Primers used for amplification of *FUT2* gene for sequencing. These are flanking sequences of exon 2 which is ORF

Primer	*Sequence (5'→3')*
FUT F	TGCCAAGTATTTACACACCTGAAG
FUT R	GATTTCTGTTACTTGCAGCCCA

RESULTS

P[4] and P[8] Genotypes were Prevalent in Study Population

A total of 150 samples were collected from the pediatric ward and outpatient department of MGM Hospital, Navi Mumbai, India, from Nov 2014 to Dec 2015. A total of 17 samples (11.33%) were found to be positive for RV RNA. Short and long electropherotypic strains were observed (Fig. 3). All genotypes were of the P[4] (n = 2, 11.76%) or P[8] (n = 15, 88.24%) type.

Expression of Le^b in Children causes Severe Diarrhea and is significantly associated with RV Infection

A total of 200 samples (100 healthy controls and 100 diarrhea patients) were screened by Lewis hemagglutination test to determine secretor phenotype using Le^a and Le^b antisera. About 11 diarrhea samples could not be typed as samples were hemolyzed. About 89 were analyzed, which included 17 rota-positive and 72 rota-negative cases. Vesikari score for each case was calculated, and for the purpose of comparison

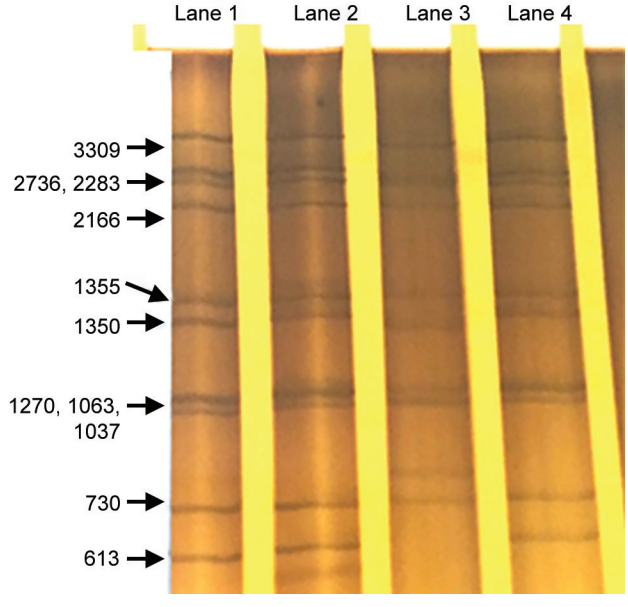
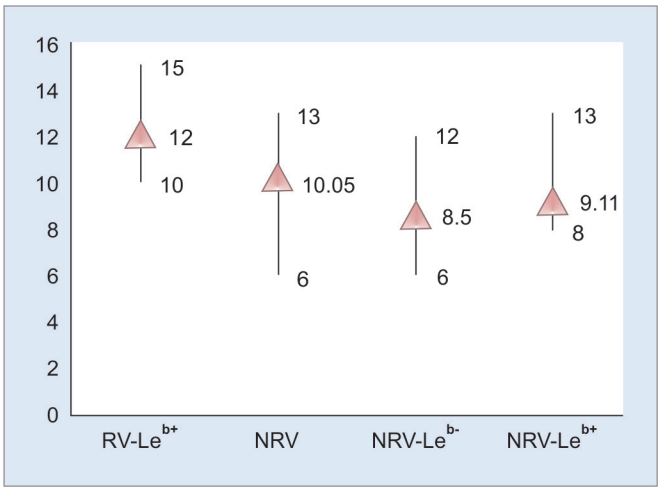
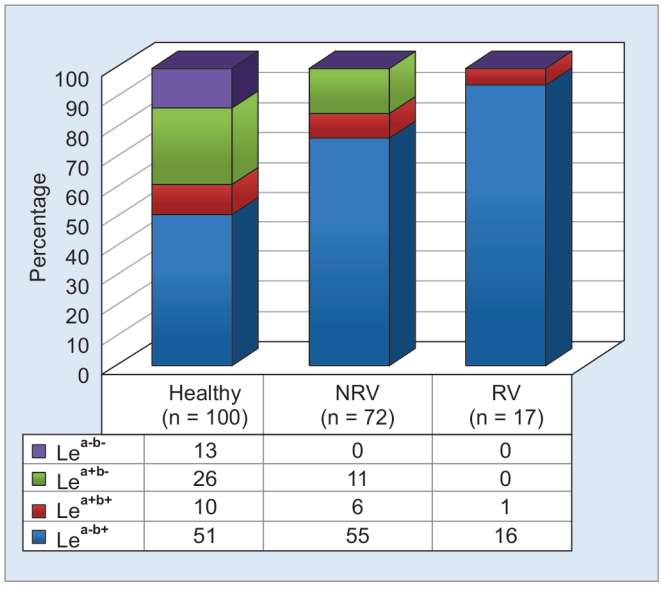


Fig. 3: Electropherotyping. RNA extracts from pediatric stool samples analyzed by electropherotyping RNA PAGE gel of selective RV-positive cases stained by silver nitrate showing three long (lane 1, 2 and 4) and one short (lane 3) phenotype



Graph 1: Disease severity according to Vesikari score. Lewis b positive from RV (RV Le B pos) infected and noninfecetd (NRV Le B pos) shows significant difference in severity as per Vesikari scoring system. Rotavirus-positive cases, all secretors had altogether severe (mean = 12) diarrhea. NRV cases had moderate (mean = 10.05) diarrhea. Nonsecretors had milder symptoms (MVS = 8.5) compared with secretors (MVS = 9.11)



Graph 2: Distribution of Le^a and Le^b antigen. Phenotypic status among 172 individuals divided into three groups of healthy individuals and diarrhea patients positive and negative for RV infection, Navi Mumbai, Maharashtra, India; December 2014 to November 2015. Frequency of nonsecretors and Lewis-negative phenotypes is higher among healthy cohort and NRV cases

between various groups, MVS was calculated (Graph 1). Diarrhea in case of RV infection was severe (MVS = 12.66) and all cases (n = 17) were Le^b hemagglutination positive. Non-RV diarrhea cases (n = 72) were moderate (MVS = 10.05), perhaps for Le^b-positive cases (n = 11) and was milder (MVS = 8.50) than secretors (n = 55) (MVS = 9.11).

Observed frequency of nonsecretors and weak secretors in healthy *vs* infected individuals, when plotted, showed significant difference in expression of Le^b antigen (Graph 2). None of the RV-positive samples were found to lack α-1/2 fucosylated glycan expression. Single sample showed weak expression of Le^b. Frequency of weak secretors and nonsecretors was found to be high in healthy population as well as in cases with NRV diarrhea. The RV has been found to cause diarrhea in secretors or weak secretor cases.

SNPs in *FUT2* Gene, G428A, and C302T associated with the Risk of P[4] and P[8] RV

The partial sequencing of the gene was carried out to monitor various polymorphisms in *FUT2*, which might be important for expression. The *FUT2* gene is 9980 bp (48695971 to 48705950 from pter) composed of two exons, 118 bp and 2995 bp. First exon is the untranslated region and the second exon codes for fucosyltransferase-2 enzyme, 343 amino acids. Using a pair of primers flanking the ORF, target region of 1272 bp was amplified (Fig. 4). Bidirectional Sanger's sequencing of target region using the same primers was carried out (SciGenome Labs Private Limited, Kakkanad, Kerala,

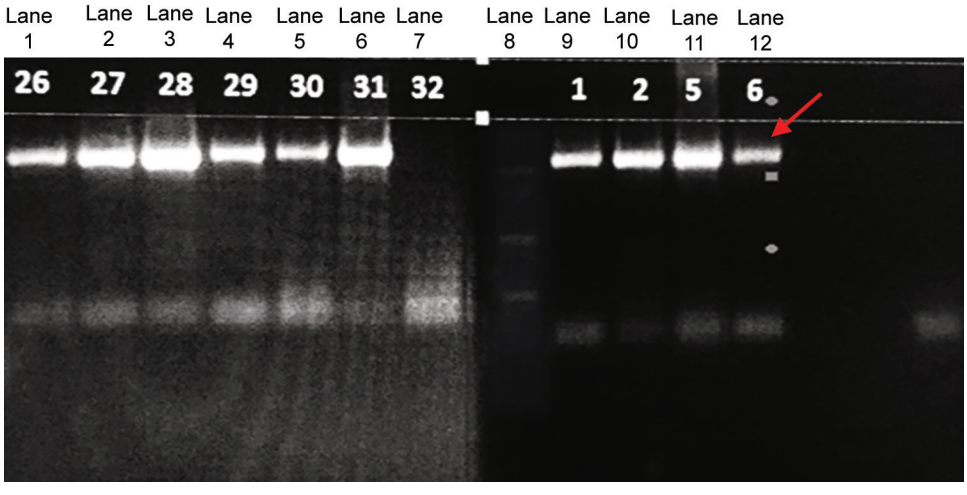


Fig. 4: A 1.5% agarose gel showing 1272 bp amplified PCR product of *FUT2* gene. Lanes 1 to 7 and lane 9 to 12 are samples from various study groups. Lane 8: 100 bp ladder

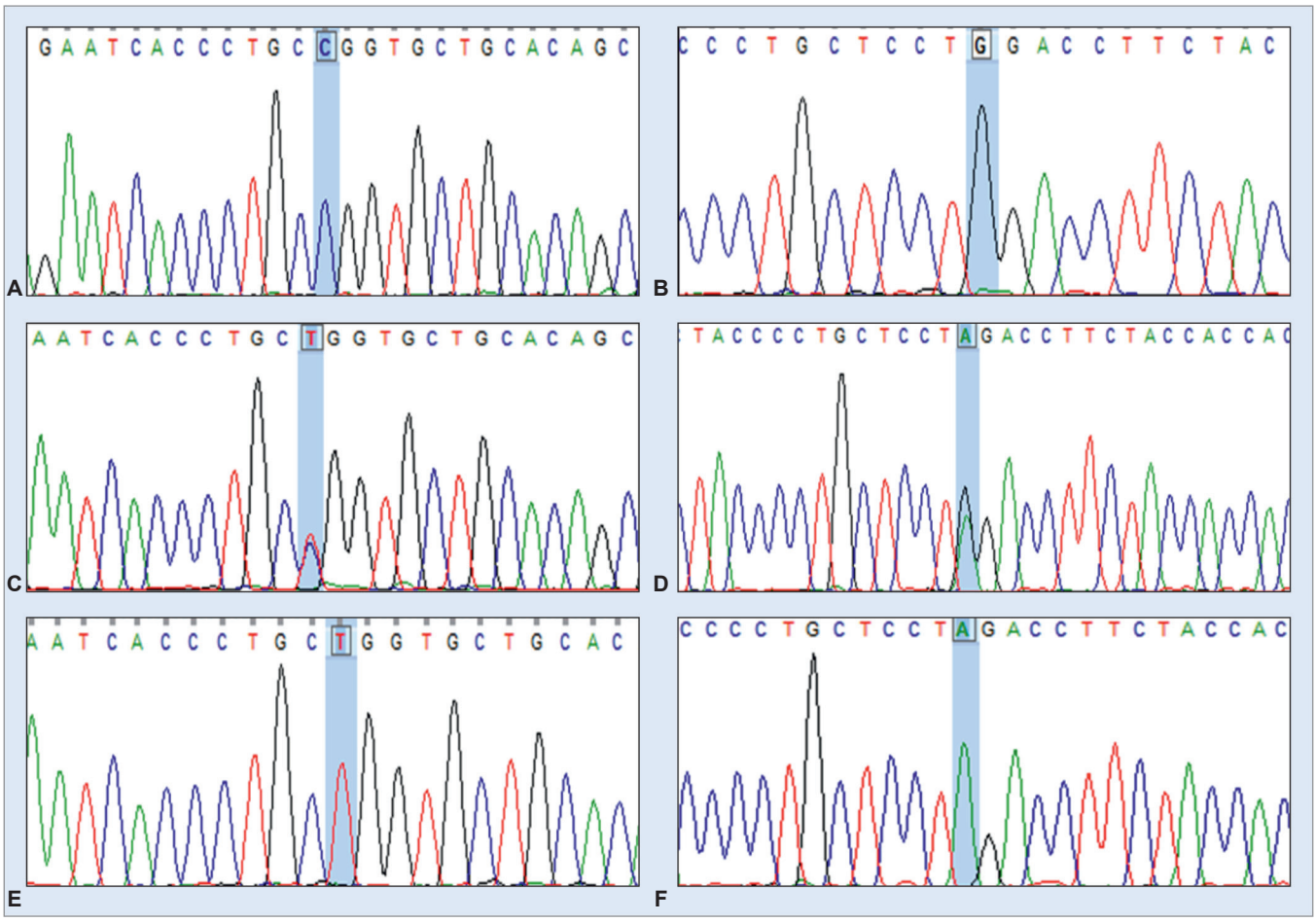
Table 4: DNA sequencing of 36 samples for *FUT2* gene exon 2 has indentified SNPs prevalent in cohort

Sl. no	*Allele*	*Reading frame*	*Amino acid*	*H-NS n = 10*	*NRV n = 14*	*RV n = 12*
1	171 A→G	GCA→GCG	No change	0 AG	7 AG	3 AG
				5 GG	1 GG	0 GG
2	216 C→T	TAC→TAT	No change	0 CT	6 CT	2 CT
				5 TT	2 TT	1 TT
3	302 C→T	CCG-CTG	P→L	0 CT	6 CT	4 CT
				2 TT	2 TT	0 TT
4	357 C→T	AAC→AAT	No change	0 CT	6 CT	6 CT
				2 TT	1 TT	3 TT
5	428 G→A	TGG→TAG	W→X	0 GA	7 GA	3 GA
				5 AA	1 AA	0 AA
6	739 G→A	GGT→AGT	G→S	1 GA	3 GA	2 GA
				4 AA	0 AA	0 AA

Data show presence (+) or absence (–) of homozygous SNPs in healthy nonsecretors (n = 10), NRV (n = 14), and RV (n = 12). Number of individuals with homozygous or heterozygous loci has been mentioned in each column. No mutant homogyzote for loci 302, 428, and 739 was found in RV-positive group (n = 12). Mutant allele at loci 739 is not restricted to RV infection and NRV group resembles same trend

India). A 1272 bp target region was performed. Totally, 36 samples were sequenced for *FUT2* gene flanking ORF, viz., 12 RV-positive (9 secretor + 3 weak secretor), 14 RV-negative (3 secretor + 3 weak secretor + 8 nonsecretor), and 10 healthy nonsecretors. All stool samples from the RV-infected patients were found to belong to Le^b-positive antigen type (secretor/weak secretor). The SNPs observed in nonsecretors are listed in Table 4. The 428 G→A and 302 C→T are critical as they are associated with compromised fucosyltransferase-2 activity.

Homozygosity at any of these two loci, 428A and/or 302T (Graph 3), results in no expression of fucosyltransfer-



Graph 3A to F: Electropherograms of 302 bp and 428 bp. Samples of secretors, weak secretors, and nonsecretors were processed for sequencing. Partial electropherograms of *FUT2* gene showing loci 302 bp and 428 bp in heterozygous and homozygous (wild type and mutant) forms. Shaded region indicates position of SNP 302/428. (A) 302 C/C (homozygous wild type allele observed in all groups); (B) 302 C/T (heterozygous allele observed in all groups); (C) 302 T/T (homozygous mutant allele restricted to nonsecretors); (D) 428 G/G (homozygous wild type allele observed in all groups); (E) 428 G/A (heterozygous allele observed in all groups); and (F) 428 A/A (homozygous mutant allele restricted to nonsecretors)

ase enzyme and, hence, lack of expression of Le^b antigen in secretion on RBCs. Sequence analysis of *FUT-2* gene in patients with positive RV infection showed concordant results with hemagglutination test based on Lewis b antisera. None of the patients with RV infection showed presence of null allele in *FUT2* gene ORF. Four weak secretor patients with RV infection were heterozygous at position 428 bp (n = 3) or at 302 bp (n=1) of the *FUT2* gene. This correlates with low expression of Le^b on the RBCs, Le^{a+}/Le^{b+}.

Statistical Analysis

Nonsecretor phenotype was not found to be associated with any of the RV-positive cases. About 16 (92.30%) were SE/SE homozygous and 1 (7.69%) was SE/se heterozygous. By contrast, there were 16 (16%) confirmed nonsecretors among the 100 individuals of our control healthy adult population. Distributions of the genotypes between the controls and rota-positive cases were significantly different (p-value = 0.04). In the other control group including U5C with gastroenteritis of unknown etiology (non-RVA), 55 (76.38%) SE/SE ($Le^{b+/+}$), 6 (8.33%) SE/se ($Le^{b+/-}$), and 11 (15.27%) se/se ($Le^{b-/-}$) were found. No significant difference was found in healthy and NRV group with p-value 0.24. The absence of null allele among patients compared with either the healthy adult control group or the NRV control group was significant, indicating that nonsecretors are resistant to symptomatic infection by RV, but they can be susceptible to other viruses causing diarrhea.

Presence of null allele at loci 302 (302 CT/TT) and 428 (428 GA/AA) was found to be significantly associated with nonsecretors. The RV-infected cases in the present study were associated with wild type alleles at nucleotide positions 302 bp and 428 bp (Table 5). Counts of heterozygotes and homozygous mutants in each group were statistically evaluated by Fischer's exact test (level of significance = 0.1) for probability calculation (2 × 2 contingency table). There was significant difference between nonsecretor cohort and RV-infected test cohort in context with nucleotide 302 bp and 428 bp of ORF at exon 2 of *FUT2* gene.

Table 5: Probability value calculation using Fisher exact test for 2 × 2 contingency table (level of significance 0.1)

	Healthy NS n = 10	*RV n = 12*
AA-*FUT2*$^{428-/-}$	5	0
GA-*FUT2*$^{428-/+}$	0	3
p-value	0.018 S	
TT-*FUT2*$^{302-/-}$	2	0
CT-*FUT2*$^{302-/+}$	0	4
p-value	0.067 S	

Significant difference was found in allelic counts for homozygous and heterozygous mutation at 302 nt and 428 nt of *FUT2* gene between two groups of nonsecretors and RV. It proves genetic difference in two groups

DISCUSSION

In cases of viral infection, host susceptibility is dependent on the successful recognition of host receptor by the ligand on the pathogen. Risk of infection, thus, could genetically predispose the host to infection from that specific pathogen. Any structural alteration mediated through mutations or polymorphisms at critical sites in the respective gene (of the host or pathogen) could result in failure of recognition of the host, leading to the development of resistance to that infection. As Collins[25] states, "most misspelled genes cause a predisposition and not a predetermination of a disease"; such misspelling could occur due to mutations or polymorphisms in the respective genes of the host or the pathogen. Various loci in the human genome have been mapped, which predispose individuals to infectious diseases, such as malaria, tuberculosis, human immunodeficiency virus, etc.[26] In recent years, there is considerable interest in the role of the secretor status and Le^b blood group antigens of the individual that serve as attachment targets for pathogens.[27] It was, therefore, of interest to study if susceptibility or resistance to RV infection is associated with secretor or nonsecretor status. The study focused on examining, such association, if any, for RV.

About 150 cases of diarrhea were screened for infection to RV, of which 17 found to be positive. P-genotyping data indicated that P[4] and P[8] are predominant in Navi Mumbai region. Studies undertaken in various parts of India showed similar results.[28,29] A few cases of P[6] have been reported in this region unlike the African population.[17] About 172 EDTA blood samples were tested for Le^b antisera-based hemagglutination test, of which 100 were from random healthy individuals. The results could be confirmed for 90 samples, viz., 66 secretors (Le^{a-} Le^{b+}), 16 nonsecretors (Le^{a+} Le^{b-}), and 8 weak secretors (Le^{a+} Le^{b+}). About 10 samples were Lewis negative (Le^{a-} Le^{b-}); hence, they were inconclusive. No reports are available in India for viral diarrhea cases and their association with RV infection. Parallel blood examination of viral diarrhea cases was carried out for 89 cases. All 17 RV-positive cases were secretors, one of which was weak secretor (p-value = 0.04) (Graph 2). Another 72 NRV cases had frequencies similar to control cases with minor deviation in frequency of nonsecretors and Lewis negative (p-value = 0.24). Attachment of RV to glycan has been previously reported by few groups considering murine, bovine, or other animal strains.[15,16,19] There are some reports suggesting that RV VP8 surface protein interacts with Lewis antigen, Le^b specifically, which is adsorbed on mucosal epithelial cells, such as enterocytes of secretor or weak secretor individuals. There are reports which indicate that human RVs recognize HBGA.[30-32] Thus, presence of HBGA on human cells could predispose the host to

RV infection. The HBGA are secretory ABO(H) antigens, which classify the population into two groups—secretors and nonsecretors. Secretor shows presence of ABO antigens in saliva, tears, mucosa as well as on the surface of epithelial tissue cells, whereas nonsecretors do not. About 20% of Caucasian population was found to be nonsecretors.[33] In India, various studies have given frequencies of nonsecretors at around 70 to 100%.[34,35] Surprisingly, of the remaining population, not all are found to be secretors, but some of them can be clubbed together to form another classification of weak secretors.[36] The larger part of Asian population reportedly show weak activity of fucosyltransferase-2 enzyme; hence, the marker Le^b shows "dim" expression. The population is defined by term weak secretor and at genome level, they are heterozygous for null allele.[37]

A major part of blood group systems is generated by a family of homological proteins (enzymes) fucosyltransferases. Secretory or nonsecretory nature of these ABO(H) antigens depends on functional status of some of these enzymes. Specific enzyme fucosyltransferase-2 coded by gene *FUT-2* or *Se* plays a major role by adding α-1→2 fucose to precursor molecule. Another enzyme fucosyltransferase-3 coded by gene *FUT-3* or *Le* regulates expression of Lewis antigens by α-1→3 fucosylation. Though both enzymes are critical for Lewis blood group system, only *FUT2* is associated with expression of Le^b antigens on epithelial cells. Role of *FUT3* is required to determine secretor status of Lewis-negative (Le^{a-}/Le^b) individuals. Secretor individual expresses fucosyltransferase-2 enzyme; hence, they show presence of Le^b antigen in saliva, tears, mucosa as well as on the surface of epithelial tissue cells. Any such mutation or variant polymorphism in *FUT-2,* which can change the active site or translation of inactive protein, ceases α-1→2 fucosylation. These individuals are nonsecretors due to absence of ABO antigens in secretions, on epithelial cells, and even on RBCs. Vesikari scoring for study groups showed that RV diarrhea was more severe (MVS = 12) in the maximum number of cases compared with NRV diarrhea (MVS = 10.05). The interesting trend was observed that among the NRV cases, individuals with secretor status (MVS = 9.11) had comparatively severe diarrhea symptoms than nonsecretors (MVS = 8.5). This indicates that other pathogens might involve Lewis antigen although to a lower extent compared with the RV. Association of HBGA with norovirus has been previously reported. It is required to perform broader studies in association with HBGA to target pediatric viral diarrhea in terms of vaccines and therapy.

There are 55 known polymorphisms of *FUT2* gene as per a recent study.[38] Some of them are nonsynonymous and, if homozygous, can confer nonsecretor status to the individual. Present study elucidates prevalence of six SNPs—171 bp, 216 bp, 302 bp, 357 bp, 428 bp, and 739 bp—for population in this region. There is a single study from France[18] carried out in year 2016, which has stated the association of *FUT2* polymorphism with resistance to RV infection. The group has performed restriction fragment length polymorphism assay to determine alleles GG/GA/AA at loci 428.[27] The ORF of *FUT2* gene was scanned to elucidate various other polymorphisms and mutations, which could be associated with RV pathogenesis. We have found G428A polymorphism in 3 out of 17 RV-positive samples. All three were heterozygous mutations Se/se^{428} and have shown weak secretor status (Le^{a+} Le^{b+}) by RBC hemagglutination test. The NRV cases showed higher frequency (8 out of 14) for presence of this polymorphic locus and a single sample was heterozygous. The rest all were homozygous mutants (se^{428}/se^{428}). The study reports another SNP C302T, which could show possible predisposition to RV P[4], P[8] for children of Indian origin. Nonsecretors from healthy as well as NRV group showed se^{302}/se^{302} allele, whereas RV-positive cases were heterozygous mutant Se/se^{302}. This leads to a change in codon (CCG→CTG). Subsequently at position 101, proline is replaced by leucine. There could be possibility that conformational changes happened in the enzyme making the active site incompatible for α-1/2 fucosylation of H precursor antigen. The se^{302} allele was reported to be restricted to Thai and Bangladeshi population.[39] We report its presence in Indian population as well. Frequency of 302T and 428A allele was found the lowest among RV (RV-positive) group (Table 6). The prevalence of these mutant alleles was predominant in NRV (N RV) cases but compared with nonsecretor cases, RV cases showed almost similar (302T—16.66 *vs.* 20%) or even less prevalence (428A—20.83% *vs.* 50%) for homozygous mutant alleles for both these loci, viz., the se^{302}/se^{302} and se^{428}/se^{428} were restricted only to nonsecretors and not observed in any case of RV-positive patient. Association of G428A and C302T with RV infection is statistically significant (p-value = 0.018 and 0.067 respectively, level of confidence = 0.1) (Table 5).

The strains circulating in Europe, America, and other White populations are different compared with India. The reason for this can be ethnicity-based host genetics.

Table 6: Distribution of alleles 302 and 428 among NRV and RV group compared with nonsecretors

	NS n = 10	*NRV n = 14*	*RV n = 12*
Allele	*Chromosome no = 20*	*Chromosome no = 28*	*Chromosome no = 24*
302 C	16 (80%)	18 (64.28%)	20 (83.33%)
302 T	4 (20%)	10 (35.71%)	4 (16.66%)
428 G	10 (50%)	19 (67.85%)	19 (79.16%)
428 A	10 (50%)	9 (32.14%)	5 (20.83%)

The present study gives plausible explanation based on host genetics for discrepant vaccine efficacy in different continent and suggests that specific population can be genetically predisposed precisely to specific strains of RV based on fucosyltransferase-2 gene.

CONCLUSION

The study shows that the risk for infection from RV P[4] and P[8] genotypes seems to be associated with secretor or nonsecretor status of the individual. The *FUT2* gene and its polymorphisms play an important role in the determination of secretor status. The *FUT2* gene alleles are at loci 428 bp and 302 bp and are found to be associated with group A RVs . Presence of 428 (AA) and 302 (TT) allele in children of Indian origin leads to nonsecretor phenotype. Both these alleles were less frequent (<20%) in the population under study. Individuals with secretor status seem to have higher risk for infection from P[4] and P[8] RVs. Our studies also indicate that alteration at 428 bp or 302 bp polymorphisms could confer resistance or low risk from P[4] and P[8] RV infection.

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ORIGINAL ARTICLE

Choledochoduodenostomy in the Present Era: Specific Indications and Outcomes

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ABSTRACT

Aim: To determine the specific indications and outcomes of patients undergoing choledochoduodenostomy (CDD) in the current era of endoscopy and interventional radiology.

Materials and methods: This retrospective study was conducted at Dr. Ram Manohar Lohia Institute of Medical Sciences, Lucknow, India, over a period of 6 years from January 2011 to December 2016. Twenty-six patients who underwent CDD within this period were evaluated. Preoperatively, all the patients underwent abdominal ultrasonography and magnetic resonance cholangiopancreatography along with routine blood investigations and were evaluated for medical comorbidities. The patients were followed up for a variable period of 6 months to 4 years.

Results: In our study, the indications for CDD included large impacted stones; choledocholithiasis with stricture; recurrent stones; and common bile duct stone with stricture and cholecystoduodenal fistula. The mean of length of hospital stay was 9.2 days. The overall morbidity was 30% without any mortality. There were no cases of residual stones, bile leak, or hemorrhage; and none developed alkaline gastritis, sump syndrome, or cholangitis in the follow-up period.

Conclusion: Choledochoduodenostomy has a definite role in the management of bile duct stones, especially in benign biliary tract obstruction. The proportion of cases requiring this approach is diminishing because of nonoperative techniques available, but it will not be eliminated by them based on current trends.

Keywords: Biliary calculi, Cholangitis, Choledochoduodenostomy, Sump syndrome.

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INTRODUCTION

In this present era of endoscopy and interventional radiology, the management of benign biliary diseases is predominantly nonsurgical. Choledochoduodenostomy (CDD), which is described as an anastomosis between the lower end of the common bile duct (CBD) and the duodenum, has limited but specific indications. This procedure had been described long back but the indications have remained the same over the years.[1] Although done infrequently, this technique has been standardized and has yielded good results. Numerous complications specific to the procedure have been described classically including ascending cholangitis, alkaline reflux gastritis, and sump syndrome, which may be the reason of this procedure being performed less frequently over the years. We reviewed our indications and outcomes of the procedure over a period of 6 years and highlight the results with a review of literature.

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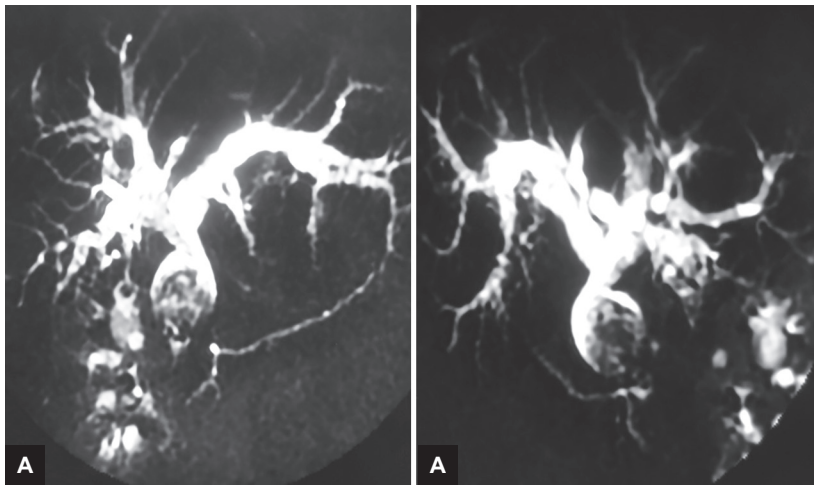
MATERIALS AND METHODS

This study was conducted at Dr. Ram Manohar Lohia Institute of Medical Sciences, Lucknow, India, which is a tertiary care referral hospital, over a period of 6 years from January 2011 to December 2016. The study design is a retrospective study in which 26 patients who underwent CDD within this period were evaluated by going through their hospital records and through telephonic interviews. Eight of these were male patients and 18 were female patients. The average age of the patients was above 60 years and the youngest patient was 43 years. All the patients underwent routine blood investigations like hemogram, renal function tests, liver function tests, and coagulation profile and specific investigations for other medical comorbidities like diabetes, hypertension, dyslipidemia, and thyroid function abnormalities in the preoperative period. All our patients had one or more medical comorbidities like diabetes, hypertension, coronary artery disease, chronic airway disease, etc., and were evaluated and deemed fit for surgery by specific physicians. All the patients had an abdominal ultrasonography and magnetic resonance cholangiopancreatography (MRCP) prior to surgery. In a few cases, MRCP was done after a failed endoscopic retrograde cholangiopancreatography (ERCP) (Figs 1A and B).

Surgical Technique

The technique of CDD begins with an initial assessment of the abdomen. The gallbladder is taken down from liver by



Figs 1A and B: Patient with MRCP following failed ERC clearance, showing bilobar intrahepatic biliary radical dilatation with dilated CBD showing a calculus within its lumen along with CBD stent *in situ*

a fundus-first method. The CBD is identified and exposed by opening the overlying peritoneum, more so at the lower end (Fig. 2). Complete kocherization of the duodenum is then performed. Lower end of the CBD is confirmed by needle aspiration of bile and stay sutures are placed using Vicryl 3-0. A vertical incision of approximately 1 cm is given at the lower end of the CBD and any stones or stent are extracted from the CBD. An intraoperative video choledochoscopy was done in each case after complete clearance of CBD identifying the proximal and distal portions. A transverse incision of 2 cm length is made over the anterior wall of the first part of the duodenum. A single-layer anastomosis is established using Vicryl 3-0 interrupted sutures keeping the stoma size around 2 cm (Fig. 3). The procedure is then completed by placing a closed-suction Jackson–Pratt drain near the anastomosis as per protocol.

Follow-up

All 26 patients were followed up for a variable period ranging from 6 months to 4 years. None of the patients were lost to follow-up. During the follow-up visits, all of them underwent a liver function test, abdominal ultrasonography, and a hepatobiliary nuclear scan, in case desired, apart from a detailed history and clinical examination. The details and outcome of the follow-up visits were systematically recorded and analyzed.

RESULTS

In our study, the indications for CDD included large impacted stones after a failed endoscopic retrograde cholangiography (ERC) clearance; choledocholithiasis with stricture; recurrent stones; and CBD stone with stricture and cholecystoduodenal fistula. The indications for CDD and their distribution among male and female patients are summarized in Table 1.

The size of CBD varied from 1.5 to 2.5 cm. The mean length of hospital stay was 9.2 days. The only postoperative complications were wound infection in four cases (15.4%), postoperative fever in one case (3.8%), and pneumonia in three cases (11.6%). No cases of sump syndrome

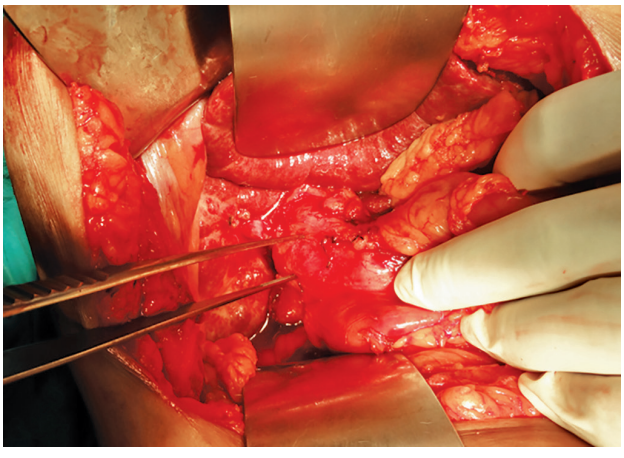


Fig. 2: Intraoperative view of the same patient with a dilated CBD 2 cm in diameter with an impacted stone

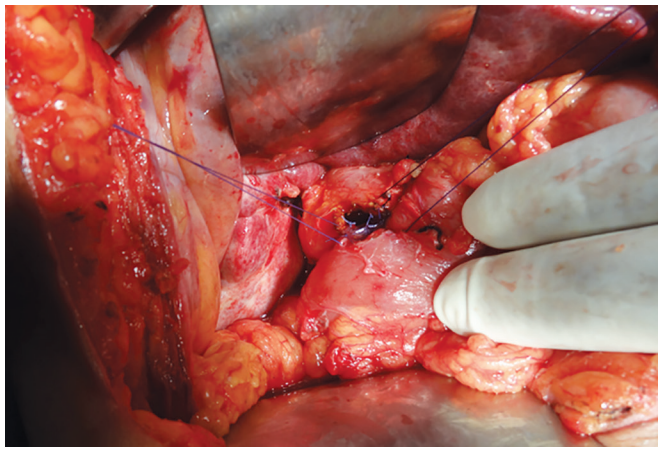


Fig. 3: Choledochoduodenostomy being performed using interrupted 3-0 Vicryl sutures with a stoma size of 2 cm

Table 1: Indications for CDD

Indications	*Male, n (%)*	*Female, n (%)*	*Total, n (%)*
Large impacted stones	4 (15.4)	8 (30.8)	12 (46.2)
Choledocholithiasis with stricture	3 (11.6)	5 (19.2)	8 (30.8)
Recurrent stones	1 (3.8)	4 (15.4)	5 (19.2)
CBD stone with stricture and cholecystoduodenal fistula	0 (0)	1 (03.8)	1 (3.8)
Total	8 (30.8)	18 (69.2)	26 (100)

Table 2: Complications following CDD

Complications	*Large impacted stones, n (%)*	*Choledocholithiasis with stricture, n (%)*	*Recurrent stones, n (%)*	*CBD stone with stricture and choledochoduodenal fistula, n (%)*	*Total, n (%)*
Wound infection	3 (11.6)	–	1 (03.8)	–	4 (15.4)
Postoperative fever	1 (03.8)	–	–	–	1 (03.8)
Pneumonia	2 (07.8)	1 (03.8)	–	–	3 (11.6)
Alkaline reflux gastritis	–	–	–	–	–
Cholangitis	–	–	–	–	–
Anastomotic stenosis	–	–	–	–	–
Bile leak	–	–	–	–	–
Sump syndrome	–	–	–	–	–

or cholangitis were recorded. The various complications encountered in each group are summarized in Table 2. Overall morbidity was 30.8% without any mortality. None of the patients developed recurrent stones, bile leak, or hemorrhage and none showed residual stones in the follow-up period, making this procedure extremely satisfactory in these patients with multiple medical comorbidities.

DISCUSSION

Open surgical procedures on the biliary tract have been widely investigated since the advent of therapeutic ERCP and more recently that of laparoscopic CBD exploration. The routine use of endoscopic sphincterotomy (ES) and CBD stone extraction, with gallbladder *in situ*, has 10 to 20% risk of developing chronic cholecystitis and empyema of the gallbladder.[2] Choledochoduodenostomy was originally performed by Sprengel in 1891[3] and was subsequently used with considerable success by German surgeons. The risk of causing recurrent cholangitis led to a decline in the popularity without detailed evidence for the same. Several studies on the long-term follow-up of CDD had good outcomes, with an incidence of sump syndrome and/or cholangitis of <5%. Most of these complications can be readily dealt with by endoscopic treatment.[1,4,5]

There has been a renewed interest in CDD in the last three decades, with several publications carefully evaluating the results, indications, advantages, complications, and shortcomings of CDD. The consensus is that CDD is a very satisfactory surgical procedure to treat a variety of obstructing lesions of the distal CBD. Most of these authors stipulate that the diameter of the CBD should be at least 16 mm for good outcomes of CDD.[4,6,7]

There are specific indications for performing CDD, most of which have remained unchanged over time. It has been recommended in the treatment of multiple CBD stones, retained or residual stones following a prior biliary intervention, primary CBD stones, lower-end CBD strictures, dilated CBD with a diameter of more than 2 cm, or failure of ERCP. Benign periampullary tumors and nonavailability of ERCP may also be considered as indications for CDD. This procedure has been favored particularly in elderly patients.[8,9]

In our study, the indications for CDD included large impacted stones, choledocholithiasis with stricture, recurrent stones, and CBD stone with stricture and cholecystoduodenal fistula. None of these patients developed recurrent stones and none showed residual stones in the follow-up period, making this procedure extremely satisfactory in these patients with multiple medical comorbidities.

There have been alternative treatments for primary or retained CBD stones, such as dissolution with deoxycholic acid and endoscopic papillotomy, but CDD has been used with increasing frequency over the past decade with good results. Although ready reflux from the duodenum into the biliary tree may lead to some derangement of hepatic function tests, this is not clinically important. The long-term results of CDD are comparable with ES.[10]

Earlier studies, such as the one conducted by Degenshein,[8] published 18-year experience with 175 consecutive CDDs, and concluded that it was a safe and effective operation for varied indications. It was emphasized that ascending infection from reflux of duodenal contents into the biliary tree, causing recurrent cholangitis, was not a problem if the diameter of the CBD used to construct the CDD measured at least 16 mm.

The results of CDD when done for the proper indications, when the diameter of the CBD used measures 16 mm or more, and the technical precautions (mobilization of the duodenum, vertical incision in the CBD, and meticulous and precise suture techniques) have been better or comparable to other alternative operative methods. There is a thought that CDD should be avoided in younger patients who have a life expectancy of 10 or more years due to long-term complication of cholangitis and the "sump syndrome." This is a rare and late, albeit overemphasized, complication of CDD. Its prevalence has been reported widely varying from as low as 0% to as high as 10%.[1,5] This complication was not seen in our series. An extensive literature review showed that the absence of this complication could be explained based on at least two important factors. Firstly, a wide tension-free anastomosis provides effective drainage of enteric contents that may enter the CBD through the CDD site. Secondly, the narrow part of CBD distal to the anastomosis prevents the entry and stasis of duodenal contents. Also in patients who have undergone a preoperative ERCP with papillotomy, the contents easily pass through the ampulla, preventing this complication altogether. Therefore, with the proper indications and meticulous technique, it can be performed even in younger patients.[4]

Choledochoduodenostomy has its own technical advantages; for instance, it maintains the normal anatomy. As compared with a routine Roux-en-Y hepaticojejunostomy, CDD is technically easier, faster, requires less manipulation of the CBD, and is more physiological. Subsequent endoscopic intervention is possible following CDD. Choledochoduodenostomy bypasses the narrowed/strictured area and is amenable to subsequent surgical intervention (hepaticojejunostomy) in case required. It is suitable for elderly patients or patients with multiple surgeries and interventions. Roux-en-Y hepaticojejunostomy in comparison requires construction of two anastomoses, is more time consuming, is technically more demanding, and alters the normal anatomy. Scarring of the duodenum and impending obstruction of the duodenum are contraindications for CDD; under these circumstances, hepaticojejunostomy is performed.[11]

Hence, from the observations made in this study and the review of literature, CDD produces good long-term results in the treatment of non-neoplastic obstructing pathology of the distal CBD. The size of the CBD with tension-free meticulous suturing techniques is essential for good outcomes. Intrabiliary stents are not necessary under these circumstances and we have not used stents or T-tubes in any of our cases. Technical experience in performing CDD is also an important consideration in assuring good results. Cholangitis and symptoms related to the "sump syndrome" do not occur, or occur very infrequently. These can be managed readily by endoscopic techniques. In the absence of local sepsis, CDD can be performed with very low rates of morbidity and near-zero mortality. The notion that CDD should be reserved only for elderly patients with limited (less than 10 years) life expectancy should be revised, and the spectrum of its indications should be broadened under appropriate circumstances to include much younger patients.

CONCLUSION

Choledochoduodenostomy has a definite role in the management of bile duct stones, especially in benign biliary tract obstruction when a permanent biliary drainage procedure is required. The size of the CBD is of critical importance and meticulous and precise suturing techniques are also essential. The proportion of cases requiring this approach is diminishing because of nonoperative techniques available, but it will not be eliminated by them based on current trends.

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ORIGINAL ARTICLE

Comparison of the Dosage Regimes of Intravenous Dexmedetomidine to supplement Spinal Anesthesia with Hyperbaric Bupivacaine in Hysterectomy

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ABSTRACT

Introduction: Intravenous dexmedetomidine (Dex) has been used in various doses with bupivacaine spinal anesthesia (SA) for various surgeries. A study was conducted to observe the effects of two doses of intravenous Dex on sensory, motor, and hemodynamic parameters and sedation during SA.

Materials and methods: In this prospective, randomized double-blind study, two groups of 35 patients, each undergoing hysterectomy, received SA with 15 mg hyperbaric bupivacaine. After subarachnoid block, group I received 1 µg/kg bolus and group II received 0.5 µg/kg bolus of Dex. The maintenance infusion of 0.5 µg/kg/hr was given to all patients. Duration of sensory block (SB) and motor block (MB), cephalad spread, quality of analgesia, and sedation score (SS) were studied with hemodynamic parameters. The data were analyzed using Student's t-test, and $p < 0.05$ was considered statistically significant.

Results: Demographic data, duration, and type of surgery were comparable. The duration of SB was significantly more in group I (133.03 ± 8.29 min) compared with group II (113.83 ± 11.18 min). The duration of MB was significantly more in group I (224.03 ± 10.32 min) than group II (200.43 ± 9.73 min). The height and quality of blocks were comparable. The SS was significantly more in group I (3.40 ± 0.55) compared with group II (3.143 ± 0.35). The incidence of bradycardia was more in group I than in group II, but that of hypotension was comparable.

Conclusion: A 1 µg/kg bolus and 0.5 µg/kg/hr maintenance dose regime of Dex with bupivacaine SA is more suitable for hysterectomy as compared with 0.5 µg/kg bolus followed by 0.5 µg/kg/hr infusion.

Keywords: Dexmedetomidine, Hysterectomy, Intravenous, Subarachnoid block.

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INTRODUCTION

Successful integration of SA in clinical practice requires willingness of anesthesiologists to supplement the block with central nervous system (CNS) depressants.[1] Dexmedetomidine proves to be a good choice for this purpose, as it has no respiratory depressant action. The intravenous (IV) Dex serves a dual purpose during SA, both by enhancing the local anesthetic effect and providing intraoperative sedation. Inadequate sedation is often implicated for failed spinal block.[2] Dexmedetomidine also causes inhibition of stress response during surgery.[3]

Intravenous Dex has been successfully used with bupivacaine SA in various dose regimes for urologic, lower limb, and gynecological surgery.[3-5] The IV route of Dex in comparison with oral or intrathecal routes gives flexibility of dose adjustment to control the degree of sedation precisely. This is very important as excess sedation is dangerous.[6] We found only one report of use of IV Dex with bupivacaine SA for abdominal hysterectomy.[7] We, therefore, chose this group for our study using 15 mg hyperbaric bupivacaine for SA. Recent work has shown that halving the loading dose of Dex eliminates the cardiovascular adverse effects, but preserves the sedative effects.[3] At 0.5 µg/kg dose, a ceiling effect of analgesia for Dex is reported.[8] Based on these evidences, we decided to compare two bolus loading doses of 1 and 0.5 µg/kg followed by a maintenance of 0.5 µg/kg/hr drip until the end of surgery. Our aim was to compare the clinical profile of the two dose regimes of Dex with bupivacaine SA for hysterectomy patients.

MATERIALS AND METHODS

After approval from the Ethical Committee, the study was conducted at the Department of Anaesthesia at Mahatma Gandhi Mission Medical College, Aurangabad, India. Study included two groups of 35 each. Patients were randomly selected with the help of closed mixed coded envelope method. American Society of Anaesthesiologists grade I and II patients, aged between 30 and 65 years, having height between 145 and 185 cm and weighing between 40 and 60 kg undergoing abdominal or vaginal hysterectomy were included in the study. Patients having valvular heart diseases, severe respiratory diseases,

poorly controlled diabetes mellitus and hypertension, neurological diseases, patients on beta blockers and second- and third-degree heart block, and patients in whom SA was contraindicated were excluded from the study.

Two regimes of Dex were used:

- *Group I*: bolus dose of IV Dex 1 µg/kg over 10 minutes (min) followed by maintenance dose of 0.5 µg/kg/hr until the end of surgery.
- *Group II*: bolus dose of IV Dex 0.5 µg/kg over 10 minutes followed by maintenance dose of 0.5 µg/kg/hr until end of surgery.

Procedure was explained to patients. Written informed consent was taken. The IV line was secured, monitors were attached, and baseline pulse rate and blood pressure (BP) were noted. Preloading was done with 10 mL/kg Ringer's lactate over 20 minutes. To make the study double blinded, one person prepared the Dex solution and the second person who was unaware of the drug gave the subarachnoid block and noted the block parameters. The patient was also unaware about drug regime. The SA was given at L3–L4 interspace in lateral position with 3 mL 0.5% (H) bupivacaine. Immediately after making the patient supine, infusion of study drug was started. Initial bolus dose was given over 10 minutes followed by maintenance dose. Sensory block was assessed using pin prick in midline every 3 minutes until peak sensory level was achieved, i.e., two consecutive readings seen at same dermatome level.

Motor block was assessed using Bromage scale—grade 0: free movement of legs 0%; grade I: just able to flex knees with free movement of feet; grade II: unable to flex knees with free movement of feet; grade III: unable to move legs or feet. The MB was assessed every 3 minutes until observation of grade III MB. During surgery, SB was assessed every 15 minutes. Highest SB level and two-segment regression time were recorded. Time from intrathecal injection to two-segment regression was taken as duration of SB and time to complete motor recovery was taken as duration of MB. Failure to achieve T6 level within 30 minutes was considered as failure of block and general anesthesia was given.

Ramsay SS was used for assessment of intraoperative sedation:

- 1—anxious agitated
- 2—cooperative tranquil
- 3—drowsy, but responds to commands
- 4—asleep, but responsive to glabellar tap
- 5—asleep with sluggish response to tactile stimulation
- 6—asleep and no response

Scores were evaluated every 15 minutes until end of surgery. In case of SS reaching 5, Dex drip was discontinued until it returned to 3. Heart rate (HR), systolic blood pressure (SBP), mean blood pressure (MBP), and oxygen saturation (SpO_2) were recorded before subarachnoid block. Immediately after giving SA, drug infusion was started and HR, SBP, MBP, SpO_2, and SS were recorded every 3 minutes for 10 minutes during infusion of bolus and then every 15 minutes until end of surgery. Duration of surgery was noted. Bradycardia (HR < 50/min) was treated with IV atropine (0.01 mg/kg) and hypotension (fall of BP 25% from baseline) with IV Inj. Mephentermine 3 mg. Injection fentanyl (0.5 µg/kg) IV was kept ready for treating intraoperative discomfort like abdominal pressure sensation or mild pain. Shivering was treated with IV inj. Tramadol (1–2 mg/kg).

STATISTICAL ANALYSIS

In order to detect a 20-minute difference in two-segment regression between the groups for type I error of 0.05 and power of 90% to ensure statistically significant results, the minimum group sample size required was 33. We took 35 patients in each group. The demographic data for categorical variables were compared using Chi-square test and *Z* test. Statistical significance in mean difference was done by using Student's t-test; $p < 0.05$ was considered to be statistically significant. Statistical analysis was done by using Statistical Package for Social Sciences, version 22.

RESULTS

The two groups were comparable regarding age, height, weight, and surgical duration as shown in Table 1. The duration of SB was significantly more in group I (133.03 ± 8.29 min) compared with group II (113.83 ± 11.18 min). The duration of MB was significantly more in group I (224.03 ± 10.32 min) compared with group II (200.43 ± 9.73 min). The maximum height of the block was comparable in both groups (Table 2). The two groups were comparable for quality of block as no patient required rescue analgesic intraoperatively. The SS was significantly more in group I (3.40 ± 0.553) compared with group II (3.143 ± 0.355). The oxygen saturation was maintained in all the cases showing no respiratory compromise. Incidence of hypotension in group I (25.71%) was comparable with group II (25.81%). The incidence of bradycardia was higher in

Table 1: Demographic data. Results are given as mean (SD)

Parameters	*Group I (n = 35)*	*Group II (n = 35)*	*p-value*
Age (years)	56.09 (5.44)	55.8 (6.49)	0.8424 NS
Weight (kilograms)	52.17 (4.91)	49.97 (6.22)	0.1053 NS
Height (cm)	156.17 (4.41)	154.83 (4.96)	0.236 NS
Duration of surgery (minutes)	109.03 (15.71)	116.14 (21.39)	0.1140 NS

NS: Nonsignificant; SD: Standard deviation

Table 2: Comparison of SB and MB parameters

Parameters	*Group I*	*Group II*	*p-value*
Maximum cephalad dermatome†	T7 (T2–T8)	T6 (T4–T7)	–
Total duration of SB (min.)‡	133.03 (8.29)	113.83 (11.18)	0.0001 HS
Duration of MB (min.)‡	224.03 (10.32)	200.43 (9.73)	0.0001 HS

HS: Highly significant; †median (range); ‡mean (standard deviation)

Table 3: Incidence of adverse cardiovascular effects

Side effect	*Group I (n = 35)*	*Group II (n = 35)*	*p-value*
Hypotension	9 (25.71%)	8 (25.81%)	0.7794 NS
Bradycardia	7 (20%)	4 (11.42%)	0.3221 NS

NS: Nonsignificant

group I (20%) compared with group II (11.4%), but the difference was not statistically significant (Table 3).

DISCUSSION

Abdominal hysterectomy can be done under SA. The SA is suitable for vaginal hysterectomy too.[9] Surgery on uterus requires a level of T4–T6.[10] Therefore, both abdominal and vaginal hysterectomy, which can be done using 15 mg hyperbaric bupivacaine 3 mL, were included in the study.[10,11] Our aim was to see whether IV Dex was useful in maintaining a higher block level for the duration of hysterectomy, which at times gets extended beyond 2 hours. Prolongation of SA by IV Dex is by its supraspinal action at locus coeruleus. Spinal and peripheral sites of action of analgesia are also known. Agonism of both alpha 2A and 2C receptors plays a role at pre- and postsynaptic levels. We found a longer duration of SB of 133.03 ± 8.29 min in group I compared with that of 113.03 ± 11.18 min in group II. The fixation time of 15 mg hyperbaric bupivacaine can be up to 30 minutes. Dex bolus, given during this period, can extend duration of SA. Same dose of bupivacaine shows regression of sensory level after 60 minutes, and IV Dex slows this regression.[7] This maintains intensity of block and patient comfort. This is of importance in abdominal hysterectomy, where head low position is given to push the viscera away from pelvic field. Dinesh et al[12] using a bolus dose of 1 µg/kg and maintenance of 0.5 µg/kg/hr of Dex with 15 mg hyperbaric bupivacaine reported two-segment regression time of 137.4 ± 10.9 min. This agrees with our observation of SB duration in group I. Whizar-Lugo et al[7] used same dose of IV Dex with bupivacaine similar to our group I, but observed a longer duration of SB (208 ± 43.5 min). The reason for this is their different endpoint of L5–S2 regression. Harsoor et al[4] used Dex doses similar to our group II and reported a SB of 111.52 ± 30.9 min with a lower dose of 12.5 mg bupivacaine.

The median height of SB in group I was T7 (T2–T8) and group II was T6 (T4–T7). This was comparable. Our observation of group I is comparable with that of Dinesh et al[12] (T6.88 ± 1.1). Whizar-Lugo et al[7] found a median block level of T4. Kaya et al[5] observed a block level of T6.4 ± 0.8 in their control group of 15 mg bupivacaine and contrary to our observation, found a higher level of T4.6 ± 0.6 with a loading dose of Dex of 0.5 µg/kg. Our findings are similar to the observation by Whizar-Lugo et al[7] and Dinesh et al[12] about getting safe level of spinal block using intravenous Dex. Like SB, our group I had longer duration of MB (224.03 ± 10.32 min) as compared with group II (200.43 ± 9.73). This probably is related to the higher dose of Dex in group I. Our MB duration in group I agrees with that of Dinesh et al[12] (220.7 ± 16.5). However, the MB duration found by Whizar-Lugo et al[7] was 191 ± 49.2 min. Abdallah et al[2] in their meta-analysis report say that MB prolongation of bupivacaine SA with IV Dex was by at least 17% compared with SB prolongation, which was at least 34%. Intraoperatively, no patient required rescue analgesia. This shows adequate density or quality of block for both the groups for hysterectomy. This probably was achieved by the 0.5 µg/kg/hr maintenance dose of Dex. A ceiling effect for analgesia is reported at 0.5 µg/kg/hr of Dex.[4] Ok et al[13] have reported prolongation of sedation (Bispectral Index score 60–80) time with increasing maintenance doses (90 min for 0.2 and 120 min for 0.4 µg/kg/hr) following a bolus dose of Dex of 1 µg/kg even for patients who receive final anesthesia with 13 mg bupivacaine. Duration of block and rescue analgesia was not studied in their report. Discussing about action of Dex, Ok et al[13] say that the distribution half-life of Dex is known to be 5 to 10 minutes and termination half-life is about 2 to 3 hours. Also, Dex has linear pharmacokinetics and dose-dependent sedation effects. If we presume other CNS actions of Dex, such as analgesia and prolongation of SB to run parallel to sedation caused by Dex infusion, then the quality of block and regression time period in our group can be explained.

The mean SS was significantly higher in group I (3.4 ± 0.55) than in group II (3.14 ± 0.35), probably because of higher bolus dose used in group I. Al-Mustafa et al[14] have observed a mean SS of 3.96, and Dinesh et al[12] observed mean SS of 4.68 using the drug doses similar to our group I. Both these studies showed higher sedation than our study, but they also found that there was no change in oxygen saturation similar to our observation. Abdallah et al[2] in their meta-analysis report say that there was no serious respiratory complication in any study. Therefore, it was concluded that the sedation seen using 15 mg bupivacaine for SA and Dex regime of 1 µg/kg bolus and 0.5 µg/kg/hr maintenance was safe. The mean duration of surgery in our study was 116.14 ± 21.39 min. Relating

this to our observations of SB and MB parameters, we conclude that 1 µg/kg bolus and 0.5 µg/kg/hr maintenance of Dex, which showed SB duration of 133.03 ± 8.29 min, was more suitable for hysterectomy under SA using 15 mg hyperbaric bupivacaine.

The cardiovascular side effects observed were as follows. Incidence of hypotension in group I (25.71%) was comparable with group II (25.81%). Hypotension observed by other workers varied from 0 to 76%.[7,9,15] Our observation of easy reversibility is supported by these workers. Harsoor et al[4] observed that hypotension did not warrant stoppage of infusion of Dex. The incidence of bradycardia was higher in group I (20%) than in group II (11.4%), but statistically it was not significant. Other workers have reported 16 to 30% incidence of bradycardia.[5,7,12,14] Easy reversibility is reported by all workers.[2] The prevalence of hypotension after routine SA is reported as 30 to 40% and that of bradycardia as 10 to 15%.[15] The higher incidences of bradycardia and hypotension seen in our patients were due to the additive effects of IV Dex. Even then, it was within safe limits, and we conclude that it should not be a hindrance to its clinical use.

CONCLUSION

The IV Dex 1 µg/kg bolus followed by 0.5 µg/kg/hr infusion significantly prolongs SB and MB and shows adequate sedation and quality of block without significant cardiovascular adverse effects as compared with 0.5 µg/kg bolus followed by 0.5 µg/kg/hr infusion. Therefore, it can be concluded that 1 µg/kg bolus and 0.5 µg/kg/hr maintenance dose regimes of Dex with bupivacaine for SA is more suitable for hysterectomy.

LIMITATION

A bigger sample size and a control group would have given more accurate results. The BIS monitoring for sedation would have been better as it does not disturb the patient.

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ORIGINAL ARTICLE

Observed Shortcomings in framing Multiple Choice Questions for assessing Medical Undergraduates: A Study

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ABSTRACT

Introduction: Multiple choice questions (MCQs) are the most common format of exams for various medical entrance competitive exams in India at both undergraduate and postgraduate levels. But this modality of evaluating a student's knowledge may have many shortcomings, which may adversely affect the selection criteria. The aim of this study is to retrospectively analyze the MCQs of Radiodiagnosis in our medical college and suggest modification/changes for better and more standardized MCQs for our medical graduates.

Materials and methods: A total of 20 MCQs set up by teachers of the Department of Radiodiagnosis of our medical college were selected and given to a group of 24 final year Bachelor of Medicine, Bachelor of Surgery (MBBS) students preparing for postgraduate entrance exams, to answer in 15 minutes. These MCQs were then subjected to detailed item analysis using ease index (EI) and discrimination index (DI).

Results: A detailed analysis of 20 MCQS set up by teachers showed that only 50% of them were phrased correctly. Rest all of them had some shortcomings which required some modification or change.

Conclusion: Multiple choice questions remain the most common format for medical entrance examinations because of its obvious advantages—cheap for evaluating a large group, examiner bias removed, a large part of the course material covered, etc. But setting a perfect MCQ requires considerable practice and sticking to certain rules of setting MCQs, which is a must for proper assessment of our students.

Clinical significance: Setting a perfect MCQ paper is necessary for proper assessment of students and for the benefit of medical education.

Keywords: Discrimination index, Ease index, Medical education, Multiple choice questions.

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INTRODUCTION

There are various modes of assessing knowledge, comprehension, and problem-solving capabilities of students like modified essay questions (MEQs) and MCQs. The MEQ is a compromise between the MCQ and the essay. A well-constructed MCQ will be unambiguous, clearly set to a defined standard, and easy to mark (usually automatically). Construction of appropriate MEQs can be difficult[1] and a major criticism of this form of assessment is that MEQs often do little more than test the candidate's ability to recall a list of facts and frustrate the examiner with a large pile of papers to be hand-marked.[2]

Multiple choice questions are a form of assessment in which the students are asked to select a single best answer. Multiple choice testing is a very common format of taking exams and is almost the most common format of exam in postgraduate entrance examinations for medical graduates all over India.

There is an increased need for self-assessment modules that include MCQs and persons with test item writing skills to develop such modules. Although principles of effective test item writing have been documented, violations of these principles are common in medical education. Guidelines for test construction are related to development of educational objectives, defining levels of learning for each objective, and writing effective MCQs that test that learning. Educational objectives should be written in observable, behavioral terms that allow for an accurate assessment of whether the learner has achieved the objectives. Learning occurs at many levels, from simple recall to problem solving. The educational objectives and the MCQs that accompany them should target all levels of learning appropriate for the given content. Characteristics of effective MCQs can be described in terms of the overall item, the stem, and the options. Flawed MCQs interfere with accurate and meaningful interpretation of test scores and negatively affect student pass rates. Therefore, to develop reliable and valid tests, items must be constructed that are free of such flaws.

Multiple choice items consist of a stem and a set of options. The stem asks a question. The options are the possible answers that the student can choose from, with the correct answer called the key and the incorrect answers called distractors.

There are several advantages of multiple choice format of exam, if questions are set properly and it can be a very effective assessment technique. Multiple choice exams require less time for a comprehensive evaluation of a student's knowledge if they are well distributed over the entire syllabus .They are a cheap format of assessment and save a lot of time if the number of students is large. They also remove various kinds of bias in evaluation as only knowledge about the topic is taken into account and other irrelevant factors are removed while marking the answer sheets.[3]

However, they are not without disadvantages. Mostly they evaluate lower domains of learning.[4] Also, even if the student does not know the exact answer, he/she has a 25% chance of getting the correct answer with only four options. This is also decided by the type of distractors provided, and the probability of getting the correct answer on guessing increases with ineffective distractors, all of the above or none of the above options. Also the MCQs need to be well distributed throughout the syllabus or otherwise the results will be more of a biased reflection of knowledge in selective areas of a subject. Additionally, it is important that questions set should not be ambiguous as it may cause confusion and pose a threat to the validity of the exam.[5,6]

Critics of this format suggest other means of examination and evaluation but because of practical difficulties in implementation, the MCQ format of exam remains the most widely used format in entrance to various postgraduate courses. We need to improve our skill of setting MCQs to make it more effective, unbiased, and a more reliable way of assessment as the future of our medical graduates depends on these entrance examinations.

The article provides an overview of established guidelines for writing effective MCQs, a discussion of writing appropriate educational objectives and MCQs that match those objectives, a brief review of item analysis, analyze their adequacy, and suggest modifications and changes if required for better and more standardized MCQs for our medical graduates.

MATERIALS AND METHODS

The study was carried out after obtaining approval from the institutional ethics committee. We collected MCQs from our Department of Radiodiagnosis set by the teachers of the department—four teachers with five questions each. A total of 20 MCQs were selected and given to a group of 24 final year MBBS students preparing for the exams. Each MCQ had four options, one key with rest three being distractors. The time limit was 15 minutes. These MCQs were then subjected to detailed item analysis using EI and DI. They were also analyzed on other points of MCQ evaluation like flaws related to stem and the options (key including distractors) associated with a particular MCQ.

Following was the question paper:

QUESTION PAPER

Time Limit: 15 minutes

1. All of the following are scoring systems for assessing severity of acute pancreatitis except
   (a) Ranson score
   (b) Glasgow score
   (c) Balthazar criteria
   (d) Bismuth classification
2. A young female presents with abdominal pain and ascites. On CT scan, small cirrhotic liver with gross enlargement of caudate lobe and nonvisualization of hepatic veins was seen. Most likely diagnosis is
   (a) Budd–Chiari syndrome
   (b) Alcoholic cirrhosis
   (c) Portal hypertension
   (d) Metabolic liver disease
3. AFP levels are elevated in all except
   (a) HCC of liver
   (b) Papillary CA of thyroid
   (c) Yolk sac tumor of testes
   (d) Pregnancy
4. Neuropathic joint occurs in
   (a) Diabetic neuropathy
   (b) Tabes dorsalis
   (c) Syringomyelia
   (d) All of the above
5. All are true about osteonecrosis except
   (a) Gradual onset
   (b) Reduced activity on scintigraphy
   (c) Self-limiting
   (d) Focal changes on MRI
6. One gray is equal to
   (a) 1 Rad
   (b) 10 Rad
   (c) 100 Rad
   (d) 1000 Rad
7. Definitive method for diagnosing bronchiectasis is
   (a) Chest X-ray
   (b) Bronchography
   (c) MRI
   (d) HRCT
8. Bone density is increased in
   (a) Osteopetrosis
   (b) Idiopathic juvenile osteoporosis
   (c) Osteogenesis imperfecta
   (d) Achondroplasia

9. Earliest diagnosis of osteomyelitis can be made on
   (a) Plain X-ray
   (b) Skeletal scintigraphy
   (c) USG
   (d) CT scan
10. Miliary shadows on chest X-ray is seen in all except
   (a) Tuberculosis
   (b) Sarcoidosis
   (c) COPD
   (d) Metastasis
11. Bilateral hip enlargement can be due to all except
   (a) Sarcoidosis
   (b) Leukemia
   (c) Tuberculosis
   (d) Congenital cyanotic heart disease
12. Kienbock's disease is due to avascular necrosis of
   (a) Femoral neck
   (b) Lateral cuneiform
   (c) Lunate
   (d) Scaphoid
13. Standard technique for imaging of the breast is
   (a) Digital mammography
   (b) Screen film mammography
   (c) Contrast USG
   (d) MRI
14. In the investigation of ureteric colic, the high accurate diagnostic modality of choice is
   (a) Spiral CT
   (b) MRI
   (c) USG
   (d) IVU
15. Which of the following is not a radiological sign of scurvy?
   (a) Wimberger's sign
   (b) Frankel's line
   (c) Pelkan spur
   (d) Looser's zone
16. Copper beaten skull radiograph is due to
   (a) Craniosynostosis
   (b) Platybasia
   (c) Cerebral tumor
   (d) Raised intracranial pressure
17. Radioactive isotopes used for permanent implant brachytherapy is
   (a) Ir-192
   (b) I-125
   (c) Cs-131
   (d) Co-60
18. Snowstorm appearance in USG is seen in
   (a) Vesicular mole
   (b) Fibroid
   (c) Endometriosis
   (d) Adenomyosis
19. HSG should be performed on
   (a) 0–5 days of menstrual cycle
   (b) 5–10 days of menstrual cycle
   (c) 10–15 days of menstrual cycle
   (d) 15–20 days of menstrual cycle
20. Annual dose limit of radiation exposure for general public is
   (a) 100 mSv
   (b) 1 mSv
   (c) 20 mSv
   (d) 5 mSv

The students were then evaluated and scored. Top four scorers formed the group of high scorers (1/6th) and lowest four formed the group of low scorers (1/6th) for calculation of EI and DI.[7] The EI and DI were calculated as follows:

Ease index: Tests how easy or tough a question is. It is calculated by the formula

$$EI = \frac{h+1}{n}$$

where

h = Number of students answering the question correctly in high scorers group

l = Number of students answering the question correctly in low scorers group

n = Total number of students in the two groups

A question is considered too easy if EI >70% and tough if EI<30%; 30 to 70% is acceptable.

Discrimination index

Differentiates between fraction of high scorers and low scorers who got that particular question right.

$$DI = \frac{h-1}{n}$$

where

h/n = Fraction of high scorers answering the question correctly.

l/n = Fraction of low scorers answering the question correctly.

If DI is <0.15 the question has to be discarded and if it is >0.25 it is recommended.[7]

There was also subjective analysis of the questions. Finally, the requisite changes were recommended in the finalized question paper.

RESULTS

Following were the results of this study:

- Item 1 resulted in an EI of 50% and DI of 0.5. Both the indices were in acceptable range.
- Item 2 gave an EI of 62.5% and DI of 0.375. Both the indices were in acceptable range.

- Item 3 had EI of 37.5% and DI of 0.375. However, there was too much use of short forms in the MCQ as AFP, HCC, and CA which is not recommended for setting ideal MCQs.
- Item 4 was too easy for the students with EI of 87.5% and DI of 0.125. Both the indices were outside the acceptable range. The question was too easy and all four students from the high scorers group and three out of four students from the low scorers group got it right, thereby making it a bad MCQ for discrimination between high- and low-performing students.
- Item 5 had similar problem with very high EI and too low DI.
- Item 6 resulted in an EI of 37.5% and DI of 0.5375. Both the indices were in acceptable range.
- Item 7 had an EI of 50% and DI of 0.25. Both the indices were good.
- Item 8 resulted in an EI of 50% and DI of 0.25. Both the indices were in acceptable range. However, option "b" proved to be an ineffective distractor with not a single student attempting this distractor.
- Item 9 had an EI of 37.5% and DI of 0.375. Both the indices were in acceptable range. Option "a" was an ineffective distractor.
- Item 10 resulted in an EI of 50% and DI of 0.5. Both the indices were in acceptable range.
- Item 11 had an EI of 25% and DI of 0.25. Ease index was too low—less than 30%—meaning it was a tough question, although the DI was in acceptable range.
- Item 12 gave an EI of 62.5% and DI of 0.375. Both the indices were in acceptable range.
- Item 13 had an EI of 62.5% and DI of 0.375. The stem of the question was ambiguous on using the term "standard technique," which does not clearly define the purpose meant.
- Item 14 was answered correctly by four students in high scorers group and one student in low scorers group, with EI of 62.5% and DI of 0.375. Both the indices were acceptable.
- Item 15 was analyzed to have an EI of 37.5% and DI of 0.375.
- The question 16 was totally ambiguous with three out of four options being correct, therefore it was set improperly.
- Question 17 was very tough with EI of 12.5% only and DI of 0.125, probably because it was a question more related to radiotherapy and thus beyond the syllabus of radiodiagnosis taught at undergraduate level.
- Item 18 gave an EI of 62.5% and DI of 0.375. Both the indices were good.
- Item 19 gave an EI of 75% and DI of 0.25. Probably the question was slightly easy but the greater problem was the overlap between options "b" and "c" as the correct answer seems to lie between the two options and should have been better framed.
- Item 20 resulted in an EI of 62.5% and DI of 0.375. Both the indices were acceptable.

DISCUSSION

The MCQ is the most common type of written test item used in undergraduate, graduate, and postgraduate medical education.[1] MCQs can be used to assess a broad range of learner knowledge in a short period of time. Because a large number of MCQs can be developed for a given content area, which provides a broad coverage of concepts that can be tested consistently, the MCQ format allows for test reliability. If MCQs are drawn from a representative sample of content areas that constitute predetermined learning outcomes, they allow for a high degree of test validity. Critics of MCQs argue that higher-level learning cannot be tested with MCQs. However, this criticism is more often attributed to flaws in the construction of the test items rather than to their inherent weakness. Appropriately constructed MCQs result in objective testing that can measure knowledge, comprehension, application, and analysis.[2] Disadvantages of MCQs are that they test recognition (choosing an answer) rather than recall (constructing an answer), they allow for guessing, and they are difficult and time-consuming to construct.

Analysis of this study revealed what all shortcomings can occur in MCQs if not framed correctly. For example, we found that:

- Questions may be too easy (MCQs 4 and 5), which may make discrimination between performers and nonperformers difficult.
- While one or two tough questions may be included in the MCQs, they should not be too tough, otherwise DI may become insignificant (e.g., MCQ 17 with DI 0.125).
- Ineffective distractors (like option "b" in MCQ 8 and option "a" in MCQ 9) should not be given as option, because these are likely to be attempted by a small number of students.
- Abbreviations may be used inadvertently in MCQs which may not be understood by students (like HCC for hepatocellular carcinoma in option "a" of MCQ 3). It is better to use full form of words.
- There should be no ambiguity in questions as in MCQ 13 (term "standard technique") and MCQ 16 (3 out of 4 options correct).
- Options given should not be too close or overlapping as in MCQ 19 (Option "c" 10–15 days, option "d" 15–20 days).
- In the present study, it is apparent that only lower domains of learning are being tested. We should test higher levels of learning as well, e.g., by giving real clinical scenarios.

- Stems of MCQs should be positive and not negative. We should ask for "correct" answer rather than "wrong" answer. Absolute and imprecise terms (like always, never, all, seldom, rarely) should be avoided.

Palmer and Devitt[8] compared overall performances of MCQs and MEQs for their abilities to test higher cognitive skills. They found MCQs better than MEQs at addressing higher levels of cognitive skills. Collins[9] suggested that test items in MCQs should relate directly to instructional objectives and should reflect different levels of learning (recall, comprehension and application, problem solving).

CONCLUSION

All in all, although the relatively small sample of this study cautions against overinterpretation, based on this study, we suggest that a well-constructed MCQ meets many of the educational requirements and suggest that this format be considered and utilized seriously when assessing students. Benefits of removing examiner bias with automated marking and potentially high reliability at low cost make MCQs a viable option for assessment in clinical medicine. This mode of examination also covers a large part of the course material at the same time. Setting a perfect MCQ requires considerable practice and sticking to certain rules.

Clinical Significance

Setting a perfect MCQ paper is a must for proper assessment of students and for benefiting medical education. This mode of evaluation, if done perfectly, will offer various advantages for both the examiner and the examinee, which may be evaluated in future studies.

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ORIGINAL ARTICLE

Effects of Early Proprioceptive Neuromuscular Facilitation Exercises on Functional Outcome and Quality of Life in Patients with Stroke

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ABSTRACT

Objective: To evaluate the effects of proprioceptive neuromuscular facilitation (PNF) exercises on functional outcome and quality of life (QoL) in patients with acute stroke.

Materials and methods: Ninety patients were recruited in this study and divided into two groups: Patients admitted within 48 hours after stroke (group I, n = 41) and patients referred from elsewhere after 2 to 3 weeks (group II, n = 49). Both groups were given PNF exercises for 4 weeks. Functional outcome was assessed by Barthel Index (BI), and QoL was assessed by Stroke-Specific Quality of Life (SSQOL) scale before and after the intervention. Patients were followed up in the outpatient department of the Department of Neurology at 1, 3, and 6 months and BI and SSQOL scores were again assessed.

Results: Group I showed significant and better recovery in functional activities ($p < 0.05$) and better QoL as compared with group II ($p < 0.05$). We also compared functional outcome and QoL in ischemic *vs* hemorrhagic stroke ($p = 0.284$) and left *vs* right stroke ($p = 0.973$) and found there was no significant difference.

Conclusion: Improvement in activities of daily living may result in better QoL. The PNF exercises are very effective in improving muscle tone, functional outcome, and QoL. The PNF exercises should be given from the first day after stroke.

Keywords: Barthel index, Proprioceptive neuromuscular facilitation, Quality of life, Stroke.

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INTRODUCTION

Stroke is the most common neurological problem in the world. It affects every aspect of life including functional outcome and QoL. Quality of life is defined as the multidimensional evaluation of physical, psychological, social, and environmental aspects.[1] Despite the progress made during inpatient rehabilitation, approximately 33% of stroke survivors still have deficits requiring additional rehabilitation in the community. The inability to access health care services may affect recovery and may result in declining cognition, poor QoL, and increased risk of medical complications.[2] Proprioceptive neuromuscular facilitation exercises are designed to promote the neuromuscular response of the proprioceptors. The PNF patterns have a spiral, diagonal direction (D1 and D2), and are in line with the topographical arrangement of the muscles, facilitating the activation of biarticular muscles.[3] The PNF techniques apply three-dimensional movement, stretch reflex, and resistance to promote functional movement.[4] Studies on effectiveness of PNF-based treatment are both conflicting and supportive.[5-8] Studies regarding application of PNF exercises in acute stroke are still lacking. Therefore, this study was designed to evaluate the effects of PNF exercises on functional outcome and QoL in the patients with stroke.

MATERIALS AND METHODS

Subjects

Ninety patients were recruited who were admitted in Department of Neurology at Dr. Ram Manohar Lohia Institute of Medical Sciences, Lucknow, Uttar Pradesh, India. Written informed consent was obtained from each patient or their legal relatives prior to their recruitment into the study. Patients were divided into two groups based on their hospital admission after stroke episode. Patients recruited in group I were those admitted to hospital within 48 hours after stroke and group II who were referred here from elsewhere after 2 to 3 weeks and did not receive PNF treatment earlier. The study was approved by institutional ethical clearance committee.

Inclusion and Exclusion Criteria

Only patients with first-time stroke and between the age of 40 and 70 years were included in the study. Patients

having recurrent stroke, aphasia, severe cardiac illness (myocardial infarction), fracture, pregnancy, National Institutes of Health Stroke Scale (NIHSS) > 20, Mini-Mental State Examination < 19, any psychiatric illness, subarachnoid hemorrhage, and amputation were excluded from the study.

Methods

Baseline data, viz., NIHSS, modified Rankin scale (MRS), and BI were recorded within 48 hours from the day of admission in both groups. Cause of stroke, whether ischemic or hemorrhagic, was recorded. Patients in both groups were given equal doses of PNF treatment, i.e., 30 minutes twice daily for 4 weeks in addition to the other medical management available at our institute. Proprioceptive neuromuscular facilitation exercises were given in cephalocaudal direction, i.e., first PNF was given for neck, trunk, scapula, pelvis, then for upper extremity and lower extremity. The patterns and techniques followed for PNF intervention were as follows:[9,10]

PNF for neck: Flexion with rotation to the left and extension with rotation to the right and vice versa, 10 repetitions of each

PNF for trunk: Rhythmic stabilization and alternating isometrics, 10 repetitions

PNF for scapula and pelvis: Anterior elevation and posterior depression; posterior elevation and anterior depression by rhythmic initiation and repeated contraction

For upper and lower extremity: D1 and D2 flexion and extension patterns

Again, functional activities were assessed after 4 weeks in each patient. Patients were discharged with instructions and to continue the exercises at home along with medication. Patients were further evaluated at 1, 3, and 6 months.

RESULTS

The data were normally distributed by applying Kolmogorov–Smirnov test. Descriptive analysis was done to calculate mean and standard deviation (SD) in both groups (Table 1). We applied independent t-test to compare the means in between groups and paired t-test to compare the means within group. A p-value of <0.05 was considered significant.

Table 1: Anthropometric factors and baseline data of the both groups

Anthropometric factors	*Group I, (n = 41)*	*Group II, (n = 49)*
Age (mean ± SD)	61.3 ± 10.44	55.29 ± 11.07
GCS	14.95 ± 0.255	14.95 ± 0.218
NIHSS	6.53 ± 3.79	6.65 ± 3.49
MRS	3.72 ± 0.653	3.68 ± 0.765
Sex: M/F	33/8	31/18
Side affected: Right/left	20/26	15/29
Type of stroke		
Ischemic/hemorrhagic	26/15	38/11

The anthropometric factors [age, Glasgow Coma Scale (GCS), NIHSS, MRS] were almost similar in both groups. Patients in the both groups were conscious and oriented to time, place, and person. All the patients were having mild to moderate stroke (NIHSS < 15). There was no significant difference in performance of functional activities in both groups. Group I showed better improvement at 4 weeks, 1, 3, and 6 months ($p < 0.05$). The QoL was assessed at 1, 3, and 6 months. There was no significant difference in QoL in both groups at 1 month, but group I showed slightly better improvement than group II after 3 and 6 months (Table 2).

In this study, we also compared the functional outcome and QoL in the patients having ischemic and hemorrhagic stroke before and after the PNF intervention. Hemorrhagic stroke patients were having slightly better scores of BI and SSQOL, but on analysis the difference was not significant (BI, $p = 0.284$; SSQOL, $p = 0.210$). We also compared functional outcome and QoL in left and right hemispheric stroke with stroke before and after the PNF intervention. There was no significant difference in BI ($p = 0.973$) and SSQOL scores ($p = 0.124$) (Table 3).

Table 2: Results of assessment of functional activities and QoL within and in between groups

	Admission	*4 weeks*	*1 month*	*3 months*	*6 months*	*p-value within group*
BI						
Group I	33.97 ± 24.53	51.22 ± 25.62	74.59 ± 24.10	85.30 ± 19.26	90.20 ± 15.30	<0.001
Group II	26.95 ± 17.31	41.09 ± 18.35	62.07 ± 20.06	75.60 ± 16.09	81.82 ± 14.39	<0.001
p-value 95% CI	0.127 (−2.03−16.09)	0.037* (0.615−19.63)	0.010* (3.11−21.92)	0.012* (2.16−17.22)	0.009* (2.10−14.64)	
SSQOL						
Group I			153.34 ± 48.60	185.57 ± 48.96	199.57 ± 48.01	<0.001
Group II			134.22 ± 39.88	162.71 ± 37.07	182.84 ± 34.96	<0.001
p-value			0.051 (−0.044−37.68)	0.016* (4.37−41.35)	0.054 (−0.77−35.04)	

CI: Confidence interval; *Significant < .05

Table 3: QoL and functional activity scores in different cohort

	n	*SSQOL*	*BI*
Type of stroke Ischemic/ hemorrhagic	90	p = 0.284	p = 0.210
Side affected Right/left	90	p = 0.973	p = 0.124

DISCUSSION

The objective was to assess whether PNF is more effective in patients if initiated within 48 hours of stroke than when initiated later. Our results are showing improvement in both groups, but group I (patients receiving PNF exercises within 48 hours after stroke) has better improvement in functional activities (BI) than group II (Graph 1). Patients in group I were able to walk with minimum support after 1 month from the time of discharge to home. The results are also showing better QoL in group I (Graph 2). Improvement in disability leads to better QoL. Early improvement in the BI in group I may be the reason of early improvement in QoL. The PNF works on the principle that resisting the strong muscle causes irradiation effect in surrounding weak muscles and muscles of contralateral side also.[4,9] This results in building up of tone in flaccid and weak muscles. We tried to apply this principle in stroke patients to generate tone and voluntary control in flaccid and weaker muscles in extremities. The PNF for neck, trunk, scapula, and pelvis was given first. When tone improved in proximal muscles, we proceeded for distal segments.

Efforts were made to find efficacy of PNF exercises in other cohorts, such as ischemic *vs* hemorrhagic stroke and left *vs* right hemispheric stroke. On analysis, we found there was equal improvement in both ischemic and hemorrhagic stroke group. Studies reported earlier state that hemorrhagic stroke has higher mortality risk than ischemic strokes.[11-20] To our knowledge, current study is the first to assess the effect of PNF exercises on functional outcome and QoL in acute stroke. Paolucci et al[21] have stated that the patients with hemorrhagic stroke have better functional prognosis but the other prognostic factors, such as stroke origin, stroke severity, age, and onset to origin interval strongly affect the functional outcome. Haacke et al[22] considered BI as an important predictor of QoL. They classified the scores as <30 = needs institutional care, 30 to 70 = needs some help, and >70 functionally independent.

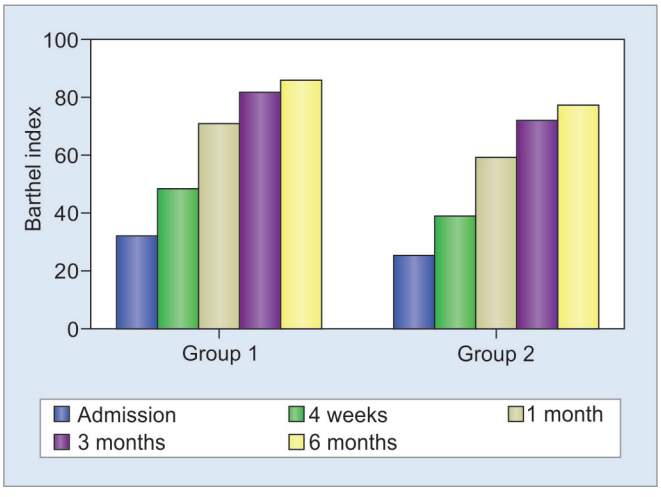
We also compared the effects of PNF exercises in left and right hemispheric stroke. Again, we found that PNF improved the functional outcome and QoL irrespective of hemisphere involvement. Our study is supported by the results of a study carried out by Nam et al[20] who found that the effect of paralysis on dominant hand and QoL in patients with subacute stroke is not significantly different from the effect of paralysis on nondominant hand. Paralysis of dominant hand had no added effect on QoL beyond the effect of stroke itself.

Clinical Significance

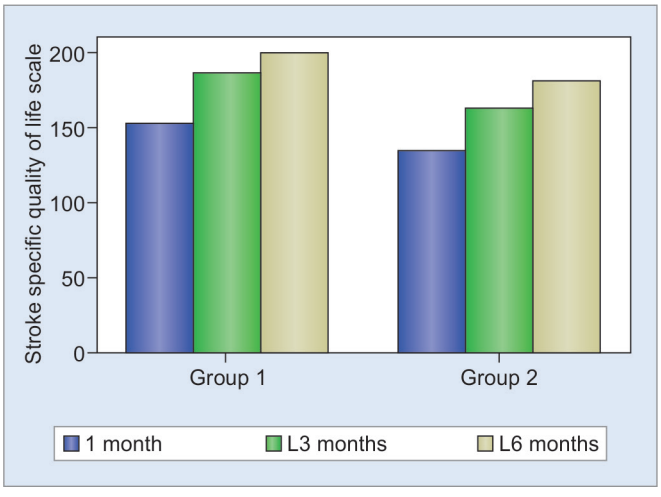
The PNF exercises are efficient to improve tone in muscles. They are very similar to our functional movements so they can be implemented to teach functional activities by the physical and occupational therapists. This may also enhance the neuroplasticity. This must be the future study.

CONCLUSION

In our study, we found that the patients with acute stroke who received earlier PNF improved more than the patients who received PNF after 2 to 3 weeks of stroke. For better and earlier improvement, PNF must be implemented from the first day after stroke.



Graph 1: Increased improvement in BI in group I than in group II



Graph 2: Improvement in QoL in group I than in group II

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REVIEW ARTICLE

Alopecia Areata: An Update

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ABSTRACT

Alopecia areata (AA) is a very common autoimmune disease that leads to unpredictable, relapsing patchy hair loss. Its chronic pathophysiology is still not fully understood. Hair follicles are not destroyed permanently due to which the potential for regrowth of hair is retained for many years, and is possibly lifelong. Clinical presentation varies from small alopecia patches most commonly on the scalp to full body involvement. Characteristic "swarm of bees" appearance on histopathology is confirmatory in acute cases. A variety of therapeutic options are available, but search for new modalities continues as there is a high relapse rate and a number of side effects associated with the available treatment options.

Keywords: Alopecia areata, Hair loss, Nonscarring alopecia.

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INTRODUCTION

Most of the dermatological conditions are underestimated as a simple cosmetic concern. Their effect on patient's quality of life and self-esteem is not taken into consideration. Alopecia areata is one such disease. Though it is a very common autoimmune disease leading to unpredictable, relapsing hair loss, its pathophysiology is still poorly understood.[1] Owing to incomplete knowledge about how the disease specifically affects only pigmented hair follicles, no curative therapy has been established.

EPIDEMIOLOGY

Alopecia areata is one of the most common causes of hair loss due to inflammation, with a worldwide prevalence of 0.1 to 0.2%.[2] It affects children as well as adults, and hair of all colors.[3] There is generally no sex predilection, but men are more frequently affected than women.[4] The disorder is more severe in males and in those with an onset in early childhood.[5] There is an increase in the overall risk of other autoimmune disorders (16%) in those affected.[6] For example, it is accompanied by lupus erythematosus in 0.6%,[7] vitiligo in 4%,[8] and autoimmune thyroid disease in 28% of patients.[9]

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ETIOPATHOGENESIS

In the normal hair growth cycle, hair follicles are continuously being recycled through various phases. During the anagen phase (I through IV), which lasts for around 1 to 8 years for scalp hair, a pigmented hair shaft is generated. This is followed by catagen phase that lasts several weeks, during which there is a rapid, apoptosis-driven organ involution. Melanogenesis is switched off and the hair shaft is transformed into a "club hair." The hair follicle then enters telogen phase, which is a quiescent phase that lasts for several months. This completes the hair cycle and then hair returns to anagen phase.[1]

In patients with AA, the hair cycle is shortened and distorted. There is a characteristic inflammatory infiltrate that attacks only (or at least primarily) the pigment producing hair follicles, predominantly those in the anagen phase. The mixed inflammatory cell infiltrate contains T cells, mast cells, natural killer cells, and dendritic cells.[1]

THEORY OF IMMUNE PRIVILEGE

The hair follicle has a crucial immunologic feature of relative immune privilege that prevents an autoimmune attack on intrafollicularly expressed autoantigens. This relative immune privilege is established mainly by suppression of the surface molecules required for presenting autoantigens to CD8+ T lymphocytes and by the generation of an overall immunoinhibitory local signaling milieu.[10-12]

Some people are genetically predisposed to abnormalities in the microenvironment of the follicle, allowing follicular autoantigens to be presented to preexisting autoreactive CD8+ T cells. When various costimulatory circumstances occur during anagen (e.g., trauma, infection, stress), the clinical phenotype of AA results.[13]

Stress is an important factor in the etiology, as it may precipitate the condition, and the condition may precipitate stress. The most frequent stressful events include the beginning of school or preschool education,

exams, change of school/group, problems with teachers, intensive studying, social problems with peers, death in the family, family financial problems, emigration of parents for work reasons, concomitant diseases, and surgical procedures.[14] Deficiency of trace elements, specially zinc, has been studied and found to be deficient in patients of AA.[15]

CLINICAL FEATURES

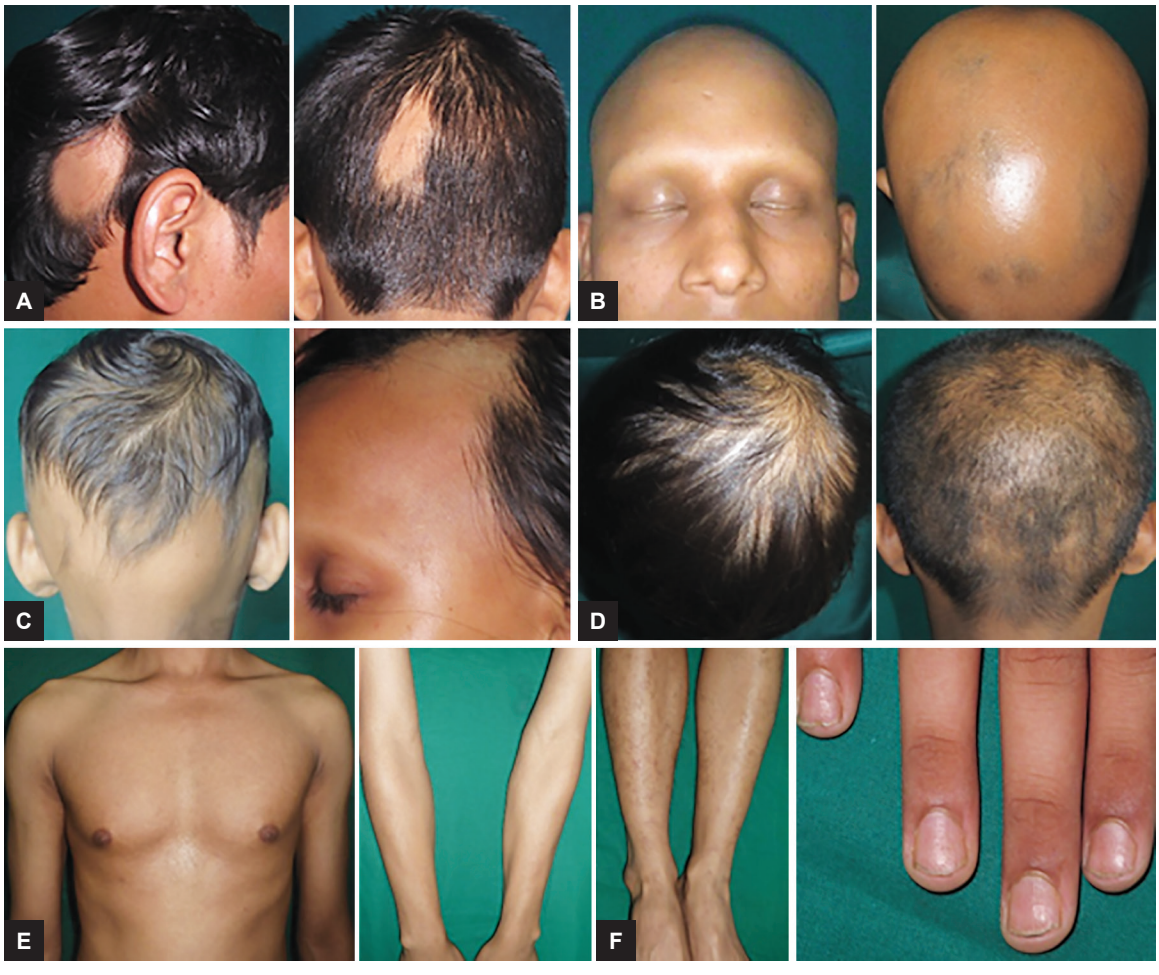
Alopecia areata manifests as well-circumscribed patches of hair loss on normal-appearing skin, most commonly on the scalp and in the region of the beard. The condition is asymptomatic with a sudden onset. Patient usually acknowledges the alopecia patch during hair grooming. Spontaneous recovery is possible but is not the rule. In severe cases, the condition can involve the full scalp or even full body.

Various clinical forms of AA include[16] (Fig. 1)

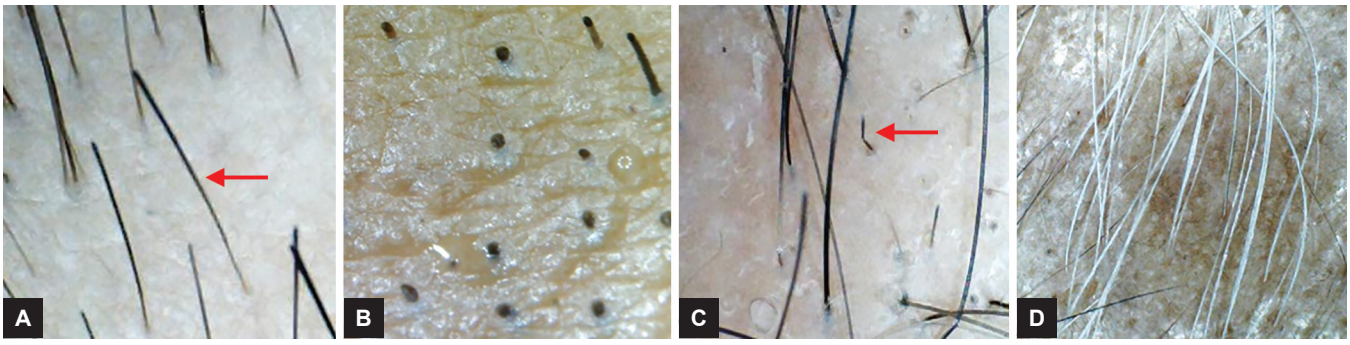
- Patchy/focal AA—hair loss occurs in patches on the scalp or on other parts of the body (e.g., face, abdomen, extremities)
- AA totalis—the loss of all hair on the scalp (including eyebrows and eyelashes)
- AA universalis—the loss of all or almost all body hair
- Alopecia maligna—a generalized long-term loss of hair, resistant to treatment
- Ophiasis or AA marginata—snake-shaped hair loss around the circumference of the head in the temporal, occipital, and frontal areas
- Sisaipho—hair loss beginning from the frontal area and extending backward
- Ophiasis inversus—the inverse pattern of hair loss, which expands from the central to the marginal area of the head
- Diffuse/reticular AA—diffuse or reticular hair loss where no separate bald patches can be distinguished
- Nail involvement is seen most commonly as regular pits on the nail plate

Dermoscopic signs[17] (Fig. 2)

- Exclamation-mark hairs—hair shaft is thinner at the root, resembling an exclamation mark
- Cadaver hairs—dystrophic hair seen as black dots
- Coudability sign—a kink is seen in the hair shaft near the root
- Growth of/sparing of white hair in alopecia patches



Figs 1A to F: Various clinical presentations of AA: (A) Patchy/focal AA; (B) AA totalis; (C) ophiasis/AA merginata and sisaipho; (D) diffuse/reticular AA; (E) AA universalis; and (F) regular shallow pits in a patient with AA



Figs 2A to D: Dermatological signs of AA: (A) Exclamation mark hair; (B) cadaver hair; (C) coudability sign; and (D) sparing of gray hair in AA patch

Table 1: Diagnostic criteria for AA

Diagnostic tool	*Diagnostic findings*
History	• Asymptomatic patchy hair loss • Overnight graying of hair • Atopy + • Other associated autoimmune diseases
Family history	• Atopy + • Presence of autoimmune diseases • AA +
Skin and hair examination	• Well-circumcised areas of alopecia • No skin atrophy in alopecia patch • Sparing of gray hair • Regrowth of gray hair • Hair pull test—positive at the margins of active alopecia patch
Nail examination	• Regular pits over nail plate
Dermoscopy	• Exclamation mark hair • Coudability sign + • Cadaver hair • Yellow dots (keratotic plugs) may be seen
Histology	• Should be done only in doubtful cases • Characteristic "swarm of bees" appearance in acute phase
Lab tests	• Not required for diagnosis

Association with atopy and other autoimmune diseases further helps in establishing the diagnosis (Table 1).

HISTOPATHOLOGY

Diagnosis of AA is usually straightforward and does not require a histopathological examination. However, in confusing cases, such as diffuse AA, a skin biopsy is usually diagnostic. In the acute phase, histological examination reveals a characteristic "swarm of bees pattern" of dense, perifollicular lymphocytic infiltrates around anagen hair follicles (Fig. 3). However, in patients with chronic disease, this pattern may be absent.[18,19]

TREATMENT

Although diagnosing AA is usually easy, treating it is not. Curative therapy does not exist, and there is a paucity of well-conducted, long-term, controlled trials evaluating

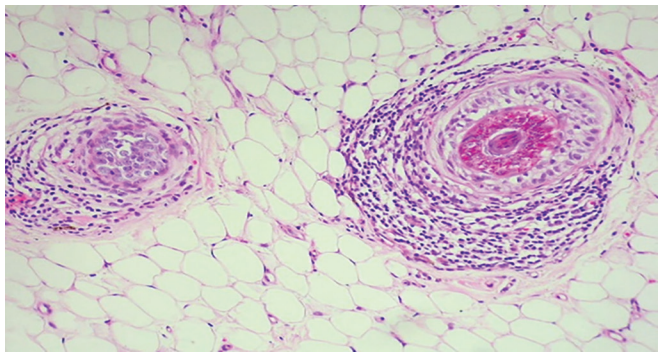


Fig. 3: Histopathology of AA (Courtesy Dr. Rajiv Joshi). Characteristic "swarm of bees appearance" denoting dense perifollicular lymphocytic infiltrate

the efficacy of therapy and its effect on the quality of life.[20] Poor prognostic factors include bald patches persisting for more than 1 year, onset or aggravation of hair loss before puberty, positive family history of AA, ophiasis pattern of involvement, associated nail changes, atopy, autoimmune disorders, and Down syndrome.[15]

Given the often unsatisfactory results, some clinicians rely on the high rate of spontaneous remission and will recommend a wig if remission does not occur.[20] Spontaneous remission occurs in 80% of patients with AA within 1 year, and not all patients require intense therapy. Therefore, watchful observation is also a therapeutic option.[21] Limited, but often helpful therapeutic options do exist for both acute and chronic, relapsing AA.

Intralesional corticosteroids (ILSs) are a time-tested modality of treatment for AA.[22] Hair regrowth is promoted by their immunosuppressive effect.[23] The time from injection to visible hair growth is 2 to 4 weeks and subsequent growth occurs at a constant linear rate.[24] Therapy should be stopped if there is no hair growth by 6 months, as such individuals may lack adequate corticosteroid receptors in their scalp tissue. Disadvantages of using ILS include transient atrophy at the injection site[25] and pigmentary changes (hypopigmentation > hyperpigmentation). Systemic side effects have also been described. The ILS near the orbit can lead to amaurosis.

Topical and oral corticosteroids are also effective. A high-potency corticosteroid can be used under occlusion for topical therapy.[26,27] Systemically, betamethasone and prednisolone are commonly used as mini pulse therapies.[28,29]

Other modalities found effective in the treatment of AA include topical minoxidil,[30,31] oral and topical psoralen and ultraviolet A (PUVA), turban PUVA, topical diphenylcyclopropenone as contact-sensitizing immunotherapy,[32] methotrexate, azathioprine,[16] platelet-rich plasma,[33] sulfasalazine,[34] narrow band ultraviolet B, and bexarotene gel.[35] Biologics are ineffective and may cause aggravation during therapy.[36]

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CASE REPORT

Pituitary Apoplexy presenting as Bilateral Ophthalmoplegia

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ABSTRACT

Pituitary apoplexy is an endocrinological emergency, usually occurring in a pituitary adenoma and is caused by sudden onset hemorrhage and ischemic necrosis of the pituitary gland. Sudden-onset headache along with vomiting is the usual presenting feature. Signs of meningism, however, occur late and are not a characteristic feature. These may be associated with other neurological features like disturbances of consciousness, vertigo, and hemiparesis. Ocular features include marked reduction in the visual acuity with bitemporal hemianopia, diplopia, and ophthalmoplegia due to ocular motor nerve palsies. Acute pituitary apoplexy is unpredictable and should be considered in any patient with abrupt neuro-ophthalmological deterioration associated with headache. We report a unique case of pituitary apoplexy with bilateral total ophthalmoplegia having complete oculomotor nerve palsy in one eye and pupil-sparing oculomotor nerve palsy in the other.

Keywords: Bilateral ophthalmoplegia, Occulomotor nerve pals, Pituitary adenoma, Pituitary apoplexy.

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Conflict of interest: None

INTRODUCTION

Pituitary apoplexy is a rare endocrine emergency that can occur due to infarction or hemorrhage in pituitary gland. This disorder most commonly occurs in a pituitary adenoma. Some well-recognized risk factors are hypertension, medications, major surgeries, coagulopathies, head injury, radiation, or dynamic testing of the pituitary. But it may also occur without any precipitating risk factors. Both neurological and endocrinological signs and symptoms are usually present and ocular manifestations may be the presenting features. The clinical presentation can mimic optic neuritis, ruptured arterial aneurysms, caroticocavernous fistula, and giant cell arteritis. Headache due to raised intracranial pressure is the commonest complaint (63–100% cases), followed by visual deficits (40–100%), cranial nerve palsies, and vomiting.[1] Here, we report a case of pituitary apoplexy with bilateral total ophthalmoplegia having complete oculomotor nerve palsy in one eye and pupil sparing oculomotor nerve palsy in the other.

CASE REPORT

A 52-year-old woman presented to our outpatient department with a 4-day history of acute onset severe headache localized in the frontal and occipital areas, swelling over both eyes and inability to open the eyes. She was conscious and oriented. On examination, her temperature was 39°C, pulse was 84 beats per minute, and blood pressure was 126/88 mm Hg. Ocular examination revealed vision of finger counting at 3 m in both eyes. She had a chin up head posture and the movements of extraocular muscles were restricted in all gazes in both the eyes except levoversion which was present in the left eye. Exodeviation was seen in the left eye (Fig. 1). However, she did not complain of diplopia. Anterior segment examination revealed edema over eyes, upper and lower lids, bilateral ptosis and 6 mm round, regular and nonreacting pupil of the left eye (Fig. 2).

Rest of the anterior segment was normal. Corneal sensations were present in both the eyes. Fundoscopy

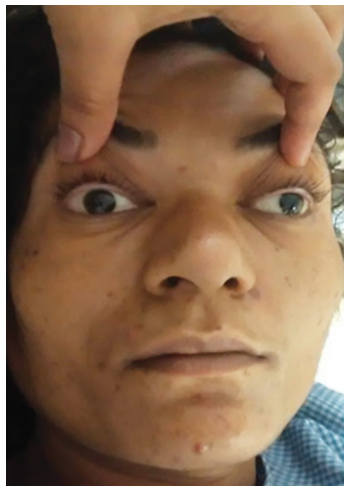

Fig. 1: Eyes in primary gaze position

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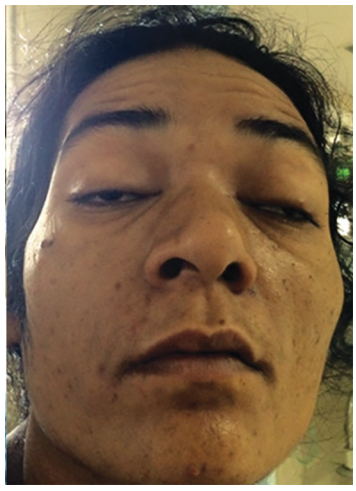

Fig. 2: Bilateral ptosis with upper and lower lid edema in both eyes

revealed healthy optic disk with clear disk margins and cup disk ratio of 0.3:1 with normal macula and periphery in both eyes. Visual fields could not be assessed as the patient was not cooperative. No other focal neurological signs were present. Differential leukocytic count was done, which showed 50% neutrophils and 42% lymphocytes. Random blood sugar was 118 mg/dL. A noncontrast computed tomography of the brain was reported to have a 2.2 × 1.7 cm sized hyperdense mass in the region of sella turcica extending above the suprasellar region and bulging above the cavernous sinus on both sides. The mass showed Hounsfield unit values of +71, suggestive of bleed within the mass (Fig. 3). Magnetic resonance imaging (MRI) of the brain using T1, T2, and Fluid Attenuated Inversion Recovery (FLAIR) sequences (Figs 4 to 6) showed 2.6 × 1.8 × 2.4 cm sized T2 hyperintense and T1 heterointense lesion within the sella causing ballooning of the sella and extending into the suprasellar cistern causing displacement of both the internal carotid arteries and elevation of the optic chiasm. Bony erosion of the inferior aspect of sella was noted. Peripheral enhancement on T1 postcontrast with a central hypointensity was noted in the left cavernous sinus, which was suggestive of left cavernous sinus thrombosis (Fig. 7).

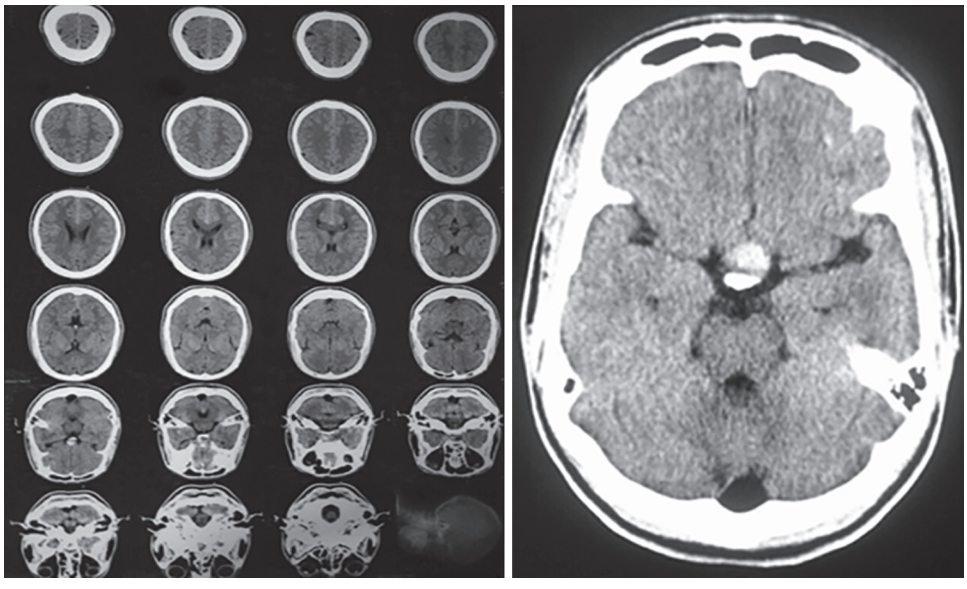

Fig. 3: Noncontrast computed tomography scan highlighting the lesion

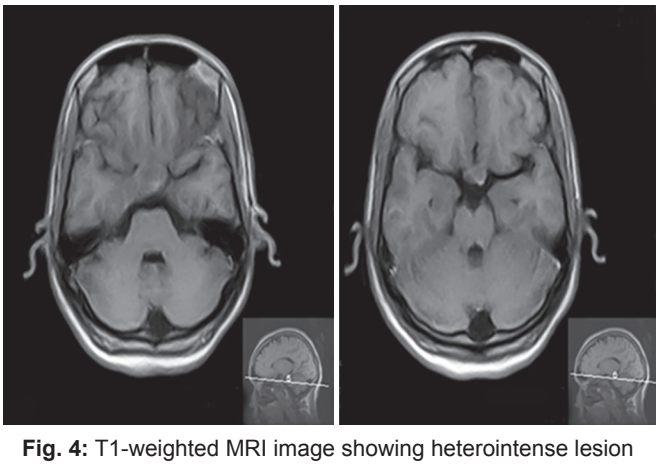

Fig. 4: T1-weighted MRI image showing heterointense lesion

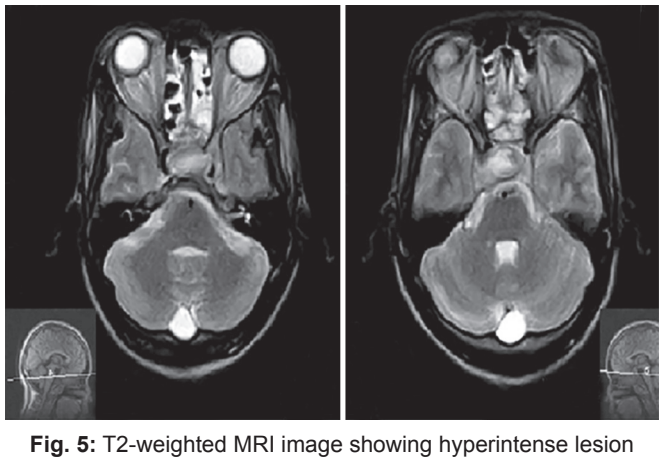

Fig. 5: T2-weighted MRI image showing hyperintense lesion

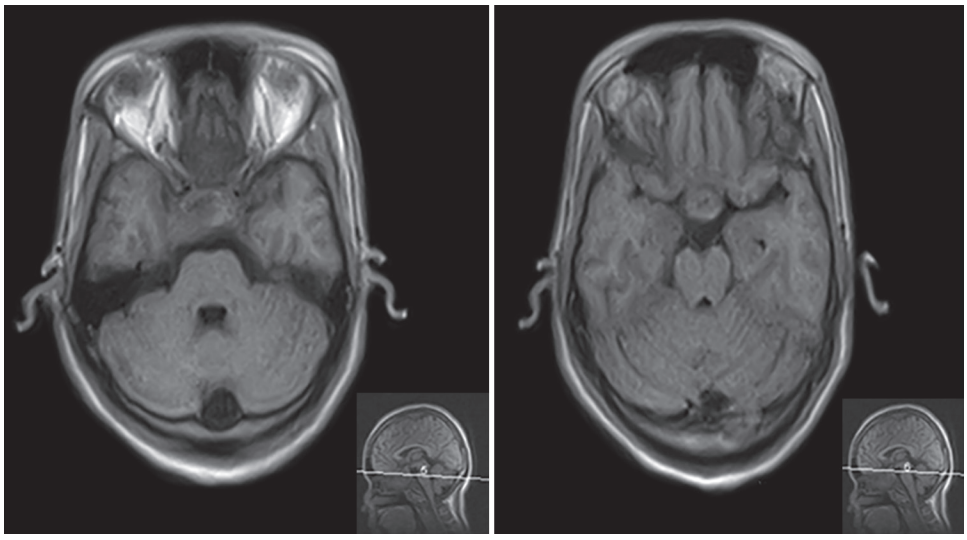

Fig. 6: Fluid attenuated inversion recovery images

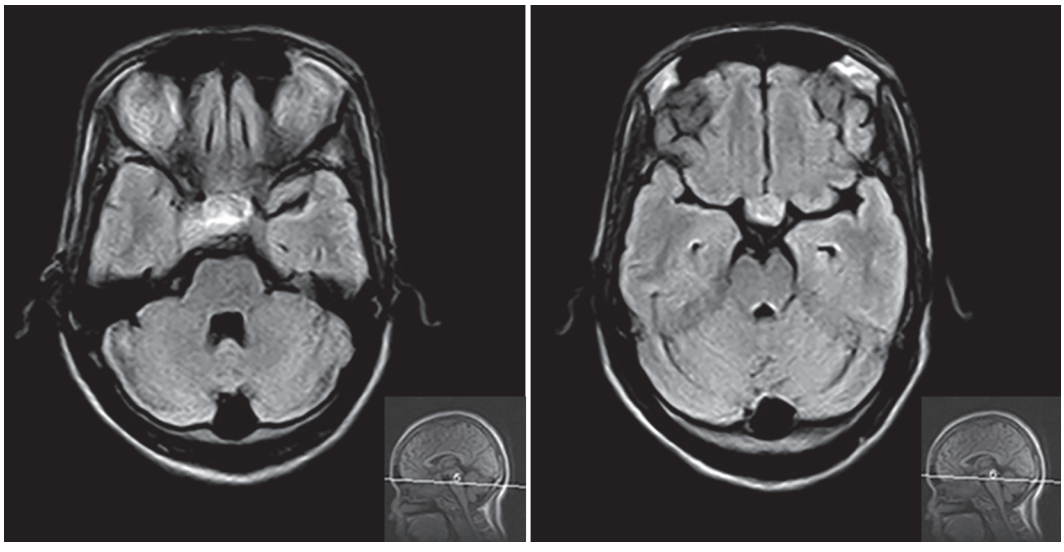

Fig. 7: T2 postcontrast images showing enhancement of the lesion

Bulging of the lateral wall of cavernous sinus was noted, more on the right side. A diagnosis of pituitary apoplexy within a pituitary macroadenoma was made based on the radiological findings. After discussion with neurosurgical colleagues, she was started on injection hydrocortisone 100 mg 8 hourly. Prolactin levels, growth hormone levels, and thyroid profile tests were advised. Patient went to another hospital thereafter for further treatment.

DISCUSSION

Pituitary apoplexy was first described by Bailey[2] in an acromegalic patient. The term "pituitary apoplexy" was coined years later by Brougham et al.[3] It is a potentially life-threatening disorder occurring due to acute ischemic infarction or hemorrhage within the pituitary gland, usually within a pituitary tumor. Most cases of pituitary apoplexy present in the fifth or sixth decade with a slight male preponderance ranging from 1.1 to 2.25:1.0.[4-7]

The exact pathogenesis of pituitary apoplexy is still unclear. Various theories have been put forward regarding the pathophysiology of pituitary apoplexy. One theory is that rapid tumor growth may outstrip arterial supply, thereby causing ischemic necrosis and hemorrhage.[8] The size of the adenoma appears to be a major factor, but even microadenomas can bleed. Another theory is that the tumor growing inside the narrow space situated between the pituitary stalk and diaphragm sellae leads to constriction of the thin vascular network and finally ischemia, necrosis, and hemorrhage on the anterior lobe and tumor tissue. But the adenoma is supplied by the inferior hypophyseal artery and the compression of the superior hypophyseal artery and its branches against the diaphragm sellae could lead to ischemia of the anterior pituitary.[9] Also the hemorrhage could be the result of invasion of the vessel wall by the tumor cells causing rupture of the vessel wall. Out of

the many risk factors causing pituitary apoplexy, hypertension is the commonest (26%) predisposing factor.[10] The earliest and most common presentation of pituitary apoplexy is headache (up to 100%), followed by ocular palsies in 70% patients. Headache is usually retro-orbital or bifrontal. Ocular motor nerve palsies and trigeminal dysfunction usually occur due to involvement of the cavernous sinus by the erosion of thin sellar wall. Third, fourth, and sixth cranial nerves are commonly involved in case of pituitary apoplexy. Decreased visual acuity and visual field defects, specifically bitemporal hemianopia, are seen in nearly 75% of the patients and are caused by upward enlargement of the intrasellar contents, leading to optic chiasmal compression.[5] Extravasation of blood or necrotic tissue into the subarachnoid space can cause meningism, resulting in fever, photophobia, and altered consciousness level.

Management of pituitary apoplexy is controversial. In some cases, a conservative approach with administration of high-dose corticosteroids along with hormone replacement therapy is indicated, while in others early trans-sphenoidal surgical decompression is advocated. Prognosis of pituitary tumor apoplexy has improved with declining morbidity and mortality because of early diagnosis, better therapeutic support, use of glucocorticoids, and refinement of surgical and postoperative techniques. Urgent surgical decompression is indicated when the patient has severe neuro-ophthalmic signs with sudden visual loss, diminished level of consciousness, or hypothalamic disturbance. On the contrary, stable patients having no visual field defects, neuro-ophthalmic signs, or mental status changes are, however, managed conservatively with a regular evaluation of electrolyte and fluid balance. In adults, hydrocortisone 100 mg intramuscular (IM) bolus, followed by 50 to 100 mg 6 hourly by IM injection or 100 to 200 mg as an intravenous (IV) bolus followed by 2 to 4 mg per hour by continuous IV infusion can be used. Surgical decompression is indicated if there is no improvement seen after 24 to 48 hours of treatment. In patients undergoing surgical decompression improvement is seen within 1 week of the surgery.

Diabetes insipidus is the commonest presentation in case of a pituitary adenoma. However, in our patient, there were no urinary complaints. Also, the signs of meningism, which is a usual presentation in a case of pituitary apoplexy, were not seen in this case. The appearance of symptoms was insidious in onset without any predisposing or any precipitating event. Bilateral ophthalmoplegia can be attributed to the cavernous sinus involvement. However, the affection of cranial nerves was different in both the eyes, with the involvement of third and sixth cranial nerve in one eye and only the third cranial nerve in the other. Also one eye presented with total third nerve palsy, while the other had pupil sparing third nerve involvement.

CONCLUSION

Pituitary apoplexy is an uncommon and underdiagnosed complication of pituitary adenomas having varied clinical presentations. It can mimic a wide spectrum of clinical conditions due to its myriad signs and symptoms. The constellation of signs must alert the clinician to the possibility of pituitary tumor apoplexy. Timely diagnosis by imaging studies and early treatment can help decrease the morbidity and mortality.

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CASE REPORT

Successful Management of an Anticipated Difficult Intubation in a Patient with Misaligned Mandibular Incisors and Canines

[1]Aaditya A Prabhudesai, [2]Kasturi H Bandyopadhyay, [3]Amiya K Mishra, [4]Tulsi Nag

ABSTRACT

The case of a 48-year-old female posted for elective surgery for cholecystitis with a history of previous failed intubation is reported. She had misaligned mandibular incisors and canine teeth, reducing the interincisor distance to 8 mm. Proper preoperative assessment and evaluation of the difficult airway helped to achieve successful uneventful intubation.

Keywords: Airway management, Anticipated difficult intubation, Fiberoptic bronchoscopy.

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Conflict of interest: None

BACKGROUND

Airway-related complications are the commonest cause of anesthesia-related morbidities and mortalities. Proper airway management is one of the most pivotal patient safety concerns in the perioperative period, thus highlighting the need for careful preoperative assessment and anticipation of difficult airway.[1] Awake fiberoptic intubation is a safe and popular mode used to bail out anesthesiologists in cases of anticipated difficult intubation.[2] A case of an anticipated difficult airway with previous history of failed intubation posted for elective laparoscopic cholecystectomy is presented.

CASE REPORT

A 48-year-old female with gallstone disease, planned for laparoscopic cholecystectomy, attended the outpatient department for preoperative evaluation and optimization. The patient had history of previous failed intubation, following which the procedure was abandoned in another hospital. The patient had no associated comorbidities and her medical, surgical, and drug history were not significant.

Systemic examination revealed no abnormal findings. All hematological and biochemical investigations showed no abnormality. Chest X-ray and electrocardiogram were within normal limits. Airway assessment revealed hyomental distance 6 cm, mentothyroid distance 7 cm, and sternomental distance 13 cm, with interincisor distance of 0.8 cm and with misaligned teeth and adequate neck extension (Figs 1 and 2). Mallampati gradation could not be assessed. Bilateral temporomandibular joint

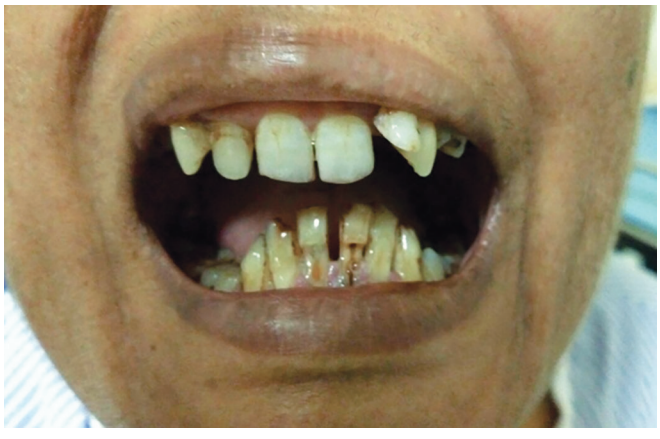

Fig. 1: Malaligned teeth

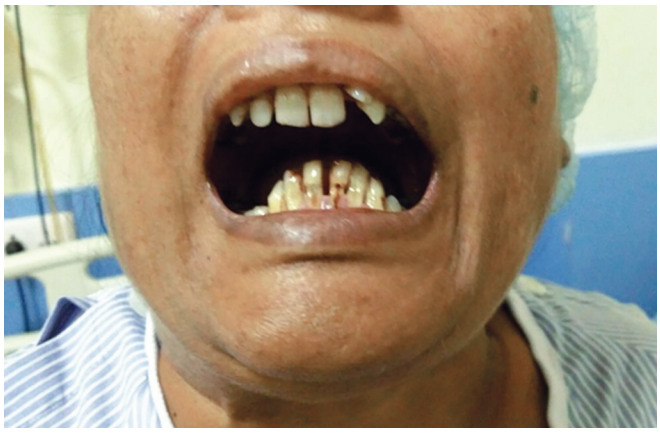

Fig. 2: Inadequate interdental space

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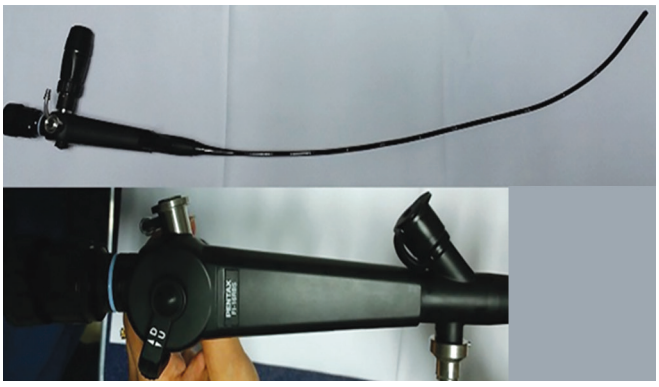

Fig. 3: Fiberoptic bronchoscope

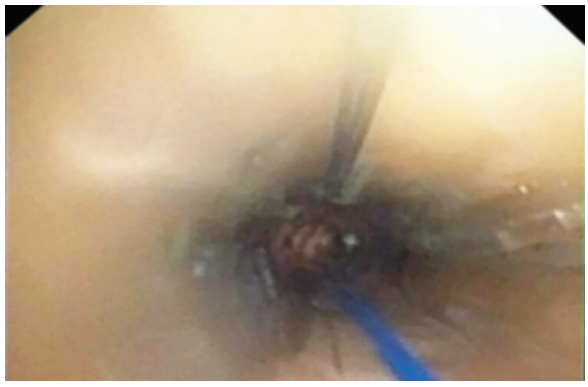

Fig. 4: Intrabronchial scope and tube

had neither pain nor tenderness or restriction of movement. We anticipated difficult intubation and opted for an awake fiberoptic intubation. After discussing and counseling the patient regarding plan of management of anticipated difficult airway using fiberoptic bronchoscope (FOB) and need for surgical airway if required as an emergency life-saving procedure, the patient was accepted as American Society of Anesthesiologists (ASA) grade I. Since high-risk procedure and complications were involved, an informed written consent was obtained.

Anesthetic Management

The patient was nebulized with 5 mL of 2% lignocaine and gargled with 4% lignocaine viscous in the preparation room. She was then taken to the operating room. The standard ASA monitors were attached recording SpO_2 97% in room air, heart rate 82/min (R), respiration rate 20/min, and BP 139/78 mm Hg. Intravenous cannulation was done and Inj. Glycopyrrolate 0.001 mg/kg intravenous (IV) antisialagogue, midazolam 0.05 mg/kg IV as anxiolytic, and injection paracetamol 1 gm IV were administered. Xylometazoline 0.1% drops were instilled in both nostrils and 10% lidocaine was sprayed twice in the hypopharynx with the help of a tongue depressor to provide topical anesthesia to the posterior pharyngeal wall. About 1 mL of 2% lignocaine each was injected just caudal to greater cornue of hyoid bone on either side to block the superior laryngeal nerve. Another 2 mL of the same drug was injected through the cricothyroid membrane for providing topical anesthesia to the infraglottic region and the trachea. After preoxygenating 15 minutes the FOB (Pentax-FI-16RBS; Fig. 3) with external diameter 5.2 mm at the tip was made ready for use. A No. 7.0 polyvinyl chloride cuffed endotracheal tube (ETT) was lubricated with water-based jelly. The bronchoscope was then passed through the nose, pharynx, and vocal cords. Just before passing through the vocal cords, another 3 mL of lignocaine was sprayed through the scope in the "spray-as-you-go" technique. The FOB was advanced through the vocal cords and after the carina was visualized the ETT was railed over the scope (Fig. 4), scope removed, and the capnometer was attached to the tube to confirm endotracheal intubation. Bilateral air entry was ensured clinically; thereafter, induction of anesthesia was done with 2 mg/kg of propofol, 2 µg/kg fentanyl, and 0.6 mg/kg of atracurium, and thereafter maintenance was done with air:oxygen (50:50) titrated dose of muscle relaxant sevoflurane 2 to 3% with multimodal analgesia. Intraoperative hemodynamic stability was maintained. The surgical procedure was uneventful. Reversal was done with glycopyrrolate 0.001 mg/kg and neostigmine 0.05 mg/kg. The patient was extubated after complete reversal of muscle relaxants, confirmation of adequate spontaneous ventilation, and return of protective airway reflexes.

DISCUSSION

The aim of the anesthetic approach in difficult intubation is to achieve a safe airway using appropriate techniques and preventing the potential airway complications that might lead to lethal catastrophe.[3] Shiga et al[4] reported that incidence of difficult intubation due to anatomical causes ranges between 1.5 and 20.2%, quoting that 600 patients die annually due to difficult and failed intubation. They concluded that the anticipation of difficult airway during the preanesthetic checkup by the anesthesiologist allows optimal plan for the difficult intubation; proper selection of airway devices, equipments, and techniques; priming the ancillary services; and arranging adequately skilled helping hands for the procedure.

This patient had misaligned mandibular incisors and canines, reducing the interincisor distance to 0.8 cm. In this difficult airway scenario, the available options were blind nasal intubation, retrograde intubation, and an awake fiberoptic intubation.[3] The success rate of intubation with fibreoptic scope after an anticipated airway or failed intubation exceeds 90%.[5] Being a visually assisted technique, airway stimulation and trauma are minimal

with FOB, hence we opted for awake FOB-assisted intubation in our case. Though more reliable, an awake intubation technique needs patient cooperation. Thus, we initially counseled her in the preoperative period, described the procedure, and used midazolam as an anxiolytic to get her best cooperation. Chances of sympathetic overactivity, laryngospasm, and bronchospasm were prevented by appropriate topical anesthesia of the airway.

CONCLUSION

The proper preanesthetic assessment and intubation technique helped us in managing difficult intubation in a patient, with misaligned mandibular incisors and canines. This article highlights the importance of comprehensive preoperative evaluation of a patient with difficult airway in order to achieve successful intubation by choosing an appropriate technique and customizing the airway device.

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CASE REPORT

Anesthetic Challenge in Corrective Hand Surgery in a Child with Russell–Silver Syndrome

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ABSTRACT

Introduction: Co-morbidities associated with Russell–Silver syndrome (RSS), viz. severe postnatal growth retardation, congenital heart disease, dysmorphic facial features, limb asymmetry, various patterns of hypogonadism, and constellation of endocrine abnormalities, present significant anesthetic challenge during surgery. We report a case of Russell–Silver syndrome in a 5½-year-old boy who underwent corrective hand surgery and the anesthetic challenges he presented. Russell–Silver syndrome has an autosomal dominant inheritance pattern with variable expressivity and its diagnosis remains primarily clinical, as no definite etiology or specific tests have been established yet. We anticipated difficulty in airway management of this child and so we ensured availability of ancillary equipments and presence of skilled anesthesiologists. This helped us to conduct surgery successfully without any anesthetic complications.

Keywords: Difficult airway, Postnatal growth deficiency, Russell–Silver syndrome.

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INTRODUCTION

Russell–Silver syndrome, also called Russell–Silver dwarfism, is a form of dwarfism characterized by intrauterine growth retardation with severe postnatal growth impairment, dysmorphic facial features including mandibular and facial hypoplasia, limb asymmetry, endocrinal abnormalities including hypoglycemia and various patterns of hypogonadism.[1] Russell and Silver were the first to describe this syndrome in 1953 and 1954 respectively.[2,3] Its incidence ranges from 1 in 3,000 to 1 in 100,000 live births, occurring in all races with equal distribution in males and females.[4]

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CASE REPORT

A 5½-year-old boy, weighing 12.5 kg, 100 cm tall, was posted for extensor pollicis longus tendon transfer of left thumb for correction of a congenital malformation. The child was diagnosed with RSS in early infancy, based on low birth weight (2.0 kg), postnatal growth deficiency, characteristic facial features like triangular face with frontal bossing, and hypoplastic mandible (Fig. 1). His height and weight were below the third percentile with delayed developmental milestones and with normal intelligence for his age (Fig. 2). He was active and playful with good effort tolerance. Parents gave history of recurrent respiratory tract infection with an episode of bronchospasm 1 year back which was treated with Salbutamol inhaler for 1 month. He had two episodes of generalized tonic–clonic seizures at the age of 4 years. Since then he had been on treatment with oral valproate.

Physical examination revealed asthenic boy with little muscle mass and scanty subcutaneous fat, narrow elongated head, small triangular face, low-set posterior-rotated ears, hypoplastic mandible, fish-like mouth with down-turned angles, pouted lips with microdontia, and normal genitalia (Fig. 3). Airway examination revealed adequate mouth opening and Mallampati[5] score II predicting ease of tracheal intubation. His left thumb was rudimentary with contracture of left first web space. He was unable to move the thumb due to weakness of dorsiflexor, abductor, and extensor muscles.

Fig. 1: Short statured asthenic boy

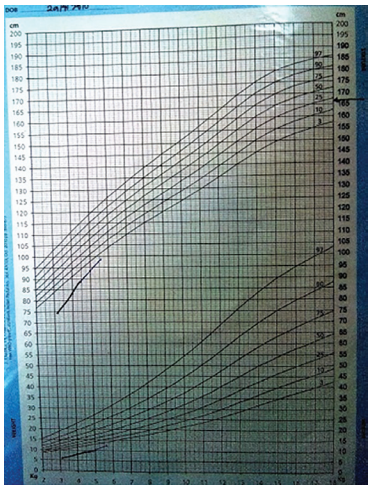

Fig. 2: Growth chart of the child

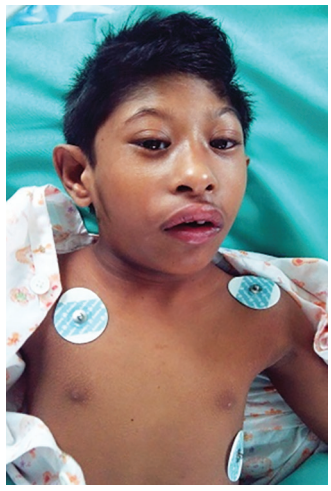

Fig. 3: Characteristic facial features

Human growth hormone stimulation analysis revealed insufficient basal (0.78 ng/mL) and post-stimulation levels (5.44 ng/mL) after ingestion of 25 µg of Clonidine. Thereafter, he had been on injection of Norditropin 1.3 mg subcutaneously daily till date. Molecular deoxyribonucleic acid analysis was inconclusive of RSS. Examination of cardiovascular system and echocardiography was within normal limits. Chest X-ray showed no abnormalities. Basic hematological and biochemical parameters were within normal limits. There was no hypoglycemia.

Anesthetic Management

The child was shifted to the operating room (OR), unpremedicated, after 8 hours of fasting for solid food, 2 hours for clear liquid, and nebulization with 2.5 mg Salbutamol 1 hour prior to surgery. Standard American Society of Anesthesiologists monitors were attached; intravenous (IV) 1% dextrose in Ringer lactate solution started via 22-gauge IV cannula and injection Glycopyrrolate IV was given as anti-sialagogue agent. Preinduction blood glucose level was 88 mg/dL. Anesthetic plan A was to provide general anesthesia with endotracheal intubation (ETI), while plan B was to secure the airway with ProSeal laryngeal mask airway (PLMA) if the former failed. Difficult airway cart was kept ready prior to induction of anesthesia.

Following preoxygenation, anesthesia was induced with IV injection of Propofol (25 mg). After confirming adequacy of bag-mask ventilation with No. 2 anatomical face mask aided by insertion of size 1 Guedel's oropharyngeal airway, injection of Succinylcholine 25 mg IV was given to facilitate ETI. Laryngoscopy with a Mackintosh size 2 blade revealed Cormack Lehane grade IIIA view. The ETI using 4.5 mm uncuffed endotracheal tube could not be achieved even after two attempts with a gum elastic bougie, hence PLMA no. 2 was inserted with confirmed position and adequate ventilation. Intermittent positive pressure ventilation was done in pressure control mode, and anesthesia was maintained with O_2:air (50:50) + Sevoflurane 1.5 to 2%, injection Fentanyl (25 µg), and titrated doses of injection Atracurium at regular intervals. Multimodal analgesia was administered with injection of Paracetamol (180 mg, IV) and infiltration of incision line with 5 mL of 0.25% Bupivacaine. Patient's core temperature was measured with esophageal probe and was maintained at 36°C with the aid of warming blankets, maintaining the room temperature at 27°C, humidifying the anesthetic circuit with a moisture exchanger, and using warm IV fluids. Intraoperative blood glucose was measured and found 118 mg/dL. Surgery lasted 3 hours. The child was reversed with neostigmine 0.05 mg/kg and Glycopyrrolate 0.004 mg/kg. He was extubated in the OR after an uneventful anesthetic emergence and recovery. He was monitored for an hour in the postanesthesia care unit. The child had an uncomplicated and smooth recovery and was discharged on the third postoperative day.

DISCUSSION

The RSS is known to have autosomal dominant inheritance pattern with variable expressivity. Maternal uniparental disomy of chromosome 7, in which a child inherits both copies of a region of the chromosome from the mother, has been shown to play a role. However, no single explanation till date has accounted for the heterogeneity of the phenotypic findings.[6] The preanesthetic evaluation and preparation to rule out various abnormalities like congenital heart disease; urogenital, orthopedic, ocular, dental anomalies; and various endocrinopathies is required.[7-11]

Diagnosis of RSS remains clinical as no definite etiology or specific tests have been established.[6] The five core clinical diagnostic criteria are intrauterine growth retardation, poor postnatal growth, preservation of

occipitofrontal circumference, classic facial phenotype, and asymmetry of extremities.

A patient fulfilling any four of these five criteria is diagnosed with RSS.[12] Our patient demonstrated four of these clinical criteria. The lack of muscle mass and subcutaneous fat renders these patients more susceptible to hypothermia. Therefore, a thermally neutral operating room, warmed and humidified respiratory gases, drapes, IV fluids, warm irrigating solutions, and wrapping of the head and extremities of the patient should be ensured. Various endocrinopathies like hypopituitarism and adrenocortical insufficiency have been reported in these patients, hypoglycemia being the commonest.[13] Hypoglycemia was found to be responsible for symptoms like seizures, tachycardia, excessive sweating, and periodic episodes of severe weakness relieved by food.[14] Our patient did not have any history of hypoglycemia but did have history of seizure, the cause of which was not obvious. Pathophysiology of hypoglycemia in RSS can be due to rapid depletion of limited hepatic glycogen stores, or due to unusually large cranial-to-body mass ratio leading to disproportionately increased utilization of glucose, and therefore the possibility of abnormal glucose homeostasis during prolonged fasting and surgical stress should be considered.[15] Perioperative glucose status monitoring is must and any unexplained tachycardia, diaphoresis, or excessive somnolence following anesthetic emergence should prompt measurement of blood glucose. Since our patient had history of recurrent upper respiratory tract infection associated with bronchospasm, congenital cardiac anomaly was ruled out.

Obtaining a good mask seal in these patients is likely to be difficult owing to facial asymmetry and small receding chin. Hence, different types and sizes of masks should be available along with airway adjuncts. Facial manifestations of RSS may also render exposure of the vocal cords difficult with conventional laryngoscopy. Since difficult intubation was anticipated, adequate size PLMA was kept ready, which eventually helped us to secure the airway. A pediatric fiberoptic bronchoscope of 3.1 mm external diameter should also be handy. Preoperative administration of anti-sialagogue agent helps in decreasing secretions in the airways and aids visibility.[16]

Since these patients have underdeveloped muscle mass, the recommended dose of any neuromuscular blocking agent may cause profound relaxation. Thus, careful titration of top-up doses along with neuromuscular blockade monitoring is advisable. Recovery from inhalational anesthetic agent is usually quick, which was also observed in our patient. The "wash-out" of volatile agents occurs at a rapid rate due to the lack of significant fat for uptake during maintenance of anesthesia and the subsequent release into the circulation during emergence. At the end of surgery, the boy was adequately reversed and extubated in the operating room.

CONCLUSION

We have presented the anesthetic implications of RSS along with our experience of managing such a case. We encountered management concerns with regard to mask ventilation, laryngoscopy, and ETI for which we were forearmed. Temperature regulation, dosing of neuromuscular blocking agents, glucose homeostasis, and emergence from anesthesia need to be managed with particular care. Awareness of concomitant anomalies associated with RSS, such as congenital heart disease, hypopituitarism, and adrenal insufficiency, is essential for proper care.

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CASE REPORT

Atypical Granular Cell Tumor of Breast

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ABSTRACT

Breast carcinomas are the leading cause of mortality and morbidity in the female population. Granular cell tumor (GCT) is a rare tumor, which commonly involves the tongue, lower extremity, skin, and digestive tract. It is derived from Schwann cells. Benign and malignant GCTs exist but the criteria that define them are poorly stated in the literature. Our case is an unusual case of atypical GCT in a 52-year-old female who presented with left breast lump. The diagnosis was confirmed by immunohistochemistry. Early diagnosis and intervention in such cases is critical to increase patient survival and reduce postoperative morbidity and mortality.

Keywords: Breast carcinoma, Epithelial malignancy, Granular cell tumor, Neoplasms.

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INTRODUCTION

Breast cancer is the most common cancer in women worldwide. About 1.7 million new cases were diagnosed in 2012. Granular cell tumor is an uncommon neoplasm that was first described by Abrikossoff in 1926 as "granular cell myoblastoma," assuming it was of myogenic origin. Subsequently, immunohistochemical and ultrastructural features are suggestive of a perineural or Schwann cell origin. However, the exact histogenesis of GCT is still unknown.[1]

CASE REPORT

A 52-year-old female came to the surgical outpatient department with a history of lump in the left breast of 4 months duration. The lump gradually increased in size. It was firm, located in the upper outer quadrant, and did not involve the overlying skin. There was no nipple discharge or skin retraction. Axillary lymph nodes were not palpable.

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Fine needle aspiration was carried out at a primary care center where it was reported as positive for epithelial malignancy, data or slides of which were not available. The patient underwent left modified radical mastectomy with axillary lymph node clearance.

We received the entire intact specimen measuring 25 × 19 × 2 cm with skin flap measuring 20 × 5 × 0.5 cm. Nipple and areola measured 1.5 cm in diameter. The tumor was located in the upper outer quadrant and measured 2 × 1 × 0.5 cm. The tumor was firm to hard and was grossly away from all resected surgical margins. The deep resection margin was 0.7 cm away. A total of 12 axillary lymph nodes were dissected. On microscopic examination, the tumor comprised of tumor cells arranged in sheets, nests, and lobules with infiltration into the surrounding breast parenchyma. Individual tumor cells were large, polygonal with mild nuclear pleomorphism and abundant granular cytoplasm (Fig. 1). There was no evidence of necrosis. All the surgical resected margins were free of tumor and the lymph nodes showed no evidence of metastatic tumor deposits.

Immunohistochemistry was carried out which showed that the tumor cells were strongly positive for S-100 stain (Fig. 2A) with a Ki-67 index of 3% (Fig. 2B). Thus, a diagnosis of atypical GCT was made.

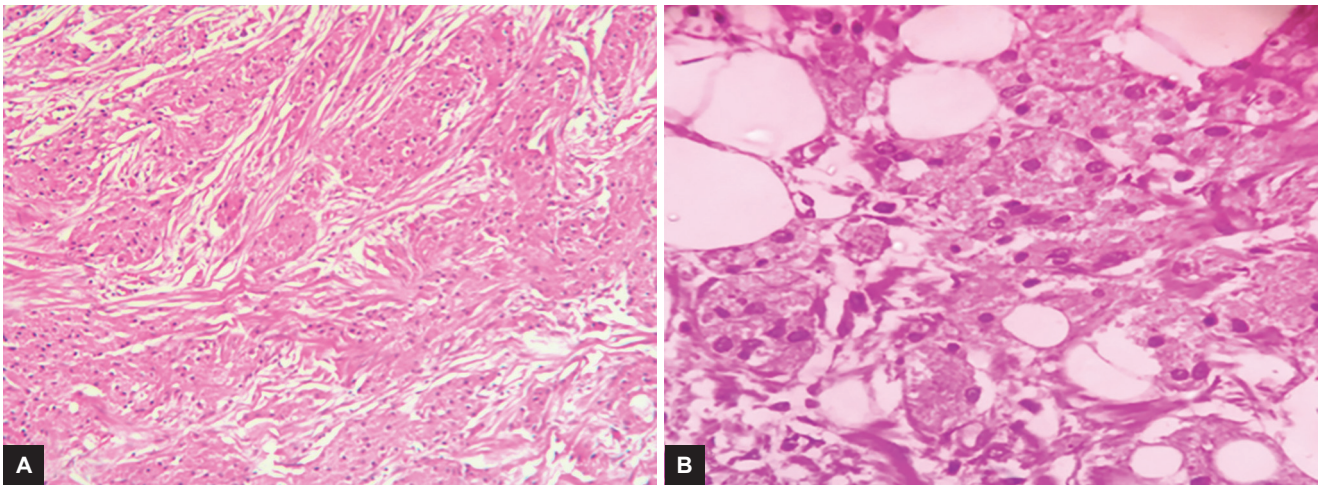
DISCUSSION

A benign GCT is an uncommon tumor that may arise anywhere in the body, most common sites of origin being the tongue, followed by soft tissues.[2] The GCT of the breast accounts for between 5 and 15% of all GCT cases. It occurs in a wide range of ages from teenagers to elderly, most commonly in women between 30 and 50 years. Afro-American women have higher incidence of GCT.

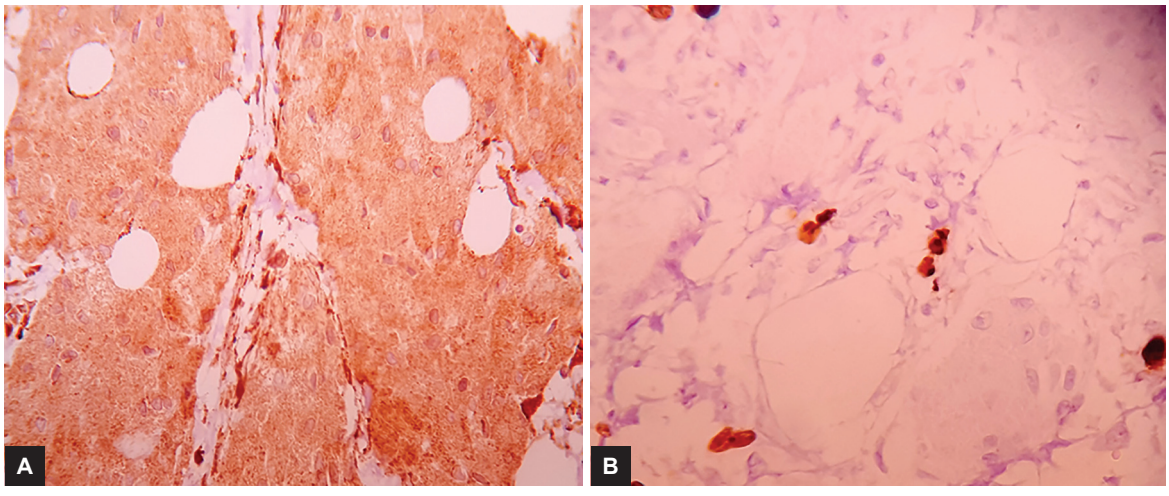
Atypical GCT of breast is quite rare. The differential diagnoses to be considered are for tumor cells with abundant granular cytoplasm, such as secretory carcinomas, benign and malignant adnexal tumors, and soft tissue tumors, such as fibrohistiocytic lesions, alveolar soft-part sarcoma, and fasciitis.[3] Immunohistochemistry can be used as a tool for confirmation of GCT in such cases.

Once the diagnosis of GCT is confirmed, it is important to establish whether it is benign, atypical, or malignant. Malignant GCT differs from benign counterpart in following clinical features[4]:

- Sudden rapid growth in a tumor of long duration



Figs 1A and B: (A) Sheets of tumor cells seen invading the surrounding breast parenchyma, hematoxylin and eosin (H&E) stain, 100×; and (B) tumor cells seen infiltrating the fat. Note the different chromatin pattern of tumor cells with few showing nucleoli, H&E stain, 400×



Figs 2A and B: (A) Strong S-100 positivity; and (B) Ki-67 immunohistochemical stain

- Larger size on presentation (median size of 4–5 cm as compared with benign tumors which in most cases are less than 3 cm in size)
- History of local recurrence
- Frequent localization to the lower limbs (unlike benign GCTs, which commonly occur in the head, neck, and tongue)

Histological criteria of malignancy was first proposed by Fanburg-Smith et al.[5] They proposed six histologic criteria for the diagnosis of atypical and malignant GCT, viz. necrosis, spindling, vesicular nuclei with large nucleoli, increased mitotic activity (>2 mitoses/10 high-power field at ×200 magnification), high nuclear-to-cytoplasmic ratio, and pleomorphism. Neoplasms that meet three or more of these criteria are classified as histologically malignant; those which meet one or two criteria are classified as atypical and those with only focal pleomorphism are classified as benign.

Our case showed a mitotic activity of 3% and focal pleomorphism with prominent nucleoli, thus being categorized into the atypical group. Insufficient tumor resection often results in local recurrence, and has a tendency to spread through both lymphatic and hematogenous routes.[6] Chemotherapy and Radiotherapy have little role in treatment. Surgical resection is the primary option.[7] Resection with adequate margins is necessary because the tumor has no capsule and is invasive.

CONCLUSION

Atypical GCT occurring in the breast is rare. More data are required to assess disease-free survival. Although rare, they should be considered in the differential diagnosis of tumors with unusually large granular cell morphology. Immunohistochemistry is confirmatory.

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CASE REPORT

Torsion of Ovarian Cyst presenting as Acute Abdomen: Report of Two Cases

[1]Jyoti Singh, [2]Vidya Kamble, [3]Sushil Kumar

ABSTRACT

Introduction: Two cases of ovarian cyst torsion are presented. The first case was a young girl, 17 years of age, who presented with acute abdomen. Ultrasonography (USG), computed tomography (CT) scan, and Doppler revealed bilateral ovarian cysts. The right-sided cyst had undergone torsion. Exploratory laparotomy confirmed the presence of twisted ovarian cyst on right side and a small dermoid cyst on left side. Right side salpingo-oophorectomy and left side cystectomy were done. The patient made an uneventful recovery.

The second case was a 27-year-old lady, primigravida with 8-week pregnancy, who came with severe pain in right lower quadrant of abdomen of 3 days duration. Ultrasonography was suggestive of live intrauterine pregnancy of 8.4 weeks gestation and two cysts in the right ovary. The Doppler scan for the cyst was suggestive of ovarian torsion. Laparotomy confirmed the diagnosis of ovarian torsion. Right-sided salpingo-oophorectomy was carried out. The patient made an uneventful recovery and she is continuing with pregnancy.

Keywords: Cystectomy, Oophoropexy, Torsion.

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INTRODUCTION

Torsion of ovary occurs when there is total or partial rotation of the adnexa around its vascular axis or pedicle. Ovarian torsion has been as reported as the fifth leading cause of gynecologic emergencies.[1,2] Ovarian torsion increases five-fold during pregnancy. Torsion of ovarian tumors occurs predominantly in the reproductive age group.[3,4] Fallopian tube may also be involved (adnexal torsion). The predisposing factors are moderate-to-large cyst size, free mobility, and long pedicle. It is most commonly seen in cases of immature teratoma (dermoid cyst) or serous cystadenomas.[1] In ovarian torsion, initially, there is venous and lymphatic blockades leading to stasis, venous congestion, hemorrhage, and necrosis, which is followed by ischemic gangrene. These patients generally present with acute abdomen.

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CASE REPORTS

Case 1

A 17-year-old unmarried girl reported with the complaints of intermittent pain of the lower abdomen of 2 days duration, which increased on straining and walking. She also had two to three episodes of vomiting the same morning. She was hemodynamically stable. Examination showed tenderness and guarding in lower abdomen.

Lab investigations: Hemoglobin (Hb) 11.8 gm%, total leukocyte count (TLC) 12,170/cu mm, platelet 2.6 lakh/cu mm, CA-125 49.4 mIU/L.

USG pelvis: Right ovary—dermoid cyst of 4.2 × 3.5 cm and a simple cyst of 4.7 × 3.1 cm dimensions. Left ovary—dermoid cyst of 3.5 × 2.3 cm dimension.

CT scan: Bilateral complex cystic adnexal masses, 5.8 × 8.6 × 9.5 on right side and 3.8 × 3.3 cm on left side, with fat and calcification.

The patient developed high fever on the next day of admission. In view of clinical findings, the USG and CT scan reports, the decision to perform exploratory laparotomy was taken.

Laparotomy findings: There was a right ovarian cyst measuring 12 × 7 × 3 cm with torsion at the pedicle. The tissue distal to torsion (ovarian cyst and fallopian tube) was necrosed and hemorrhagic. Left-sided dermoid cyst was 5 × 3 × 2 cm in dimension; left fallopian tube and uterus were normal (Fig. 1).

Considering gangrene of right-sided adnexal mass, right-sided salpingo-oophorectomy was carried out. The left-sided dermoid cyst was enucleated to conserve the ovarian tissue for future fertility. Postoperatively, the patient made an uneventful recovery and was discharged home after 5 days. Histopathological examination (HPE) showed bilateral mature cystic teratoma with gangrene of right ovarian tissue and fallopian tube.

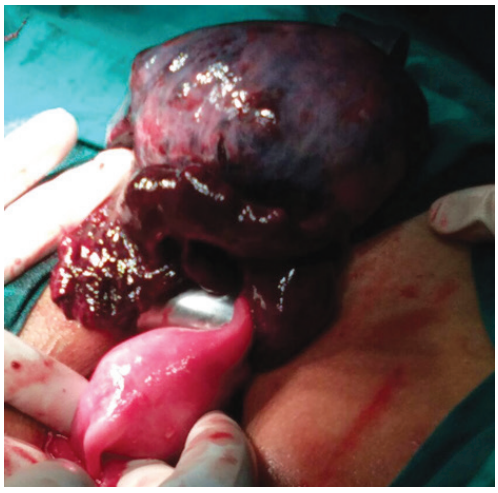

Fig. 1: Right ovarian cyst with torsion of pedicle involving fallopian tube with uterus

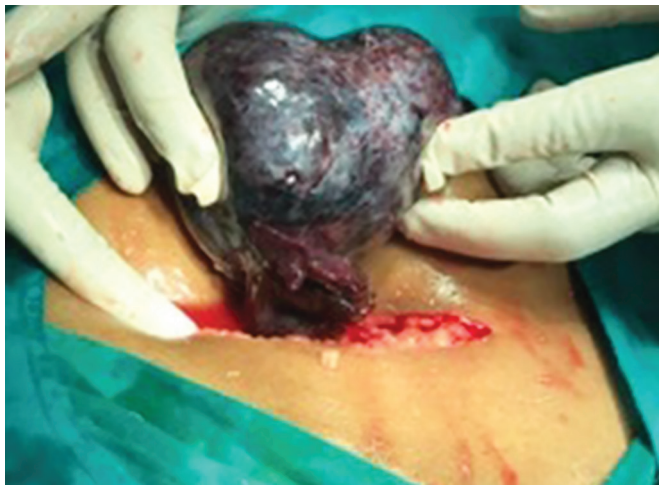

Fig. 2: Right side ovarian cyst with torsion of pedicle involving fallopian tube

Case 2

A 27-year-old lady, married, primigravida with 8-week pregnancy came to the outpatient department with complaint of severe pain in the right iliac fossa, radiating to back of 3 days duration. The pain was not relieved by pain killers. Clinical examination showed tachycardia and tenderness over right iliac fossa. Other vital parameters were normal.

Lab investigations: Hb 12.6 gm%, TLC 10,700/cu mm, platelet 4.15 lakh/cu mm.

USG pelvis: Live single intrauterine pregnancy with 8-week gestation, right ovary having two ovarian cysts measuring 5.9 × 5.6 cm and 4.6 × 3.7 cm respectively.

Doppler scan: Right side ovarian cyst with decreased vascularity suggestive of ovarian torsion. In view of provisional diagnosis of ovarian torsion leading to acute abdomen, decision of exploratory laparotomy was taken despite risk to early pregnancy.

Laparotomy findings: Right ovarian cyst was 10 cm × 12 cm in size. There was torsion involving the fallopian tube (Fig. 2). The right fallopian tube and right ovarian mass were hemorrhagic, necrosed, and gangrenous. Uterus, left fallopian tube, and left ovary were normal. Right side salpingo-oophorectomy was carried out. The patient made an uneventful recovery. The USG done on postoperative day 7 confirmed a viable pregnancy. The HPE report showed hemorrhagic cyst with no viable tissue, suggestive of corpus luteal cyst, since there was no corpus luteum despite early pregnancy. Patient made an uneventful recovery and was discharged 11 days after surgery. She was advised progesterone support up to 12 weeks of pregnancy.

DISCUSSION

Ovarian torsion affects the females of reproductive age from 15 to 45 years.[1,4] Ovaries measuring size of 6 cm or more have higher chances of undergoing torsion.[5] One in 10,000 pregnancies are complicated with adnexal torsion, mostly in first trimester.[5] Almost 70% of ovarian torsion takes place on the right side. The left-sided ovarian torsions are less common because the sigmoid colon occupies most of the space on the left side of the pelvis.[4] Chances of ovarian torsion increase in pregnancy. Ovarian torsion usually involves the fallopian tube, infundibulopelvic ligament, round ligament, utero-ovarian ligament, and broad ligament. Types of ovarian cysts that can undergo torsion are dermoid cyst, cystadenomas, corpus luteal cyst, and endometriomas. The cysts may be unilateral or bilateral, but torsions are mostly unilateral.[3,4] Predisposing causes of torsion are large cyst, ovulation induction, and pregnancy.[4] Clinical parameters are less specific for diagnosis of ovarian torsion. Ultrasonography is the primary method for evaluating ovarian torsion. It is readily available, non-invasive, cost effective, and accurate. Doppler findings are dependent on the degree of vascular compromise. Absence of venous and arterial flow is the most specific finding. Ovarian torsion can also be diagnosed with CT and magnetic resonance imaging (MRI). The CT carries the risk burden of radiation exposure. The MRI is not readily available in the emergency settings. Laparoscopy is another modality that can be used for both confirmation of diagnosis and treatment. The treatment of ovarian cyst depends on size and type of cyst, age, symptoms, and fertility, USG, and Doppler findings. It can be either conservative or surgical. For conservative treatment, timely diagnosis and treatment may help in salvaging ovaries. The ovaries may be preserved in adolescents and the women of reproductive age group desirous of having children.[3] Detorsion alone or with cystectomy may preserve ovarian function and fertility. In cases of enlarged ovaries, torsion can be recurrent and, in these

cases, oophoropexy[3] (fixation of ovary to pelvic wall) is an option. The laparoscopic plication of utero-ovarian ligament is another option available to the gynecologists to prevent recurrent torsion.[5] In case of gangrenous ovaries, the surgical method is the treatment of choice.[4] Laparotomy or laparoscopy can be performed for removal of affected ovaries.

CONCLUSION

Two patients of torsion of ovarian cysts presenting as acute abdomen are presented. Their clinical features, diagnostic modalities, and management are discussed. Early diagnosis can salvage the ovary, but once gangrene sets in, salpingo-oophorectomy becomes inevitable.

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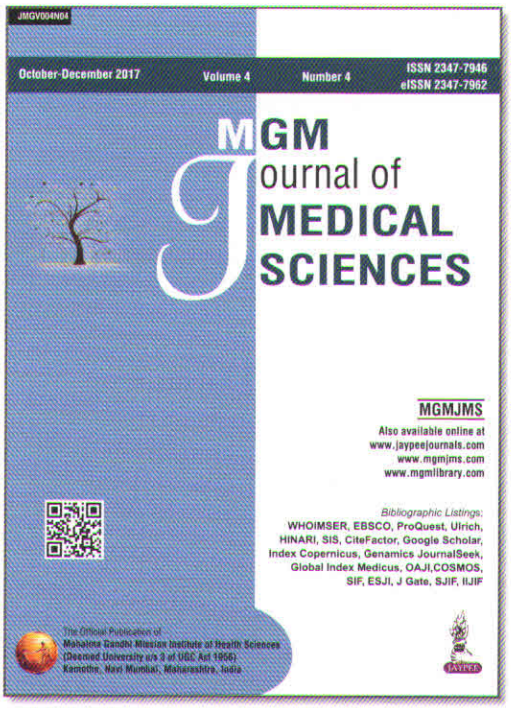

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