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Journal of the College of Physicians and Surgeons Pakistan

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EDITORIAL

Environment and Neurological Diseases: Growing Evidence for Direct Relationship

Mohammad Wasay and Adeel Khoja

Exposure to air pollution has been implicated in a number of adverse health outcomes. There is increasing evidence that point towards association of airborne pollutant exposure with respiratory, cardiovascular, and neurovascular pathology.[1] However, the latter receives the least attention due to paucity on literature examining the impact of polluted environment on cerebrovascular disease. It is important to note that airborne pollutants, such as particulate matter (PM), have the ability to extend beyond the respiratory system and enter the central nervous system (CNS).[2] The World Health Organization (WHO) has estimated that environmental pollution affects health outcome of every nine out of ten people in the world.[3]

Recent studies have shown air pollution and PM to be associated with neuroinflammation and production of reactive oxygen species (ROS).[4] Although the mechanisms regarding entry of PM into CNS are not well understood, PM can be trans-located along the pathway of olfactory nerve into the olfactory bulb and can cross permeable brain barriers.[5] Inhaled pollutant particles are also implicated in neuronal damage through microglial activation and increase in production of cytokines by immune cells of the brain.[3] A study has shown exposure to diesel exhaust particles, a common component of urban air pollution, causing microglial activation, dopaminergic neurotoxicity and increase in ROS production.[6] These findings suggest strong pro-inflammatory and immune upregulating capacities of air pollution; which may contribute to the pathogenesis of neurodegenerative diseases.

Air pollution is a prevalent proinflammatory stimulus to the CNS and a risk factor for neurodegenerative diseases such as Parkinson's disease (PD) and Alzheimer's disease (AD).[5] An alarming finding by Braak *et al*., confirmed the presence of aggregated α-synuclein in targeted areas of PD involvement in young cohorts exposed to high air pollution.[7,8] The process of accumulation and fibrillation of soluble α-synuclein (pathologic hallmark of PD) and Aβ42 (pathologic hallmark of AD) can start in early years despite having no other risk factors for neurodegenerative diseases.[7] Moreover, carriers of particular alleles have a higher risk of developing AD, if they live in a polluted environment.[7] In a study by Chen *et al*. carried out on individuals greater than 40 years of age exposed to high concentration of PM_{10} (above 65 $\mu g/m^3$), had 35 times higher chances of developing PD as compared to those exposed to low concentrations of PM_{10} (below 54 $\mu g/m^3$).[9] Having a residence in an urban area, it can lead to 9% increase risk of developing PD as compared to those living in rural areas.[10]

The impact of living in a polluted environment on stroke is under-recognised, yet substantial.[4] According to the Global Burden of Disease Study 2013, 29.2% of the global stroke burden is attributable to air pollution.[11] Furthermore, a meta-analysis of 94 studies found stroke hospital admissions to be correlated with high concentrations of carbon monoxide, sulfur dioxide and nitrogen dioxide.[12] The European Study of Cohorts for Air Pollution Effects reported that even a small increase of about 5 $\mu m/m^3$ in $PM_{2.5}$ concentration in the air was associated with 19% increase risk of stroke.[4]

Ground level ozone (O_3), an urban air pollutant, is also associated with delirious effects on CNS and cognitive impairment.[1,5] Short- or long-term exposure to O_3 may induce an inflammatory response or generate oxidative stress leading to lipid peroxidation in the brain, dopaminergic neurotoxicity and memory deterioration.[2,5] Moreover, high concentration of O_3 in the environment was associated with a 211% increase risk of AD onset.[13] A recent study showed that people living in a polluted environment having high concentrations of O_3 had a 34% faster rate of cognitive decline annually on the Mini Mental Status Examination (MMSE) as compared to those exposed to lower concentrations of O_3.[1]

Increasingly, studies have shown that PM can enter the brain and has been associated with neurovascular pathology.[5] PM exposure was also reported to cause 138% increase in risk of AD onset,[13] increase in hospital admissions for migraines by 3.3%, and for headaches by 3.4%.[14] Two fractions of PM predominantly affecting CNS are $PM_{2.5}$ and ultrafine particulate matter (UFPM); both being inhaled on a consistent basis as constituents of air pollution.[5] Histopathological examinations of postmortem brain tissue from individuals living in highly polluted areas have observed PM to be accumulated in

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neurons in the olfactory bulb and in blood vessels present in the frontal lobe and trigeminal ganglia.[7] This provides insight into PM being able to pass through the tissue barriers in the lung and brain, subsequently accumulating in the neurons and vessels.

Major roadway proximity was associated with diminished verbal memory, psychomotor speed, language, and executive functioning.[4] A population based cohort study of 2.2 million individuals, demonstrated a significant association between major roadway residence and dementia incidence.[15] A study targeting urban population showed that residents who were exposed to high traffic-related air pollution had 40% more chances of developing dementia or AD.[16] The study also reported that increase in incidence was greater than 70% in a subpopulation excluding younger adults.[16] These studies highlight the significance of urban air pollution with cognitive decline and neurodegenerative diseases.

These findings may have important implications for Pakistan as according to a recent World Bank report, Pakistan's urban air pollution is among the most severe in the world.[17] Notable contributors to this pollution are, including but not limited to; burning of biomass solid fuels and untreated wastewater from urban and industrial sources.[18] The potential role of air pollution in the incidence of neurodegenerative disorders and its adverse impact on health cannot be neglected.

The above discussion highlights the need for additional robust observational and experimental studies highlighting harmful air pollutants and their association with common neurological disorders. Reliable data collections and formidable interventions are key components for reducing air pollution, environmental hazards and neurodegenerative diseases. Safety measures and effective strategies to curb air pollution need to be implemented alongside increase in public awareness.

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ORIGINAL ARTICLE

Evaluation of Microleakage in Single-Rooted Teeth Obturated with Thermoplasticized Gutta-Percha Using Various Endodontic Sealers: An *In-Vitro* Study

Maham Muneeb Lone[1], Farhan Raza Khan[2] and Muneeb Ahmed Lone[3]

ABSTRACT

Objective: To compare apical microleakage of extracted, single-rooted teeth obturated with thermoplasticized injectable gutta-percha using two different endodontic sealers (calcium-hydroxide and resin based).

Study Design: An experimental study.

Place and Duration of Study: The Aga Khan University Hospital (AKUH), Dental Clinics and Laboratory from June to September 2015.

Methodology: The study was conducted using extracted teeth. After access cavities were made, cleaning and shaping of root canals was done in 70 teeth. Teeth were randomly allocated into two groups and obturated with thermoplasticized injectable gutta-percha (Obtura II) using two sealers (Sealapex *vs.* AH plus). After immersing the teeth in 2.0% methylene blue, they were split longitudinally, viewed under light microscope (magnification X4) and images were taken by a camera connected to microscope. The extent of dye penetration was assessed from apex to its coronal part and recorded in millimeters. Independent sample t-test was used to compare microleakage in the two groups. Pearson correlation coefficient was used for inter-examiner reliability of dye penetration measurements. A p-value of <0.05 was taken as statistically significant.

Results: Teeth obturated with Obtura II gutta-percha with AH plus sealer had a mean dye penetration of 1.20 ±0.79 mm. This was significantly better than Obtura II with Sealapex sealer (p=0.003).

Conclusion: Obtura II-AH plus sealer was a better combination for obturation as it showed a lesser degree of microleakage. Obtura II with Sealapex group showed higher microleakage, so this combination should be avoided in single-rooted teeth.

Key Words: *Thermoplasticized injectable gutta-percha. AH plus sealer. Dye penetration. Microleakage.*

INTRODUCTION

The ultimate objective of the root canal treatment is to achieve maximum eradication of microorganisms from the root canal space and to form an impervious apical, lateral and coronal seal to prevent re-colonisation by the disease causing microorganisms.[1] Poor apical sealing of the root canal space accounted for as many as 60% of endodontic treatment failures.[2]

In contemporary dental literature, the historic term, 'hermetic' seal has been replaced by bacteria-tight or fluid-impervious seal, when defining the ideal apical seal expected in endodontic therapy.[3] A core obturation material along with a sealer placed by varying techniques is employed to provide this seal. Warm condensation techniques using gutta-percha have also been employed in clinical practice to fill in the root canal space with varying success. A wide variety of sealers are available for use with the gutta-percha to effectively fill in any voids present around the core material and to flow into the difficult to reach intricate areas, i.e. accessory and lateral canals.[4] Calcium hydroxide based sealers are considered to retard the growth of microbes in the root canal space, thus decrease the chances of root canal re-infection, but studies have found a neurotoxic effect in case the sealer comes in direct contact with nerve tissues.[5] A paradigm shift in dentistry has taken place over the years with the advent of adhesive materials for restoration of teeth. Similarly, resin based sealers have been introduced in an attempt to achieve bonding of the root filling with the root dentine, thereby forming a monoblock to better seal the root canal space.[1]

In endodontics, microleakage is described as clinically imperceptible movement of microorganisms, ions, fluids and molecules between the root canal dentinal walls and obturation material or in spaces within the obturation material.[6] The most prevalent technique for the evaluation of microleakage is the use of dyes. The dye penetration test is employed because of the ease and

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simplicity of its methodology. In endodontic microleakage studies, an aqueous solution of 2% methylene blue is commonly used. Inconsistent results of *in-vitro* studies regarding the sealing ability and microleakage of obturation materials has been the reason for continuous research to develop a material with near ideal properties to seal the root canal space.

The present study was carried out to ascertain which endodontic sealer, when used along with the thermo-plasticized gutta-percha as the core filling material, would result in the best sealing of the tooth-obturation material interface.

The objective of the study was to compare the apical microleakage of extracted, single-rooted teeth obturated with thermoplasticized injectable gutta-percha using two different endodontic sealers (calcium hydroxide, and resin based).

METHODOLOGY

This *ex-vivo*, experimental study was conducted at the Dental OPD and Juma Building Research Laboratory of the Aga Khan University Hospital. Extracted single-rooted teeth were selected for the study sample. Roots with cracks or fractures, root decay, resorptions, open apices or already endodontically treated teeth were excluded from the sample.

Ethical Committee approval was taken before the commencement of the study (3271-Sur-ERC-2014). All procedures were done by the investigator (MML). Extracted teeth that satisfied the inclusion criteria were cleaned of any debris with ultra-sonic scaler, disinfected by immersing in 5.25% sodium hypochlorite (NaOCl) and then placed in normal saline at 37°C until the experiment.

After preparing endodontic access openings, patency was established and glide path made by ISO number 8, hand K-files (Mani Inc., Japan). Working length of the canals was measured by placing a 15-K file from the coronal reference point to 1 mm short of apex. Cleaning and shaping of the root canal was carried out by ProTaper rotary system (Protaper rotary files, DENTSPLY, USA) using a torque control motor (X-Smart plus, DENTSPLY, USA) using shaping and finishing files. EDTA (RC Prep, Premiere Dental Inc.) used with each file helped in minimizing friction during instrumentation, removing inorganic debris and smear layer from the dentinal tubules. In between each file, 5.25% NaOCl was used intermittently to flush dentinal debris out of the canal space. Once prepared, teeth were then dried using F3 paper points. Before obturating the teeth, they were randomly allocated into two sets to be obturated with thermoplasticized gutta-percha using Sealapex sealer in Group I and AH Plus sealer in Group II.

Canal walls were coated with freshly mixed sealer using a paper point. A rubber stopper was placed 4-5 mm short of working length on a 23-gauge Obtura II needle. At the initiation of every obturation, a new gutta-percha pellet was placed into the Obtura II gun. When the temperature of the unit touched 200°C, the premeasured needle was positioned in the canal before expressing 3-4 mm of gutta-percha passively into the canal. Vertical pressure with an endodontic plugger was applied for compaction in the apical area. Increments of 3-4 mm were placed in a similar manner, filling the canal to the orifice. Access cavities were then sealed using Cavit. The teeth were then placed at 37^{o}C, 100% humidity, for one week for the sealer to set completely. After 7 days, the teeth were air dried and except for 1-2 mm around the apex, 2 layers of nail polish were applied to the rest of the root. Specimens were then immersed for 10 minutes in 2.0% methylene blue dye at room temperature, removed, washed and dried (Figure 1). Slow speed diamond saw was used to obtain longitudinal sections of the roots; cutting in the buccolingual direction. After sectioning, the split root segments were observed under a light microscope at X4 magnification. The digital images were captured by a camera connected to the microscope (Olympus CX41 microscope, OLYMPUS CORPORATION, JAPAN) (Figures 2a and 2b). The outcome variable (microleakage around root filling in apical area) was recorded by measuring the amount of dye penetration from coronal side to the apex, (in millimeters).

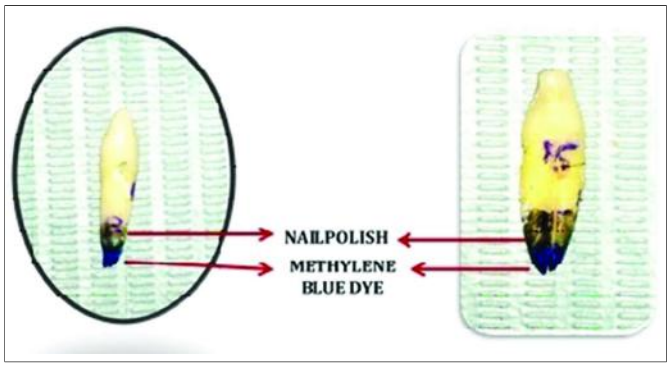

Figure 1: Specimen after application of two coats of nail polish and immersion in methylene blue dye.

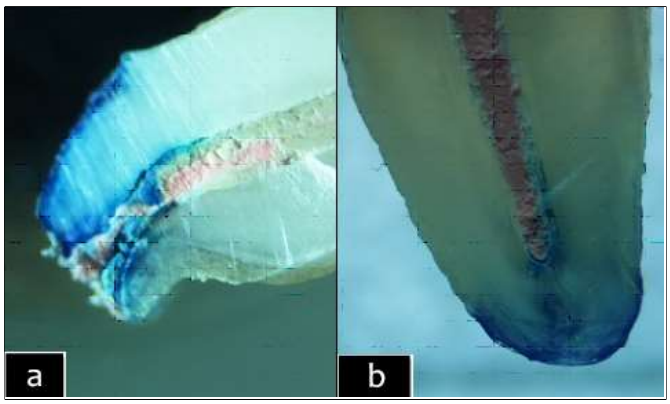

Figure 2: Digital images of specimen as seen under the microscope at 4X magnification:
a. Group I (Obtura II and Sealapex).
b. Group II (Obtura II and AH Plus).

Table I: Apical dye penetration in millimeters according to the arch type.

Study Group	n	Minimum (mm)	Maximum (mm)	Mean (mm)	Standard deviation	p-value
Maxillary	30	0.18	4.40	1.58	1.06	0.87
Mandibular	40	0.00	3.68	1.54	1.04	

Independent sample t-test was applied at 5% level of significance.

Table II: Comparison of dye penetration in the experimental groups.

Study Group	n	Minimum (mm)	Maximum (mm)	Mean (mm)	Standard deviation	p-value
Obtura II & Sealapex	35	0.00	4.40	1.91	1.15	0.003
Obtura II & AH Plus	35	0.00	3.45	1.20	0.79	

Independent sample t-test was applied at 5% level of significance.

Data analysis was done using SPSS 20.0 (by MML and FRK). Mean and SD of the continuous variables were computed. Frequency distribution of the categorical variables was determined. To assess inter-examiner reliability 7/70 (10%) samples were selected at random and assessed by a second evaluator. The inter-examiner reliability was then calculated using Pearson product moment correlation test. Independent sample t-test was used for comparison of microleakage in the two groups. A p-value of <0.05 was considered as statistically significant.

RESULTS

There were 35 specimens in each of the two experimental groups, giving a total of 70 readings. The two groups had 30 maxillary and 40 mandibular teeth. The mean length of maxillary and mandibular teeth was 21.95 ±2.23 mm and 19.69 ±1.89 mm, respectively. As shown in Table I, maxillary teeth exhibited slightly higher dye penetration compared to the mandibular teeth, but the difference was not statistically significant (p=0.87). The mean microleakage around Obtura II-Sealapex group was 1.91 ±1.15 mm. This was higher than the Obtura II-AH plus group, i.e. 1.20 ±0.79 mm (Table II). A statistically significant difference was observed between the two groups (p =0.003). For inter-examiner reliability, 7/70 samples (10%) were randomly selected and assessed for dye penetration by a second evaluator blinded of the original set of readings. The correlation coefficient turned out to be 0.78, (p=0.03) showing an excellent correlation among the measurements done by the two independent evaluators.

DISCUSSION

The present study evaluated sealing ability of two different sealers; AH Plus (resin based) and Sealapex (calcium hydroxide in salicylate base); when used in combination with thermoplasticized injectable gutta-percha as the core obturation material.

Out of the two groups, the higher mean apical leakage in this study was recorded in the Obtura II-Sealapex group. Sealapex has been used along with gutta-percha since 1980's to obturate the root canal space. Numerous studies have been conducted on the microleakage associated with Sealapex and have reported varying results. The seal provided by Sealapex has been found to be adequate at the time of obturation, but deteriorate over time.[7,8] This can be attributed to the higher solubility of the sealer when in contact with tissue fluids. In comparison to the resin based sealers, Sealapex has shown a higher apical leakage, similar to the results of this study.[8]

Obtura II used with AH plus sealer exhibited lesser amount of microleakage values. This can be attributed to the properties of the resin based AH plus sealer. The relatively better mechanical properties of AH plus compared to Sealapex, and more importantly the adhesive nature of the resin based sealer that results in a better bond to dentin explains the better sealing ability of the apical area with this sealer.[9,10] Rather than undergoing setting shrinkage as seen with some sealers, AH plus sealer undergoes up to 1.0% setting expansion suggesting better adaptation and subsequently lesser leakage at the tooth-filling interface. The low solubility of AH plus can also be a reason for its enhanced sealing ability.[11,12]

In contrast, some studies have concluded that both the AH plus and Sealapex sealers resulted in comparable microleakage values with no statistically significant difference in the two groups.[5] Such inconsistencies in outcomes may be as a result of different methodologies and varying sample size in different studies. Pommel *et al*. assessed the apical leakage on the same teeth by three dissimilar methodologies, and concluded that there was a strong influence of the testing technique on the test results.[13]

In the present study, linear measurements from the apical foramen to the coronal level of dye penetration were recorded to quantify the seal provided in the two experimental groups. Various other techniques reported in literature for microleakage assessment of obturated root canals include fluid filtration, bacterial and glucose penetration, radioisotope penetration and scanning electron microscopy. According to Wu *et al*.,[14] over 80% of leakage studies related to endodontics have employed radioisotope penetration or dye penetration.

When using dyes, 2.0% methylene blue is favored for microleakage evaluation for its cost effectiveness and easier to perform with minimum of armamentarium. Methylene blue was favored as its molecular size is analogous to a bacterial by-product, i.e. butyric acid, that is said to leak from diseased root canals, leading to periapical irritation.[15,16]

In this study, incremental obturation was carried out, with the thermoplasticized gutta-percha compacted vertically in between every increment so as to decrease the voids within the gutta-percha mass and at the gutta-percha-dentine interface that might otherwise form because of shrinkage of α-gutta-percha on cooling.

In this study, the amount of dye penetration was measured in millimeters on calibrated digital images captured by a camera connected to the microscope. This quantitative measurement aids in precise assessment of microleakage as evaluated by the penetration of dye through the apical foramina. Comparable methodology for measurement of dye penetration has been done in several other studies.[15,17-20] Variations to the methodology have been suggested and performed whereafter immersion of obturated root canals in the dye, the teeth were treated with nitric acid to demineralize the tooth, thereby achieving transparency and permitting a better three-dimensional evaluation of dye penetration.[4,5,21,22] Instead of longitudinal section, a few authors have suggested transverse sectioning of the roots at predetermined distances from the apex to assess the dye penetration at each level.[23,24] Transverse sectioning is considered as more damaging than longitudinal sectioning. Although it allows a better visualization of the dye penetration, the objective measurement of dye penetration in these sections is more difficult to measure with accuracy. The tracer dye may also be lost as a result of coming in contact with the different solutions used for clearing of teeth.[25]

The present study addressed a relevant research question regarding the decision making of obturation. For obturation of single-rooted teeth where thermo-plasticized obturation is planned, we recommend AH plus as the preferred sealer.

CONCLUSION

Within the limitations of this study, Obtura II with AH plus sealer was a superior combination than Obtura II with Sealapex in terms of dye permeability.

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ORIGINAL ARTICLE

Is Hepatovenocaval Syndrome a Different Entity from Budd-Chiari Syndrome in Children?

Nadia Waheed, Huma Arshad Cheema, Hassan Suleman, Iqra Mushtaq, Zafar Fayyaz and Nadeem Anjum

ABSTRACT

Objective: To differentiate between clinical and demographic spectrum, and outcome in hepatovenocaval syndrome (HVCS) and Budd-Chiari syndrome (BCS).

Study Design: Descriptive study.

Place and Duration of Study: Division of Pediatric Gastroenterology, Hepatology and Nutrition, The Children Hospital, Lahore, from January 2014 to January 2017.

Methodology: All children less than 18 years of age, presenting with ascites and visible veins over abdomen, flanks and back were enrolled in the study. Real time Doppler Ultrasonogram was performed in all children for documentation of intra-hepatic part of IVC obstruction along with or without hepatic venous obstruction. Children meeting inclusion criteria underwent liver profile, coagulation profile, diagnostic paracentesis for SAAG gradient, and Gadolinium enhanced multiphasic MR scan. Liver biopsy and venography was performed in selected patients.

Results: A total of 92 children presented with ascites, among them 58 children met our inclusion criteria. Intrahepatic IVC obliteration, i.e. HVCS, found in 67% (n=39) and hepatic venous outflow obstruction, i.e. BCS was found in 33% (n=19) children. Children with BCS were older than HVCS with mean age of 9.5 ±2.58 versus 4.12 ±0.977 years. HVCS group had 14 boys and 24 girls with a ratio of 1:1.8, while BCS had a ratio of 1:0.9 with 10 boys and 9 girls. No etiological factor was found for HVCS, while most of patients with BCS had a procoagulant disorder. Caudate lobe hypertrophy was a consistent feature in BCS, while IVC obstruction was found in HVCS persistently. Orthotopic liver transplant was needed in three cases (7.6%) of HVCS and four (20.96%) of BCS cases. Antibiotic therapy has a good role in HVCS, while anticoagulation and diuretics had good result in BCS.

Conclusion: Hepatovenocaval syndrome (HVCS) mostly affected younger children, especially girls. BCS usually affected older age groups with pro-coagulant disorders who responded to anticoagulation and diuretic. Further studies are needed to compare both conditions.

Key Words: *Budd-Chiari syndrome. Hepatovenocaval syndrome. Ascites. Intrahepatic vena cava obstruction. Liver transplant.*

INTRODUCTION

Hepatovenocaval syndrome (HVCS) is the term used to describe the disease of hepatic portion of the inferior vena cava (IVC). It is a chronic disease with insidious onset characterised by complete obliteration of hepatic portion of inferior vena cava and development of cavo-caval collaterals. It is a different entity from Budd-Chiari syndrome (BCS), which is hepatic venous outflow obstruction.[1]

It is proposed that the hepatic portion of inferior vena cava gets affected by recurrent thrombophlebitis.[2] Initially, there is localised thrombophlebitis in IVC, followed by resolution which transforms the lesion into stenosis or complete obstruction. Gradually, it extends to involve the whole of the hepatic portion of inferior vena and hepatic veins too. The pertinent feature of hepato-venocaval syndrome is the occurrence of recurrent acute exacerbation precipitated by clinical or subclinical bacterial infection.[3] During acute exacerbation, fresh thrombus is deposited at the site of the lesion in inferior vena cava resulting in ischemic damage to the liver. This presents with mild derangements of liver functions, ascites and fever. This acute exacerbation may be subclinical and frequently unrecognised or mis-diagnosed.[4] When thrombus gets resolved, it either converts into fibrous band or organised and cause stenosis of that part of IVC, development of collaterals anastomosis, so that obstructive signs are not evident. Deep cavo-caval collaterals form as dilated ascending lumbar, azygos, and hemiazygos veins, best visualised by cavogram.[5] Superficial cavo-caval collaterals with upward flow are seen as dilated superficial veins in the body trunk. Intra-hepatic collaterals also develop between obstructed and patent intra-hepatic veins. Recurrent acute exacerbation results in ischemic damage to the liver ending in liver cirrhosis.[6]

Primary Budd-Chiari syndrome is an obstruction of hepatic veins or the terminal portion of the inferior vena cava.[7] This is a rare well known entity as compared to hepatic vena-caval syndrome. It is caused by multiple

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factors, including acquired and inherited thrombophilia. Budd-Chiari syndrome has variable presentation depending upon rapidity of hepatic venous obstruction and formation of collaterals.[8] When all hepatic veins get thrombosed at a time with no time for collateral formation, it presents with rapid development of ascites causing abdominal pain, tender hepatomegaly, deranged liver function tests and renal function tests.[9] Chronic form is the most common form characterised by progressive ascites resembling chronic liver disease. Fulminant form of Budd-Chiari syndrome is an uncommon presentation; it is characterised by fulminant hepatic failure along with ascites, tender hepatomegaly, jaundice, and renal failure.[10]

As both conditions may share the initial presentation, the aim of this study was to differentiate clinical and demographic spectrum, and outcome in hepatovenocaval syndrome and Budd-Chiari syndrome.

METHODOLOGY

It was a descriptive study conducted at Division of Pediatric Gastroenterology, Hepatology and Nutrition, The Children Hospital, Lahore, from January 2014 to January 2017. All children of either gender, age less than 18 years, presenting with ascites and visible vein over flanks, abdomen and or back, were enrolled in the study, and underwent Doppler ultrasound. All children with ascites underwent real time Doppler examination. Children with obstruction or thrombosis of hepatic part of vena cava were included as HVCS, and of hepatic veins were included as BCS in the study. Children with ascites of causes other than either HVCS or BCS were excluded from study. Informed written consents were taken from children's guardian after explaining the purpose, risks, and benefit of the study.

Bio-data, socioeconomic status, onset and duration of symptoms and detailed general physical and systemic examinations were noted on a proforma. All laboratory tests were performed to all patients within hospital and free of cost. Gadolinium enhanced multiphasic MRscan were performed in children meeting inclusion criteria. Children with normal coagulation profile underwent percutaneous liver biopsy. Diagnosis of liver cirrhosis was based on transformation of the liver parenchymal echo-texture from normal to uniformly coarse with irregular edges, confirmed by biopsy. Venography was performed in some of the patients when there was doubt whether non-visualisation of IVC and/or hepatic vessels are due to compression of vessels by hepatic cirrhosis or obliteration of vessels. Procoagulant deficiency was evaluated in all patients. Thrombophilia profile including antiphospholipid antibody, protein C, protein S, antithrombin III (AT-III) assays, serum homocysteine levels, factor V Leiden mutation, paroxysmal nocturnal hemoglobinuria profile, lupus anticoagulant, JAK-2 mutation were performed in all children. Deficiency of Protein C, S and AT-III in the presence of chronic liver disease were defined as patient having positive family history with low level of factors and/or significant low levels of one factor with normal level of other factors. Antibiotics were added to patients with HVCS for six weeks, three weeks intravascular and three weeks per oral. Patients with BCS were started on diuretic therapy and those with procoagulant disorder were started on heparin, followed by warfarin.

All qualitative data were expressed as frequencies and percentages, while quantitative data were expressed as mean and standard deviation. Data were analysed on SPSS version 20.

RESULTS

Ninety-two children were enrolled in the study and 58 children met the inclusion criteria. HVCS was documented in 67% (n=39) children and BCS was documented in 33% (n=19) children. In BCS, children were older as compared to HVCS with mean ages 9.5 ±2.58 and 4.12 ±0.977 years, respectively ($p<0.05$). HVCS had female dominance with M:F ratio of 1:1.8 (14 boys to 25 girls) while in BCS both genders had almost equal distribution, i.e. 1:0.9 (10 boys to 9 girls, Table I).

Majority of patients with HVCS presented with end-stage liver disease characterised by clubbing (89.7%, n=35), palmar erythema (87%, n=34) height and weight below third centiles (94.8%, n=37), and deranged synthetic functions of the liver. Radiological findings in both syndromes are shown in Figure 1. Venocavography was performed in four patients, one diagnosed as BCS while three were diagnosed as HVCS. Venocavography in BCS revealed obliteration of hepatic veins and IVC was compressed by caudate hypertrophy but it was patent. In

Table I: Demographic, clinical & laboratory parameters in HVCS & BCS.

Parameters	HVCS1 (n=39)	BCS2 (n=19)
Age (years)	9.5 ±2.58	4.12 ±0.977
Male	10	14
Female	09	25
M:F	1:0.9	1:1.8
Mode of presentation		
Fulminant	0	5.2% (1)
Acute	0	0
Sub-acute	0	63% (12)
Complication of CLD3	100% (39)	31.5% (6)
Consanguinity	10% (4)	80% (15)
Failure to thrive	76.9% (30)	58% (11)
Poor Socio-economics	85% (33)	31.5% (6)
Pro-coagulant disorder	0	47% (9)
Protein C deficiency	0	5% (1)
Protein S deficiency	0	11% (2)
Anti-thrombin III deficiency	0	26% (5)
Factor V mutation	0	5% (1)

1. HVCS-Hepatovenocaval syndrome
2. BCS-Budd Chiari syndrome
3. CLD Chronic liver disease

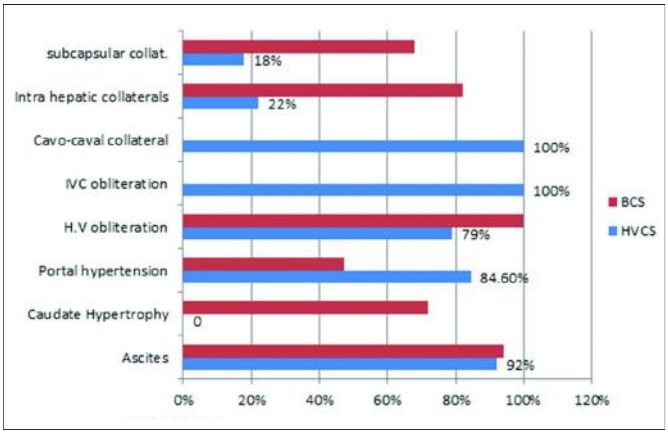

Figure 1: Radiologic findings in HVCS and BCS.

HVCS intra-hepatic part of IVC, it was obliterated along with hepatic veins; and infra-hepatic part was connected to supra-hepatic part of IVC via cavo-caval collaterals.

Liver biopsy (percutaneous) was performed in 10 cases, five cases each of BCS and HVCS. In hepatovenocaval syndrome, there was periportal inflammation of variable degrees and fibrous bands between portal tracts; some of them were having cirrhotic nodule formation. None of them showed hepatocellular carcinoma. In BCS, histo-pathology revealed dilated sinusoids and peri-sinusoidal congestion. There was peri-venular inflammation and ballooning degeneration of hepatocytes. All cases of HVCS had turned into chronic liver disease at time of presentation with waxing and waning ascites, deranged coagulation profile, and coarsening of liver texture on USG.

Three (7.6%) HVCS children underwent successful orthotropic liver transplant. Fifteen (38.46%) were lost to follow-up. Twenty-one (53.84%) patients of HVCS are still on liver supportive treatment.

On the other hand, in BCS, one (5.26%) child presented with fulminant BCS; and three (15.7%) having chronic liver disease (CLD) with Child Pugh C, underwent orthotropic liver transplant. Nine (47.36%) presented with sub-acute form having pro-coagulant disorder, were started on anti-coagulant therapy and did well. Six (31.57%) cases of BCS were lost to follow-up.

DISCUSSION

Hepatovenocaval syndrome and Budd-Chiari syndrome are vascular diseases of the liver. Budd in 1846 described a syndrome of abdominal pain, hepatomegaly, and ascites. Later, Chiari explained the etiology of interruption of blood flow anywhere between hepatic veins to the IVC draining into the right ventricle. So the term Budd-Chiari syndrome was coined. Initially, BCS was used to describe all types of vascular diseases including sinusoidal obstruction and veno-occlusive syndrome. Afterwards, a debate started over the nomenclature of Budd-Chiari syndrome depending upon involvement of hepatic veins, terminal venules, and vena cava.[11] Obliteration of hepatic portion of inferior vena cava once thought as a type of Budd-Chiari; but now, it is considered as separate entity.[12]

In this study, more cases of HVCS were found in children as compared to BCS. Shrestha found hepatovenocaval syndrome to be more prevalent in Nepal and other developing countries, while Budd-Chiari syndrome is the problem of Western world.[13] All children with HVCS were of younger age group and presented with end-stage liver disease. The youngest was three years of age presented with hematemesis due to portal hypertension. Doppler USG showed no flow in hepatic IVC. Later, venocavography confirmed that hepatic part of IVC was completely obliterated and infra-hepatic part of IVC was connected to supra-hepatic part deep para vertebral cavo-caval venous collaterals. This is consistent with Shrestha's findings who reported that HVCS affects in early childhood.[14] Although literature shows that in HVCS, both genders are equally affected; but in this study, there was a female preponderance. All of our children with HVCS presented with chronic liver disease as mentioned in another study; but none of them having hepatocellular carcinoma.[14] While in BCS, children were older as compared to HVCS with no gender predilection and had different mode of presentation, most common being sub-acute BCS.

No etiology was found for HVCS and all cases were labelled as idiopathic. This is in accordance to the known data,[16] though others have shown their relation to the poor socioeconomic conditions.[16] In BCS, procoagulant disorders were found to be frequent, and anti-thrombin-III deficiency was the commonest.

In HVCS, ascitic fluid is exudative with high protein content and neutrophil count.[17] All children with HVCS in this study, had ascites with high SAAG gradient as seen in BCS; this is because all of the patients with HVCS had presented with established CLD. Doppler studies, multiphasic MRI scan and histopathological findings are quite different in both groups. Development of cavo-caval collateral anastomosis was main finding in HVCS, while sub-capsular spider web was found in BCS. In this study, caudate lobe hypertrophy was a consistent finding in BCS, not present in HVCS. Literature shows that in 95% cases of BCS, Doppler ultrasonography was diagnostic showing caudate lobe hypertrophy, absence of visualisation of one or more hepatic veins and presence of intra-hepatic or sub-capsular hepatic venous collaterals.[18]

Liver biopsy performed in a few cases showed marked differences in both syndromes. In HVCS, the histopathology revealed chronic liver disease but no sinusoidal dilatation and peri-venular congestion as we found in BCS; also none of the biopsy result was

consistent with hepatocellular carcinoma (HCC), as reported in literature.[19] All children with HVCS had developed chronic liver disease at the time of presentation with portal hypertension, deranged coagulation profile, and coarse liver texture. They had established obstruction of hepatic part of IVC; with the use of antibiotics, their Child score improved and the disease remained as compensated CLD for long. Studies have shown that it is the acute exacerbation that leads to hepatic decompensation.[20] Prophylactic antibiotics will help or not, in maintaining compensated liver disease, needs further studies.

CONCLUSION

BCS usually affects older children and procoagulant disorder is main underlying etiology. Caudate lobe hypertrophy is most consistent imaging finding for BCS. HVCS was found in younger age group with female dominance. Stenosis of hepatic portion of IVC is best documented on multiphasic MR scan of liver. HVCS is preventable cause of liver cirrhosis. If it is picked up early, further damage to the liver can be arrested.

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ORIGINAL ARTICLE

Shortened Preoperative Fasting Time to Allow Oral Rehydration Solution Clear Liquid up to Two Hours before Elective Major Surgery in Adults

Jay N. Shah, Shantabir Maharjan and Rajan Gurung

Abstract

Objective: To generate evidence of feasibility to allow clear liquid 2 hours before elective surgery.

Study Design: Cross-sectional observational study.

Place and Duration of Study: The Department of Surgery, Patan Hospital, Patan Academy of Health Sciences, Nepal, from October to December 2016.

Methodology: One hundred consecutive adult elective major surgery patients of American Society of Anesthesiologist criteria 1 or 2 were enrolled. The protocol was discussed with patients, nurses, anesthetists and surgeons to allow 500 ml clear liquid (ORS) up to 0600 hours on the day of surgery to maintain minimum of 2 hours (h) nil per os (NPO) before surgery. Compliance, discomfort, nausea and vomiting were observed. Institutional review committee approved the study. Microsoft excel was used for descriptive analysis.

Results: All 100 patients completed the protocol of shortened fasting time. Two patients had incomplete records and were excluded from analysis. Among the 98 patients analysed, age was 48 ±12.38 years with 74 females (75.51% of 98). There were 68 gastrointestinal, 20 urosurgery and 10 others surgeries. There was no discomfort, nausea or vomiting reported due to ORS 2-h before elective surgery.

Conclusion: Preoperative clear liquid up to 2-h before elective surgery in adults is feasible and safe in our set-up to shorten the fasting time.

Key Words: *Clear liquid. Major elective surgery. Nil per os (NPO). Oral rehydration solution (ORS). Preoperative NPO. Shortened fasting time.*

INTRODUCTION

Preoperative fasting was advocated by Mendelson in 1946 to prevent aspiration.[1] Malt in 1986 questioned the midnight nil per os (NPO) practice allowing water 3 hours (h) before surgery.[2] Fasting guideline was modified by Anesthesiologist Societies of America in 1999 and 2011,[3] to allow clear fluids water, fruit juices without pulp, clear tea, black coffee. Similar fasting guidelines were introduced by societies in Europe,[4,5] and Scandinavia,[6] etc. to allow free oral intake of clear liquid up to 2-h before elective surgery.

In Asia, midnight NPO is the norm. A nationwide study from Japan found 9-h median fasting.[7] Audit of 152 ASA-I elective non-obstetric surgery patients at a tertiary care teaching hospital in India reported mean fasting of more than 12-h for clear fluid and 14-h for solid,[8] despite other Indian researchers,[9] having questioned the midnight NPO practice. Sri Lankan study also reports fasting of 13-h for solids and 12-h for liquids.[10] Shortened fasting guideline is yet to become a reality in Asia. Reports are limited in the Asian region on protocol and practice of enhanced recovery after surgery (ERAS) and fast tract surgery (FTS).[11-13] ERAS is a multidimensional approach of care during perioperative period to achieve early recovery, maintain optimum organ function, reduce stress and discomfort to the patients. ERAS protocol includes, but is not limited to counseling, nutrition, analgesics, and anesthetic regimens and early mobilisation.

Shortened fasting allowing oral clear liquid improves subjective well-being of patients by decreased thirst, nausea, vomiting, anxiety; and carbohydrate clear fluid decreases insulin sensitivity and metabolic response to surgical trauma.[14-18] The 'oral rehydration solution (ORS) with glucose and electrolyte is easily available, inexpensive and safe to shorten the preoperative fasting time.[19,20] At our tertiary care university teaching hospital, midnight NPO is routine, like most other institutions. In this study, the aim was to generate local evidence for acceptance, compliance, and feasibility of shortened preoperative fasting to allow clear liquid in elective surgery patients as a component of ERAS protocol.

METHODOLOGY

This cross-sectional observation was conducted in the Unit Two, Department of Surgery, Patan Hospital, Patan Academy of Health Sciences (PAHS), Nepal, from

Department of Surgery, Patan Hospital, Patan Academy of Health Sciences, Lalitpur, Kathmandu, Nepal.

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October to December 2016 with the intention to include 100 consecutive adult patients, age above 14 years, admitted for elective surgeries. Patients were counselled about preoperative fasting by the admitting surgeon in the Outpatient Department (OPD) before admission. Informed consent was obtained in the OPD at the time of admission during routine patient counselling for surgery. Admitting surgeon wrote the NPO instruction in 'Dr's order sheet' for nurses in the patient chart. The hospital policies require all mentally capable adult patients, 14 years or above as per hospital policy to provide consent themselves. The consent is further counter-signed by patient family member and the doctor who obtain the consent. Patients were advised not to take food by mouth after 2100 hours before surgery. They were advised to take ORS, a glucose and electrolyte containing clear fluid till 0600 hours to ensure 2-h of NPO before first list of surgery at 0800 hours.

The protocol of shortened fasting time was discussed in the morning department conference, attended by surgeons and students. This was further briefed to the anesthetist and surgery ward nurses about the change in midnight NPO. Written notice was posted in surgery ward so that all nurses and doctors were familiar with these changes in preoperative fasting time. Nurses were requested to obtain a sachet of ORS (locally popular as 'Jeevan Jal') from the hospital pharmacy and dissolve in a liter of bottled clean water. Nurses instructed and ensured that patients consumed 500 ml of ORS between 2100 hour and 0600 hour before surgery.

All elective surgery patients of American Society of Anesthesiologist (ASA) criteria 1 or 2 were included. Patients who did not give consent, with known diabetes mellitus, ASA 3 or more, gastro-intestinal obstruction, pregnant, were excluded. The study was approved from the Institutional Review Committee of PAHS. Other perioperative protocols remained the same as per Hospital policies for the particular surgeries.

Data were collected as per predesigned proforma together with the consent, mentioning change in preoperative NPO for the study and surgery, and were kept in patient's chart during the admission in the OPD and later re-checked by ward nurses in the surgery department.

Two coauthors (SM and RG) were in-charge to oversee that the proforma was completed daily for each patient fulfilling the inclusion criteria. Proforma included: study variables of patient age, gender, ASA grade, types of surgery and anesthesia. Other variables specific to changes in NPO were recorded by nurses for patients' compliance to drink ORS and complain of nausea, bloating, vomiting before shifted to operation theatre. Anesthetists recorded vomiting or aspiration during induction of anesthesia. In-hospital mortality was recorded. Microsoft excel was used for descriptive analysis as percentage, mean and standard deviation (SD).

Table I: Preoperative clear liquid (ORS- oral rehydration solution) up to 2-h before elective surgery in adults (n=98).

Variables		N (98*)	%
Surgeries	**Abdomen GI**	68	69.4
	Cholecystectomy	54	
	Oesophageal cancer	1	
	Gastric cancer	1	
	Ileostomy closure	1	
	Others		
	Uro-surgery	20	20.4
	Open pyelolithotomy	2	
	MPCNL	6	
	TURP	6	
	URSL	4	
	Others	2	
	Miscellaneous surgery	10	10.2
	Inguinal hernia repair	6	
	Others	4	
Patients	Male	26	24.5
	Female	74	75.5
	Finished 500 ml ORS before 0600 h	98	100
	Before shifting to OR nausea, bloating, vomiting	0	0
	In OR- nausea, vomiting during induction	0	0

Note: OR = Operation room; GI = Gastrointenstinal; MPCNL = Mini percutaneous nephrolithotomy; TURP = Trans urethral resection of prostate; URSL = Ureterorenoscopy lithotripsy.
**Out of 100 patients, two were excluded from analysis because of incomplete data entries.*

RESULTS

There were total of 98 patients' records for final analysis (out of 100, data were incomplete in two thus were excluded). Youngest patient was 15 and oldest 65 years of age; mean being 48 $\pm$12.38 years. Female were 74 (75.51% of 98). Type of surgical procedure is given in Table I. All 98 patients completed the planned protocol to drink 500 ml ORS before 0600 hours on the day of surgery to maintain at least 2-h NPO before surgery. In the surgery department, there was no discomfort reported by patients after ORS ingestion before going to operation theater. In the operation theatre, the incidence of vomiting or aspiration during induction of anesthesia was nil. There was no in-hospital mortality in this series.

DISCUSSION

All 98 patients out of 100 (two had incomplete data thus were excluded from analysis) completed the planned ingestion of ORS safely without discomfort, before and during induction of anesthesia. There was no recorded incidence of bloating, nausea, and vomiting after ingestion of 500 ml of ORS between 2100 and 0600 hours before the elective surgery. Similarly, there was no remarkable nausea, vomiting during the induction of anesthesia in those patients who were encouraged and allowed ORS until 2-h before surgery as part of ERAS to change the existing protocol of routine midnight NPO. This finding adds local evidence to the confidence of the nursing staff, surgeons, anesthetists and patients that there is no increased risk of nausea, vomiting; and we

can safely allow clear liquid at least until 2-h before elective major surgery in healthy patients of ASA-1 and ASA-2 without diabetic.

ORS was used as oral clear fluid to standardise the study. The compliance from health professionals and patients in this study was good and all patients completed the protocol to ingest 500 ml of ORS during 2100 and 0600 hours before the day of elective surgery. The Japanese multicenter randomised trial, including six hospitals and 300 patients (150 in each group), reported that patients safely consumed one liter ORS containing balanced glucose and electrolytes from 2100 hours till 2-h before surgery.[19] They concluded that compared to the patients who fasted from 2100 hours, gastric fluid volume immediately after anesthesia induction was similar, 14 ml in ORS vs. 23 ml in fasting group (p=0.30), and ORS group reported less thirst and hunger before surgery (p<0.001, and 0.01). They concluded ORS to be safe and feasible until 2-h before surgery and physicians should use this practice to maintain the body fluid and electrolytes to improve the patient's comfort. Other studies have also reported ORS as safe and effective alternative to shorten preoperative fasting time and replace water, electrolytes and body fluid with reduced thirst, hunger, anxiety, and shortened hospital stay.[13,20]

In western countries, most protocols have hospital stay and cost as endpoints; but in Asian societies, social acceptance by patients and their families play important role. Satisfaction of family members is major consideration, because they are the one who take care of patients during hospital admission and also after the discharge. The main objective of present study was to observe the acceptance of change in preoperative feeding from routine midnight NPO to allowing ORS till 2-h before elective surgeries. All other protocols of surgery remained same as per existing hospital policies. In this study, complete ERAS protocol was not analysed, rather the objective was to reproduce the safe introduction of already practised preoperative NPO in most of the western countries,[3-6,11-20] to allow clear liquid until 2-h before elective surgery.

Cultural and social acceptance and hospital care in Asian countries are different. Besides hospital stay and cost, the team dynamics, patient satisfaction, reduced workload of nurses play important role in modification of clinical practices, like 'modified-ERAS'.[13] Evidence-based practice and local modification of internationally accepted guidelines are a continuous process of good clinical practice. In developing countries like Nepal, the cost of hospital stay constitutes only 2-5% of total costs of surgeries. For example, the average total cost, admission to discharge, for laparoscopic cholecystectomy is approximately $250. And, one day hospital stay in general ward is $2.5 including doctor's visit and nursing care. Out of pocket payment is the norm.[21] This is likely the scenario in the public hospitals in developing countries. Simply thinking in line to 'reduce hospital stay', do not have same value of 'cost reduction' like in developed economy. Patients tend to stay in hospital longer due to lack of support system of transportation, community nurses and general practitioner. We need to continuously upgrade our clinical practices for the benefit of patients. Modification of ERAS protocol for local adaptation is feasible without compromising safety. For example, in line with the concept of evidence-based medical practice and ERAS, in case of cholecystectomy, routine antibiotic prophylaxis are not given in low risk laparoscopic surgeries and allow clear liquid and stop IV fluid four hours after laparoscopic cholecystectomy.[22,23] Simiarly, with aim to change the 'routine' clinical practice, and extension of ERAS, we have now stopped 'routine hospital visit after day case inguinal hernia surgery in children' to decrease the unnecessary discomfort to the children and parents and to add to the benefit of the day case surgery.[24]

In the present study, the gastric fluid or postoperative blood glucose was not monitored just for research purpose to add extra cost to the patients. The safety of ORS has been well documented by Japanese researchers to shorten fasting time.[13,19,20] Taniguchi *et al*. reported successful results of preoperative to shorten fasting time, fluid management, increased patient satisfaction and improved nursing services as modification of ERAS protocol in their ESSENSE (essential strategy for early normalisation after surgery with patient's excellent satisfaction) project.[13] They further argued that ORS was good substitute for high cost commercial glutamine drink. There have been studies in Asian population on use of commercially available carbohydrate drinks containing glutamine to decrease insulin resistance and improve glucose metabolism.[11,12,17,18,25]

The gap between clinical practice and evidenced-based guidelines depends on many factors. The organisational, financial, cultural as well as perception of patients and health workers all need to be taken in to consideration to modify clinical practices for the benefit of patients.

The clinical implication based on locally generated evidence is obvious than protocol generated in Western advanced countries. After successful observation of present study, the authors have now changed their practice in surgery department to allow clear liquid (water, black tea with sugar, ORS) till 0600 hours to maintain 2-h NPO before the first list of the day. It still needs to be worked out as how to decrease the fasting time for cases later in the day. The successful observation of change in midnight NPO practice to allow oral clear liquid in the form of ORS, can be further modified with other 'clear liquid' until 2-h before elective

surgeries to shorten fasting time in line with the internationally accepted guidelines and evidence-based medicine.

CONCLUSION

Preoperative clear liquid, oral rehydration solution (ORS), up to 2-h before elective surgery in adults is feasible and safe to decrease the fasting time in line with international guidelines.

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ORIGINAL ARTICLE

Effectiveness of Vascular Markers (Immunohistochemical Stains) in Soft Tissue Sarcomas

Namra Naeem, Sajid Mushtaq, Noreen Akhter, Mudassar Hussain and Usman Hassan

Abstract

Objective: To ascertain the effectiveness of IHC markers of vascular origin like CD31, CD34, FLI1 and ERG in vascular soft tissue sarcomas including angiosarcomas, Kaposi sarcomas, epithelioid hemangioendothelioma and a non-vascular soft tissue sarcoma (Epithelioid sarcoma).

Study Design: Descriptive study.

Place and Duration of Study: Shaukat Khanum Memorial Cancer Hospital and Research Centre, Lahore, from 2011 to 2017.

Methodology: Diagnosed cases of angiosarcomas (n=48), epithelioid hemangioendothelioma (n=9), Kaposi sarcoma (n=9) and epithelioid sarcoma (n=20) were selected. Immunohistochemical staining as performed on formalin fixed paraffin embedded sections. The sections were stained for the following markers: CD34 (VENTANA clone Q Bend 10), CD31 (Leica clone 1 A 10), FLI1 (CELL MARQUE clone MRQ-1) and ERG (CELL MARQUE clone EP111).

Results: A complete panel of CD34, CD31 and ERG was applied on 8/48 cases of angiosarcomas with triple positivity in 6 cases. Eight cases showed positivity for only CD31 and ERG and 2 cases showed positivity for only ERG. A complete panel of CD34, CD31 and ERG was applied on 3/9 cases of epithelioid hemangioendothelioma with positivity for all markers in 2 cases. Combined positivity for ERG and CD34 was seen in 2 cases and on 4 cases only CD31 immunohistochemical was solely applied with 100% positivity. FLI1 was not applied on any case. Among 9 cases of Kaposi sarcoma, ERG, CD34 and CD31 in combination were applied on only 1 case with triple positivity. Remaining cases show positivity for either CD34, CD31 or FLI1. Majority of cases of epithelioid sarcomas were diagnosed on the basis of cytokeratin and CD34 positivity with loss of INI1. The other vascular markers showed negativity in all cases.

Conclusion: Among these four markers, ERG immunohistochemical stain is highly effective for endothelial differentiation due to its specific nuclear staining pattern in normal blood vessel endothelial cells (internal control) as well as neoplastic cells of vascular tumors and lack of background staining.

Key Words: *Immunohistochemical stains. Angiosarcoma. Kaposi sarcoma. Epithelioid hemangioendothelioma. Epithelioid sarcoma.*

INTRODUCTION

Soft tissue sarcomas are rare and diverse group of neoplasms, both anatomically and histologically. They arise in the connective tissues throughout the body and account for 1% of adult and 15% of childhood malignancies.[1] Subtyping of soft tissue sarcomas is based on morphology, immunohistochemistry and genetic testing. Assigning a soft tissue sarcoma to vascular group is based primarily on morphology and it is not difficult to suspect in many cases. However a large number of tumors require immunophenotype for diagnosis.[2,3] Advance developments in immunohistochemistry have improved the diagnostic accuracy of soft tissue sarcomas.

Commonly available markers to ascertain vascular origin are CD31, CD34, FLI1 and ERG.[3,4] None of these markers is entirely specific for vascular origin and show positivity in majority of other sarcomas and carcinomas.[5-8]

The aim of this study was to ascertain the effectiveness of these markers vascular soft tissue sarcomas including angiosarcomas, Kaposi sarcomas, epithelioid hemangioendothelioma and non-vascular soft tissue sarcoma (epithelioid sarcoma).

METHODOLOGY

It was a descriptive study carried out on cases of angiosarcomas (n=48), epithelioid hemangioendothelioma (n=9), Kaposi sarcoma (n=9), and epithelioid sarcoma (n=20) selected from the archives of Shaukat Khanum Memorial Cancer Hospital and Research Centre at the Department of Histopathology from 2011 to 2017. For the sample selected separately for these tumors, their immunohistochemical profiles were reviewed and analysed for the comparison of vascular markers used in their diagnosis.

The Immunohistochemical stains on these cases were performed on formalin fixed paraffin embedded sections. The sections were stained for the following markers: CD34 (VENTANA clone Q Bend 10), CD31 (Leica clone 1 A 10), FLI1 (CELL MARQUE clone MRQ-1) and ERG (CELL MARQUE clone EP111). The sections for Leica,

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CELL MARQUE and VENTANA were respectively deparaffinised in Bond dewax solution and EZ prep solution. Antigen retrieval was performed in Bond ER2 (Ph 9.0) and Cell Conditioning 1 (Ph 8.0) in automated stainer (Bond 111) and VENTANA Benchmark XT. Staining was performed with Bond Polymer detection kit and Ultra view DAB detection kit. The sections were developed with diaaminobenzidine tetrahydochloride and counterstained with hematoxylin. The prepared slides were reviewed by two histopathologists having special interest in soft tissue pathology, including the primary author.

All the findings were entered and analysed by SPSS-20. Mean and standard deviations were calculated by applying descriptive statistics for numerical study variables such as age; while qualitative variables like gender, site of sarcomas, and staining outcomes were given in the form of frequency and percentage.

RESULTS

A total of 86 patients, presenting with sarcomas, were taken. Of these 86 patients with sarcoma, 45 (52.32%) were males while 41 (47.68%) were females. The mean age of the study cases was 42.39 ±16.58 years (range; 4 years to 80 years). Among these 86 patients, angiosarcomas were noted in 48 (55.8%) patients including 27/48 (56%) females and 21/48 (44%) males with a mean age of 49.14 ±9.18 years (range 22-77 years).

Kaposi sarcoma 9 (10.4%) patients included 6/9 (67%) males and 3/9 (33%) females with an age range of 40 to

Table I: IHC staining of angiosarcomas (n=48).

IHC stains	No. of cases on which IHC applied	No. of positive cases	No. of negative cases	Percentile positive
CD34	23	15	8	65.22%
CD31	37	28	9	75.68%
ERG	18	18	0	100%
FLI1	9	8	1	88.89%

Table II: IHC staining of epithelioid hemangioendothelioma (n=9).

IHC stains	No. of cases on which IHC applied	No. of positive cases	No. of negative cases	Percentile positive
CD34	5	5	0	100%
CD31	7	6	1	85.71%
ERG	4	4	0	100%
FLI1	0	0	0	-

Table III: IHC staining of Kaposi sarcoma (n=9).

IHC stains	No. of cases on which IHC applied	No. of positive cases	No. of negative cases	Percentile positive
CD34	8	8	0	100%
CD31	4	3	1	75%
ERG	2	2	0	100%
FLI1	1	1	0	100%

Table IV: IHC Staining of epithelioid sarcoma (n=20).

IHC stains	No. of cases on which IHC applied	No. of positive cases	No. of negative cases	Percentile positive
CD34	12	11	1	91.67%
CD31	2	0	2	0%
ERG	2	0	2	0%
FLI1	1	0	1	0%

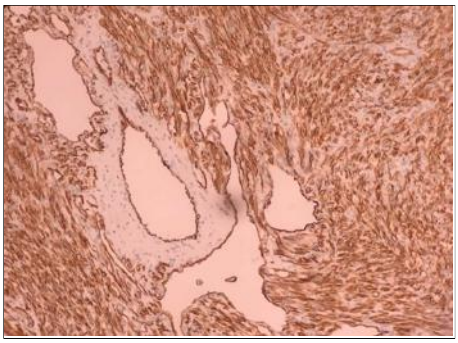

Figure 1: Immunohistochemical stains in vascular tumors CD34 expression in Kaposi sarcoma at 20X magnification. Note the characteristic membranous expression in tumor cells and blood vessels.

Figure 2: CD31 expression in epithelioid hemangioendothelioma at 10X magnification.

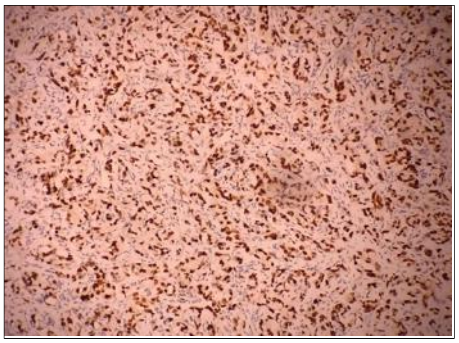

Figure 3: ERG expression in angiosarcomas at 20X magnification. Note the characteristic nuclear pattern of expression in tumor cells.

80 years; while their mean age was 56.82 ±8.94 years. Nine patients (10.4%) of epithelioid hemangioendothelioma with a mean age of 32.54 ±13.62 years (range 4-60 years) included 5/9 (56%) females and 4/9 (44%) males. For epithelioid sarcoma, Twenty patients (23.25%) with 14/20 (70%) males and 6/20 (30%) females were selected with age range of 16 to 54 years and their mean age was 30.11 ±6.82 years.

The cases of angiosarcomas (n = 48) were diagnosed in soft tissues of head and neck region in 25/48 (52%), abdominopelvic region in 12/48 (25%) and upper and lower extremities in 11/48 (23%) of the study cases. For epithelioid hemangioendothelioma, 4 (44.4%) cases were of abdominal region, 3 (33.3%) cases from neck region and 2 (22.3%) cases from upper extremity. The majority of cases, i.e. 5 (55.55%) of Kaposi sarcoma were from lower limb with 2 (22.22%) cases from thoracic region and one case (11.11%) from oral cavity and one (11.11%) from upper extremity. The results of immunohistochemical stains on these four tumors are summarised in Tables I to IV.

For ERG and FLI1, nuclear staining was considered positive. For CD31 and CD34, the cases with membranous/ cytoplasmic staining were considered positive. The staining of CD34, CD31 and ERG are shown in Figures 1-3, respectively.

The results of IHC stains show that among the vascular markers, ERG that has been recently introduced in SKMCH and RC in 2016 as an effective vascular marker, show positivity in all cases of angiosarcomas, Kaposi sarcomas and epithelioid hemangioendothelioma on which it has been applied. However, the two cases of epithelioid sarcoma were negative for ERG making it useful to distinguish this sarcoma from epithelioid angiosarcomas.

A complete panel of CD34, CD31 and ERG was applied on 8/48 (16.6%) cases of angiosarcomas with triple positivity in 6 (12.5%) cases. Eight cases show positivity for only CD31 and ERG, and two cases show positivity for only ERG.

A complete panel of CD34, CD31 and ERG was applied on 3/9 (33.3%) cases of epithelioid hemangioendothelioma with positivity for all markers in two (22.2%) cases. Combined positivity for ERG and CD34 was seen in two cases and on four cases only CD31 immunohistochemical was solely applied with 100% positivity. FLI1 was not applied on any case.

Among the nine cases of Kaposi sarcoma, ERG, CD34 and CD31 in combination were applied on only one case with triple positivity. Remaining cases show positivity for either CD34, CD31 or FLI1.

Majority of cases of epithelioid sarcomas were diagnosed on the basis of cytokeratin and CD34 positivity with loss of INI1. The other vascular markers showed negativity in all cases.

DISCUSSION

Substantial developments in immunohistochemistry, cytogenetics and molecular genetics have caused significant change in the diagnosis and classification of soft tissue sarcomas.[9,10] Soft tissue sarcomas showing endothelial differentiation include angiosarcomas, epithelioid hemangioendothelioma and Kaposi sarcoma.[2]

Angiosarcomas are most common vascular tumors with a peak incidence in 7th decade affecting males more than females. Morphologically, it shows well formed anastomosing vessels to solid sheets of epithelioid to spindle cells without vasoformation. It is associated with radiation exposure, 8q24 mutation, KDR (VEGFR2) mutation and myc amplification.[2]

Epithelioid hemangioendothelioma is a rare tumor with peak incidence in 4th to 5th decade affecting females more than males. It shows angiocentric and infiltrative growth pattern with epithelioid cells arranged in cords and small nests lacking well formed vascular channels. It has indolent behaviour and mutation in WWTR1 CAM1A1 fusion gene on chromosome 1p36.[11,12] Kaposi sarcoma is a low grade neoplasm showing proliferation of vessels, spindle cells and inflammatory cells. It is associated with human herpes virus 8 (HHV8).[13] Epithelioid sarcoma is a rare aggressive cytokeratin positive sarcoma that coexpress CD34. The tumor is multinodular with central necrosis surrounded by polygonal eosinophilic cells and peripheral spindle cells. It shows mutation on chromosome 22q and loss of SMARCB1 INI1.[11,14]

These tumors show positivity for vascular markers CD31, CD34, FLI1 and ERG.[3,4] CD34 (hematopoietic progenitor cell antigen) is a surface glycoprotein and is normally expressed by hematopoietic stem cells, small vessels excluding lymphatics, fibroblasts, interstitial cells of Cajal and dendritic cells.[15] CD31 (Platelet endothelial cell adhesion molecule 1) is expressed in endothelial cells, granulocytes, macrophages and platelets, and T lymphocytes.[16] FLI1 (Friend leukaemia integration-1) is a nuclear transcription factor and is involved in cellular proliferation and tumorigenesis.[17]

Erythroblast transformation specific related gene (ERG) is an ETS family transcription factor – a sensitive marker for endothelial differentiation. It is involved in endothelial cell migration and angiogenesis. It is constitutively expressed in malignant vascular tumors, Ewing's sarcoma (translocation in EWS-FLI1 fusion gene), prostatic adenocarcinoma (translocation in TMPRSS2-ERG fusion gene) and a subset of acute myeloid leukemia. It has a specific nuclear staining pattern and is highly sensitive and specific for diagnosis of vascular tumors.[5,6,18]

All the vascular markers are multispecific because they are expressed in normal as well as neoplastic tissues

with background staining in cells lowering their diagnostic accuracy. The diagnostic utility of CD31 as a vascular marker is partly limited due to its staining in platelets adherent to vessels as well as background macrophages and myofibroblasts lowering its sensitivity.[19] CD34 considered as a less specific vascular marker as compared to CD31, in addition to showing positivity in vascular tumors, also shows positivity in solitary fibrous tumor, dermatofibrosarcoma protuberance and acute myeloid leukemia.[7] FLI1 is also expressed in Ewing's sarcoma, desmoplastic small round blue cell tumors and a subset of melanomas.[8] FLI1 also shows expression in small lymphocytes. Although ERG also shows positivity in prostatic adenocarcinomas and is negative in epithelioid sarcomas, this negativity is helpful in distinguishing this tumor from epithelioid angiosarcoma, although recently its positivity in few cases of epithelioid sarcomas has been described in two studies.[6,20] It shows positivity only in few bone marrow precursors and fetal mesenchyme. Moreover, the specific nuclear staining pattern of ERG is highly characteristic as compared to membranous staining pattern of CD34 and CD31.[5]

In a study conducted by Sullivan *et al*., the utility of ERG, CD34 and CD31 was assessed in 25 cytology cases of angiosarcomas with 100% sensitivity for ERG and CD31 and 60% for CD34.[3] In this study, the percentile positivity of ERG is 100% as compared to 75% and 65% of CD31 and CD34, respectively.

Miettinen *et al*. conducted a study showing positivity of ERG in 96 out of 100 cases of angiosarcomas, 42 of 43 epithelioid hemangioendotheliomas and all 26 Kaposi sarcomas. Eight cases of epithelioid sarcomas were also tested with negativity in all cases.[2] This study results showed positivity of ERG on all the cases of vascular sarcomas; on which, it was applied with negativity in epithelioid sarcomas. This further augments its sensitivity and specificity as a vascular marker.

In one study by Stockman *et al*., ERG and FLI1 expression was observed in cases of epithelioid sarcomas with positivity in 19 of 28 cases and 28 of 30 cases, respectively.[6] These findings contradict with the results of the present study. However, the positivity of ERG in epithelioid sarcomas has been published in two studies in the literature rendering the requirement of a panel of immunohistochemical stains including cytokeratin, CD34, CD31, ERG and INI1 to differentiate it from its histologic mimic epithelioid angiosarcoma. Generally, epithelioid angiosarcoma shows positivity for CD31; while epithelioid sarcoma shows positivity for CK, and CD34 with negativity for CD31 and INI1.

This study results add to the diagnostic utility of ERG as a reliable vascular marker because it is exclusively expressed by endothelial cells unlike the specific limitations and drawbacks of other vascular markers. Moreover, ERG antibody is a nuclear transcription factor, making it diagnostically specific as compared to other markers directed against cytoplasmic determinants. Therefore, ERG should be considered as a novel and robust immunohistochemical stain that can be used as a primary marker for diagnosis of vascular neoplasms.

CONCLUSION

Among the studies markers, ERG immunohistochemical stain is highly effective for endothelial differentiation due to its specific nuclear staining pattern in normal blood vessel endothelial cells (internal control) as well as neoplastic cells of vascular tumors and lack of background staining. It can either be used alone or in conjunction with other vascular markers (CD34 and CD31) to corroborate in the diagnosis of vascular soft tissue sarcomas. The immunohistochemical panel for vascular tumors if correctly selected, may help to improve the diagnostic accuracy with limited and, therefore, less expensive panels.

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ORIGINAL ARTICLE

Carbamazepine Verses Valproic Acid as Monotherapy in Epileptic Patients

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Abstract

Objective: To evaluate the effectiveness of carbamazepine and valproic acid as monotherapy in epileptic patients of Pakhtun population.

Study Design: An experimental study.

Place and Duration of Study: The outpatient Neurology Department, Lady Reading Hospital, Peshawar, from August 2014 to April 2016.

Methodology: Epileptic patients placed on carbamazepine and volproic acid were inducted. Carbamazepine and valproic acid plasma levels were determined using reverse phase high performance liquid chromatography. Plasma levels of vitamin B6 and homocysteine were determined at baseline before the switching to carbamazepine and valproic acid therapy, and on the sixth month of the therapy. Hemoglobin (Hb) level were also determined through blood analyser. Clinical response (number of seizures per week) was evaluated on third and sixth month of the therapy.

Results: There were 79 patients in carbamazepine group and 82 in the valproic acid group. Mean age of patients was 18.08 ±8.6 years in carbamazepine group and 17.5 ±7.04 years in the valproic acid group. Median dose of carbamazepine was 400 mg/day at baseline and at 6th month of therapy. Median dose of valproic acid was 500 mg/day and 750 mg/day of valproic acid at baseline and end study point. Difference in homocysteine, vitamin B6 and Hb levels were statistically significant ($p<0.05$) in carbamazepine cohort compared to valproic acid cohort. The frequency of seizures in the carbamazepine was 0.85 times more compared to valproic acid at the end study point.

Conclusion: Hyperhomocysteinaemia and lower vitamin B6 and Hb levels was found in the carbamazepine cohort. The frequency of number of seizures/week was higher in the carbamazepine cohort compared to valproic acid cohort.

Key Words: *Carbamazepine. Homocysteine. Hb. Non-responder. Valproic acid. Vitamin B6.*

INTRODUCTION

Carbamazepine (CBZ) and valproate (VPA) are considered first-line drugs in the treatment of epilepsy in most part of the world. VPA is the drug of choice for generalised tonic-clonic seizure, absence seizure and myoclonic seizures; and the treatment of choice for simple partial, complex partial and secondary generalised tonic-clonic seizures is CBZ.[1] Variable response to CBZ and VPA are linked to types of epilepsies in clinical practice.[2-4] The unpredictable response to CBZ and VPA may be due to polymorphisms in genes, which are responsible for pharmacokinetics, pharmacodynamics of drugs and ethnicity of individuals.[5] Thus, genetic and ethnicity disparities are also considered one of the keen reasons that might be accountable for unpredictable response to therapy in patients.[6] However, it is believed that the other elements are still to be explored.[7] However, narrow therapeutic index of the agents, and capricious pharmacokinetic characteristics of patients impose a hurdle to find a relationship between the dose and the wanted effects of anti-epileptic agents (AEDs). Changes in blood levels of CBZ and its metabolites is associated with different factors such as genetic, age, sex, race, variable absorption rates, auto induction, disease-state, and co-medication.[8] Although, carbamazepine and valproic acid are less expensive and reduce the treatment gap, but they have been associated with various hematological abnormalities and some other toxicities.[9] It has been demonstrated that folic acid can reduce the blood cell abnormalities which are associated with the use of carbamazepine during treatment.[10] Carbamazepine and valproic acid affect homocysteine, vitamin B12 and folic acid levels by microsomal enzyme induction.[11,12] It has been demonstrated that CBZ is powerfully related with Stevens-Johnson syndrome and toxic epidermal necrolysis in some patients due to genetic makeup of the individuals.[13] Similarly, AEDs are also responsible for hyperhomocysteinemia and deficiency of vitamin B6 in the patients of most population.[14] Clinical responses of AEDs are variable in different populations. The current study was focused to investigate that whether our study supports the existing data available about the current practice of preferring CBZ and VPA therapy. As such type of study is not carried out in Pakhtun population, this study will add insight from a new population to current practice of carbamazepine and valproic acid therapy.

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The objective of the study was to evaluate the effectiveness of carbamazepine and valproic acid as monotherapy in epileptic patients of Pakhtun population.

METHODOLOGY

Ethics Board of the University approved the study *via* approval No. DIR/KMU-EB/AC/000047 that complied with Helsinki Declaration. The study was also approved by Research Committee *via* approval No. DIR/KMU-AS&RB/000099. Patients included in the study were those who were switched to carbamazepine and valproic acid as monotherapy for the first time during their treatment. These patients were divided into carbamazepine and valproic acid groups on the basis of types of seizures and severity of epilepsy; and an optimal dose of carbamazepine was 200-800 mg/day, and 100-1500 mg/day of valproic acid. At the end of the follow-up period, there were 62 patients on carbamazepine as monotherapy, and 58 patients on valproic acid as monotherapy. Interviews of the patients were carried out for the estimation of seizures frequencies/week before the initiation of the CBZ and VPA therapy. The dose was adjusted on the basis of their seizures' control and types of epilepsies. Patients were educated to visit the out-patient Neurology Department of the Hospital, on scheduled basis for monitoring of their clinical prognosis and adherence to carbamazepine and valproic acid therapy. The enrolled patients were evaluated on 3rd and 6th month of the CBZ and VPA therapy for better epilepsy management and determining their plasma levels for CBZ and VPA. The doses of CBZ and VPA in patients with poor seizure controlled were escalated, based on patients' response by their respective neurophysicians. Blood samples from each patient were taken at optimal dose after morning doses on steady state concentrations for plasma level monitoring after 3rd month and 6th month of the CBZ and VPA therapy.

Patients were included in the study after explaining the aim of the study in the local language upon personal consent and surrogate consent. Patients who were on other antiepileptic agents, comorbidities, and not willing to participate in the study were excluded.

Sample size for target population (N_{cbz}=62 and N_{vpa}=58) was predicted at 95% CI with standard deviation of 8 and marginal error of 1 using G power 3.1. The alpha score was 0.025 and Z score was 1.95. Expecting 20% dropout, the total patients included in the study was 79 and 82 respectively. The sampling formula was used as "$n=Z^2$ x P (1-P)/D2". Plasma level of CZ and VPA were analyzed using (RP-HPLC, HPLC LC-20AT Shimdzu Kyoto, Japan) coupled with UV detector SPD-20A/20AV (Shimdzu Kyoto Japan) with a slight modifications.[15] Detection of CBZ and VPA was performed in a UV range of 220 and 210 nm.

Vitamin B6 levels were determined using immunoassay-based kit (Alpha Diagnostic International USA). Homocysteine levels were also determined using enzyme-linked immunosorbant assay (ELISA, Kit Abbott Laboratories Ltd., Pakistan) techniques. The collected blood was analysed using blood analyser (Model Sysmex KX-21N™, Asia Pacific Re. Ltd. Japan).

Seizures' frequency/week was recorded at start of the therapy and these patients were then reassessed for control of seizure at 3rd and 6th month of CBZ and VPA monotherapy. The patient compliance was confirmed by counting the pills remaining in the strip at follow-up period. Response to the CBZ and VPA was assessed as freedom from seizures after initiation of CBZ and VPA therapy at the third and the sixth month.

Data were analysed using Stata version 12 and Graphpad Prism version 6. Differences in the number of gender, age, types of seizure in CBZ and VPA cohort were determined using Chi-square test with Yates' correction, $P \leq 0.05$. Fluctuations in plasma levels of CBZ, VPA, homocysteine, vitamin B6, and Hb were compared between CBZ and VPA cohort, using students unpaired t-test with Welchs' correction for high difference in SD at end point of the study. The likelihood of non-response to carbamazepine and valproic acid was determined, using Fisher's exact test. The impact of candidate predictors (age, gender, dose, plasma level, homocysteine, vitamin B6 and types of seizures) was evaluated with clinical outcome of carbamazepine and valproic acid, using multivariate linear regression models.

RESULTS

The mean age of patients in the carbamazepine group was 18.08 ±8.6 years, and 17.5 ±7.04 years in the valproic acid group. Female patients were more (n=35, 56.45%) in the former and male patients were more 44 (75.86%) in the latter. Significant ($p<0.05$) dissimilarity was established in the distribution of gender, and types of seizures (p=0.002) between CBA and VPA cohorts (Table I). Median dose of carbamazepine was 400 mg/day and of valproic acid was 750 mg/day.

Table I: Demographic and clinical features of enrolled epileptic patients.

Variables	Carbamazepine (n=62)	Valproic acid (n=58)	p-values
Gender			
Male, n (%)	27 (43.55)	44 (75.86)	0.0004^
Female, n (%)	35 (56.45)	14 (24.14)	
Age (year)			
Mean (range)	18.0±8.6 (1-42)	17.5±7.04 (5-35)	0.77*
Types of Seizure			
Generalised tonic clonic seizure, n (%)	48 (77.4)	29 (50.00)	0.002*^
Generalised tonic seizure, n (%)	1 (1.6)	1 (1.72)	
Atonic seizure, n (%)	2 (3.2)	0 (0)	
Simple partial seizure, n (%)	3 (4.8)	7 (12.07)	
Complex partial seizure, n (%)	3 (4.8)	5 (8.62)	
Secondary generalised complex seizure, n (%)	6 (9.7)	2 (3.45)	
Myoclonic seizure, n (%)	0 (0)	2 (3.45)	
Absence seizures	0 (0)	12 (20.68)	
Daily dose (mg/day)			
Median (range)	400 (200-800)	750 (100-1500)	0.005

*^=χ^2 test with Yate's correction; *=unpaired student "t' test. Mann-Whitney test was used to fine median of dose.*

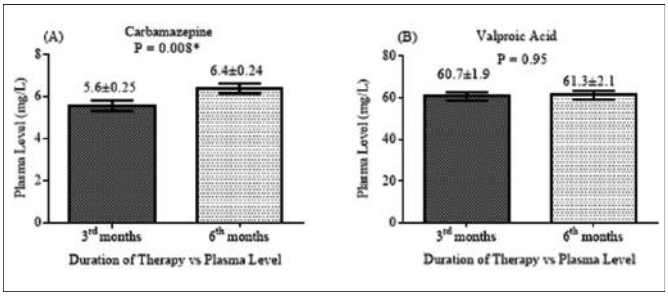

Figure 1: Plasma level of carbamazepine and valproic acid. Student paired "t" test was used.

The variation in plasma levels of carbamazepine was significant ($p<0.05$) between 3rd month and 6th month of therapy (Figure 1A). The plasma level of carbamazepine was high as compared to 3rd month of therapy. However, the difference in the plasma level of VPA was not substantial ($p>0.05$) between 3rd month and 6th month therapy (Figure 1B).

The mean level of homocysteine was 9.6 ±0.41 mmol/L in carbamazepine group and 8.6 ±0.25 mmol/L in valproic acid group. The difference in the mean level of homocysteine between carbamazepine and valproic acid groups was noteworthy ($p=0.04$). Similarly, mean level of vitamin B6 was 36.2 ±2.3 mmol/L in the carbamazepine group and 44.3 ±3.3 mmol/L in the valproic acid group. The difference in the mean level of vitamin B6 was significant between carbamazepine group and valproic acid group ($p=0.03$). Mean level of hemoglobin (Hb) was 12.6 ±1.0 g/dL in carbamazepine group and 13.3 ±1.0 g/dL in the valproic acid group. The difference in mean level of Hb between carbamazepine cohort and valproic acid cohort was significant ($p<0.001$). Mean level of Hb was low in the carbamazepine group compared to valproic acid group.

The likeliness of non-response in patients treated with CBZ and VPA as monotherapy was not significant. Poor response was 0.88 times likely to occur (34/62; OR 0.88, 95% CI 0.43-1.8, $p=0.85$) in patients that were treated with carbamazepine compared to VPA. Predictors (age, gender, dose of carbamazepine, dose of valproic acid, plasma level of homocysteine, vitamin B6, plasma level of carbamazepine and valproic acid) did not affect the clinical response of CBZ, (5, 62=1.3, $p=0.29$, $r^2=0.15$). Similarly, these predictors did not affect the clinical outcome of VPA (5, 58) = 1.5, $p=0.20$, $r^2=0.16$).

Eleven patients had adverse reactions [anemia (n=6), Steven's Johnson syndrome (n=3), drowsiness (n=1) and confusion (n=1)] in carbamazepine treated patients (n=62). Eight patients experienced adverse reaction like anemia (n=4) and tremor (n=3) in 58 VPA treated patients.

DISCUSSION

It was evident that plasma level of CBZ and VPA were within recommended reference range (4-12 mg/L for CBZ and 40-100 mg/L for VPA). Bauer reported that clinical response is related to steady state concentration of the agent when plasma level is within therapeutic range.[16] Rise in the plasma level of homocysteine and decline vitamin B6 level was high in carbamazepine-treated patients compared to valproic acid-treated patients. These findings were in line with the observations of Vincenzo *et al*. that carbamazepine highly affect the homocysteine and vitamin B6 level.[17] Recent study about vitamin B6 levels demonstrated that its levels are not affected by AED therapy,[18] whereas Mintzeret established that B6 deficiency is mostly associated with enzyme inducer AEDs.[19] Hb level was also lower in carbamazepine-treated patients than in patients treated with valproic acid. It has been found that monotherapy of AEDs has no prominent association with Hb and some other blood profile.[20] However, carbamazepine, valproic acid and other AEDs is responsible for bone marrow suppression leading to blood problem(s) like thrombocytopenia, leucopenia and aplastic anemia via anti-folic activity.[21] The anti-folate properties of carbamazepine result in decrease of Hb level and increase in homocysteine level, which lead to affect other metabolic pathways.[22]

It was found that carbamazepine and valproic acid was equal in efficacy. However, non-responding patients were more in carbamazepine cohort compared to valproic acid in this study. Clinical efficacy is related to steady state concentration of drugs. However, Ebid reported that therapeutic steady state did not assure clinical effectiveness of a medicine.[23] Reduction in seizure frequency is associated with vitamin B6, magnesium, vitamin E, manganese, taurine, dimethylglycine and omega-3 fatty acids so far.[24] Insufficiency of vitamin B6 level leads to reduction in the synthesis of GABA, which is an inhibitory neurotransmitter in CNS. Carbamazepine markedly reduces vitamin B6 level and raises homocysteine level; so, that may be a reason why non-responder patients are high in carbamazepine therapy. It has been reported that vitamin B6 and hyper-homocysteinemia have a role in the pharmacogenomics of carbamazepine. It has been found that the impact of combined predictors on clinical outcomes of carbamazepine and valproic acid was not significant.

CONCLUSION

A rise in homocysteine level, and decreased vitamin B6 and Hb levels were observed in carbamazepine cohort as compared to valproic acid cohort in Pakhtun population, which is according to the literature conducted in other population of the world. The number of seizures/week was also slightly high in carbamazepine as compared to valproic acid cohort.

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ORIGINAL ARTICLE

Ultrasound Predictability of Lower Uterine Segment Cesarean Section Scar Thickness

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ABSTRACT

Objective: The objective of this study is to find out association between scar thickness, assessed sonographically, and intraoperative findings (IOF).

Study Design: Descriptive study.

Place and Duration of Study: Department of Gynecology and Obstetrics, Sharif Medical and Dental College, Lahore, from June 2016 to April 2017.

Methodology: A total of 70 pregnant patients were included in this study. Transabdominal ultrasound was done for scarred uteri. Sonographic findings were co-related with intropeative findings. All the given data were entered on SPSS version 23. Age was expressed as mean ± SD. Parity, gestational age, and interval between cesarean sections were expressed as frequencies with percentages. Statistical analysis was done by using Chi-square test for categorical data for association between sonographic scar thickness and intraoperative findings. The statistical significance was set at p-value $\leq$0.05.

Results: The age range of the patients was 20-36 years with a mean of 27.91 ±3.690 years. Gestational age at the time of cesarean section was between 27-40 weeks of gestation with a mean of 37 ±2.126 weeks. The interval from previous cesarean was 10 months at the minimum, and 6 years at the maximum with a mean of 2.29 ±1.0 months. Mean scar thickness was 2.5 mm. Association between scar thickness (<1-3 mm) and intaoperative findings of dehiscence and rupture showed a p-value of <0.001.

Conclusion: Sonographic assessment of a uterine scar has a practical application to determine the thickness of previous scar, and assess its integrity.

Key Words: *Uterine rupture. Vaginal birth after cesarean section (VBAC). Cesarean section. Scar thickness. Ultrasound.*

INTRODUCTION

Lower segment cesarean section (LSCS) rates are raising throughout the world.[1] Women with previous one cesarean can undergo either the trial of vaginal birth or elective repeat cesarean section in their next pregnancy.[2] Uterine scar dehiscence is one of the complications associated with previous LSCS, in which there is disruption and separation of previous scar. The incidence reported for uterine scar dehiscence was between 0.2-4.3% of all pregnancies associated with previous cesarean section.[3]

A successful vaginal birth after cesarean (VBAC) is associated with fewer complications than an elective repeat cesarean section. However, elective repeat LSCS is better to be done than failed trial of labour after LSCS.[4] Advantages of VBAC include avoidance of major abdominal procedure, decrease risk of postpartum hemorrhage, and purpeural infections and reduction in the recovery time after delivery. It also reduces the further risk of repeat cesarean section, lessen the chances of cesarean hysterectomy, bowel and bladder damage, and need for blood transfusion and abnormal placental conditions in future, e.g. placenta previa and placenta accreta.[5] Disadvantages of VBAC are risk of uterine rupture due to dehiscence of previous cesarean section scar. It can be life-threatening due to excessive hemorrhage.

There are different methods to check the integrity of previous cesarean section scar. These include post-operative echographic evaluation of uterine wound, interval hysterography, and MRI imaging. Ultrasonography can be used to check the integrity of previous scar; and it can be helpful in the prediction of uterine rupture during labour, and detect the lower uterine thickness.[6,7] The lower uterine segment (LUS) thickness is categorised into 4 grades.[8,9] Grade 1 is a well formed LUS. Grade 2 is a thin uterine scar but no uterine contents are visible. Grade 3 is scar dehiscence. Grade 4 is dehisced or ruptured scar.

Different studies had given varying cut-off values for the safe trial of VBAC, but still there is no consensus on a safe limit. The cut-off value of LUS scar thickness in different studies range between 2-3.5 mm, above which the chances of uterine rupture during labour is less likely. Factors associated with uterine scar rupture during labour include number of LSCS, inter-delivery interval, prior vaginal delivery, age of the mother, gestational age at delivery, and birth weight.[10]

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The objective of the present study was to find out the association between scar thickness, assessed sonographically, and intraoperative integrity.

METHODOLOGY

This descriptive study was conducted in Obstetrics and Gynecology Department of Sharif Medical and Dental College (SMDC) Lahore from June 2016 to April 2017. Ethical approval was taken from institutional ethical committee of Sharif Medical and Dental College, Lahore. Patients were recruited from outpatient and inpatient departments of SMDC. Inclusion criteria were patient with previous LSCS, singleton pregnancy, and cephalic presentation and after the age of viability admitted either for elective LSCS or emergency LSCS. Exclusion criteria were patients with twin gestations, previous spontaneous vaginal deficiencies (SVDs) and placenta previa. History and examination of all included patients were done. Age, gravidity, parity, time interval between previous LSCS, number of LSCS were asked specifically in history. General physical examinations and abdominal examinations were done in these patients and scar tenderness was elicited.

The LUS scar thickness was assessed from the Radiology Department of Sharif City Hospital by transabdominal ultrasonography with a partially full bladder after taking informed consent. Ultrasound was done 24 hours prior to undergoing elective LSCS or, in case of emergency LSCS, prior to surgery without contractions. The LUS was assessed both in longitudinal and transverse plane under magnification to find out the weakened area or rupture. At least two measurements were recorded and the lowest measurement was taken. These sonographic findings were related with the intraoperative LUS appearance.

During surgery, the LUS scar was identified by the surgeon and the scar was scored according to normal, thinned out, dehiscent or rupture scar.

All the given data were entered on Statistical Package for Social Sciences (SPSS) version 23. Age was expressed as mean ±SD. Parity, gestational age, and interval between cesarean sections were expressed as frequencies with percentages. Statistical analysis was done by using Chi-square test for categorical data. Scar thickness was taken as a dependent variable; while sonographic findings and intraoperative findings were taken as independent variables. The statistical significance was set at p-value $\leq$0.05.

RESULTS

The age range of the patients in present study was 20-36 years with a mean of 27.91 ±3.69 years. Gestational age at the time of cesarean section was between 27-40 weeks of gestation with a mean of 37 ±2.12 weeks. The interval from previous cesarean was 10 months at the minimum and 6 years at the maximum with a mean of 2.2986 ±1.00903. Regarding gravidity, 40 (57%) patients were 3rd or 4th gravida, 19 (27%) patients were second gravida and 41 (58%) patients were para 2 and 3; while 24 (34%) patients were para 1. Only one (1.4%) patient was having parity of more than 5 with a mean parity of 2.01 ±0.95.

Regarding scar thickness, which was assessed sonographically, 12 (17%) patients were having a scar thickness of more than 3 mm, 15 (21%) patients were having thickness of 3 mm and there were 25 (36%) patients in which scar thickness was between 2.5-3 mm; while 6 (8.5%) patients had scar thickness of 2.5 mm. Between 2-2.5 mm thicknesses, there were 12 (17%) patients. Scar thickness of 18 (26%) patients were between 1.5-2 mm. Minimum scar thickness was 0.9 mm, which was found in one (1.4%) patient only. Mean scar thickness was 2.5 mm.

Regarding intraoperative findings, in 20 (28.6%) patients scar was found to be normal; while 31 (44.3%) patients had thinned out scar intraoperatively. Eighteen (25.7%) patients were found to be having dehiscent scar and in only one (1.4%) patient scar was found to be completely ruptured and that patient has a scar thickness of 0.9 mm on ultrasound.

Regarding the association of scar thickness with the intraoperative findings, only one (1.4%) patient had scar thickness of <1 mm and scar was ruptured intraoperatively. In 26 (37%) patients, scar thickness was between 1-2 mm; and out of 26 (37%) patient scar of 1 (1.4%) patient was normal, 10 (14%) has thinned out, in 15 (21%) cases it was dehiscent, and none was rupture. Between 2.1-3 mm scar thickness, scar was normal intraoperatively in 14 (20%) cases, 16 (23%) scars were thinned out and 3 (4.2%) were dehiscent. In cases with scar thickness of more than 3 mm, 5 (7%) scars were

Table I: Distribution of cases for intraoperative findings in relation to the sonographic scar thickness (n=70).

Introperative findings	Scar thickness (mm)				Total
	<1mm	1-2	2.1-3	>3	
Normal	0 (0.0%)	1 (5.0%)	14 (70%)	5 (25%)	20 (100%)
Thinned out	0 (0.0%)	10 (32.3%)	16 (51.6%)	5 (16.1%)	31 (100%)
Dehiscent	0 (0.0%)	15 (83.3%)	3 (16.7%)	0 (0.0%)	18 (100%)
Rupture	1 (100%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (100%)
Total	1 (1.4%)	26 (37.1%)	33 (47.1%)	10 (14.3%)	70 (100%)

Chi-square test=96, p-value <0.001 at df 9.

found to be normal, 5 (7%) were thinned out, and no scar was found to be dehiscent or rupture. This is shown in Table I.

DISCUSSION

The risk of uterine scar dehiscence or rupture is a life-threatening condition for both mother and baby. The frequency of uterine rupture is 0.5-0.8% after one LSCS.[1] Measuring lower uterine segment thickness before surgery in patients with scarred uteri can identify those patients who are at risk of dehiscence or rupture. In this study, sonographic findings were compared with the intraoperative findings to assess the relationship between the two, and support our findings in using ultrasound as a diagnostic modality in cases of previous LSCS.

The age range of the patient in our study was 20-36 years with a mean of 27.91 ±3.690 years. Gestational age was between 27-40 weeks with a mean of 37 ±2.126 weeks. These findings are comparable to the findings by Jastrow *et al*.,[2] Cheung *et al*.,[3] Micheal *et al*.,[4] and Martin *et al*.[5] who performed the study in third trimester as well. While it differs from the findings of Qureshi *et al*., who started assessing lower segment as early as 16 weeks of gestation.[6] Many studies in literature assessed the LUS even before conception.[7,8] The benefit of performing ultrasound at later gestation is that LUS has been developed and the presenting part is engaged in the pelvis.

Many studies have been done to assess the scar thickness by ultrasound. The lower segment scar is visible in only 30% of the patients.[9] Studies have suggested that there is an inverse relationship between scar thickness, assessed sonographically, and risk of uterine rupture.[10,11] The risk factors associated include induced labour,[12,13] reduced inter-delivery interval,[14] the number of previous cesarean, type of closure of uterus,[15] previous vaginal delivery, maternal age, gestational age at delivery, and fetal birth weight.[16,17]

Sonographic and intraoperative analyses show a p-value of 0.001, which is significant. Based upon this findings, ultrasound can be used to assess the LUS and this modality can be used in third trimester in those cases where trial of labour after previous LSCS is intended. This finding is supported by the findings of Mohammad *et al*.[18] Risk of thinning, dehiscence or rupture of scar is based upon the ultrasound appearance of LUS and its measurement. Suzuki *et al*. and Fukude *et al*. also supported these findings.[19,20]

Transabdominal ultrasound was used in our study to assess lower uterine segment thickness or thinness. Suzuki *et al*., Fukude *et al*., and Rozenberg *et al*. also used transabdominal ultrasound to assess previous scar.[19-21] While Gotoh *et al*. used tranvaginal ultrasound to assess the lower segment scar.[22]

Based upon the results of this study, sonographic assessment of previous scar has a practical application to predict the thickness and thinness of previous scar. It can be performed in patients with previous scar reliably, to assess its integrity. One of the drawbacks of using this technique is that it is operator dependent. To overcome this problem, standardisation of technique is important. Further studies are needed to define a safe cut-off at which trial of labour after LSCS can be given.

CONCLUSION

Sonographic assessment of a uterine scar has a practical application to determine the thickness of previous scar, assess its integrity.

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ORIGINAL ARTICLE

Childhood-Onset Systemic Lupus Erythematosus: A Cohort Study

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ABSTRACT

Objective: To determine the clinical and immunological characteristics and short-term outcome of children with systemic lupus erythematosus (SLE).

Study Design: A descriptive cohort study.

Place and Duration of Study: Paediatric Rheumatology Clinic, The Aga Khan University Hospital, Karachi, from January 2011 to December 2015.

Methodology: Clinical and immunological profile and short-term outcome of children less than 16 years of age admitted in the paediatric ward, with the diagnosis of SLE was studied. Demographic data, clinical presentation, laboratory findings, immunological profile and treatment regimens of these children were evaluated.

Results: Thirty-two children, satisfying the criteria of American College of Rheumatology (ACR) for SLE, were enrolled during the study period of five consecutive years. A female predominance was observed with 28 (87.5%) patients being female (F:M 7:1). Mean age at symptom onset was 10.5 $\pm$2.7 years; and 8.8 $\pm$2.1 years in females and males, respectively. The mean age at diagnosis was 11.3 $\pm$2.8 years in females and 9.4 $\pm$1.9 years in males. Prolonged fever was the most common non-specific symptom found in 27 (84%), followed by pallor in 13 (41%) patients. Twenty-two (69%) children were found to be anemic and 18 patients (56%) having signs of arthritis at presentation. Renal involvement was observed in 15 (47%) patients. The most common laboratory finding was anemia, found in 22 (69%) of cases. The most common immunological markers were serum anti-neutrophil antibodies (ANA), positive in 28 (88%) patients, followed by anti double-stranded DNA antibodies, raised in 26 (81%) of cases. Out of 32, 12 patients were lost to follow-up. Of the remaining 20 children who were followed for four years, ten (50%) went into remission.

Conclusion: Childhood-onset SLE encompasses a wide variety of manifestations with a female preponderance. Fever, arthralgia and pallor are the most frequent clinical manifestations among the children. Hemolytic anemia (HA) is the most common laboratory abnormality, with ANA and anti ds-DNA antibodies positivity in the majority of padiatric patients.

Key Words: *Childhood-onset. Systemic lupus erythematosus. Lupus nephritis. Immunological profile. Outcome.*

INTRODUCTION

Systemic lupus erythematosus (SLE) is a multisystem, chronic, episodic, autoimmune rheumatic disease with complex etiology, characterised by immune complex deposition and autoantibody production mainly anti-nuclear antibodies (ANA). SLE causes immuno-logically mediated tissue damage that commonly affects the skin and musculoskeletal systems, but can affect every organ including kidney, central nervous system, heart, lungs.[1,2] The disease is commonly described in adolescent girls and adults, but it is believed that 20% of SLE patients have the onset of disease in their childhood.[3] Childhood-onset SLE (cSLE) is a ubiquitous disease, which is characterised by diverse manifestation of the disease. There is varying epidemiologic information regarding SLE among countries in Asia. Prevalence rate usually falls within 30-50/100,000 population. India, Japan, Saudi Arabia showed a lower prevalence of 3.2-19.3/100,000. Incidence rate as reported from various studies, varies from 0.9 to 3.1/100,000 per annum.[4]

Previous studies have suggested that age at disease onset and age at diagnosis may influence disease expression in terms of initial clinical diagnosis, pattern of organ involvement, and serologic findings.[5] Moreover, during recent years a more widespread awareness of cSLE, as well as the development of newer diagnostic techniques, has led to the recognition that the course and overall prognosis of cSLE is less grave than previously thought.[6] In addition, studies that compared cSLE with adult-onset SLE (aSLE) demonstrated higher disease activity and severity in the former group.[7] Childhood-onset SLE is a more severe disease due to the higher incidence of nephritis and needs more aggressive treatment with immunosuppressive agents.[8]

The studies pertaining to childhood-onset SLE in Pakistan are limited. This study was undertaken to determine the common clinical presentations, immunological traits and short-term outcome in the children who have received the standard care.

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METHODOLOGY

This cohort descriptive study was carried out on children under the age of 16 years, presenting to the pediatric rheumatology section, The Aga Khan University Hospital, Karachi, from January 2011 to December 2015, who were diagnosed as SLE. Detailed history from patients and their parents/guardians was taken. A complete clinical examination, including musculoskeletal examination was performed by the treating physician. For the purpose of this study, childhood-onset SLE was defined by the presence of any 4 of 11 criteria of the American College of Rheumatology (ACR) used to diagnose SLE in children aged 16 or less (Table I).[9] Children with other possible, more similar, diagnoses such as undifferentiated connective tissue disorder with 3 or few ACR criteria, isolated cutaneous lupus erythematosus, neonatal lupus erythematosus, drug-induced lupus, and other autoimmune diseases were excluded.

A retrospective review of files was done and information was collected via a structured proforma prepared for the study. Data pertaining to gender, age at onset of symptoms and age at diagnosis, systems and organ involvement were collected. Laboratory parameters including hematological findings, e.g. hemoglobin, leucocytes, platelets, inflammatory markers, erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP), presence or absence of immunological indicators like anti-nuclear antibody (ANA), extractable nuclear antigen (ENA), double stranded DNA (ds-DNA) were collected. Pathological findings, including the renal biopsy results in children with lupus nephritis were also noted. In addition, different therapeutic regimen offered to these children and their short-term response was also observed.

Data were entered, validated and analysed using Statistical Package for Social Sciences (SPSS) version 19.0. Mean and standard deviation were expressed for numerical variables, while frequencies and percentages were expressed for categorical variables. As per Ethical Review Committee (ERC) guidelines, the study protocol was granted exemption (1935-Ped-ERC-11).

The Chi-square test was applied with the p-value of <0.005 being taken as statistically significant.

RESULTS

A total of 32 patients were enrolled during the study period of five consecutive years. A female predominance was observed, 28 (87.5%) of the patients being females (F:M 7:1). Mean age of onset of symptoms varied among males and females.

Mean age at symptom onset was 10.5 $\pm$2.7 years, and 8.8 $\pm$2.1 years in females and males, respectively. The mean age at diagnosis was 11.3 $\pm$2.8 years in females and 9.4 $\pm$1.9 years in males. Consanguinity was present among 15 47% of the parents of the patients.

Prolonged fever was the most common non-specific presenting symptom in a majority of the children. Prolonged fever presented in 27 (84%) children followed by pallor in 13 (41%), and anorexia and fatigue in 10 (31%) each. Other symptoms included alopecia (n=10, 31%), weight loss (n=9, 28%) and myalgia (n=8, 25%).

The most common specific clinical manifestation in these children, based on the ACR criteria for the diagnosis of SLE, was anemia, which was present in 22 (69%) patients. Arthritis is the second common manifestation after anemia; 18 (56%) patients have polyarthritis at presentation, large joints mainly knee, ankle, wrist and elbow joints are involved in most of the patients. Malar rash was found in eight (25%) patients while photosensitivity and discoid rash were seen in 3 (9%). Ten (31%) patients had muco-cutaneous ulcers at presentation. Renal involvement was observed in 15 (47%) patients who presented with hematuria, proteinuria, edema and hypertension. Nine of them, who had heavy proteinuria, underwent renal biopsy. Five (16%) patients exhibited neuropsychiatric symptoms; out of which, two had new onset seizures, two had psychosis; while one already had seizure disorder whose symptoms were exacerbated with the onset of the disease. Pericardial effusion was found in four (12.5%) patients, out of which one required peri-cardiocentesis due to cardiac de-compensation.

Various laboratory parameters in patients with SLE are shown in Table II. Anemia (hemoglobin <10 g/dl) was the most frequent hematological abnormality observed in 22 patients (69%); out of which 13 (41%) patients were severely anemic (Hb $\leq$6 gm/dl) and required urgent transfusion of packed RBC's. Direct coombs was positive in 18 (56%) who had evidence of hemolysis with raised reticulocyte count. The remaining had microcytic hypochromic anemia. Leucopenia (white cell counts <4,000/mm^3) was seen in 7 (22%) patients; 12 (37%) had thrombocytopenia (platelet count <150,000/mm^3) at admission. It was observed that acute phase reactants, including ESR and CRP, were also raised in most of the patients with the frequency of 25 (78%) and 22 (69%), respectively. Low serum complements C3 & C4 were observed in 25 (78%) and 11 (34%) patients, respectively. Immunological markers demonstrated that overall 28 (88%) patients had ANA positivity, followed by anti ds-DNA in 26 (81%) children; anti smith antibody was elevated in 11 (34%) children. Laboratory and immuno-logical profile is summarised in Table II.

Out of 15 patients who presented with renal manifestations, nine underwent renal biopsy. A majority of them (six), were of WHO class IV, while three were of class I, III and V each. Class III was treated with azathioprine, and class V with cyclosporine. Patients with class IV received monthly cyclophosphamide infusion for a period of seven months followed by quarterly infusion for

Table I: ACR criteria for diagnosing SLE.

ACR criteria
Malar rash
Discoid rash
Serositis; pleuritis, pericarditis
Oral ulcers
Arthritis; non-erosive
Photosensitivity
Neurological disorders; seizure, psychosis
Blood disorders; hemolytic anemia, leucopenia, lymphopenia,
Renal disease; proteinuria, RBC casts
Positive antinuclear antibodies
Immunological abnormalities (positive anti ds-DNA, anti-Sm, etc.)

Table II: Laboratory and immunological profile of children with c-SLE.

Laboratory results	Number (%)
Leokopenia (<4,000/mm^3)	7 (22)
Anti-SM	11 (34)
Serum complement C3	11 (34)
Thrombocytopenia (<150,00/ mm^3)	12 (37)
Coombs (positive)	18 (56)
Anemia (HB <10g/dl)	22 (69)
Elevated CRP	22 (69)
Serum complement C3	25 (78)
ESR (>20 mm/hr)	25 (78)
Anti DS-DNA	26 (81)
ANA	28 (88)

Table III: Comparative analysis of our study and two multicenter studies.

Variables	The present study (n=32) Percent	Brazilian multicenter study[18] (n=847) Percent *¥	French multicenter study[19] (n=155) Percent Ý
Demographic data			
Gender			
Female	87.5	84	
Male	12.5	16	
Mean age of diagnosis (years)	10.35	9.35	11.5
Non-specific symptoms			
Fever	84	62	60
Weight loss	28	27	-
Clinical manifestations, based on ACR criteria			
Arthritis	56	68	62
Malar rash	25	54	39
Discoid rash	9	7	6.5
Photosensitivity	9	42	13
Oral ulcers	31	32	10.5
Serositis	12.5	30	-
Renal disorders	47	51	58
Neurological symptoms	16	28	20
Laboratory findings			
Hemolytic anemia	69	20	27
Leukopenia	22	23	35
Thrombocytopenia	37	14	28
Immunological profiles			
ANA	88	99	97
Anti ds-DNA	81	67	93
Anti-Sm	34	38	32

** Mean of the percentages in the three age groups ranging from <2 years till <18 years.*
¥ Represented white (patients with white European ancestors), African-Latin Americans (patients born in Latin America with at least 1 African ancestor), Asian (patients with Asian ancestors), and other/unknown.
Ý Represented white, black, or North African children, but was significantly higher number of Asian children.

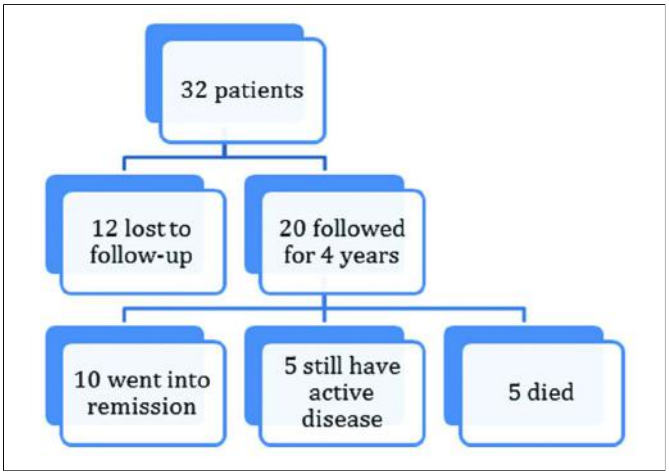

Figure 1: Follow-up results of the studied cohort of children with SLE.

two and half years. Mycophenolate mofetal was used in patients who refused the cyclophosphamide infusion.

Several anti-inflammatory and immunosuppressant drugs were given to these children. Majority (91%) were treated with hydroxychloroquine. The other commonly prescribed drugs were steroids and NSAIDS given in 88% and 75% children, respectively. Immunosuppressive like cyclophosphamide, azathioprine and mycophenolate mofetal were used in children with lupus nephritis and with neurological manifestations. Other therapeutic measures included blood transfusion in 41% and anticonvulsant medication in 9% of the children.

Out of 32 patients, 20 (62.5%) were followed for 4 years (Figure 1). Twelve patients (37.5%) were lost to follow-up due to certain reasons, the major one being due to lack of accessibility as they were from the remote areas. Other reasons included prolonged nature of therapy and financial constraints.

During the follow-up period, out of 20 patients, 10 (50%) patients showed a good response to therapy and were in clinical and biochemical remissions. Five (25%) children, who had lupus nephritis, had active disease and were on treatment. Five (25%) children died during the therapy; two of them due to disseminated tuberculosis, one because of chronic renal insufficiency and two died because of severe sepsis.

DISCUSSION

There is compelling evidence that the presentation of childhood SLE (cSLE) is not only diversified in terms of disease manifestations, but it tends to be more serious, with renal involvement, and is associated with a more aggressive clinical course and an increased need for heavy immunosuppressive therapy.[2,10-12]

In this descriptive observational study, the authors found that the prevalence of cSLE is quite high in the female population, with a female to male ratio of 7:1, which is nearly similar to most previous reports on cSLE.[5,7,13,14]

Males present early as compared to females; mean age at onset of disease in males was 8.8 ±2.1 years compared to females whose mean age was 10.5 ±2.7 years. In addition, we observed that fever and pallor were the most common non-specific manifestations. Furthermore, we also observed the high frequency of renal manifestations in our population. Most of the previous studies have underlined the frequent and debilitating involvement of renal pathology in cSLE.[6,8,15-17] Coomb's positive hemolytic anemia was the most common laboratory derangement; whereas, ANA positivity followed by anti ds-DNA elevation were the most frequent immunologic findings.

A comparative analysis of two multicenter studies on clinical features, organ involvement, laboratory and immunologic profile of c-SLE patients showed comparable results with minor contrasts with this study as shown in Table III.[18,19]

In addition to the age at onset of the disease, inter-ethnic differences have also influenced the disease course in the pediatric population. Mortality is higher in certain racial and ethnic groups,[20] including poorer prognosis in African-American and Hispanic patients. Severe nephropathy is significantly more frequent in Asian patients.[21]

Arthritis and fever were the two most common manifestations, in all ethnicities including American, African, European and Asian; as evidenced in other multicenter studies.[22] However, this study results had significant differences in frequencies of other manifestations as compared to these multicenter cohorts in different races. Malar rash has occurred in around 58 to 60% of American and European children and is the one of the most common manifestations at the time of presentation.[23]

Similarly, hemolytic anemia (HA) was found to be the most common laboratory abnormality in up to 69% (22 children), which is quite close to occurrence of HA as part of cSLE in African children in 82%, compared to only 20% and 27% children of American and European descent.[5,21]

Although the racial difference had presented with substantial variation in symptomatology and laboratory findings, immunological profiling did not reveal such remarkable disparities. ANA is positive in up to 88% of patients in this study and analogous results have been demonstrated in children of all other races and range from 92-99%.[4,5,21] Anti ds-DNA antibodies did not show comparable results among different ethnicities with positivity in 81% in this study; whereas, it was found positive in up to 67% and 85-95% in American and European ancestry.[21]

A comparison of cSLE between this study and an Indian series showed analogous findings representing disease progression in Asian population.[24]

Limitations of the study include small sample size and single center study.

Strength of the study is that it is among the few reported studies from the region and can be used as a parent study to explore the disease further. In addition, owing to the rarity of SLE overall and especially in the pediatric population, we did not come across any comparative work done on cSLE in Pakistan.

CONCLUSION

Childhood-onset SLE encompasses a wide variety of manifestations with a greater female preponderance. It is associated with an aggressive disease course as compared to adult SLE. Biopsy proven lupus nephritis is the most common complication reported. Hemolytic anemia is the most common laboratory derangement and ANA and anti ds-DNA are positive in the majority of c-SLE patients. A detailed history, thorough review of systems, complete physical examination, complete blood count, urinalysis, and a high index of suspicion help make the correct diagnosis of SLE in patients. Early diagnosis and early treatment can improve the prognosis of children with SLE.

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ORIGINAL ARTICLE

Effects of Lower Limb Cycle Training on Echocardiographic Parameters of Left Ventricle in Dilated Cardiomyopathy Patients

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Abstract

Objective: To determine the effect of lower limb ergometric training on echocardiographic parameters of left ventricle in dilated cardiomyopathy (DMC) patients.

Study Design: Randomized control trial.

Place and Duration of Study: Rawalpindi Institute of Cardiology, Rawalpindi, Pakistan, from September 2016 to February 2017.

Methodology: Clinically stable patients with DCM (n=60), were randomly allocated into an interventional group with two-month interventional program and a non-trained control group (n=30 each). Treatment protocol for interventional group was lower limb ergometer exercise for 8 weeks, 4 days/week. Pre and post-treatment echocardiography was done in both groups at baseline and after 8 weeks. SPSS 21 was used for data analysis.

Results: The median (IQR) age of the patients was 51 (18) years in interventional group and 62 (11) years in control group. Male to female ratio was 18:9 in control group and 17:12 in Interventional group. Statistically significant results were detected within the groups regarding ejection fraction (EF), left ventricular internal dimension systole (LVIDS) and left ventricular internal diastolic dimension (LVIDD) ($p < 0.001$).

Conclusion: Exercise training with lower limb ergometer was effective in improving the ejection fraction and left ventricular dimensions in patients with dilated cardiomyopathies.

Key Words: *Dilated cardiomyopathy. Lower limb ergometer. Echocardiography. Ejection fraction. Left ventricular dimension.*

INTRODUCTION

Dilated cardiomyopathy (DCM) is becoming a primary health issue worldwide. Hospitalisation as primary diagnosis with DCM, and the number of visits to emergency departments and admissions in critical care unit have markedly increased.[1,2] As a result of improved diagnostic techniques in the last decade, the prevalence and rate of patients with dilated cardiomyopathies have also increased significantly.[3] In Pakistan, DCM is the second most commonly reported cause of heart failure and the commonest type of cardiomyopathy.[4-6] This pathology primarily involves the left pumping chamber of the heart.[7] Common associated symptoms are shortness of breath, pedal edema and swelling of upper and lower extremities and ascites.[8] As the disease progresses, remodelling of left ventricle occurs.[9] Reverse remodelling indicates improvement of heart function, which is manifested by the decrease in dimensions of left ventricle and normalisation of shape of the heart.[10] As a result of certain pharmacological and non-pharmacological treatments in patients with dilated cardiomyopathies, there is an impressive improvement regarding reverse remodelling of left ventricle of the heart.[11]

The major screening tool for the assessment of DCM is echocardiography.[12] Transthoracic echo (TTE) is the most commonly performed type of echocardiography in these patients.[13] The hallmark of DCM on echocardiography includes a combination of reduced EF (<40% by definition) and impaired contractility of myocardium.[14] In DCM, exercise training is beneficial and supported by literature to improve quality of life and myocardial function.[15] CET (cycle ergometer training) effects function of the whole body.[16] It has been demonstrated relatively easy, safe, low impacted; and causes no related harmful effects.[17] There is evidence for improved left ventricular echocardiographic parameters on exercise training with cycle ergometer.[18-20]

The chief purpose of this study was to examine the effect of exercise training with cycle ergometer on EF, LVIDS and LVIDD in patients with dilated cardiomyopathies.

METHODOLOGY

It was a single blind randomised controlled study conducted on clinically stable patients with DCM. Selection of patients was made through non-probability purposive sampling, then they were allocated to experimental group (n=30) and control group (n=30),

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accordingly *via* coin toss method. Only stable patients with DCM were included six weeks after discharge from hospital. Patients of both genders were included with LVEF 20-40% and ages between 30-70 years. Patients with all other disorders of heart or any medical pathology, uncontrolled diabetes, hypertension or infections, respiratory complications and unstable and disoriented patients were excluded. Study was conducted in the Physiotherapy Department of Rawalpindi Institute of Cardiology (RIC), Rawalpindi from September 2016 to February 2017. Ethical approval was obtained from Ethical Committee of RIC and Riphah International University (RIU).

A self-structured evaluation sheet was constructed which included demographics, risk stratification, and echocardiography. The demographics included data for age and gender. Risk stratification included mild, moderate, and high risk categories; and medical supervision was suggested on the basis of risk stratification. Echocardiography parameters included EF and LVIDS, LVIDD. Lower limb ergometer exercise (static bicycling) along with conventional treatment protocol was given to interventional group for 8 weeks, 4 days/week with training% or intensity 40-60% (40% at start and increased to 3-5%/week). Goal was progressive increase in duration from 5 to 30 minutes and resistance of ergometer was kept at zero.

In the control group, conventional treatment protocol was followed, patients were counselled to take proper sleep, avoid supine lying or maintain semi-fowlers position, or heavy work, not to perform push, pull or avoid weight lifts etc., eat a heart-healthy low-fat low-salt diet, quit smoking (if smoker) and maintain a healthy weight. In-bed activities included 15 repetitions of active ankle pumps four times a day, 15-20 repetitions of AROMS of extremities three times a day and 15-20 repetitions of deep breathing exercises four times a day. Walk was advised for 10-15 minutes or below the level of fatigue and symptoms, three times a day.

Median (IQR) of age and the descriptive statistic frequency of gender and risk stratification were analysed. At baseline data of EF and LV dimensions, Kolmogorov-Smirnov test of normality was applied. Wilcoxon test was applied on both groups to analyse and compare the outcomes after 8 weeks of intervention. Level of significance was considered as p-value <0.05. SPSS version 21 was used for data analysis.

RESULTS

Total number of patients who completed the study was 56; 27 in the control group and 29 in the experimental group. In control group, male to female ratio was 2:1, while 12 (41.4%) females and 17 (58.6%) males were included in the experimental group. The mean age was 54.07 ±12.5 years in experimental group and 59.6 ±10.33 years in the control group. In both groups, high risk patients were more in number with 96.5% in experimental and 96.2% in control group, respectively (Figure 1).

Within the group, comparison of medians and interquartile through Wilcoxon tests showed statistically significant improvement in EF in interventional group as compared to control group after 8 weeks training. Median and interquartile of left ventricular dimensions also showed statistically significant outcomes with the p-value of <0.001 (Table I).

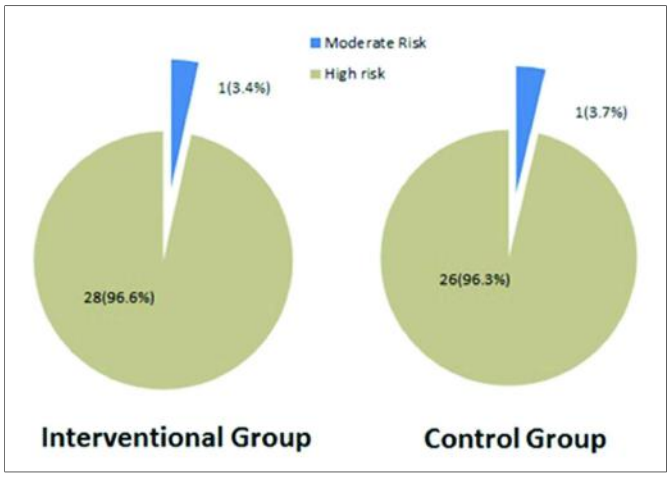

Figure 1: Pie chart showing frequency of risk stratification in both groups.

DISCUSSION

This randomised control study compared two groups – experimental and control – for the presence or absence of effect of intervention programme on echocardiographic parameters (EF and LV dimensions) in DCM patients. According to the results, there was marked improvement of EF in the experimental group patients and significant decline in LV dimensions after eight weeks of training.

These results are supported by a few previous researches, such as a study conducted on DCM patients to assess cardiac volumes and functions which were measured through cardiac magnetic resonance imaging

Table I: Inferential statistics of echocardiography parameters (comparison of both groups).

Variable	Group A Interventional Median (IQR)		Interventional	Group B Control Median (IQR)		Control
	Baseline	Week 8	P-value	Baseline	Week 8	P-value
EF (percentage)	22.5 (10)	30 (15)	<0.001	25 (10)	20 (7.5)	<0.001
LVIDS (mm)	54 (13)	46 (12)	<0.001	52 (9)	55 (9)	<0.001
LVIDD (mm)	65 (12)	59 (16)	<0.001	67 (11)	69 (9)	<0.001

(MRI) at rest and after seven minutes of exercise. There was improvement in resting LVEF, and LVEF after performing acute physical activity. Sedentary patients showed the greatest improvement, according to results of this study.[19] Another research was conducted on DCM patients to measure oxidative metabolism in both the right and left ventricles. Its results showed that training increased the EF and reduced the LV end-systolic diameter.[20]

Other few researches indirectly supports these results such as effects of exercise training on left ventricular volumes: and function in patients with non-ischemic cardiomyopathy were studied and the results showed that left ventricular systolic function (i.e. EF) were unchanged with training, suggesting that training in patients with DCM does not lead to further myocardial damage.[21] Another study was conducted to evaluate persistent recovery of normal left ventricular function and dimension in idiopathic dilated cardiomyopathy during long-term follow-up in DCM patients, who were taking tailored medical treatment (without any exercise intervention programme). Results of the study showed systolic dysfunction and increased LV dimensions.[18] Current study results are indirectly supported by these findings, as improvement with exercise training has been reported on patient's echocardiography results.

A prospective study was conducted on patients with DCM, in which they were randomly allocated to two groups named as training and control groups. Both groups were matched for outcomes as functional and clinical characteristics. A pulsed Doppler echocardiography, cardiopulmonary exercise test, and angiography with radionuclide scan was performed prior to and after completion of exercise training programme of eight weeks. Results of the study demonstrated significant increase in peak Vo2 after training program in training patients only.[22] The current study also showed improvement in patient's clinical characteristics in the perspective of improved LVEF and decreased LV dimensions.

There was no accessible literature on DCM rehabilitation in Pakistan and a few researches were conducted internationally. The present study has provided a basis for DCM rehabilitation and exercise training as a treatment option for these patients in Pakistan. As a future recommendation, long-term follow-up of the patients should be carried out with inclusion of other variables. The study needs to be conducted at the larger scale with wider timeframe so that more generalisability of results could take place.

CONCLUSION

Based on the results of statistical data analysis, it is concluded that exercise training with lower limb ergometer was effective in improving left heart function (EF and LV dimensions) in patients with dilated cardiomyopathies.

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ORIGINAL ARTICLE

Clinical Profile and Outcome of Paraphenylene Diamine Poisoning

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ABSTRACT

Objective: To determine the clinical presentation, complications, and outcome of paraphenylene diamine (PPD) poisoning in patients presenting to Nishtar Hospital, Multan.

Study Design: Descriptive study.

Place and Duration of Study: Medical Unit II and III, Nishtar Hospital, Multan, from April 2015 to September 2016.

Methodology: All adult patients admitted with history of paraphenylene diamine ingestion were evaluated for clinical features, complications, and outcomes on a pre-designed proforma.

Results: Out of 122 patients, 95 were females (77.9%) and 101/122 patients had ingested the poison with an intention of suicide or self harm (82.8%). The mean age of presentation was 23.21 ±8.2 years. Cervico-facial edema was the chief presenting complaint, seen in 116/122 (95%) of patients, with median of 2 (interquartile range=3) for time of onset after ingestion of poison. Tracheostomy was needed in 95/116 (82%) patients with cervico-facial edema. Other common complaints were dark urine in 95/122 (77.9%) and pain in limbs in 98/122 (80.3%) patients. Rhabdomyolysis was evident in 91/122 (74.5%) patients at admission. Acute kidney injury developed in 37/122 (30.3%) patients. Among these, 16 (43.2%) patients required haemodialysis. Myocarditis was diagnosed in 33/122 (27%) patients. The median for hospital stay was 9.50 days (interquartile range=6.25). The mortality was 34/122 (28%). Ventricular arrhythmias were the commonest cause of death in 25/34 (73.5%), followed by renal failure in 5/34 (14.7%), asphyxia in 2/34 (5.88%), and aspiration pneumonia in 2/34 (5.88%) patients.

Conclusion: Paraphenylene diamine is an emerging domestic poison in Pakistan, with a high morbidity and mortality.

Key Words: *Paraphenylene diamine poisoning. Acute kidney injury. Cervico-facial edema.*

INTRODUCTION

Poisoning is one of the preferred means of suicide and deliberate self harm. A common method of self-poisoning worldwide is pesticide ingestion, that is responsible for high mortality in such cases.[1] In the developing world, rodenticides and aluminium phosphide are also commomly used poisons particularly in populations from rural areas. Other frequently used domestic poisons include dettol (chloroxylenol), bleach, acids, kerosene oil, and hair dye (paraphenylene diamine).[2] Among these, paraphenylene diamine (PPD), commonly known as *'Kala Pathar'* (black stone) by the local population, is emerging as a major poison. Recently, a large number of cases of hair dye poisoning have been reported from Africa and South-East Asia.[3-5]

Paraphenylene diamine is a skin and body dye, and is used for coloring hair, palms; and forming temporary tattoos. PPD in its raw state is available as powder as well as in rock form. PPD is a common ingredient of commercial hair dyes, and, in Asia and Africa, it is also mixed with henna to form black henna. This darkens its color and reduces the time of application. While henna is a natural product and is seldom associated with adverse effects, PPD, a coal tar derivative, on oxidation is converted into a highly toxic and allergic compound. Acute allergic reactions following absorption from skin are well described.[6] Transdermal absorption of PPD during prolonged occupational exposure has been linked to interstitial fibrosis and chronic renal failure.[7]

When ingested, PPD is highly toxic, and toxicity is largely dose dependant. The lethal dose has been estimated to be 7-10 grams.[8] Acute ingestion of PPD results in angioneurotic edema causing massive cervico-facial swelling leading to asphyxia, and rhabdomyolysis; and intravascular hemolysis leading to acute renal failure. Toxic hepatitis, myocarditis and convulsions are other manifestations.[9]

PPD ingestion has been associated with a high mortality rate, in early stages due to asphyxia and ventricular arrhythmias, and, later in the clinical course, due to renal failure secondary to rhabdomyolysis. Management is largely supportive, as no antidote is available. The mainstay of treatment is urgent tracheostomy, followed by forced alkaline diuresis, steroids and antihistamines. Early gastric lavage has also been found to reduce mortality.[10] Dialysis is needed in cases developing renal failure.

The aim of this study was to describe the clinical presentation, complications, and outcome of PPD poisoning in patients presenting to Nishtar Hospital, Multan.

METHODOLOGY

This descriptive study was conducted at the medical Units II and III of Nishtar Hospital, Multan, from April 2015 to September 2016. The institutional ethical review

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board had approved the study. All adult patients admitted through emergency department, in medical wards II and III, with history of ingestion of PPD poisoning were included. Patients not showing clinical or biochemical evidence of poisoning and patients with pre-existing renal or cardiac disease were excluded from the study. Data was collected from 170 patients. Serial laboratory measurements were not available in 48 patients, so finally, a total of 122 patients were fully analysed.

After taking informed consent, a pre-designed proforma was filled. The patients and/or attendants were interviewed about the time, intent of poisoning, and form of ingestion of PPD. The time elapsed from ingestion to development of cervico-facial edema, and time from ingestion to hospitalisation was noted. The clinical features at presentation as well as the laboratory measurements of total leukocyte count (TLC), creatinine phosphokinase (CPK), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and creatinine during hospital stay were recorded. The complications developing during hospital stay, hospitalisation time and patient outcome were documented as well. Female patients reporting gestation and any pregnancy loss was noted. All patients received standard treatment in the form of gastric lavage, forced alkaline diuresis, antihistamines and parenteral steroids. Tracheostomy was done for severe cervico-facial edema causing respiratory compromise.

Rhabdomyolysis was defined on the basis of CPK above 1000 U/L. Acute kidney injury was defined on the basis of increase in serum creatinine by 0.3mg/dl within 48 hours or increase in serum creatinine to 1.5 times of the baseline value. Myocarditis was diagnosed on the basis of symptoms (acute chest pain, palpitations, syncope) and ECG changes (ST segment elevation or depression, T wave inversions, ectopic beats, atrioventricular and bundle branch blocks, atrial fibrillation, ventricular tachycardia, ventricular fibrillation).

The data collected was analysed on the statistical package for social sciences (SPSS) version 20. Frequencies and percentages, mean and standard deviations were calculated. Median and interquartile ranges were calculated for non-parametric data sets. Association between renal failure and late hospital presentation (defined as more than 5 hours after ingestion), and association of form of ingestion with severity of cervico-facial edema was analysed by applying Chi-square test. A p-value equal to or less than 0.05 was taken as significant.

RESULTS

The total number of patients in this study was 122. The mean age of presentation was 23.21 ±8.2 years. Out of 122 patients, 27 (22.1%) were males, and 95 (77.9%) were females. In 101 (82.8%) patients, PPD was ingested as a means of suicide or deliberate self harm, 12 (9.8%) cases were accidental, and 9 (7.4%) cases were homicidal. Out of 122 patients, 55 (45.1%) ingested the powdered form of poison, and 55 (45.1%) took it in dissolved form; while in 12 (9.8%), the form of ingestion could not be ascertained. The median value for time from ingestion of poison to arrival at Nishtar hospital was 6 hours (interquartile range=6), ranging from 30 minutes to 96 hours.

Table I: Clinical features of PPD poisoning at presentation.

Clinical feature	Number of patients (N=122)*	Percentage
Cervicofacial edema	116	95
Dark urine	95	77.9
Pain in limbs	98	80.3
Dysphagia	88	72.1
Respiratory distress	104	85.2
Tachycardia	33	27
Hypotension	10	8.2
Chest pain	22	18
Palpitations	11	9
Anuria	2	1.6
Oliguria	9	7.4
Syncope	6	4.9
Nasal Regurgitation	7	5.7
Convulsions	4	3.3

**Most of the patients had multiple clinical features simultaneously.*

Among the clinical manifestations, cervico-facial edema was the chief presenting complaint, seen in 116/122 (95%) patients, with median of 2 hours (interquartile range = 3) for time of onset. Of patients presenting with cervico-facial edema, 21 (17.2%) patients had mild cervico-facial edema, 30 (24.6%) had moderate and 65 (53.3%) had severe cervico-facial edema with protrusion of massively swollen tongue. Other clinical features on admission are presented in Table I. Out of 116 patients with cervico-facial edema, 95 (82%) needed a tracheostomy. Out of these, 65 (68.4%) patients had a tracheostomy reversal in mean time of 8.03 ±2.7 days. The rest of 30 patients either expired or left the hospital against medical advice. Three out of 122 (2.5%) patients required ventillatory support. The effect of form of ingestion on severity of cervico-facial edema is presented in Table II.

Rhabdomyolysis was evident in 91/122 (74.5%) patients at admission. The CPK peaked during the first three days of hospital admission in 84.4% (n=103/122) patients and had a median peak value of 53560 U/L and interquartile range of 85696. Among the laboratory parameters at admission, mean TLC was 19.26 ±7.32 $X10^3$/dl, median CPK was 12700 U/L (interquartile range=33877), median AST was 546 U/L (interquartile range=1422), and median ALT was 112.35 U/L (interquartile range=233.5). The median peak AST was 2554 U/L (interquartile range=3298) and median peak ALT was 745 U/L (interquartile range=843). Renal failure developed in 37/122 (30.3%) patients. Among these, 16 (43.2%) patients required haemodialysis, while 21 (56.7%) were managed conservatively. Among the

Table II: Effect of form of ingestion on development of cervicofacial edema and mortality .

Cervicofacial edema	Form of ingestion				
	Powdered	Dissolved	Chi-square values		
			χ^2-value	df	p-value
Severity of edema					
Mild (N=20)	11 (55%)	9 (45%)	12.00	6	0.062
Moderate (N=30)	15 (50%)	15 (50%)			
Severe (N=54)	24 (44.4%)	30 (55.5%)			
Absent (N= 6)	5 (83.3%)	1 (16.6%)			
Edema onset					
<6 hours (N=98)	48 (49%)	50 (51%)	0.37	2	0.54
≥6 hours (N=12)	7 (58.3%)	5 (41.6%)			
Tracheostomy					
Yes (N=84)	39 (46.4%)	45 (53.6%)	3.37	2	0.186
No (N=26)	16 (61.5%)	10 (38.4%)			
Clinical outcome					
Discharge (N=80)	44 (55%)	36 (45%)	13.19	4	0.01
Expired (N=26)	10 (38.4%)	16 (61.5%)			
Left against medical advice (N=4)	1 (25%)	3 (75%)			

Table III: Causes of mortality in PPD poisoning.

Cause of death	Number of patients (N=34)	Percentage
Ventricular arrhythmias	25	73.5%
Renal Failure	5	14.7%
Aspiration Pneumonia	2	5.8%
Asphyxia	2	5.8%

patients who developed renal failure, 71% (88/122) had presented late (more than 5 hours after ingestion) while 28% (34/122) arrived early at the hospital (within five hours of ingestion). Myocarditis was diagnosed in 33 out of 122 patients (27%). However, ECG changes suggestive of myocarditis were seen in a total of 81 (66.4%) patients at presentation. The most common ECG changes were ST segment depression or elevation in anterior chest leads, present in 52/81 (64.2%) cases.

The median for duration of stay in the hospital was 9.50 days (interquartile range=6.25). Out of 95 female patients with PPD ingestion, 6 (6.3%) had a pregnancy. Among these, only one had a pregnancy loss (16.6%). Following recovery, 84 (68.9%) patients were discharged while 4 (3.3%) patients had left the hospital against medical advice. The mortality in the current study was 34/122 (27.9%). Ventricular arrhythmias were the most common cause of death (Table III).

DISCUSSION

During the last few years, there has been an alarming increase in the use of PPD for intentional poisoning. The easy availability at a low cost and salty taste (as against bitter taste of most poisons) are the factors favouring the use of this new poison. Most of the time, the poison is available at home as it is commonly used for dyeing hair. Most patients present with cervico-facial edema. Catheterisation in the emergency department reveals dark brown urine. It was observed during the current study that even in the absence of a clear history of poisoning, simple bedside observation of cervico-facial edema and brown color urine in the Foley catheter's tubing is highly suggestive of PPD poisoning. This study was conducted to highlight the emerging epidemic of hair dye poisoning in Pakistan.

Most patients in the current study were young females who ingested the poison with the intent of suicide or self-harm. This was similar to the studies recently done in India by Jain *et al*. and Rawat.[11,12] In the current study, cervico-facial edema was the most frequent symptom at presentation (95%) that mostly (81%) required emergency tracheostomy. Dark urine (77.9%) and pain in limbs (80.3%) were other common complaints. A prospective study by Jain *et al*., comprising of 1020 patients with hair dye poisoning in India, has reported the development of severe cervico-facial edema in 73.03%, dark urine in 53.82%, and muscular pain in 47.05% patients.[11] The slightly lower incidence of these features are possibly the result of inclusion of patients with branded hair dye ingestion in the study, which are less toxic. Other studies from India and Pakistan have reported similar presenting features.[12,13] According to a recent study from Pakistan, PPD poisoning has become the most common indication for emergency tracheostomy.[5] Although recent studies have mentioned increased severity of cervico-facial edema in patients who had prolonged contact of the poison with oral mucosa by ingesting powdered form of PPD,[12] no significant difference was found between form of ingestion of poison and the severity of cervico-facial edema in the current study.

The mean time of arrival at the hospital in the present study was 9.23 ±12.6 hours. This is comparable to the mean arrival time of 8.9 ±10.9 hours stated in a study in India.[14] This delay in reporting to hospital may be due to the social taboo of self poisoning/suicide and is one of the main factors causing high mortality due to asphyxia and myocarditis. Almost all patients who ingested the poison with suicidal intention admitted that they had heard about the poison from someone in the family or neighbours committing suicide using the same method. The amount ingested was variable, and could not be quantified.

Acute kidney injury is a well known complication of PPD poisoning. It occurs due to the toxic effects of myogobin released after rhabdomyolysis as well as the direct toxicity of the poison itself. The most common renal lesions are acute tubular necrosis and pigment casts.[15,16] Complete renal recovery occurs in upto 77% patients, average time needed for recovery being 6 weeks.[16] In the current study, renal failure developed in 30.3% patients. This was similar to the rate of renal failure stated by Jain *et al*. and Reddy *et al*.[11,15] Delayed presentation to hospital was not found to be a significant factor in causing renal failure (p=0.06). Among patients with renal failure, dialysis was required in 43.2%. In previous similar studies, the patients requiring dialysis range from 8.62 to 33.3.[11,17]

In the current study, myocarditis was seen in 33 out of 122 patients (27%). However, 66.4% patients had ECG changes that ranged from sinus tachycardia to ST-T changes in precordial leads and extrasystoles, in the first 24 hours of admission. Tiwari *et al*. reported the incidence of similar ECG changes in 90% of patients with hair dye poisoning.[18] Another prospective study from northen India, comprising 1,595 patients with hair dye poisoning, has reported myocarditis in 15% of patients with hair dye ingestion. Nine percent of these patients suffered from ventricular arrthymias.[19]

The mean time for tracheostomy reversal was 8.03 ±2.7 days in this study. The mean duration of hospitalisation was 8.75 ±4.94 days. Given the increasing number of patients admitted with PPD ingestion, this is a significant burden on the healthcare services.

Of the pregnant females with PPD poisoning, the rate of pregnancy loss was 16.6%. This was close to pregnancy loss of 8.33% reported by Elgamel *et al*. in 2014.[3]

The mortality in the present study was 27.9%, with most of deaths (73.5%) due to ventricular arrthymias. The reported mortality of PPD poisoning in earlier studies is similar and ranges from 21.5 to 37.5%.[14-16] A low reported mortality of 3.5% in a Sudanese study can be explained by relatively early arrival to hospital and use of branded hair dyes by most of these patients, that contain a very small amount of PPD.[3]

A limitation of this study was that myocarditis was not confirmed by cardiac enzymes and echocardiography and is possibly under-reported. Since the most common cause of death in these patients is ventricular arrthymias due to myocarditis, further studies are needed for the early detection and prompt treatment of PPD induced myocarditis.

CONCLUSION

This study highlights the importance of PPD as newly emerging domestic poison in our area, resulting in a significantly high morbidity and mortality.

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ORIGINAL ARTICLE

Relationship of Low Temperature with Testicular Torsion

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ABSTRACT

Objective: To determine whether there is a correlation between seasonal temperature change and frequency of testicular torsion.

Study Design: An observational study.

Place and Duration of Study: Departments of Urology, Hitit University Corum Training and Research Hospital, Corum and Ankara Training and Research Hospital, Turkey, from June 2005 to December 2014.

Methodology: Patients who had been diagnosed with testicular torsion and operated in the last 10 years were retrospectively reached through the hospital records. The seasons and the seasonal average temperature occuring in this region were recorded.

Results: The median (IQR) age of the patients was 14 (10.8 - 17.0) years. Testicular torsion was on the right side in 18 (60%) and left side in 12 (40%) patients. Twenty-four (80%) patients underwent surgical detorsion and bilateral testicular fixation while 6 (20%) patients underwent orchiectomy. There were 14 (46.6%) cases in the winter months, 7 (23.3%) in the spring months, 4 (13.3%) in the summer months, and 5 (16.6%) in the fall months. Acute testicular torsion in the winter to be statistically significant ($p<0.05$).

Conclusion: Acute testicular torsion was seen more commonly in cold season with low temperature.

Key Words: *Season. Testicular torsion. Relationship. Cold temperature.*

INTRODUCTION

Testicular torsion, which manifests in all age groups but especially seen with one peak in the neonatal period and the second peak around puberty, is a common cause of urological emergencies.[1] It requires immediate and mandatory intervention. If there is no intervention when the rotation of the spermatic cord disrupts blood flow to the testicle, testicular necrosis can develop.[2] Differential diagnosis includes similar acute conditions such as the torsions of the appendix testis or epididymis, or infections like epididymitis, epididymo-orchitis and occupant massive pathologies like hydrocele, spermatocele, tumor and acute scrotal edema.[3]

There are several hypotheses surrounding the emergence of acute testicular torsion. Commonly, testicular torsion occurs as a result of bell-clapper testicle deformity, in which the testicle can freely move in the tunica vaginalis. It is elevated by cremasteric contraction and simultaneous rotation can occur.[4] Intense exercise, testicular trauma and even sexual dreams are among additional causes.[5] However, many cases arise in the absence of these explanatory factors.[6]

Although the topic has been addressed by several studies, the hypothesis regarding the relationship between the hyperactive cremasteric reflex in cold weather and testicular torsion has yet to be definitively proven.[7,8]

In this study, the aim was to examine whether there is a correlation between the seasonal temperature change and frequency of testicular torsion in Turkey.

METHODOLOGY

Thirty patients diagnosed with acute testicular torsion in Corum Training and Research Hospital and Ankara Training and Research Hospital between June 2005 and December 2014, were identified through the hospital information system and confirmed with operational notes. The patients were divided into 4 groups according to the seasons of diagnoses: winter, spring, summer and fall. The average temperature changes of the region of study over the past 10 years were recorded from the State Meteorological Service online records. Patients suffering from intermittent torsion, testicular appendix torsion, spontaneous or patients that underwent manual detorsion were excluded from the study.

Statistical analyses were conducted with the R package software. The defining statistics were presented as median (IQR) for continuous variables, and frequency numbers with percentages for categorical data. Chi-square test was utilised to compare the percentages of more than two groups. Spearman's test was utilised for correlation analysis. The statistical significance level was evaluated to be $p<0.05$.

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RESULTS

The median (IQR) age of the patients was 14 (10.8 - 17.0) years. The median (range) duration from onset of symptoms to time of presentation was 21 (7 - 142) (median (IQR) hours. The distribution of acute testicular torsion among seasons were found to be 14 (46.6%) in the winter, 7 (23.3%) in the spring, 4 (13.3%) in the summer and 5 (16.6%) in fall. Data elucidated a statistically significant difference between testicular torsion percentages with regard to seasons (p=0.0126, Table I).

Table I: Proportion test results according to seasons.

Groups	N (30)	Groups Median (IQR) Temperature (°C)	Percentage (%)	P
Testicular torsion				
Spring	7	9.5 (6.7, 12.1)	23.33	0.0126*
Summer	4	22 (19.0, 26.8)	13.33	
Fall	5	11.3 (8.1, 13.5)	16.67	
Winter	14	1.4 (-2.3, 5.2)	46.67	

**Proportions difference is significant (p<0.05).*

In the 3-month period between December-February when the median temperature was 1.4°C (-2.3, 5.2), 14 cases were observed (46.6%). In the 3-month period between March-May when the median temperature was 9.5°C (6.7, 12.1), 7 cases were observed (23.3%). In the 3-month period between June-August when the median temperature was 22°C (19.0, 26.8), 4 cases were observed (13.3%), and in the 3-month period between September-November when the median temperature was 11.3°C (8.1, 13.5), 5 cases were observed (16.6%). There is a significant and strong negative correlation between temperatures and percentages (Spearman's r=-1.000, p=0.033).

DISCUSSION

The increase in the cremasteric reflex with cold weather and the rotation of the elevated testicle is considered to be a contributing factor to testicular torsion and the relationship with seasons has been evaluated in international studies.[9] Most published studies of testicular torsion address the incidence in areas at higher latitudes. However, some data support the controvercial findings. These differences are probably due to study design and patient selection bias.[10]

The seasonal relation with testicular torsion was first evaluated by Sparks nearly half a century ago with 19 young patients diagnosed with testicular torsion in England. He concluded that the episodes are more common when the country faces a primarily cold climate.[3] In another study, Shukla *et al*. reported 40 out of 46 cases occurred in those months when the temperature was below 2°C. They arrived at the conclusion that the cold weather induces contraction in the cremasteric muscle, which results in the occurrence of torsion.[6] Similarly, in a study consisting of 275 children, the prevalence of testicular torsion was higher between the months of October-February compared to other 4-month period.[7] Also the results by Chiu *et al*. suggest the torsion was significantly associated with seasonality.[11] All the studies mentioned above have attributed testicular torsion to a hyperactive cremasteric reflex that resulted from low temperature. Similarly, an increased incidence of testicular torsion is seen with decreasing atmospheric temperature and humidity, suggesting a possible etiological role.[12]

On the other hand, Preshaw *et al*. evaluated a series of 272 cases in Canada, divided into 6-month period and reported that there was no relationship between cold weather and testicular torsion.[4] Driscoll *et al*., after examining 134 patients over a period of 10 years, reported no association between months and prevalence of testicular torsion.[8] In one of the largest series on this topic, Cost *et al*. investigated the role of seasonal variation.[13] Of the 2,876 patients with torsion of testicles, 792 (27.5%), 827 (28.8%), 616 (21.4%) and 641 (22.3%) presented in the winter, spring, summer and fall, respectively (p >0.05).

The data from Bingol identified other contractility and histological differences in the cremaster muscles, especially in children with different inguinal pathologies like cryptorchidism.[14] We think that the physiopathology of testicular torsion is more complex than postulated. Probably pharmacokinetic, ultrastructural differences in the cremaster muscle play a role in that complex process. There is a great variability in cremasteric reflexes and several different ways of cremasteric reflex provoking.[15,16] Also the frequency of triggering the cremasteric reflex has a very wide range in the literature.[17,18] Further studies concentrating on physiology of this reflex should be performed.

There was a significant difference between testicular torsion frequency percentages among seasonal periods, and there was a significant and strong negative correlation between temperatures and percentages of testicular torsion distribution. (Spearman's r=-1.000, p=0.033). It is possible that the ambient temperature has to drop to a certain critical level for cremasteric contractions to be stimulated.[19] In the acute scrotum, torsion should be the first conceivable pathology, especially in winter period. Moreover, warmer external genitalia undergarments will be a good option, especially for young males around puberty in this period. But there is a very thin borderline, conversely the amount of "tight" clothing, can be related to the torque that causes the retracting testis to undergo torsion.

There are some limitations of this study. First, its design is retrospective looking at one time point. Second, the sample size in this study was very small because only surgically confirmed cases were included. Third, it is

clear that our regional median seasonal temperatures are not enough to demonstrate whether cold weather influences the incidence of torsion. Obtaining the correct ambient temperature might be more useful rather than determining the seasons and median seasonal temperature at diagnosis. Finally, the authors did not determine the exact place of the torsion as indoor or outdoor. In places with extreme weather conditions where indoor-outdoor temperatures differ more, it will probably be difficult to evaluate the association between climatic condition and testicular torsion.[20] But the central region of Turkey (Middle Anatolia), where the study was performed, has a temperate climate compared to many countries and seasonal changes are less radical than some countries at higher latitudes. Indoor versus outdoor temperatures tend to differ less than many areas of the world. Studies evaluating this topic with more constant climatic variations might be more informative.

CONCLUSION

Acute testicular torsion is seen more commonly in cold seasons when low temperatures occur; so, there might be a seasonal predilection for occurrence. Seasonal weather changes may be an initiating event for testicular torsion. But yet the complex physiology of the cremasteric reflex is not fully understood. There is a need for prospective multicenter clinical studies and reviews of current mechanisms of torsion which should be helpful in both counselling and treating men for initial management of this acute pathology.

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REVIEW ARTICLE

Molecular Pathogenesis of Chewable Tobacco

Saeeda Baig, Zile Rubab and Wasfa Farooq

ABSTRACT

In Pakistan, extensive use of several precarious chewable tobacco formulations has made oral cancer the second leading malignancy. Selection of literature was done by a survey of studies published from 1990 to 2017 mainly, from PUBMED and few from other search engines, on *naswar*, *gutka*, *areca* nut and betel quid, which included published reviews, original articles and other data sources on chewable tobacco, its epidemiology, pathological implications, and psychological effects. These studies have revealed that the chemicals in these formulations bind and mutate DNA of oral mucosa through down regulating cellular repair pathways and upregulating genetic networks associated with pathogenesis. *Areca* nut, having aercoline (the major alkaloid) causes carcinogenicity, mutagenicity, and genotoxicity of oral mucosa through increased production of growth factors and corticotrophin-releasing hormone, and genetic alteration in expression of CASP8, APAF-1, BAX, BAD, and upregulation of caspas-3. *Gutka* addiction leads to precancerous lesions resulting in characteristic facial abnormalities, following trismus. *Naswar,* in addition to oral cancer, causes adverse cardiovascular events by reducing glutathione per oxidase (GPx) and super-oxide dismutase (SOD), serum levels of HDL, whereas, increasing the ratio of cholesterol, LDL, triglycerides and LDL-C/HDL-C. Betel quid (*Paan*), causes psychoactivity affecting central and autonomic nervous systems leading to dependence with decreased cognition, euphoria, sweating, salivation, palpitation, heightened alertness and zest to work. Metabolically, cardio-acceleration, cortical desynchronisation of EEG, elevated plasma noradrenaline and adrenaline were found. This review highlights the corrosive effects of various most popular chewable tobacco formulations; and damage done by their cocktail of carcinogenic substances and added ingredients, leading to oropharangeal cancer.

Key Words: *Chewable tobacco. Precancerous lesions. Oropharangeal cancer.*

INTRODUCTION

The colossal popularity of chewable tobacco, among the youth of underprivileged, lower socioeconomic communities, poses the threat of oral cancer epidemic in our population. The popularity is irrespective of racial ethnicity; however, ethnic priority on the chewable type has been observed.[1] These chewable formulations including *naswar*, *gutka*, *paan* or betel quid with *areca* nut, contain tobacco in raw or processed form. Tobaccos, as well their constituents, have been found to cause dependence. Once addiction is established, other carcinogens in chewable formulations make the oral mucosa rough and lesionous with leukoplakia, erythroplakia and trismus; also making it susceptible to viruses like HPV to enter epithelial basal cells. These viruses start proliferating in the desquamated epithelial cells leading to oral cancer.[2-4]

The relationship of chewable tobacco products with oral squamous cell carcinoma is now well established.[3-6] These products are a concoction of multiple precarious compounds individually, which have been proven as carcinogenic. Studies have found that South and Southeast Asia harbours 58% of the total worldwide oral squamous cell carcinoma and have attributed this to the wide use of smokeless tobacco formulations.[3] Usually, they are a blend of betel leaf, *areca* nut, lime and some with noxious stuff (crushed glass, blood, *khat*, etc.), added for savour and aroma. In this regard, among Indian population, studies have estimated that 49% of oral cancers among males and 90% among females are attributed to chewing habits.[7] Studies from Pakistan reported 8.5 to 10 times increased risk of oral cancers in persons habitual of betel, *areca* and tobacco chewing.[8]

This review highlights the corrosive effects of various types of most popular chewable tobacco formulations and the damage done by their cocktail of carcinogenic substances and added ingredients, leading to oropharangeal cancer.

Selection of literature was done by a survey of studies published from 1990 to 2017, mainly from PUBMED and few from other search engines, on *naswar*, *gutka*, *areca* nut and betal quid, which included published reviews, original articles and other data sources on chewable tobacco, its epidemiology and pathological implications, and psychological effects.

DISCUSSION

Studies have found that South and Southeast Asia harbours 58% of the total worldwide oral squamous cell carcinoma; and this has been attributed to wide use of

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smokeless tobacco formulations. Possibly, this is the underlying reason for the ranking of Karachi South as having highest incidence of oral cancer in both genders worldwide.[4]

Paan or betel quid was recognised as an essential component of elite class of Central and South Indian culture for hundreds of years. However, it was not accepted in Punjab, Sindh, Balochistan and KPK before the Partition of India. In 1947, *Paan* culture migrated to Pakistan along with migrants who spread out to settle in all provinces with majority in Karachi, Sindh. On the other hand, *Naswar*, made from fresh tobacco leaves, is part of Pathans' culture of Khyber Pakhtoonkhaw (KPK), probably because tobacco is grown in KPK (approximately on 30,800 hectares). Over the decades, these few cultural chewing and dipping habits evolved into development of multiple dangerous delectable products which crept into different ethnicities who, although glued to their history, heritage, and culture, adopted these habits.[9]

Oral squamous cell carcinoma (OSCC) occurs in habitual eaters of these products in Pakistan. A study, conducted at Johns Hopkins University on patients with newly diagnosed oropharyngeal cancer, showed that 90% had tumors on the tonsil or base of the tongue; independently associated with a family history of squamous-cell carcinoma of the head and neck and poor oral hygiene.[10] These findings are consistent with other reports.[11] Until specific genetic markers for the risk of an HPV-associated cancer are identified, familial aggregation due to shared environmental exposures cannot be ruled out as an explanation for these findings. Because of low level of education and ignorance about carcinogenicity of chewable products, these people are ultimately forced to substance abuse under lot of peer pressure.[12] As pointed out by other studies, these habitual eaters are least concerned about oral health. Their poor dentition, irregular tooth brushing and infrequent dental visits result in the form of oral lesions, which may serve as a surrogate for chronic infection, inflammation, precancerous lesions; and ultimately lead to squamous cell carcinoma.[13-15]

The main risk factors evaluated for possible cause of OSCC in this review, were chewable tobacco formulations namely *gutka*, *niswar*, *paan* (betel quid) and *areca* nut *(chalia).*

***Gutka* causes facial abnormalities – characteristic *gutka* face and *gutka* speech:** *Gutka* is most popular among the various tobacco formulations since 1975, the year it appeared in market. Ranganathan *et al*. pronounced trismus as *gutka* syndrome or *areca* nut chewer's syndrome.[6] Chronic users' oral cavity is constantly corroded by the use of *gutka,* leading to fibrosis of the sub-mucosal layers resulting in characteristic facial abnormalities, following ultimately

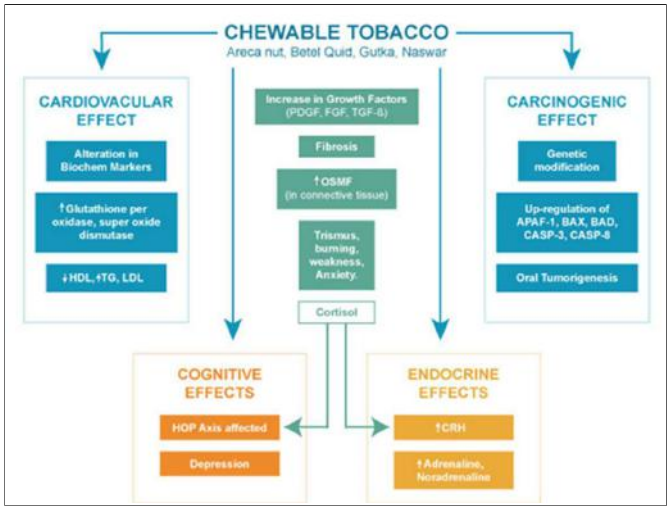

Figure 1: The molecular pathogenesis pathway affected by various chewable formulations. HDL, high density lipoprotein; TG, triglycerides; LDL, low density lipoprotein; PDGF, platelet derived growth factor; FGF, fibroblast growth factor; TGF-β, transforming growth factor beta; OSMF, oral submucous fibrosis; HOP axis, hypothalamo-pituitary-adrenal axis; CRH, corticotropin-releasing hormone; APAF1, apoptotic protease activating factor 1; BAX, Bcl-2-associated X protein; BAD, Bcl-2-associated death promoter protein; CASP-3, caspase 3; CASP-8, caspase 8.

into locking of the jaw (trismus) and a follow-on distinct *gutka* face and *gutka* speech.[16] Other studies also confirmed *gutka* as a major cause of trismus.[17] Widespread popularity of *gutka* exists mostly due to its frequent availability and a very low-price.[18] It has a strong flavour and does not carry a health warning compared to cigarettes. *Gutka* is well known for the cause of the worst observable oral lesions such as oral submucosal fibrosis, oral cancers, leukoplakias and other head and neck malignancies in the Southeast Asia.[8,19] Looking at the alarming popularity of *gutka,* a preliminary study conducted in 2012, showed 18% HPV frequency in *gutka* addicted subjects. These *gutka* addicts (78%) presented with single and multiple lesions such as ulcers (25%), rough mucosa (62%), sub-mucosal fibrosis (24%), leukoplakia (20%) and erythroplakia (10.6%).[20] In India, *gutka*, referred to as the new flavoured chewable, has been pronounced as one of the current epidemiological risk factors involved in causation and development of oral malignancy (Figure 1).[21]

Naswar causes oral warts: *Niswar* can be homemade, is prepared from crushed moist tobacco leaves, slaked lime, indigo, flavoured with cardamom and menthol.[19] It is not chewed rather placed in oral vestibule from few minutes to several hours either under the lower lip or tongue, or inside the cheek. After continuous exposure, this place in the mouth may develop an oral wart, which is considered as harmless and healthy.[9] Until recently, there was scarcity of studies available on *naswar* regarding its contents, health hazards or carcinogenicity. A latest survey among *naswar* users showed poor level of awareness about the existence of oral and lung cancer (10.41%). Majority were of the opinion that it is harmless or mentioned one or two form of oral (non-

serious) or tooth problems.[22] A recent study in 2017 in Khyber Pakhtunkhwa, showed that *Naswar* consumption contributes 68% to oral cancer in men and 38% in women in Pakistan.[23] Assessment of adverse cardio-vascular events in *Naswar* users showed altered levels of various biochemical markers. Glutathione per oxidase (GPx) and super oxide dismutase (SOD), levels as well as serum HDL-C were significantly reduced ($p<0.01$); whereas, serum total cholesterol, LDL-C, triglycerides and LDL-C/HDL-C ratio were significantly increased ($p<0.01$) compared to controls.[24] *Naswar* is the second most common form of tobacco addiction product among low socioeconomic areas of Karachi.

Searching through studies around the world about chewable tobacco, a product *toombak* was found in Sudan similar to *naswar*. *Toombak* is a major risk factor for cancer of the oral cavity in Sudan, where *toombak* dipping is common in 40% of males.[25] Epidemiological similarities of *toombak* with *naswar* include its dipping method and aged women users, comprising 10% of the users. *Toombak* use and infection with high risk Human Papilloma Virus (HPV) were extensively investigated and linked to the etiology of oral cancer in Sudan.[26]

Nass, another similar form of chewable tobacco, is marketed in northern Pakistan, Iran and the Central Asian Republics with the same mode of dipping. The manufacturing procedure is also quite similar, except it is only partially cured, with addition of ash, cotton or sesame oil and lime (Figure 1).[19]

***Areca* nut contains aercoline – the major causative factor of oral submucous fibrosis (OSMF):** *Areca* nut has the maximum popularity among the youth (less than 15 years), whether sold independently or mixed with ingredients.[9] It is an integral part of betel quid and many other chewable tobacco formulations. *Areca* nut, through a large number of animal studies, has been proven as a component with high carcinogenicity, mutagenicity, and genotoxicity. It contains four major alkaloids: arecoline, arecaidine, guvacoline, and guvacine. Out of these, arecoline has been identified as the mediator of OSMF. Arecoline along with high copper content in *areca* nut mediates OSMF by increasing collagen formation through differential mechanisms of carcinogenesis. Mechanisms include increased production of number of growth factors of various origins such as connective tissue, platelet-derived fibroblast, or transforming growth factor-beta.[27,28]

The development of OSMF due to chewing habit of raw *areca* nut is within 6-10 years. Once OSMF is established, a progressive depression sets in. This is associated with critical weakening, restricted mouth opening/eating/gulping, difficulty in conversation, and burning sensation in the mouth, which consequently affects the serum cortisol level and leads to anxiety and depression. Depression has an effect on the hypothalamo-pituitary-adrenal axis, leading to increased production of corticotrophin-releasing hormone and consequently increases in the serum.[29] Other genetic modifications include expression of CASP8, APAF-1, BAX, BAD, upregulation of caspas-3 etc.

These results show that oral tumorigenesis occurs due to upregulation of caspase-8 which, encoded by the CASP8 gene, is a central mediator in the extrinsic apoptotic pathway via death receptors. Deregulation of caspase-8 in OSCC has been reported as involved in cell proliferation rather than apoptosis during the initial stage of arecoline N-oxide (ANO) mediated.[28] The risk of cardiovascular disease was evaluated in betel nut chewers. It was found that these chewers develop arterial stiffness and are subject to subclinical atherosclerosis with an odds ratio of 2.29 (OR 2.29, 95% CI=1.05-4.99).[30] Thus malignant transformation in OSMF induced by *areca* nut proves its role as carcinogen, mutagen, and genotoxic (Figure 1).

***Paan* or betel quid – the psychoactive substance:** Betel quid habit, endured on long-term basis, affects largely the central and autonomic nervous systems. This was revealed through several studies which showed cardio-acceleration leading to hyperthermia, widespread cortical desynchronisation of EEG, elevated noradrenaline and adrenaline in plasma.[31] According to the diagnostic criteria of substance dependence in Diagnostic and Statistical Manual of Mental Disorders-IV (DSM-IV), it has been suggested that long-term betal quid chewing can lead to betel quid dependence (BQD), which can lead to decreased cognitive functions, such as attention and inhibition control,[32] producing a sense of well-being, euphoria, warm sensation of the body, sweating, salivation, palpitation, heightened alertness, and increased capacity to work.

Betel quid chewing has been claimed to produce a sense of well-being, euphoria, warm sensation of the body, sweating, salivation, palpitation, heightened alertness and increased capacity to work. These effects suggest that betel quid chewing affects predominantly the central and autonomic nervous systems. Several studies have been conducted to reveal the central and autonomic effects of betel quid chewing leading to its dependence causing rejection of inhibition control.[31,32] Several mechanisms were found involved, like cardiovascular system, integumentary system, endocrine system; disrupt balance between prefrontal cortex and subcortical areas, establishing dependance.[32] These studies have confirmed several effects claimed by betel quid users. The effects of betel quid chewing appeared to be habit-related and dose-dependent. Although arecoline has been thought to be responsible for several effects of betel quid chewing, the present data suggest a role also played by sympathetic activation.

Studies around the world including United States,[5] China,[33] Taiwan,[34] and Vietnam[35] conducted in Asian

communities particularly South Asian living in the West, identified *Paan*/betel quid as an independent factor with or without tobacco in the causation of oral cancer, predominantly oropharyngeal cancer. These findings are of significance around places where South Asians and *paan*/betel quid is used and sold freely.[36] In Taiwan, high-risk HPV has been found associated with betel quid chewing, and cigarette smoking (Figure 1).[34]

CONCLUSION

Analyses of various studies suggest that chewing habit prior to carcinogenesis leads to psychiatric morbidity, which is a social setback for the individual and the society as a whole. Later, the other carcinogenic ingredients in *gutka*, *naswar*, *paan* and *areca* nut corrode the squamous cells, making opportunistic viruses such as HPV to invade the basal epithelium and develop into oral cancer. Global chewing-cessation programmes should be developed to help prevent oral cancer epidemic.

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CLINICAL PRACTICE ARTICLE

Role of Percutaneous Cholecystostomy Tube Placement in the Management of Acute Calculus Cholecystitis in High Risk Patients

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ABSTRACT

Objective: To evaluate the utility of percutaneous cholecystostomy tube in patients with acute calculus cholecystitis, who are considered unfit for immediate surgery.

Study Design: Observational study.

Place and Duration of Study: The Aga Khan University Hospital, Karachi, Pakistan, from January 2010 to December 2014.

Methodology: All adult patients who underwent percutaneous cholecystostomy tube placement for acute calculous cholecystitis were included. These patients were divided into two groups for further analysis. Group-I consisted those who had interval cholecystectomy after tube placement and Group-II were those who had no further treatment. Recurrence of symptoms, infections and operation related complications were noted.

Results: Sixty-five patients met the inclusion criteria. Mean age was 58.5 years. Forty-four patients (67.7%) were males. Forty-three patients underwent interval cholecystectomy (Group-I) and 22 did not (Group-II). Mean operative time was 134.9 ±57.8 minutes. Five (11.6%) patients were converted to open cholecystectomy, two (4.6%) developed CBD injury, and seven (16.2%) developed surgical site infection. In Group-II, three patients (13.6%) developed recurrence of symptoms and 19 (86.4%) remained symptom-free. Catheter related problems occurred in four (18%) patients. Mean follow-up was 19 ±8 months.

Conclusion: Percutaneous cholecystostomy is a good alternative for patients unfit to undergo immediate surgery. Recurrence of symptoms after tube removal are in a low range; therefore, it can be considered a definitive management for high risk patients. Laparoscopic cholecystectomy after tube placement becomes technically challenging.

Key Words: *Cholecystostomy tube. Laparoscopic cholecystectomy. CBD injury. Conversion rate. Acute cholecystitis. High-risk surgery. Symptom recurrence.*

INTRODUCTION

Acute cholecystitis is one of the commonest surgical emergencies. In the majority of cases, a stone impacted in the neck of the organ is the cause; resulting in distension, subsequent inflammation and bacterial infection. Standard of care for patients presenting early in the course of their illness is an urgent surgical intervention, which is laparoscopic cholecystectomy.[1,2] However, immediate surgery may not be possible in some patients due to presence of significant or uncontrolled comorbidities making simple laparoscopic cholecystectomy, a high risk undertaking in such situations.[3,4] In these circumstances, percutaneous tube cholecystostomy is used as a temporizing measure as it allows for source control of the infection without any increase risk of a major surgical intervention.[5,6] At present, this can be done very effectively under ultrasound guidance. Combined with intravenous antibiotics, most of these patients can be managed successfully from the gall-bladder infection perspective. Once the sepsis resolves, definitive treatment in case of calculous cholecystitis is still cholecystectomy. However, recent international literature suggests that percutaneous cholecystectomy may be a valuable option for definitive treatment in selected high-risk patients with acute calculous cholecystitis.[7,8] For patients having acalculous cholecystitis, tube cholecystostomy is considered definitive treatment;[8] nevertheless for calculous cholecystitis, it is still debatable as to the need of subsequent surgery after percutaneous tube cholecystostomy, its timing and whether interval surgery is technically more challenging.[9]

Only few studies have evaluated the efficacy and safety of percutaneous tube cholecystostomy in the treatment of acute cholecystitis, from this part of the world.[10,11] Also none of these studies specifically divulged the role of percutaneous tube cholecystostomy in calculous cholecystitis. This study presents a retrospective review of patients, diagnosed to have acute calculous cholecystitis and treated with percutaneous cholecystostomy during a 5-year study period, at a tertiary care hospital in Pakistan.

The objective of this study was to evaluate the utility of percutaneous cholecystostomy tube in patients with

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acute calculus cholecystitis, who were considered unfit for immediate surgery.

METHODOLOGY

A retrospective chart review, from January 2010 to December 2014 was undertaken at the Aga Khan University Hospital, Karachi. The study proposal was exempted by the Hospital Ethical Review Committee (3743-Sur-ERC-15). All patients above 16 years of age, who were diagnosed to have acute calculus cholecystitis and underwent percutaneous cholecystostomy tube placement, were included in the study. Patients were identified by the medical records department using the ICD-9 coding system. Patients with cholecystostomy tube insertion were divided into two groups, i.e. those who had interval cholecystectomy within 6 - 8 weeks of tube placement were put in group I and those who had no further treatment were put in group II. The severity of cholecystitis was assessed according to Tokyo guidelines.[12] The inflammatory process was graded into grades I, 2 and 3, according to the severity of inflammation and organ dysfunction.

Data was analysed using SPSS version 20. Qualitative variables were reported as percentages or proportions and Chi-square test was used for comparative analysis. Quantitative variables were reported as mean (standard deviation) and independent sample t-test was used for analysis. However, data which did not follow the normal distribution was reported as median (interquartile value) and Mann-Whitney U-test was used. A p-value of less than 0.05 was considered statistically significant.

RESULTS

Sixty-five patients were identified and included in the study. There were 44 (67.7%) males and 21 (32.3%) females. The mean age of patients was 58.5 ±12 years. *E. coli* was the most common organism in bile cultures seen in 16 (24.6 %) patients. In 44 (67.7%) patients, bile cultures were negative. Forty-one (63.1%) patients were in the ASA III or IV category at presentation and 24 (36.9%) were in the ASA II. Grade II was most common severity of cholecystitis, seen in 41 (63.1%) cases, followed by grade III in 22 (34%) patients. Patients were followed up for a mean of 19 ±8 months in the medical or surgical outpatient clinics. All patients had their tubes removed as shown in Table I.

Forty-three patients (Group I) underwent an interval cholecystectomy. The remaining 22 (Group II) were managed non-operatively; of these, 3 patients expired during their index admission and were excluded from further analysis.

All 43 patients in Group I had attempted laparoscopic cholecystectomy. In five patients, this had to be converted to an open procedure due to difficult dissection, giving a conversion rate of 11%. The median operative time was 120 minutes (interquartile range = 91-75 minutes). Two patients had CBD injuries during laparoscopic procedure (4.5%). One was managed with intraoperative CBD repair over T-tube, the other with post-procedure ERCP and stenting. Seven patients developed surgical site infection.

Table I: Demographics.

Variable	N=65 n (%)
Age * (year)	58.5 ±12.6
Gender	
Male	44 (67.7)
Female	21 (32.3)
Comorbids	53 (81.5)
DM II	38 (58.5)
Hypertension	33 (50.8)
Ischemic heart disease	15 (23.1)
CKD	6 (9.2)
Other comorbids	14 (21.5)
ASA level	
I / II	24 (36.9)
III / IV	41 (63.1)
Hospital stay** (days)	5 (4 - 7)
Grade of acute cholecystitis	
I	5 (7.7)
II	39 (60.0)
III	21 (32.3)
Follow-up* (months)	19 ±8
Cholecystogram	44 (67.7)

**mean, standard deviation; **median, (interquartile value).*

Table II: Comparison of Group I and II.

Variable	Group I N=43 n (%)	Group II N=22 n (%)	p-value
Age * (years)	55.3 ±11.4	64 ±11.5	0.006
Gender			
Male	31 (72.1)	13 (59.1)	0.401
Comorbid	35 (81.4)	18 (81.8)	1.000
ASA Level			
III or IV	24 (55.8)	17 (77.3)	0.109
Grade of acute cholecystitis			
Grade I or II	31 (72.1)	13 (59.1)	0.401
Grade III	12 (27.9)	9 (40.9)	
Duration of tube placement** (days)	45 (40 - 58)	38 (26.25 - 57)	0.068, U=306
Hospital stay** (days)	5 (4 - 7)	5.5 (4 - 9.25)	0.253, U=391

** Mean standard deviation; ** median (interquartile value*).

Of the 19 patients in Group II, during follow-up, three developed symptoms that needed intervention. Two patients had attacks of pancreatitis, one at 6th month and the other in the 2nd year. One needed an ERCP and laparoscopic cholecystectomy; the other was managed with ERCP and papillotomy alone. The third patient developed acute cholecystitis 4 years after removal of tube. This was managed successfully with antibiotics as the patient refused intervention.

On comparing the two groups apart from age (Group I=55.3 vs. Group II=64 years, p=0.006), no other significant

difference was found with regard to gender, comorbids, ASA level, grade of acute cholecystitis, duration of tube placement, and hospital stay (as shown in Table I).

DISCUSSION

For the majority of patients presenting with acute calculus cholecystitis, an urgent laparoscopic cholecystectomy would be the treatment of choice.[2] In patient deemed unfit to undergo immediate surgery, due to associated medical conditions, the acute infection can be successfully aborted in over 90% of cases by percutaneous tube decompression of the distended gallbladder and judicious antibiotic usage.

The need for an interval cholecystectomy in acute calculus cholecystitis, successfully treated non-operatively, is unclear. There are no randomised studies addressing this area, the general consensus of opinion in literature being that surgery is offered to patients fit to undergo the procedure. In patients having acalculus acute cholecystitis, tube cholecystostomy can be used as definitive treatment.[8]

In this study, 65% of patients with acute calculus cholecystitis initially managed with a percutaneous tube cholecystostomy, subseqently underwent surgery. All had attempted a laparoscopic procedure. In 89%, the procedure was successfully completed as planned. In 11%, the procedure had to be converted due to dense adhesions and distorted anatomy. The expected conversion rate for elective laparoscopic cholecystectomy is about 1-4%.[13,14] The average procedure time for this group was also more than expected for elective procedures on the gallbladder 130 $\pm$58 minutes compared to average time in literature of 60-90 minutes. Similarly, the incidence of CBD injury in this group was 4.8% (2/44), a proportion much higher than seen in elective gallbladder surgeries.[15]

Out of the 19 patients in group II, 15.7% (3/19) developed symptoms related to gallstones. In two, interventions were needed, the third successfully managed with antibiotics. In a retrospective review, it is not possible to identify the exact reason why elective surgery was not offered in this group but it is probably safe to presume that the patients' health status and willingness to undergo the operation were important factors.

In literature the proportion of this subgroup, i.e. who relapse after tube removal, is variable from 10-40%.[16,17] There are no seemingly identifiable risk factors for relapse at present that could form the basis for selective intervention in this group. For the present, it appears as if the decision to operate was based on the subjective assessment of the involved surgeon and the fitness of a given patient to undergo surgery. There is an accumulating evidence in literature *albeit* at present of a weak nature, that the proportion relapsing with significant symptoms may not be large.[16,18-20] This area needs prospective randomised trials to guide the decision-making process in these cases.

Acute cholecystitis presenting in patients with uncontrolled medical conditions can be effectively managed with radiologically placed percutaneous tube cholecystostomy and antibiotics in over 90% of cases. Patients offered subsequent elective surgery need to be counselled as to the likely difficult nature of the operation, its higher incidence of conversion to an open procedure, and complication rate.

CONCLUSION

Percutaneous cholecystostomy is a good alternative for patients unfit to undergo immediate surgery. Recurrence of symptoms after tube removal are in a low range; therefore, it can be considered a definitive management for high risk patients. Laparoscopic cholecystectomy after tube placement becomes technically challenging.

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Clinical Spectrum and Outcome of Cerebral Venous Sinus Thrombosis in Children

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ABSTRACT

Objective: To determine clinical spectrum, neuroimaging finding and outcome of cerebral venous sinus thrombosis (CVST) in children.

Study Design: Descriptive and cross-sectional study.

Place and Duration of Study: Department of Paediatric Neurology, Children's Hospital and Institute of Child Health, Lahore, from 2015 to 2016.

Methodology: Data was collected in a predesigned proforma by non-probability purposive sampling technique from all enrolled 32 patients fulfilling the inclusion criteria. We analysed descriptively the clinical presentation, neuroimaging findings, associated risk factors and outcome. Chi-square test was used to check the association between demographic variables and findings at five percent level of significance.

Results: Out of 32 patients enrolled, 75% (24) patients were male; median age was 2.5 years. Fever was found the most common presentation followed by headache and lethargy. Neuroimaging showed superior sagital sinus thrombosis in all (100%), while 25% (8) have additional thrombosis of internal cerebral veins. Ischemic infarction was found in 11 (35%), while hemorrhagic infarction was found in 9 (29%) patients. Death occurred in 6.25% of children.

Conclusion: Infections were the common cause of CVST in children followed by anemia and dehydration. Mortality trend was low with earlier diagnosis and aggressive treatment. Anticoagulant treatment along with adequate hydration, antibiotics and correction of anemia can lead to a better outcome. A large local and regional prospective multicenter studies for pediatric cerebral venous sinus thrombosis is suggested to evaluate the risk factors and plan guidelines for managing this condition in children.

Key Words: *Cerebral venous sinus thrombosis. Neuroimaging findings. Outcome. Children.*

INTRODUCTION

Cerebral venous sinus thrombosis (CVST) is an uncommon but serious neurological condition characterised by occlusion of venous system of brain by the blood clot. All age groups can be affected, but it commonly affects relatively younger age groups.[1] Its occurrence is 0.67 per 100 000 children per year.[2] In adults, the incidence of CVST has been estimated to be as high as 3-4 cases per million; while the incidence in children and neonates is more and has been estimated to be as high as 7 per million.[3-4]

Various causes have been mentioned in medical literature. However, even with thorough investigation, 20-25% of cases revealed no cause.[5] It is relatively under recognised condition in children with wide spectrum of etiological conditions and variable clinical menifestations.[6] Dehydration, infectious processes in head and neck region, iron deficiency anemia, and hyper-coagulable states are conditions that increase the risk of CVST in children.[7,8]

A combination of Magnetic Resonance Imaging (MRI) and Magnetic Resonance Venograghy (MRV) is useful for diagnosis of this condition. MRI detects signs of CVST, like ischemic and hemorrhagic infarction; whereas, MRV localises the disease as filling defect within the cerebral venous system.[9] General treatments along with specific treatments, like anticoagulant and thrombolytic therapy are the treatment modalities.

Most of the population-based studies on CVST have been found in adult population for developed countries. There is scanty local and regional data available in children.[10] It is likely that diagnosis is often delayed or might be missed as well, due to lack of awareness and vague clinical features. Early suspicion and recognition is crucial to reduce its mortality and morbidity.

The aim of the study was to describe clinical presentation, underlying causes of cerebral venous sinus thrombosis, neuroimaging findings and its outcome in children so that diagnostic and management protocol can be developed for prompt diagnosis of this potentially fatal but treatable entity, especially in developing countries like ours.

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METHODOLOGY

This descriptive cross-sectional study was conducted in the department of Paediatric Neurology Children's Hospital and Institute of Child Health, Lahore for a period of one year (2015-2016) after approval from Institutional Review Board (IRB). Patients of both gender and age from 1 month to 18 years presenting with headache, any focal neurological deficit, seizure, papilledema, coma and confirmed CVST by magnetic resonance venography (MRV) as filling defect were enrolled while patients of neonatal age group and those having arterial ischemic stroke on MRI and in whom venous sinus thrombosis was not confirmed on venography were excluded. Written informed consents from the parents were taken.

History and neurological examinations were conducted by consultant neurologists. All the patients were subjected to MRI and MRV, keeping higher index of clinical suspicion. CT brain was not done as it provides lesser anatomical details and higher radiation exposure. Additional investigations, including all baseline investigations and prothrombotic profile were carried out according to suspected cause of cerebral venous sinus thrombosis. Primary data was collected through purposive sampling on proforma for variables like age, gender, history examination findings, neuroimaging findings, investigation, treatment given and immediate outcomes within 2 weeks after treatment.

Descriptive analysis was done by using frequency tables. Chi-square test was also used to calculate association between demographic variables, neuro-imaging findings and outcome at 5% significant level. The data was analysed by using SPSS version 20.

RESULTS

Table I represents clinical and neuroimaging findings. Eight (25%) out of 32 patients were females while 24 (75%) were males. The male to female ratio is 3:1. Twenty-four (75%) patients were less than five years while rest were between five to eleven years. Fever was found the most common feature followed by headache and lethargy.

On MRI, cerebral ischemic infarction was found in 11 (35%) patients while hemorrhagic infarction was found in 9 (29%) patients. Superior sagital sinus was found to be most commonly involved in our patients. Twenty-four (75%) patients had anemia while twenty (62.5%) had thrombocytosis. Ten (31.3%) patients had protein C, S, and antithrombin 3 deficiency while two (6.3%) had raised level of antiphospholipid antibodies level.

Table I: Clinical and neuroimaging findings.

Examination	Numbers (n)	Percentage (%)	Neuroimaging	Numbers (n)	Percentage (%)
Fever	28	87.5	Superior sagital sinus	32	100
Headache	22	68.8	Internal cerebral veins	8	25.0
Seizure	12	37.5	Straight sinus		
Coma	24	75	Right	30	93.8
Focal neurological deficit			Both	2	6.3
Hemiplegia	12	37.5	Transverse sinus		
Cranial nerve palsies	12	37.5	Right	20	62.5
Papilledema	12	37.5	Left	2	6.3
			Both	10	31.3
			Sigmoid sinus		
			Right	22	68.8
			Left	6	18.8
			Both	4	12.5

Table II: Association between demographic variables, neuroimaging findings and outcome.

Categories	Age[5]			Gender[6]		
	<1-5 years n=24	6-11 years n=8	p-value	Male n=24	Female n=8	p-value
Internal cerebral veins						
Yes	8 (25%)	0	0.070	6 (18.75%)	2 (6.25%)	0.667
Transverse sinus						
Right	12 (37.5%)	8 (25%)		12 (37.5%)	8 (25%)	
Left	2 (6.25%)	0	0.041	2 (6.25%)	0	0.041
Both	10 (31.25%)	0		10 (31.25%)	0	
Complete recovery						
Yes	2 (6.25%)	2 (6.25)	0.254	4 (12.5%)	0	0.295
Partial recovery						
Yes	20 (62.5%)	6 (18.75%)	0.476	18 (56.25%)	8 (25%)	0.149
Death						
Yes	2 (6.25%)	0	0.556	2 (6.25%)	0	0.556

[5]Age is taken as categorical variable having <1-5 and 6-11 years. [6]Gender is taken as categorical variable having male and female category.

Table III: Predisposing factors.

Predisposing factors	Numbers (n)	Percentage (%)
Cause Identified	30	93.75%
Cause Not identified	2	6.25%
Infections	20	62.5%
Otitismedia/mastoiditis	4	20%
Boil on nose	1	5%
Meningitis	13	65%
Sepsis	2	10%
Hypercoagulable states	2	6.25%
Severe dehydration	8	25%
Anemia	24	75%

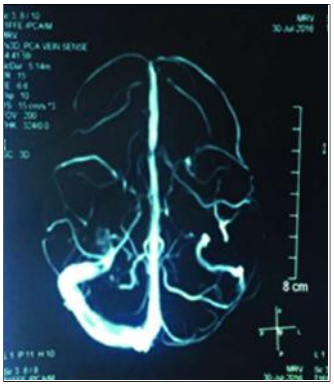

Figure 1: MRV showing filling defect in left transverse signus.

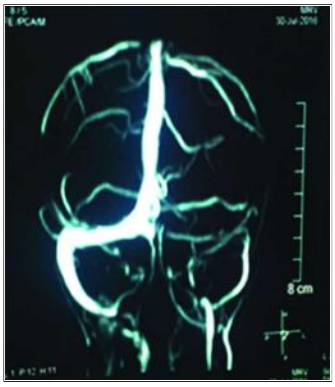

Figure 2: MRV showing filling defect in left transverse sinus and sigmoid sinus.

Patients were categorised in two age groups, like one month to five years, and six to eleven years as shown in Table II. Association between age, gender and sigmoid and transverse sinus thrombosis was found to be significant (p=0.041). Hemoglobin (Hb) level [anemia= 24 (75%), normal=8 (25%)] was significantly associated with transverse sinus at five percent level of significance (p=0.041); while hydration status [mild dehydration=20 (62.5%), moderate=10 (31.25%), severe=2 (6.25%)] was associated with transverse sinus thrombosis (p=0.001) and sigmoid sinus thrombosis (p=0.006). Association between platelets count [thrombocytosis= 20 (62.5%), normal=12 (37.5%)] and internal cerebral veins thrombosis was also found to be significant (p=0.012). Association between sigmoid sinus and boil on nose was found to be significant at five percent significance level; while other infections had not significant association with any particular sinus.

Concerning treatment, neurological care and LMW (low molecular weight) heparin were given to all patients while warfarin and aspirin were given to 30 (93.8%) and 26 (81.3%) patients, respectively.

Concerning outcomes, conscious level of 24 (75%) patients was improved within 2 weeks of hospital stay, while neurological deficit of 28 (87.5%) persisted. Two (6.25%) patients died.

DISCUSSION

Clinical spectrum of CVST in the studied patients was identical to what has been previously reported. Unconsciousness at presentation was found in 75% of these patients, which can be due to underrecognition and delayed presentation. It was noted that non-specific presentation, like headache and lethargy were more common in this study (68.8%), which endorses that CVST in children often has vague clinical presentation as mentioned in most of the studies.[11] So threshold for neuroimaging should be low for earlier diagnosis of CVST.[12] Male predominance (75%) was also observed similar to IPSS group (60%).[13] Infections including head and neck infection, CNS infection and sepsis were also found in 62% of patients in contrast to one study which described that aseptic causes of CVST predominate in the present modern antibiotic era.[14]

It was observed that there were some differences in profile of predisposing factors of this condition from Western data like iron deficiency anemia as a predisposing factor of CVST in 75% of patients of the study population, which is considerably higher as compared to previous studies. Dehydration is also found to be commonly encountered predisposing factor. No risk factor could be found in 6% of patients in our study consistent with IPSS group study, i.e. 9%. It means that CVST can be idiopathic.

Anticoagulation treatment with low molecular heparin and warfarin is considered safer in children having cerebral venous sinus thrombosis.[15] So, all patients are treated with low molecular weight heparin for 7-10 days followed by overlap of warfarin for 3-5 days. Warfarin and aspirin continued for 3-6 months. This study provides encouraging data regarding efficacy of anticoagulation and better outcome in children of CVST but more data is needed for children. Role of thrombolytic therapy in children with CVST is supported by only a few case series. One study described the comparison of direct thrombolytic therapy to heparin therapy in adults and found to be effective.[16] But none of the presently reported patients underwent direct thrombolysis due to lack of expertise in this field at the study institution.

Two patients died having age <1-5 years, and both had multiple sinus involvement like right transverse, straight as well as sigmoid sinus thrombosis reflecting that multiple sinus involvement and younger age have a poor outcome. In this study, mortality was 6.25%, which is low as compared to reported by Khealani *et al*.[17] Four males (12.5%) were completely recovered, while 26 patients were partially recovered with abnormal neurological status at discharge, further indicating the morbid nature of the condition.

Although it has a limited number of patients, however, it provides a snapshot of clinical spectrum of CVST in children presenting to a single tertiary care centre. Therefore, there is a need for further multicentre studies with a bigger sample size over longer study period.

CONCLUSION

In this study, infections were the most common predisposing factors for CVST in children followed by anemia and dehydration. Mortality was relatively low in this study despite lack of direct thrombolysis. Non-specific presentation was common. Early recognition and anticoagulant treatment along with adequate hydration, antibiotics and correction of anemia can lead to a better outcome.

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Tensionless Purely Laparoscopic Intra-Gastric Surgery using an Innovative Mucosal Flap-Valve Mechanism: The Jategaonkar Technique

Priyadarshan Anand Jategaonkar[1], Smita Priyadarshan Jategaonkar[2] and Sudeep Pradeep Yadav[3]

ABSTRACT

Cooperative laparoscopic endoscopic intra-gastric surgery, with its ability to acquire advantages of both these technologies, is a recent development in the field of minimally invasive surgery. However, the pre-requisition of its multi-disciplinary approach together with several technical constraints have, plausibly, limited its widespread acceptance. The ever-increasing number of modifications being reported in today's literature largely underscores the inability of any one to be the best. We illustrate a novel, simple-to-learn, rapid and potentially cost-effective technique of intra-gastric surgery using just the routine laparoscopic instruments, and name it the 'Jategaonkar technique'. We have practised it in seven consecutive patients without any complications; and hence, recommend it for regular application. Such a method is yet to be described in the literature.

Key Words: *Laparoscopic intra-gastric surgery. Laparoscopy. Trans-gastric surgery. Innovation. Mucosal flap.*

INTRODUCTION

Laparoscopic endoscopic cooperative surgery (LECS) is an established, much sought after alternative to conventional laparoscopy for dealing with select gastric as well as duodenal lesions.[1-3] Its salient advantage is the precision in optimising surgical margin without compromising gastric morphology/motility or oncological principles.[2] However, to accomplish this benefit, apart from the obligatory advance laparoscopic skills, the surgical team has to pull through numerous technical difficulties – the prominent among them being flawless selection of ergonomic positions for abdominal as well as gastric port-sites while retaining a steady pneumogastrium, along with an undeterred inter-disciplinary cooperation. These issues deserve further attention, given that the stomach continues to have limited luminal working space for efficient laparoscopic maneuverability, even after achieving adequate pneumogastrium. In this context, we describe an innovative, technically simple method of purely laparoscopic intra-gastric surgery and name it the Jategaonkar technique. To our knowledge, this is the first such modification reported.

Technique: Routine laparoscopic instruments are implemented in the entire procedure. For better conceptualisation, the stomach is arbitrarily divided into proximal and distal halves by an imaginary line coinciding with the mesenterico-axial axis. Under general anesthesia, the patient is positioned supine. The surgeon stands on the patient's left for distal gastric lesions and vice versa. Herein, a representative case of a 41-year male diagnosed to have distal gastric adenomatous polyp infeasible to endoscopic polypectomy (Figure 1), who subsequently underwent its excision by our technique, is discussed in detail.

Once pneumoperitoneum (14 mm Hg) is attained by per-umbilical Veress needle, the next critical step is to plan the abdominal ports as illustrated in Figure 2. Keeping all the ports in intra-peritoneal state, the stomach is then rapidly air-insufflated using 50cc syringe via pre-placed naso-gastric tube. Once the stomach is optimally distended (about 500-600cc atmospheric air generally suffices), the mucosal flap-valves are tailor-made for all the three gastric ports in a peculiar fashion. For this, serosal incision is carefully made and the underlying mucosa identified as pearly-white glistening layer. Subsequently, the sub-mucosal tissue plane entered at an angle of about 90° and around 1.5 cm tunnel is meticulously dissected, precluding injury to the mucosa at any place. It is, at this point, that a "mucosotomy" is made (to access the stomach lumen) just enough for inserting the laparoscopic instrument.

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This concludes the formation of airtight, unidirectional mucosal flap-valves harboring a peculiar Z-shape path sheltered beneath the gastric serosa (Figure 3).

With the laparoscopic cannulae stationed in the peritoneal cavity itself and the working instruments as well as the 10 mm laparoscope introduced directly through the designated mucosal flap-valves into the optimally inflated stomach, the pneumoperitoneum is released completely. Then, intra-gastric excision of the lesion is performed by two-hand technique using either ultrasonic scissors (if available) or standard suture-loop ligation and excision method (Figure 4). After checking hemostasis, pneumoperitoneum is regained and the specimen is extracted either antegradely through the working ports or retrogradely through the camera port and sent for histopathology. At this step, it is important to keep the specimen gradually withdrawn until the gastric port-site and then extract it along the same Z-path used for the instruments so as to shutter-close the mucosal flap-valve at the end. In this case, the specimen measured 4x3x2 cm in size. The stomach is then completely deflated by the naso-gastric tube suction.

Interrupted/continuous polyglactin acid (VicrylTM; Ethicon, Somerville, NJ) sutures are employed to close all the gastric port-sites and their integrity tested by air-insufflation test (Figure 4). Abdominal port-sites sutured by standard way to conclude the procedure. The naso-

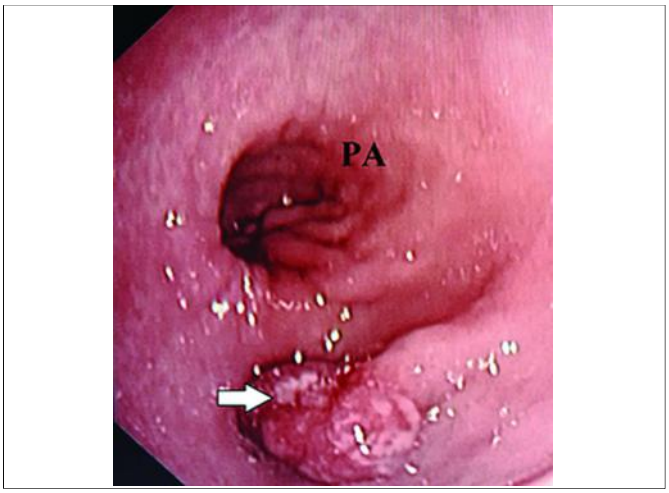

Figure 1: Gastroscopic findings. Note a large, pedunculated polyp in the distal stomach. It caused recurrent "ball-valve" obstruction of the pyloric antrum (PA). Also note the previous biopsy sites (white arrow).

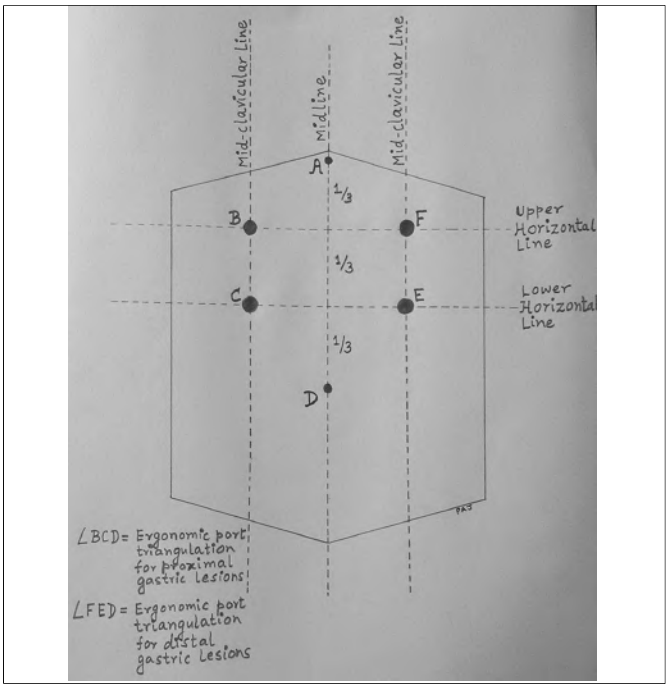

Figure 2: Scheme for abdominal trocars. Ports A, B, C, D (C-camera, B, D-working) and A, F, E, D (E-camera, F, D-working) are recruited for proximal and distal gastric lesions, respectively. A is for liver retraction. B, C and F, E are 10 mm ports contemplated to be used interchangeably for camera. Note the desired laparoscopic triangulation principle continued throughout.

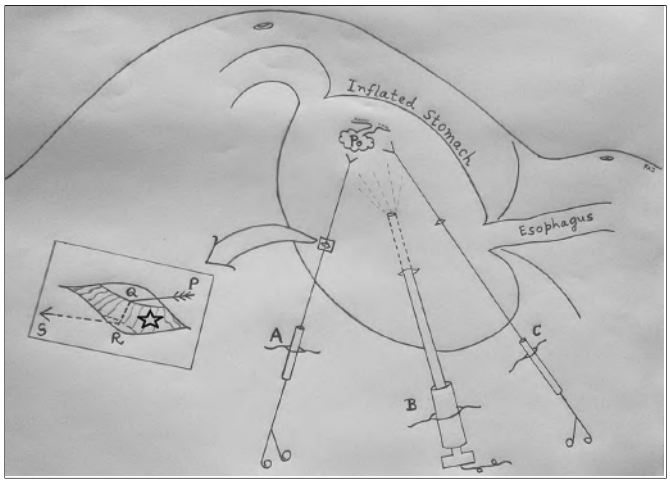

Figure 3: Gastric ports. Note their congruence with the abdominal trocars (A, B, C). Inset, the process of making gastric mucosal flap-valve resulting into a fail-safe shutter mechanism and a Z-shape path (P, Q, R, S) developed on the pliable gastric mucosa (star). Also note that none of the laparoscopic cannulae need to be reached up to the stomach wall for sustaining pneumogastrium; it practically rules out the need for gastropexy and makes the procedure "tensionless". *Po*, polyp.

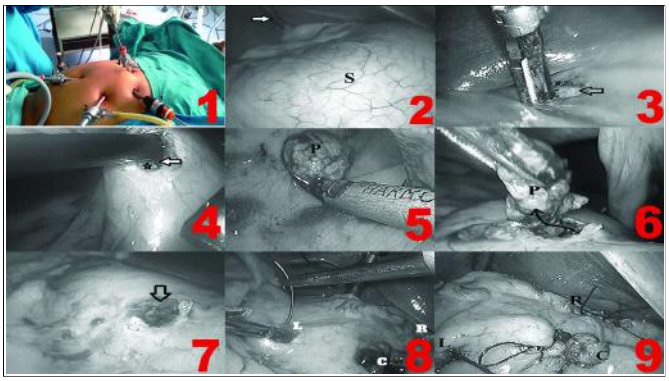

Figure 4: Operative steps. 1, the abdominal trocars in position; 2, liver retracted away (white arrow) and stomach (S) air-insufflated without using any bowel clamps; 3, "on roads" to create gastric port: serosal incision to expose the underlying mucosa (arrow); 4, completion of mucosal flap-valve (star) that permits just the working instrument (arrow); 5, intra-gastric view-the polypectomy (P); 6, Polyp (P) removal along the pre-determined zigzag path; 7, the typical mucosal flap design (arrow) lends the port leak-proof and the gastric distension persists unabated; 8 and 9, ports closure. Note the surgeon-friendly triangulation between left-hand working (L), camera (C) and right-hand working port (R). Also note the absence of gastric content leak from port-sites at any step.

gastric tube removed in the operating room itself and oral liquids commenced in the evening. Patient discharged on the second postoperative day.

DISCUSSION

While the final extirpative steps remain the same, the main initial steps of LECS demand committed surgical steps (that consumes considerable operating-room time) and customised instrumentation for its successful execution. Noted among them are cuffed laparoscopic ports, some form of gastropexy for stabilising the stomach, laparoscopic bowel clamps designed to obstruct the esophago-gastric/duodeno-jejunal junctions and a dedicated team of expert gastro-intestinal endoscopists.[1,4] This, particularly for a resource-poor country, could be genuinely a trying task to fulfill satisfactorily. Hence, for wider and easier absorption of LECS, tactful improvements in the available methodologies are much needed.[5]

In this regard, the presently described technique has following advantages. The strategic precision of the abdominal port-sites have an ergonomic triangulation for efficient laparoscopic manipulations, and the ability to reach pan-gastric locations with ease. The uniquely devised mucosal flap-valve that functions in many ways: it offers only one-way instrumental admission but disallows luminal gas egress, virtually preserving pneumogastrium during the entire operation; it is simple to master even by a novice; it makes use of relatively thicker gastric mucosa (for bold dissection in the sub-mucosal tissue plane, yet, safeguarding the flap) and abundant gastric blood supply (so the ports heal faster even with sub-optimal closure); it prevents spillage of the gastric contents, thus, averting life-threatening peritonitis; it curbs undue spillage of malignant cells, a known critical complication during early gastric cancer excisions,[3] thereby limiting the trans-peritoneal spread of the disease; the specimen extraction along its in-built Z-shape path prevents loss of pnuemogastrium even at the very moment; it circumvents fixed points gastropexy for direct gastric porting (as practised regularly by other researchers)[1,4] thereby maintaining a uniform, globular, undistorted stomach contour amenable to dynamic gastric manipulations yielding at every instrumental movement. Hence, the authors call it tensionless. With this, the authors experienced an enhancing overall operative comfort. Such a modification also prevents the otherwise likely ischemic insults at the gastric cuffed port-sites, especially during prolonged operation. No special equipments are utilised at any stage. This could save operating room time, resources and overall expenditure. In contrast to LECS, it precludes the necessity of having and coordinating with an expert endoscopic team, thereby reducing the procedural duration, the technical jargon, and the associated cost. It avoids trans-esophagial withdrawal of lesion and possible iatrogenic esophagial perforation. This technique may be tried efficiently even for obese individuals when requisite laparoscopic set is handy. In fact, one of the seven patients was a 37-year male with body-mass index of 34.67 kg/m^2.

However, it has certain limitations. Firstly, creation of the gastric mucosal flap-valve, especially judging the angle of entry into the sub-mucosal plane, could be tricky for beginners in their learning curves. Here, there seems a narrow margin of error for causing inadvertent mucosal flap perforations where the technique may fail abruptly. Secondly, though not noticed in this series, there remains a possibility of intermittent seepage of pneumogastrium into the esophagus and/or small bowel leading to peroperative stomach deflation and postoperative paralytic ileus. However, in this aspect, we have observed that the rapid inflation of stomach through the naso-gastric tube (as described above) tends to accentuate the angle of His and the duodeno-jejunal flexure to isolate the stomach into a perfect gas-filled chamber conducive for intra-luminal surgery. Thirdly, it may have restricted technical acceptability for lesions arising from the anterior wall of stomach. Fourthly, till now, its feasibility is limited to benign pathologies only. Lastly, it is a small-volume series with just the initial experience. However, presently, the authors are studying this technique prospectively at their institution for which greater number of patients is being encouraged and enrolled to strengthen the available experience.

Being a novel and investigational technique, while excluding carcinomas and submucosal lesions, the authors judiciously selected uncomplicated cases having benign pedunculated gastric polyps of < 5 cm size that were not amenable to endoscopic excision. As such, the authors have successfully applied this skill in 7 cases of large gastric adenomatous polyps – 3 antral, 2 fundic and 2 juxtra esophago-gastric junction – and got encouraging results. The average procedural time and blood loss were 34 minutes and 3cc, respectively. The mean time to create a mucosal flap-valve was 1.2 minutes, and the learning curve completed in just three cases. There were no complications in our series. All these 7 patients are under regular follow-up over last 1.5 years and remain asymptomatic. Moreover, during this period, as a protocol, everyone underwent post-procedural review – upper gastrointestinal endoscopies at 3-month intervals for a year; those revealed no mucosal ulcerations or recurrences at the sites of polypectomies. Yet, these individuals have been kept under long-term follow-up.

In conclusion, though further large-volume multi-centric studies are required to endorse these findings, the Jategaonkar technique seems feasible, safe, time-saving and cost-effective alternative for intra-gastric surgery.

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Percutaneous Non-Stenting Approach for Distal Simultaneous Multivessel Acute Coronary Occlusions

Muhammad Nasir Rahman and Bilal Hussain

ABSTRACT

The occurrence of distal coronary lesions causing simultaneous occlusion of two coronary arteries in the setting of ST elevation myocardial infarction is a rare occurrence. This can occur due to simultaneous plaque rupture at more than one site or embolisation in coronary arteries. We describe a case of a middle-aged man who presented with acute inferoposterior lateral wall ST elevation myocardial infarction with simultaneous occlusion of distal left anterior descending artery and distal left circumflex artery on angiogram. The patient was treated with intracoronary streptokinase, followed by glycoprotein (GP) IIb/IIIa inhibitor and Factor X inhibitor (Rivaroxaban) with full resolution of flow in the distal vessels. Thus, coronary lesions, not amenable to stenting, can be dealt percutaneously, using a combination of old and newer pharmacological agents without stenting.

Key Words: *Simultaneous coronary occlusions. Intracoronary streptokinase.*

INTRODUCTION

The simultaneous occlusion of two coronary arteries in the setting of ST elevation myocardial Infarction is a rare occurrence and is estimated to occur in 2.5% of all primary PCI patients.[1]

Occasionally, such multivessel occlusions of coronary vessels can be at areas where stenting might not be feasible due to very small vessel or very distal vessel occlusion. These lesions pose a unique challenge to the interventional cardiologist. We, herein, report such a case of a patient with simultaneous distal occlusion of two coronary arteries in the setting of ST elevation myocardial infarction that were dealt with intracoronary streptokinase upfront separately in each vessel for restoration of flow, followed by glycoprotein (GP) IIb/IIIa inhibitors infusion and then Factor Xa inhibitor (Rivaroxaban) with full recovery of patient.

CASE REPORT

A 57-year gentleman presented to the Aga Khan University Hospital, Karachi, Pakistan in May 2015 with Killip I Inferoposterior lateral STEMI (Figure 1A). ST segments did not show any response to sublingual nitrates in emergency room. His baseline investigations are shown in Table I. He was transferred to the cath lab for emergent coronary angiogram that showed simultaneous occlusion in distal left circumflex artery (LCx) and apical left anterior descending artery (LAD). As the patient had borderline blood pressures, intracoronary nitroglycerin (IC GTN) was not given to rule out spontaneous coronary spasm. Due to the very distal location of the lesions, it was decided to use intracoronary streptokinase (SK) for revascularisation. 250KU intracoronary SK was injected sequentially into the LAD and LCx over 3 minutes via aspiration catheter. Post-intracoronary SK, TIMI III flow was seen both in distal LAD and LCx (Figure 2). The ECG changes settled

Table I: Investigations at admission.

Investigations	Result	Normal Lab value
Hemoglobin	14.8 g/dl	12.0-15.0 g/dl
Platelets	290x10^9/L	150-300x10^9/L
Creatinine	0.9 mg/dl	Less than 1.2 mg/dl

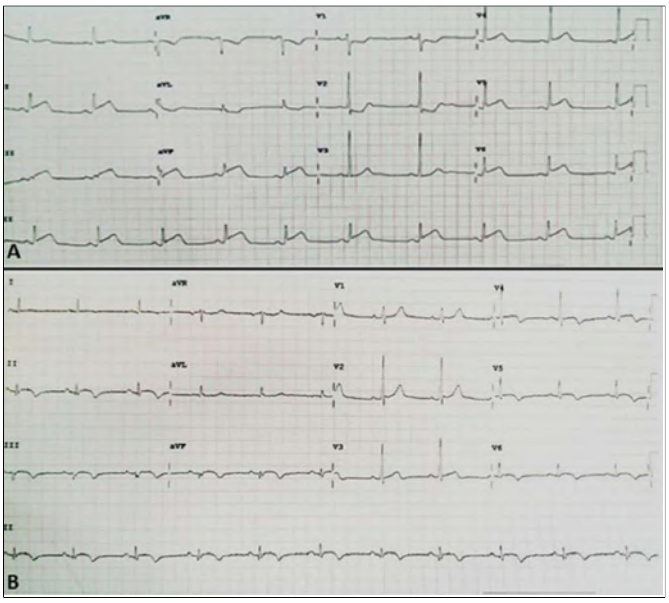

Figure 1: Electrocardiograms (ECG): (A) at presentation; (B) after intra-coronary streptokinase.

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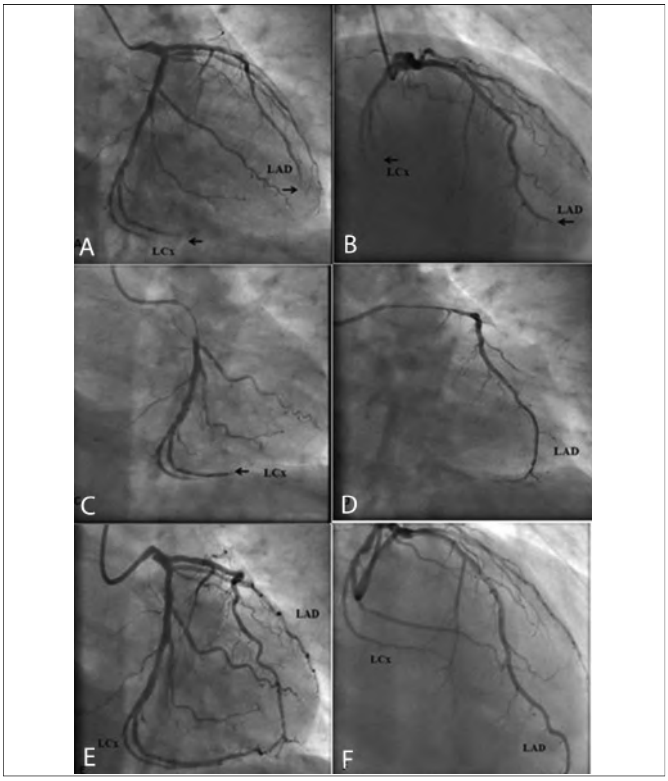

Figure 2: Coronary angiogram: (A) RAO caudal view showing occlusion of distal LAD and LCx branches; (B) RAO cranial view showing occlusion of distal LAD and LCx branches; (C) Intracoronary streptokinase injection into left circumflex artery; (D) Intracoronary streptokinase injection into left anterior descending artery; (E) RAO caudal view after intracoronary streptokinase showing flow in distal LAD and LCx; (F) RAO cranial view after intracoronary streptokinase showing flow in distal LAD and LCx.

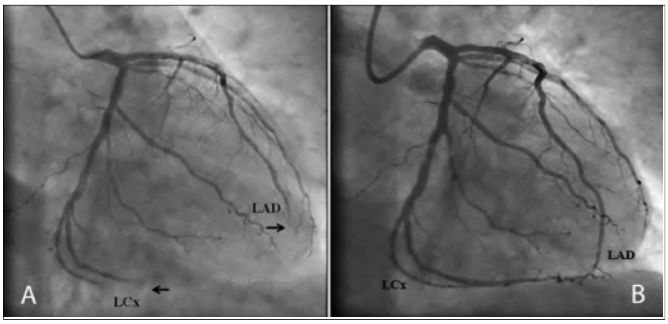

Figure 3: (A) Coronary angiogram pre-intracoronary streptokinase; (B) Post-intracoronary streptokinase.

(Figure 1B) and the patient became pain-free. Patient was started on GP IIb/IIIa inhibitor infusion for 24 hours and then on tablet Rivaroxaban 10 mg twice daily. The patient remained symptom-free during hospital stay. A trans-esophageal echocardiograph (TEE) was done which showed no evidence of vegetation or clot. A relook angiogram was done prior to discharge that showed TIMI III flow in LAD and LCx. The patient was discharged on dual antiplatelets (DAPTs) and Rivaroxaban 15 mg once daily. Rivaroxaban was continued for 3 months and then he was kept on DAPTs. At one year follow-up, the patient is asymptomatic with full recovery of left ventricle function on echocardiograph.

DISCUSSION

Review of literature shows that simultaneous occlusion of more than one coronary vessel causing STEMI is unusual and often lethal associated with cardiogenic shock and high mortality.[1,2] Simultaneous occlusion of more than one coronaries may occur due to plaque rupture, embolisation, coronary spasm, hypercoagulable states such as malignancy, thrombocytosis and antithrombin III deficiency.[3,4] Most of these patients are hemodynamically unstable with high incidence of cardiogenic shock and chances of life-threatening ventricular arrhythmias.[5]

A review of literature on the use of thrombolytics in the setting of STEMI with simultaneous multivessel occlusions on coronary angiogram shows four cases which were dealt with intracoronary (IC) thrombolytics (Table II). In these cases, use of IC thrombolytics was reserved with stenting, mostly for proximal coronary artery lesions. The most common combination for simultaneous occlusions of vessels was LAD and right coronary artery (RCA). Urokinase appeared as the thrombolytic agent of choice.[6-8] To our knowledge, there is no reported case where an IC thrombolytic therapy with SK was used for distal occlusions in the setting of STEMI caused by simultaneous occlusion of more than one coronary vessels.

Though, IC SK was used in the pre-stenting era, but its use was associated with complications such as systemic

Table II: Review of case reports of patients with ST elevation myocardial infarction with simultaneous occlusion of two coronary arteries treated with intracoronary thrombolytics.

Study	Year	Age	Presentation	Vessel involved	Treatment	Dose of intracoronary thrombolytics	Immediate results of intervention	Outcome
Hamada [11]	1989	59	Inferior STEMI	Proximal left anterior descending and right coronary artery	Intracoronary urokinase to LAD and RCA	720,000 IU to RCA 480,000 IU to LAD	60% residual stenosis in RCA, 50% residual stenosis in LAD	Alive
Yoshitomi [12]	1998	34	Anterolateral STEMI	Proximal left anterior descending and proximal left circumflex coronary artery	Stenting of LAD and Intracoronary prourokinase to LCx	3,000 IU to LCx	30% distal stenosis in LAD and LCX	Alive
Hosokawa [13]	2001	33	Inferolateral STEMI	Proximal left anterior descending and mid right coronary artery	Stenting of RCA and Intracoronary tisokinase in LAD	6,400,000 IU	TIMI III flow in RCA, LAD showed residual thrombus	Alive
Turgeman [14]	2007	44	Anteroinferior STEMI with cardiogenic shock	Proximal left anterior descending and proximal right coronary artery	Thrombus aspiration followed by intracoronary urokinase in the left and right coronary system	125,000 IU	TIMI III flow in LAD and RCA	Alive

*RCA = Right coronary artery; LAD = Left anterior descending artery; LCx = Left circumflex artery; IU = International units; STEMI = ST elevation myocardial infarction.

bleeding and bleeding at the puncture site. These complications were more pronounced if dose of SK exceeded 200,000 IU. The other major cardiac complications of IC SK administration were reperfusion arrhythmias. With the advent of stenting, the use of IC SK became limited. However, in 2007, Murat in his study used IC SK after stenting in patients undergoing primary percutaneous intervention.[9] The dose used by Murat *et al*. was 250 KU, at which the chances of SK associated complications were significantly low. However, in that study IC SK was used after stenting to achieve better flow. The authors used this dose of SK (250KU) separately for each vessel upfront without any complications. This case showcases the fact that this dose of SK can be used safely in more than one vessel occlusion.

This case report is an unusual occurrence of STEMI with simultaneous mutlivessel occlusions that might have occurred because of possible simultaneous plaque rupture in multiple coronary arteries or due to embolisation of a proximal clot. In the patient, the TEE did not show any evidence of proximal clot; however, the possibility of clot embolisation from a proximal source cannot be ruled out completely. An intravascular ultrasound (IVUS) might have helped in delineating the etiology. However, due to the distal location of lesions, it was not performed during the angiogram. The history and laboratory workup did not suggest any hyper-coagulable state. Another differential was spontaneous coronary spasm in the distal vessels; however, patient did receive sublingual nitrates in emergency room, but due to low blood pressure during the procedure, IC GTN could not be used. Furthermore, we found in our procedure that flow was only restored after fibrinlolytic therapy suggesting a thrombotic event rather than spasm. Other possibility in this case was spontaneous plaque rupture occurring at more than one distinct site simultaneously leading to ST-elevation myocardial infarction. Therefore, to prevent re-occlusion of arteries, we decided to keep our patient initially on GP IIb/IIIa inhibitors and then on Rivaroxaban for few months.

Rivaroxaban, a coagulation Factor Xa inhibitor, is approved for use in nonvalvular atrial fibrillation for the prevention of stroke and systemic embolism. However, in patients with acute coronary syndromes, low-dose Rivaroxaban is known to reduce the risk of the composite end-point of death from cardiovascular causes, myocardial infarction, or stroke.[10] As spontaneous plaque rupture at multiple sites was suspected in our patient, we used a higher dose of Rivaroxaban. This approach helped in our patient's management without recurrence of any clinical events and full recovery of left ventricular function.

This case is unique in which there was simultaneous occlusion of two distal vessels, which were dealt with old (SK, DAPTs and GP IIb/IIIa) inhibitors and new pharmacological agents (Rivaroxaban) combined with percutaneous intervention for full recovery of patient.

Though rare, ST-segment elevation myocardial infarction with simultaneous multiple coronary artery occlusions may occur. In patients with distal coronary lesions not amenable to stenting, the use of IC SK initially and then followed by GP IIb/IIIa inhibitors and newer anti-coagulants based on clinical condition and risk factors of patients can provide good immediate angiographic results with full recovery of left ventricular function in these patients on follow-up.

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CASE REPORT

Iridogoniodysgenesis: A Challenging Case

Afshan Ali, Mahmood Ali and Farah Akhtar

ABSTRACT

Iridogoniodysgenesis is a rare autosomal dominant disorder affecting anterior segment of the eye. Fifty percent cases of iridogoniodysgenesis have glaucoma, which is particularly difficult to manage. We report here a case of 40 years old man with this rare disorder, presenting to our glaucoma department. It was characterised by iris hypoplasia and juvenile glaucoma. To stop fluctuation in his intraocular pressure (IOP) and to save his vision from glaucomatous damage, our team had to do three different surgical procedures, i.e. trabeculectomy with F5U, diode laser cycloablation and aqueous shunt procedure, over a period of 10 months. This case report discusses management of glaucoma in this particular patient and challenges faced during the treatment. Regular follow-up and timely intervention can save such patients from complete blindness. To authors' knowledge, this is the first reported case of iridogoniodysgenesis in Pakistan.

Key Words: *Iridogoniodysgenesis. Glaucoma. Intraocular pressure.*

INTRODUCTION

Anterior segment dysgenesis is a group of developmental disorders of anterior segment of the eye affecting cornea, iris, lens, trabecular meshwork and Schlemms canal. According to phenotype, it has many subtypes, aniridia, posterior embryotoxin, iridogoniodysgenesis, Reiger's anomaly, Axenfeld anomaly and Peters anomaly.[1]

It has autosomal dominant pattern of inheritance. Management is offered according to patient symptoms. Most important and challenging part of management is treating glaucoma in these patients to prevent permanent loss of vision.[2]

CASE REPORT

A 42 years old male, presented to glaucoma outdoor department with complaint of gradual painless decrease in vision in his right eye for the last 6 months. He was known glaucomatous for last 20 years. Family history for glaucoma was positive. Surgical history included trabeculectomy in the same eye 15 years back. Drug history included many antiglaucoma drugs; he was currently on beta blockers. Patient had no known systemic illness.

Examination of right eye showed visual acuity of 4/60 in right eye. Intraocular pressure (IOP) was 10 mmHg with morning dose of beta blocker. There was diffuse bleb in superior conjunctiva. Cornea was clear. Gonioscopy showed closed angle with iridotrabecular adhesions in three quadrants while open angle in one quadrant. Iris was hypoplastic, dark chocolate brown in color with loss of normal architecture, and smooth surface (Figure 1). Pupil was dilated and nonreactive. There was grade 2 nuclear sclerosis. Optic disc showed glaucomatous changes with cup disc ratio of 0.8. Left eye had no perception of light with IOP of 15 mmHg. Cornea was opaque with bullous keratopathy. Rest of the details could not be seen due to hazy view. Based on history and examination, clinical diagnosis of iridogoniodysgenesis was made. General physical examination was not remarkable.

Optical coherent tomography (OCT) of right retinal nerve fibre layer showed thinning with average thickness of 70 um. Left eye testing was not possible due to hazy medium. Pachymetery showed thicker cornea with measurement of 621um.

A plan was made to perform lens matter aspiration with intraocular lens (IOL) implantation in his right eye which was done successfully. Post-operative vision of patient improved to 6/60 maintaining IOP of 10 mmHg with topical beta blocker. On one month post-operative visit, patient complained of pain. His vision was 6/60 and IOP was 30 mmHg. He was put on maximum antiglaucoma therapy; but after 2 weeks, no change in IOP was observed. So we decided to do his second trabeculectomy

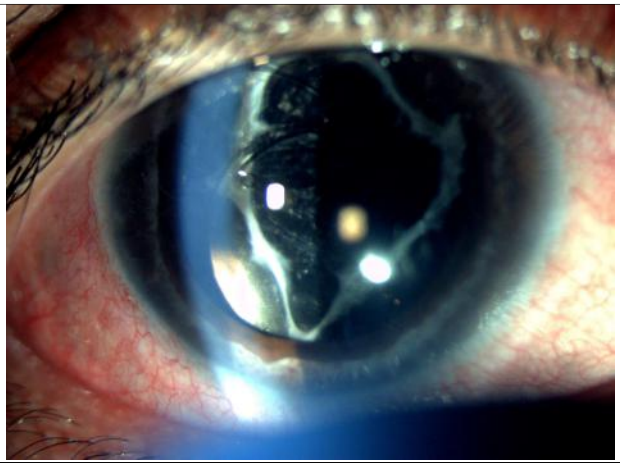

Figure 1: Dilated pupil with chocolate brown hypoplastic and atrophic iris.

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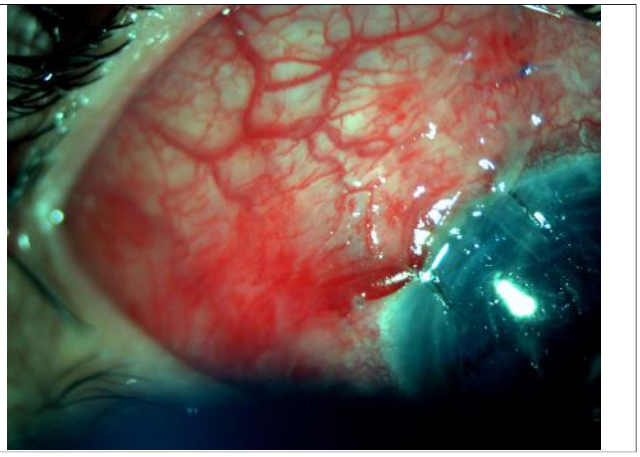

Figure 2: One week postoperative picture showing ahmad glaucoma valve with tube in anterior chamber.

with 5 fluorouracil to save vision in his only eye. On first post-operative day, bleb was flat, IOP was down to 20 mmHg without any antiglaucoma drugs. Patient was put on topical steroids and antibiotics. On further visits, bleb vascularisation started and IOP started rising. Steroids were tapered and antiglaucoma drugs were started again. After 2 months, IOP was back to 32 mmHg with maximum antiglaucoma drug therapy.

This time it was decided to do diode laser cycloablation of inferior 180 degree conjunctiva of same eye. On first postoperative day IOP was 8 mmHg with visual acuity of 6/60. Patient was put on topical steroids which were tapered and stopped afterwards. Patient was stable for 2 months when his IOP spiked to 32 mmHg. Antiglaucoma drugs were started again without much effect. After 3 months, to save his current vision of 6/60, next plan was to do ahmed glaucoma valve implantation in the same eye. On first day, IOP was 8 mmHg without any antiglaucoma drugs. Patient was put on steroids and antibiotics. On further visits, examination showed vision of 6/60 with IOP of 18 mmHg without any antiglaucoma drugs (Figure 2). Steroids were tapered. On 6 months follow-up, he is maintaining vision of 6/60 with IOP of 18 mmHg with no drugs.

DISCUSSION

To authors' knowledge, this is the first reported case of iridogoniodysgenesis in Pakistan. Etiology is related to defect in neural cell migration, resulting in abnormal anterior segment of eye. Such abnormality can obstruct aqueous outflow resulting in high IOP and glaucomatous damage in 75% of cases. It is broadly divided into two types, iridogoniodysgenesis anomaly and iridogoniodysgenesis syndrome. Anomaly is caused by mutations in FOXC1 gene on 6p25, and is usually not associated with systemic features.[3] Syndrome is caused by mutations in PITX2 on 4q25,[4] and is characterised by systemic involvement like maxillary hypoplasia, dental, periumbilical anomalies and hypospadias in males.[5,6]

Patients usually present with gradual decrease in vision, photophobia or constriction of visual fields. Clinical features include iris stromal hypoplasia which makes pigmented epithelium visible giving it striking chocolate brown color or dark slate grey color and trabecular meshwork maldevelopment. Angles have iridotrabecular or iridocorneal adhesions. IOP typically shows large fluctuations. Cornea can also be involved showing diameters larger than usual. Juvenile glaucoma is seen in most of the cases. It can appear at any age from birth to late adulthood.[7,8] Family history is present in such cases. Diagnosis is made clinically. Physical examination is done to rule out any systemic involvement.

Management depends on signs and symptoms of patient. Most of the patients present with glaucoma. Both medical and surgical options are available. Any systemic involvement requires multidisciplinary approach.[9] These cases are difficult to manage and need multiple interventions to keep IOP in normal range. Follow-up is for life. Patients who have not developed glaucoma yet, need lifelong screening for the disease but have better prognosis.

This case report highlights difficulties encountered in management of these cases. Condition can be easily missed due to its rarity. Repeated and timely surgical interventions are required in such cases to stop fluctuations in IOP for preservation of vision. Lifelong follow-up is required to save patient from blindness due to secondary glaucoma. Awareness regarding screening of family members at risk is also very important.

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CASE REPORT

A Novel Frameshift Mutation in *ESCO2* Gene in Roberts Syndrome

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ABSTRACT

Roberts syndrome is a very rare autosomal recessive inheritance pattern genetic disorder characterised by symmetric bilateral extremity deformities, midfacial defect, and severe intellectual deficit. These patients also grow slowly prenatal and postnatal. RBS is caused by mutation in the *ESCO2* gene. With these clinical and radiological findings, the case was diagnosed as Roberts syndrome. Full gene sequencing of the *ESCO2* gene for the patient was done. In this patient, a novel frameshift mutation was identified in the *ESCO2* gene.

Key Words: *Roberts syndrome. ESCO2. Novel mutation.*

INTRODUCTION

Roberts syndrome (RBS) is a very rare autosomal recessive inheritance pattern disorder. RBS is characterised primarily by bilateral extremity malformations, craniofacial deformities, profound growth deficiency of prenatal onset, and a wide range of mild to severe growth retardation after birth. Extremity deformities, including bilateral symmetric hypomelia or varying from tetra-amelia to tetraphocomelia, lead to mesomelic shortening. The upper extremity is affected more severely than the lower extremity. Other extremity malformations include oligodactyly with hypoplasia or thumb aplasia, clinodactyly, syndactyly and flexion contractures of knees, ankles, wrists, or elbows. Head and face deformities include cleft palate with or witout cleft lip, midfacial capillary hemangioma, prominent premaxilla, microbrachycephaly, encephalocele, a small chin, malar flattening, widely spaced eyes, bluish sclera, down-slanted palpebral fissures, exophthalmos, shallow eyeball, prominent eyes, corneal blur, immature ala nasi, pointed nose, thin nares, and malformed ears.[1-3]

Microcephaly, severe mental defect in some and borderline to mild mental deficiency in others.

Mortality is increased among heavy affected patients. Most individuals have been stillborn or have died in early infancy. Slightly affected patients may survive longer. However, people with RBS may have, heart, kidney and genital abnormalities.[3]

This disorder was initially described by Roberts in 1919.[4] RBS is rare. Although the exact frequency of this syndrome is unknown, approximately 150 affected individuals of different racial and cultural from society have been reported.[3]

RBS is caused by mutation in the *ESCO2* gene, the protein product of which is necessary for the establishment of sister chromatid adherence in the course of S phase.[5]

This report describes the clinical, radiological and molecular features of a Turkish patient with Roberts syndrome.

CASE REPORT

The case was of an eight-day male neonate. The patient presented with absence of sucking, difficulty in feeding, limitation of movement in extremities, and very much deformities in the face and extremity. The neonate was born to 27-year healthy mother's first pregnancy from a term (39 weeks) gestation and was small for gestational age (2020 gram). The parents were healthy second-degree relatives (cousins). Maternal obstetrical history for infection, smoking, alcohol use, teratogenic drug intake, diabetes mellitus, X-ray exposure during pregnancy, or familial risk factors were negative. The medical history, revealed that although multiple congenital abnormalities had been determined on fetal ultrasonography, there was no history of congenital abnormality in the family.

On physical examination, his height, weight, and head circumference were below 3 percentile (41 cm, 2590 gram and 32 cm, respectively). There was microcephaly, sparse hair, midfacial capillary hemangioma, clear and flat forehead, prominent premaxilla, shallow orbits and prominent eyes, hypertelorism, malformed ears with hypoplastic lobules, pointed nose, thin nares, micrognathia, high palate short neck (Figure 1a). On cardiological examination, the cardiac pulse was 136/minute and it was rhythmic. There was a 2/6 systolic ejection murmur, auscultated in mesocardia focus. Echocardiographic evaluation detected small apical muscular ventricular septal defects and patent foramen ovale. On genital examination, he had 1 ml of testicles bilaterally and 3.0 cm penile length.

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Table I: Normal sequence.

Amino acid number	293	294	295	296	297	298	299	300	301
The bases that constituent amino acid	AGA	GTT	TCT	TCA	AAG	GAA	CAT	AAA	GTT
Amino acid	R	V	S	S	K	E	H	K	V

Table II: Mutant sequence.

Amino acid number	293	294	295	296	297	298	299	300	301
The bases that constituent amino acid	AAG	AGT	TTC	TTC	AAA	GGA	ACA	TAA	
Amino acid	K	S	F	F	A	G	T	X	

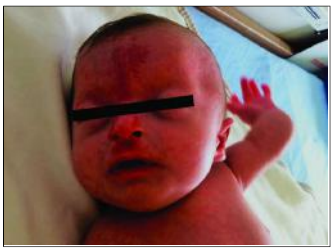

Figure 1a: Photo of the patient.

Figure 1b: Photo of the patient.

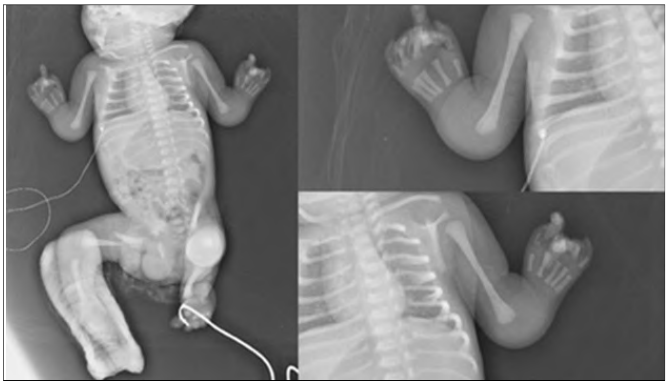

Figure 2: Radiographic images of the patient.

On the upper extremity examination, there were flexion contractures of bilateral wrists and bilateral symmetric hypomelia. On the lower extremity examination, there were bilateral pes equinovarus, flexion contractures of bilateral knees and syndactyly (Figure 1b). Whole-body X-ray; shows absence of ulna and radius, reduction in the femurs, absence of the fibulas; and there was midline fracture of the left femur (Figure 2). There were no other skeletal abnormalities. The trans-fontanel and the abdominal ultrasound were normal.

With these clinical and radiological findings, the case was described as Roberts syndrome. Full gene sequencing of the *ESCO2* gene for the patient and their normal parents was done. Molecular studies of *ESCO2* have confirmed the diagnosis. In the patient, a homozygous 1-base insertion (c.877_878insA) leading to a frameshift mutation (p.R293Kfs*8) was identified a novel mutation in the *ESCO2* gene. Parents were heterozygous, presenting one normal variant and the c.877_878insA mutation of *ESCO2* gene.

Arginine (R), the amino acid at position 293 of the *ESCO2* gene, was into to Lysine (K) amino acid as a result of frameshift (p.R293Kfs*8). The reason of this frameshift is addition of adenine nucleotide between the bases of 877 and 878 (c.877_878insA). Arginine 293 amino acid is encoded by AGA (877. base A (Adenine), 878. base G (Guanine), 879. base A (Adenine). Because an Adenine base is inserted between 877 (A) and 878 (G) bases, the triplet base sequence has changed to the AAG format. The hindmost 'A' nucleotide become first nucleotide of next amino acid, because the intervening base converts AGA known as 293 base sequence to AAG-A. As a result, triplet base sequence of the amino acids is disrupted. The AAG base sequence codes for Lysine (K) amino acid. As seen mutant table, chancing nucleotide sequences generate early stop codene (TAA, X stop codene) that is eight amino acid later from amino acid in insertion. While normally the protein of this gene composes of 601 amino acid, it has 300 amino acids because of the stop codene (Tables I and II).

ESCO2 mutation in this study was not found in public SNP databases (dbSNP136, 1000 genomes, the NHLBI Exome Sequencing Project Exome Variant Server, or The Exome Aggregation Consortium). Insica prediction methods, PolyPhen-2, and Mutation Taster indicated this mutation would be harmful.

The patient has been planned to be administered an individualised treatment, which increases the quality of life. The patient was planned to undergo surgical correction for extremity anomalies and to provide special training for developmental delays and learning difficulties. Psychomotor development and physical growth will be periodically followed. Speech evaluation and treatment will be carried out in the future. The patient was also planned for follow-up in terms of ophthalmologic, cardiac and renal disorders.

DISCUSSION

This very rare, autosomal recessive disorder characterised severe defects in midfacial and limb development contains features that overlap with RBS, such as intrauterine-onset growth deficiency, severe mental retardation, microcephaly, brachycephaly, prominent premaxilla micrognathia, cleft lip with or without cleft palate, short stature, flexion contractures of various joints, petty hands and feet.[6]

Patients show shortness in their upper extremities, craniofacial abnormalities, 3 < percentile of lenght, weight and head circumference. Also the parents were healthy second-degree relatives (cousins). The relationship is the most remarkeble finding in cases reported in the literature, which supports the recessive inheritance.

Roberts-SC phocomelia syndrome is determined with ultrasonographic and cytogenetic investigations in the prenatal stage.[7] Diagnosis rate in ultrasonography would be in the range of 41-65%.[8,9] Therefore, ultrasonography in the early gestational weeks is useful to diagnose some anomalies, such as congenital abnormalities. In the presented case, mother had undergone an anomaly scan in pregnancy period and multiple anomalies were determined in the baby. Despite this, mother wanted to continue the pregnancy. This syndrome is commonly seen in society of inbreeding, which may affect a few individuals from the same family. It is very important to inform the parents about the course and characteristics of this syndrome.

In 2005, the gene responsible for the disease was determined by Vega *et al*. and eight different mutations in a gene called "*ESCO2*" were reported in 18 affected individuals from 15 families.[5] The *ESCO2* protein product is a member of a conserved protein family that is required for the establishment of sister chromatid cohesion during S phase and has assumed acetyl-transferase activity.[5]

In this patient, a homozygous 1-base insertion (c.877_878insA), leading to a frameshift mutation and premature stop (p.R293Kfs*8), was identified a novel mutation in the *ESCO2* gene. Transcripts harbouring this frameshift mutation might be sensitive to nonsense-mediated mRNA decay.[10]

These patients should be treated by a team of pediatric, genetic, ophthalmologist, cardiologist, nephrologist, neurologist, child development, rehabilitation, general surgery, and orthopedic specialists. It is very important that family should be informed about antenatal diagnosis and preimplantation genetic diagnosis.

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CASE REPORT

Berardinelli-Seip Congenital Generalised Lipodystrophy

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ABSTRACT

Berardinelli-Seip congenital lipodystrophy (BSCL) syndrome is a rare genetic disorder caused by dysregulation of glycemic and lipid metabolism. We report five BSCL cases with typical clinical pictures and complications. These, to the best of our knowledge, represent the first case series from Pakistan. BSCL is characterized by marked atrophy of adipose tissue, acromegaly, acanthosis nigricans and tall stature. We could not perform genetics studies in any patient owing to non-availability of genetic laboratory in Pakistan. All the cases presented hypertriglyceridemia. One case developed hyperinsulinism controlled with metformin. There is no curative treatment and the current approach is low-fat diet and management of insulin resistance and diabetes. Recently published studies showed that leptin-replacement therapy is promising in the metabolic correction of complications of BSCL. This highlights the importance of further research in BSCL treatment.

Key Words: *Lipoatrophy. Insulin resistance. Hypertriglyceridemia.*

INTRODUCTION

Berardinelli-Seip congenital lipodystrophy (BSCL) is a rare autosomal recessive disorder characterised by generalised lack of subcutaneous adipose tissue, insulin resistance, acromegaloid features, hepatomegaly, and hypertriglyceridemia.[1] Additional features are mental retardation, hypertrichosis, precocious puberty, nephropathy, bone cysts, and hypertrophic cardiomyopathy.[2]

Berardinelli from Brazil described the first patient in 1954. Later somewhere in 1959, Seip described series of patients from county of Rogalandon, Norway. So, the syndrome was coined as Berardinelli-Seip syndrome.[3] The estimated world-wide prevalence is 1 in 10 million population.

The exact pathophysiology of lipodystrophies is still unknown. However, murine models of lipoatrophic diabetes suggest that it is primarily a genetic defect of fat development, resulting in diabetes and dyslipidemia.[4] The absence of adipose tissue causes leptin deficiency; and leptin plays an important role in the metabolism of fats and glucose. It has been demonstrated that exogenous administration or transgenic overexpression of leptin results in marked improvement in insulin sensitivity, glycemic control, dyslipidemia and hepatic steatosis in mice. The adinopectin is another fat-derived hormone, which has been shown to be involved in insulin resistance.[5]

On the basis of mutational and haplotype analysis, BSCL families have been classified into three types: BSCL1, BSCL2 and BSCLX.[6] BSCL1 has less severe phenotype, starts in 2nd-3rd decade of life, and is more common in Africa and North America. It is linked to 9q34. BSCL2 is more prevalent in Portugal, Lebanon and Norway. It starts in neonatal life and is linked to 11q13. Some families appear unlinked to 9q34 or 11q13. They are classified as BSCL3. It is associated with a severe phenotype. We had the opportunity to diagnose and manage five children with BSCL. We present our experience to highlight the need for recognition of this condition and create awareness among pediatricians.

CASE REPORT

Case 1: The first patient was diagnosed in 2009 from Lahore, Punjab, at 3 years of age. She was referred to us by a general physician for her hepatomegaly assessment. On examination, she was having generalised lipoatrophy involving limbs, abdomen and chest, allowing for the diagnosis of BSCL. Her height was higher than her target height. She had hirsutism and acromegaloid appearance. The triglycerides (TGs) were raised and liver biopsy showed steatosis. Lipoatrophy and muscle hypertrophy increased markedly during the years. At 8 years of age, she developed acanthosis nigricans and her resting insulin levels were higher than normal. She was advised to consume a low-fat diet and intake of saturated fats; and cholesterol were reduced. She was also advised to increase daily physical activity. Metformin has been added to her medicine. Our patient showed improvement in HbA1c.

Case 2: Second patient from Multan presented at 8 months of age with chronic diarrhea. He had generalised

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Table I: The clinical and the biochemical features of five cases.

Parameters	Case 1	Case 2	Case 3 (Figure 1)	Case 4	Case 5
Age	10 years	8 months	3 years	5 years	12 years
Sex	Female	Male	Male	Female	Female
Lipoatrophy	Present	Present	Present	Present	Present
Hirsutism	Present	Present	Present	Present	Present
Acanthosis nigricans	Present	Absent	Present	Absent	Present
Hepatomegaly	Present	Present	Present	Present	Present
Acromegaloid features	Present	Present	Present	Present	Present
Insulin resistance	Present	Absent	Present	Absent	Present
Triglyceride level	375 mg/dl	397 mg/dl	456 mg/dl	375 mg/dl	419 mg/dl
Precocious puberty	Absent	Absent	Present	Absent	Absent
Cardiac involvement	Absent	Absent	Absent	Absent	Absent
Bone cyst	Absent	Absent	Absent	Absent	Absent
Liver biopsy	Micro-vesicular steatosis	Micro-vesicular steatosis	Micro-vesicular steatosis	Micro-vesicular steatosis	Micro-vesicular steatosis

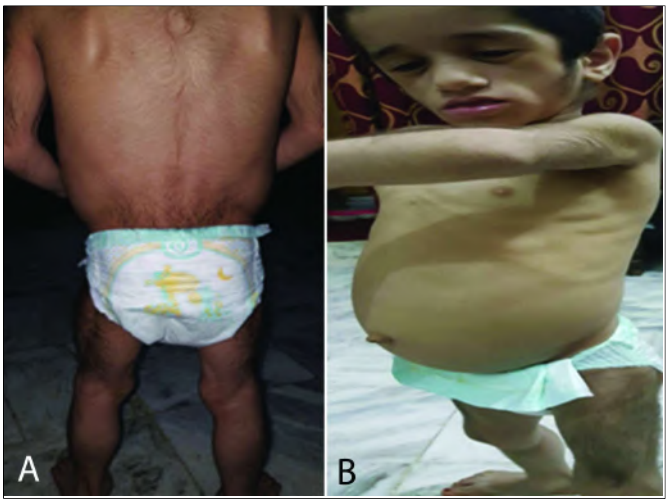

Figure 1: (A) Hypertrophied calf and thigh muscles; (B) Acromegaloid features, hirsutism and hepatomegaly.

lipoatrophy and muscle hypertrophy. His height was at 95th centile and weight at 25th centile. His liver was enlarged 4 cm below the coastal margin. Laboratory data revealed normal random blood sugar (BSR), raised TGs, normal liver function tests (LFTs), and mildly raised level of insulin at resting state. Liver biopsy showed mild steatosis with no glycogen content.

Cases 3 and 4: Two siblings, a brother and a sister from Lahore aged 3 years and 5 years respectively, were referred from Department of Developmental and Behavioral Sciences for the investigation of hepatomegaly and raised TGs. Both of them were taller for their ages and had lipoatrophy, muscular hypertrophy and hypertrichosis since neonatal age (Figure 1). Sister had moderate mental retardation, while brother had motor delay only. For that, they were on follow-up of Developmental Sciences Department. Both of them had hepatomegaly but LFTs were normal. Insulin levels were much higher in sister as compared to brother. Echocardiography was normal in both.

Case 5: A 12-year girl was diagnosed as insulin-dependent diabetes mellitus (IDDM) since the age of 7 years and was on endocrinology follow-up. She never went in diabetic ketaacidoss (DKA) since the diagnosis. But, now her BSR was not in control despite maximum units of insulin for her age. She was referred to us for hepatomegaly and markedly raised TGs. On examination, she had generalised lipoatrophy and muscle hypertrophy. Acanthosis nigricans was present on nape and axilla bilaterally. We performed liver biopsy, which showed micro-vesicular steatosis. She was already on high dose of insulin; metformin was also added. She was advised low fat diet and increased physical activity.

The relevant clinical and biochemical features of all five children are shown in Table I.

DISCUSSION

In this series, all five children had lipoatrophy since neonatal age that gradually increased with age. All had acromegaloid features and hypertriglyceridemia. Hepatomegaly was found at different ages with similar neuropsychology that is microvesicular steatosis. There was no ambiguity in the diagnosis of BSCL disease in any of these cases because all of them had major diagnostic features of the disease as described in literature,[7] i.e. lipoatrophy, acromegaloid features, hepatomegaly, elevated serum TGs and insulin resistance. In case 1, hepatomegaly and hypertriglyceridemia were suggestive of glycogen storage disease. But, lipoatrophy and hyperinsulinism favoured BSCL. Subsequent cases (cases 2, 3, 4 and 5) were diagnosed and treated as BSCL syndrome in the setting of lipoatrophy.

The features of BSCL syndrome are mainly due to fat deficiency, which leads to failure of the tissues to respond to insulin resulting in hyperinsulinemia. Lipodystrophy is present since birth but age at which hyperinsulinemia affects individuals is not well established. Approximately 25-35% develop diabetes mellitus (DM) between ages 15 and 20 years.[8] As seen in our case, the girl had hyperglycemia at younger age. She was treated as IDDM; but later, it proved to be type II DM due to insulin resistance. She had acanthosis nigricans and never went in DKA.

In all individuals with BSCL, the liver is affected in the form of abnormal liver functions, hepatic steatosis and cirrhosis.[9] Hepatomegaly with hepatic steatosis was present in all patients in our case series. Hypertrophic cardiomyopathy is reported in 20-25% of affected individuals and is a significant cause of morbidity from cardiac failure and early mortality.[10] Our patient showed no evidence of cardiac involvement.

Acromegaloid features consist of prognathism, enlarged hands/feet, big ears, prominent orbital ridges, macro-genitosomia, gigantism, muscular hypertrophy, and advanced bone age; all are thought to be a result of insulin cross-reacting with IGF-1 receptors.[11]

Management of BSCL syndrome includes reducing the TGs levels, improving the insulin resistance and controlling the diabetes. Patients with BSCL, as exemplified in our case 5, are quite resistant to insulin therapy.[12] Maximum daily dose of insulin may not suffice to control blood glucose levels. Metformin is helpful in reducing their appetite and improving insulin resistance, resulting in better glycemic control and hepatic steatosis. Our patient showed improvement in HbA1c on a combined regimen of insulin and metformin. Recombinant leptin is a break-through in the management of congenital generalised lipodystrophy. This medication improves insulin sensitivity, decreases TGs levels, and helps control energy homeostasis. This results in less food intake and lower fasting blood glucose levels as well as lower HbA1c levels. Patients with BSCL must have a multidisciplinary follow-up. They should consume a low-fat diet, with reduction of saturated fats and cholesterol intake. It is also important to practice daily physical activity.

BSCL syndrome is a rare disease which causes important metabolic abnormalities, with complications and a fatal outcome if optimal therapeutic, preventive measures, and a multidisciplinary follow-up are not adopted. Pediatricians should keep this possibility in mind while evaluating patients with similar clinical features.

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Clinicopathological Characteristics of Prostate Cancer in Patients Presenting to a Tertiary Care Private Sector Hospital

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ABSTRACT

Prostate cancer (PCa) is one of the leading cancers in older males. The aim of this study was to evaluate the clinico-pathological characteristics of prostate cancer and rate of detection of prostate cancer by DRE (digital rectal exam) in patients presenting to a tertiary care private sector hospital in Islamabad, and determine the awareness about PCa in these patients. There were 226 patients who presented from December 2009 to December 2015 having mean age of 68.63 ±8.76 years and median Prostate Specific Antigen (PSA) value of 19.12 ng/mL (IQR=35.8). Median prostatic volume was 49 (IQR=22) gram/cc in the subjects. DRE was abnormal in 164 (72.56%) patients and normal in 62 (27.43%) patients only. Majority of patients presented relatively late, which may be due to lack of screening programme or public awareness.

Key Words: *Epidemiology. Clinicopathological characteristics. Prostate cancer.*

Prostate cancer is one of the leading cancers in older males, as most of the cases are diagnosed in men over 65 years of age.[1,2] It has been the second common cause of mortality in males after lung cancer.[3] The reason for high incidence of prostate cancer in the West may be ascribed to a combination of genetic and environmental risk factors; but it might also be due to improved health coverage leading to diagnosis of new cases.[3]

The incidence of prostate cancer is said to differ even in various parts of the same country.[4,5] So different countries and guidelines have made their own preventive and screening programmes based on incidence and prevalence of the prostate cancer, clinico-pathological variations, education of people, their understanding of the disease process and awareness about its treatment.[4,5]

Lack of a tumor registry at national level is an impediment in understanding our own national epidemiology of the disease. There are only a few studies at local city level from Karachi. This study was done to evaluate the clinicopathological characteristics of prostate cancer, and rate of detection of prostate cancer by DRE (digital rectal exam) in patients presenting to a tertiary care private sector hospital in Islamabad. Most of the patients in Shifa International Hospital come from the Northern Province and upper Punjab. So this study would give knowledge about the frequency of prostate cancer detection by DRE, clinico-pathological details and the stage and grades at the time of presentation in the hospitals in the north eastern areas of Pakistan.

This was a cross-sectional observational study conducted from December 2009 till December 2015. Trans-rectal ultrasound (TRUS) guided biopsy was done in all patients presenting with suspected prostate on digital rectal examination (DRE). In patients with normal DRE, having lower urinary tract symptoms, and age greater than 50 years, PSA levels were checked. If PSA levels were more than 4ng/mL, TRUS biopsy was again done to rule out prostate cancer. All procedures were done under local anesthesia (Xylocaine gel was used prior to the procedure) in left lateral position, and 12 core biopsies were taken under ultrasound guidance. Specimen was sent in six containers of formalin. Number and length of core biopsies was measured. They were submitted in 6 separate cassettes and processed for 18 hours approximately. Stained with hematoxylin and eosin for light microscopy. On microscopy, primary and secondary Gleason's grade were noted. We collected data regarding age of the patient, PSA levels, Gleason score on histopathology, PSA density, mean prostatic volume, organ confined disease, locally advanced, and metastatic disease. The data was compiled and analysed in SPSS version 16. Frequencies and percentages were determined for findings of DRE, and biopsy results. Mean ± SD and median (IQR) were calculated for quantitative variables, i.e. age and serum PSA level, respectively.

A total of 226 patients diagnosed with prostate cancer at our institute were included in the study. Their data was collected from charts review. Mean age was 68.63 ±8.76 years and median PSA value was 19.12 ng/mL (IQR=35.8). Median prostatic volume was 49 (IQR=22) gram/cc. Out of the 226 patients with prostate cancer, 15 (6.63%) were smokers. One hundred and eleven

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Table I: Our clinicopathological findings compared with other studies.

	Hsing *et al.* West Africa	Belinda F *et al.* Jamaica	Deep Parkash *et al.* Karachi	Yasmin *et al.* Karachi	Our data
No of Ca prostate Pts.	65	63	151	206	226
Mean age	62 years	59.9 ±8.2 years	64.7 ±8.04 years	67 ±10.12 years	68.63 ±8.76
Main presenting symptom	screening	screening	screening	------	Irritative LUTS
Abnormal DRE	40 (61.5%)		128	------	164(72.56%)
Serum PSA	202.12 ng/mL	8.9 ng/mL (range 1.5-1059 ng/mL)	23.8±10.5	------	median PSA=19.12 ng/mL (IQR=35.8)
Mean PSA density	6.38 ±20.6ng/mL/cc	------	------	-------	1.50 ng/mL/cc
Prostatic volume	38.43 cc	------	------	--------	median volume=49 cc (IQR=22)
Gleason score <7	23 (35.4%)	54%	------	66.8%	73 (32.30%) patients
Gleason score ≥7	42 (64.6%)	46%	------	26.3%	153 (67.70%) patients
Organ confined	56 (86.15%)	------	------	51%	112 (49.5%) patients
Local invasive	9 (13.8%)	------	------	28.8%	66 (29.2%) patients
Metastatic disease	None	------	------	8.2%	48 (21.3%) patients

(49.11%) had obstructive lower urinary tract symptoms (LUTS), 59 (26.10%) had irritative LUTS; in 23 (10.17%) it was found incidentally, 20 (8.84%) had hematuria, while in 12 (5.30%) patients it presented with metastasis.

Stage-wise, it was confined locally in 112 patients, locally advanced in 66 patients, and advanced in 48 patients. Seventy-three (32.30%) patients had less than 7 Gleason score, 78 (34.51%) patients had score of 7, while 75 (33.18%) patients had Gleason score more than 7. DRE was abnormal in 164 (72.56%) patients and normal in 62 (27.43%) patients only. Family history was positive in 12 (5.30%) patients.

There has been wide geographical variation in the incidence of prostate cancer in high- and low-risk countries.[2] In a study done by Pinnock *et al*., it was found that prostate cancer was a matter of concern in all groups including those at low-risk as well. Most men believed that screening for prostate cancer should be offered. The reason for barriers to meaningful health action included issues such as not discussing frankly about such sensitive health issues and the poor relationships of the patients with doctors. This study suggested a dire need for community and professional education and for promotion of health policies for focusing on preventable morbidity.[6]

Currently used tools to detect prostate cancer include serum total PSA test, the DRE, TRUS, and TRUS-guided needle biopsy. Some studies have shown that inter-observer variation is not that much different if the prostate is examined in a systematic way.[7]

In a study done by Hsing on more than 1,000 Ghanaian men, 65 patients had prostate cancer. Out of these confirmed Ca prostate cases, 61.5% had positive DRE at screening. In 60% individuals, clinical stage was T2 or greater and in 65% of cases Gleason score was 7 or greater.[8] In the present study, 153/226 (67.7%) cases had Gleason score of 7 or above. In a Pakistani study, it was concluded that Karachi falls into a low-risk region for prostate cancer, although it was noted with an increasing incidence and a marginal down-staging, according to the records till the early years after year 2000.[2] In that study, 208 patients had prostate cancer with mean age of 67.1 ±10.1 years. Approximately half the cases presented as localised cancers (51.0%) and a third (37.0%) at an advanced stage.[2] In present study, mean age was 68.63 ±8.76 years and median PSA was 19.12 ng/mL (IQR=35.8). There were 112 (49.5%) patients with localised prostate cancer, which was comparable to a study in Karachi.[2] Their study had not commented in terms of Gleason scores categorisation of their patient's clinicopathologically, while we had categorised our patients according to Gleason scoring. In one study in Austrailia, it was found that there was no significant difference in the rates of insignificant and high-risk prostate cancer between men >55 years, and >55 years on biopsy.[9]

In this study, on a subsection of a Pakistani population, perhaps the awareness about prostate cancer is too low as in our institute there were more than 50% subjects who presented late with locally advanced or metastatic prostate cancer. Most of the patients had lower urinary tract symptoms of duration ranging from one to 6 years. A markedly high rate of detection by DRE can be attributed to late presentation due to lack of public awareness and screening programme. It also shows the importance of DRE and PSA monitoring, in older patients having urinary symptoms, at general surgeons and physicians level in periphery to detect it at earlier stage. The study participants included a number of patients with urinary symptoms and their DRE were significantly abnormal (164, 72.56%). Only 15/226 (6.6%) knew something about relation of prostate to urinary symptoms. Only 8/226(3.53%) knew something about PSA, prostate and prostate cancer relation, and only 3/226 (1.3%) knew about following PSA and concern for cancer prostate and its treatment and follow-up at time of presentation. There is also a need to improve public attitude regarding urinary symptoms in older age and knowledge about prostate cancer in Pakistan.[10]

Larger multicenter studies and registry record-keeping is needed to have a country-wide epidemiology of disease and the trends of Gleason scores, grades, and stages of the prostate cancer at the time of presentation in hospitals in Pakistani population.

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Cardiac Rehabilitation as a Continuum of Care and Current Situation in Pakistan

Sir,

We have read with interest, "Do We Need Cardiac Rehabilitation in Heart Failure?" by Kinsara with interest.[1] Author has correctly highlighted the role of cardiac rehabilitation in improving the quality of life (QOL) in patients with heart failure. We would like to add that cardiac rehabilitation is a recognised field and there is evidence that cardiac rehabilitation has a role beyond heart failure including other cardiac diseases and cardiac surgery procedures.

The prevalence of cardiovascular diseases is increasing at an alarming pace, and it is becoming a leading cause of death in middle- and low-income countries. World Health Organization (WHO), in its global action plan to prevent and control non-communicable disease 2013-2020, has emphasised the need for rehabilitation including cardiac rehabilitation.[2] Over the last three decades, cardiology has evolved from only acute management of cardiac events to a multidisciplinary team approach that begins with life-saving measures, medical management and then simultaneously introducing patient centered rehabilitation plan.[3] This comprehensive cardiac rehabilitation programme is different from the acute cardiology and is managed by rehabilitation medicine physicians (physiatrists) who lead a multi-disciplinary team often consisting of physical therapists, occupational therapists, respiratory therapists, psychologists, social workers and recreational therapists. Each team member makes assessment and treatment and is initiated under the supervision of rehabilitation medicine physician. This rehabilitation assessment goes beyond the routine medical and physical management and aims to get the patient back to his pre-disease/ surgery functional levels considering his/her current health status, home environment and social support. The aim of this approach is to devise a patient-specific plan to improve the survival and QOL of the patient. Studies worldwide have demonstrated an improvement in QOL and decreased disease burden after cardiac rehabilitation intervention.[4] Cardiac risk factor reduction, improved physical, social and psychological health, reduced disability adjusted life years, decreased recurrence and mortality rate are proven outcomes of a standard cardiac rehabilitation programme. Many international cardiology societies have developed guidelines and proposed cardiac rehabilitation as a continuum of care for a patient with cardiac disease.[5] Two meta-analyses reported reduction in recurrence and mortality in coronary artery disease patients while a meta-analysis of 63 randomised control trials reported a 17% reduction in recurrent myocardial infarction after 12 months, which further improved to 47% reduction after 2 years in patients who underwent cardiac rehabilitation.[6]

Despite the evidence, the availability of cardiac rehabilitation and its utilisation is minimal.[7] Barriers to underutilisation include inconsistent physician awareness and referral, and poor patient compliance. Ali *et al*. reported an attendance of 36.2% for patients enrolled for cardiac rehabilitation at a cardiac institute in Karachi.[8] In Pakistan, only few cardiac hospitals offer integrated cardiac rehabilitation and none of the cardiac institutes in our country offers a formal cardiac rehabilitation programme supervised by a trained rehabilitation medicine physician.

We propose that considering the increasing burden of cardiovascular diseases in the country, efforts should be made to establish formal cardiac rehabilitation units in all major cardiology institutes in the country. These units should be managed by rehabilitation medicine physicians qualified in cardiac rehabilitation and should be part of the core cardiology team managing a patient with a major cardiac disease. This will ensure that patients with cardiac diseases, interventions and surgery have more productive and better QOL.

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Sofosbuvir Causing Diabetes Mellitus: Is there a Link?

Sir,

Chronic hepatitis C infection affects 170 million individuals worldwide. Since the introduction of new oral direct acting antiviral (DAA) drugs, the treatment of chronic hepatitis C infection has been revolutionised.[1] Although metabolic derangements along with insulin resistance are widely recognised with HCV infection, there are few case reports of how treatment with the newer oral antiviral agents leads to diabetes in previously non-diabetic chronic hepatitis C infected patients, after successful eradication of the infection itself.[2]

Here, we describe a case of a 50-year female with a body mass index (BMI) of 32.8 kg/m^2 with chronic hepatitis C virus infection, mixed genotype 3 and 6, diagnosed and started on three drug regimens with sofosbuvir, 400 mg QD, Daclatasvir Dihydrochloride, 60 mg QD, and Ribavarin 400 mg TID. She achieved end-of-treatment response (ETR) at 6 months but thereafter presented with history of progressive leg pains. On testing, her random blood sugar was 494 mg/dl with an anion gap of 12 and no urinary ketones. Prior to starting antiviral agents, her HbA1c was 5.50% and three months post-treatment, her HbA1c was 11.40%. Her HbA1c pre- and post-treatment were done by our laboratory by immunoturbidity method and data from this laboratory is traceable to NGSP and is DCCT certified. There was no history of gestational diabetes mellitus (DM) or pre-diabetes, except that her father was a known diabetic. Other etiologies for her raised blood sugars were excluded, including exposure to certain drugs, pancreatitis or any ongoing acute infection.

In recent past, there has accumulated conflicting data on effects of oral DAAs with few studies favouring good glycemic control following treatment; whereas, others showing no effects on HbA1c levels pre- and post-treatment.[3,4] Our patient did not have any comorbities or a pre-diabetic range HbA1c level prior to treatment with DAAs and it was only after completion of treatment and achieving ETR that there was a sudden rise in HbA1c levels. Therefore, we could not completely rule out the possibility that the DAAs might have led to overt hyperglycemia in our patient or this could simply be a coincidence. It is not possible to pinpoint the precipitating factor on the basis of just one case presented here. However, it provides food for thought for future studies on this subject.

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Peripheral Osteochondroma of Buccal Mucosa

Sir,

Osteochondroma is regarded as the most common tumor of bone that commonly affects the long bones; however, its occurrence in jaw bones is very rare.[1,2] Peripheral Osteochondroma (POC) of long bones is usually derived from the joint capsule or para-articular soft tissue without bony attachment.[3] A 38-year male presented to the Department of Oral Medicine and Radiology with the chief complaint of a painless swelling on his right upper posterior region of jaw since 1 year. The growth was without any discharge and was not associated with any systemic changes. Intra-oral examination revealed an oval-shaped sessile growth of right buccal mucosa, 3 x 3 cm in dimension (Figure 1). The color of the overlying mucosa was normal without any evidence of ulceration. On palpation, it was a firm to hard growth. Based on all the clinical findings, a provisional diagnosis of fibroma was made, and complete surgical excision of the lesion was planned. The patient was referred to the Department of Oral Surgery. Surgical excision of the lesion was done under local anesthesia and the tissue specimen was sent to the Department of Oral and Maxillofacial Pathology. The follow-up period of one year was uneventful.

Microscopical examination of the tissue specimen revealed mature bony trabeculae lined by osteoblasts and enclosing osteocytes surrounded by a cap of mature cartilaginous stroma (Figure 2). Serial section showed well circumscribed, mature chondroid areas enclosing chondrocytes with central ossification (Figure 3). The overlying epithelium was normal stratified squamous epithelium separated by the lesional tissue with fibrocellular connective tissue stroma. Based on all the microscopical features, a final diagnosis of POC was rendered.

POC of oral cavity is a rare neoplasm and it should not be confused with osteocartilagenous choristoma. The later is devoid of peripheral capsule surrounding lobules of mature cartilage with central areas of mature ossification. Clinically, osteocartilaginous choristoma presents as a painless, slow growing tumor, commonly found on the tongue followed by buccal mucosa and palate.[2] A complete surgical excision is the treatment of choice for osteocartilaginous choristoma.[4] The present case was also treated with the same approach and no recurrence was noted.

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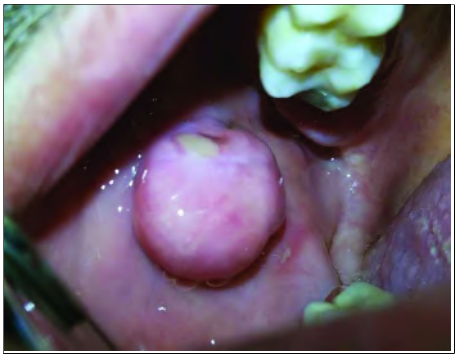

Figure 1: Clinical picture of the swelling.

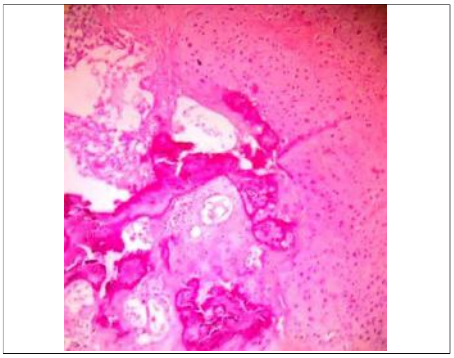

Figure 2: Low power view shows a mature bone formation and marrow surrounded by the cap of mature cartilaginous tissue. (Hematoxylin and eosin staining X20).

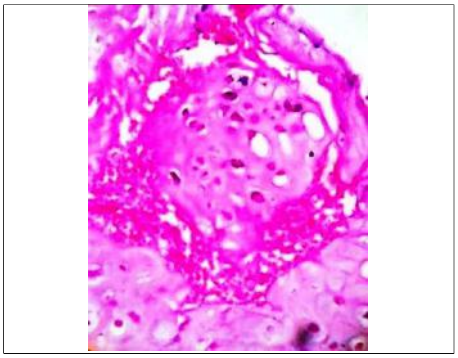

Figure 3: Well circumscribed mature chondroid areas enclosing chondrocytes with central ossification. (Hematoxylin and eosin staining X40).

Spontaneous Neck and Upper Airway Hematoma

Sir,

A 51-year lady was referred to our hospital with unknown coagulopathy-related disorder. On physical examination, ecchymosis and swelling over anterior neck were noted (Figure 1A). No stridor or shortness of breath was auscultated. Laryngoscopy showed diffuse hematoma extending from tongue base to valleculla, epiglottis, hypopharynx, arytenoids and bilateral vocal cords (Figure 1B). Laboratory data showed prolonged PT of 33.2 seconds (reference range: 8-12 seconds), aPTT of 49 seconds (reference range: 23.9-35.5 seconds) and an elevated INR of 3.2 seconds (reference range: 0.90-1.10 seconds). Coagulation factor assay test revealed the results of factor IX, 62%; factor II, 40%; factor VII, 30%; and factor X, 32%. She had no prior history of hematological disorders, but she had inadequate dietary intake associated with alcohol abuse for the past few months. Under the impression of vitamin K deficiency, vitamin K supplementation therapy was initiated for one week. The patient's symptoms resolved and the INR was corrected.

Vitamin K is necessary for the synthesis of functional forms of coagulation factors II, VII, IX, and X in the liver. In patients with actively using alcohol, vitamin K deficiency may exacerbate deficiencies of vitamin K-dependent factors (II, VII, IX, and X) and cause hemorrhage.[1] Vitamin K deficiency, as a cause of upper airway hematoma is rare; but, it can be life-threatening due to causing acute airway obstruction. Close monitoring of such patients should be strict, because emergency airway intervention may be required.[2]

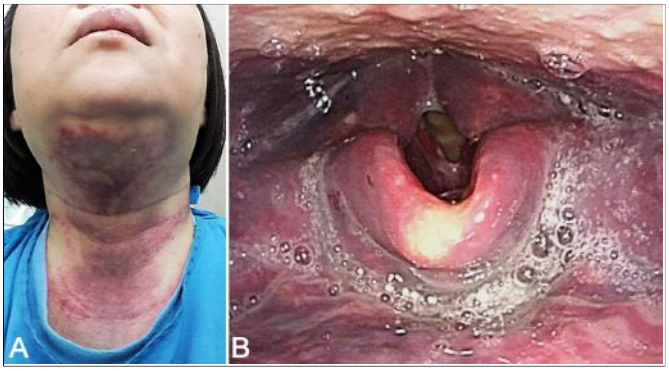

Figure 1: (A) swollen anterior neck with diffuse ecchymosis. (B) Submucosal hemorrhage extending from the base of the tongue to the valleculla, epiglottis, pyriform sinuses and bilateral vocal cords.

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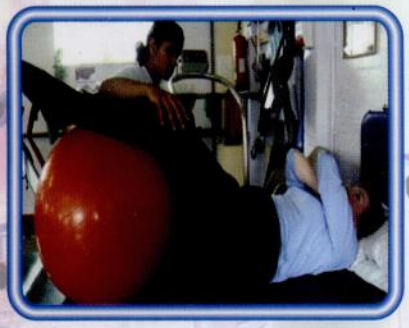

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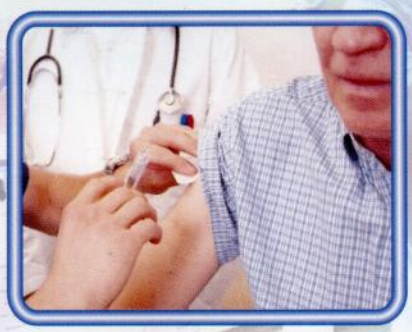

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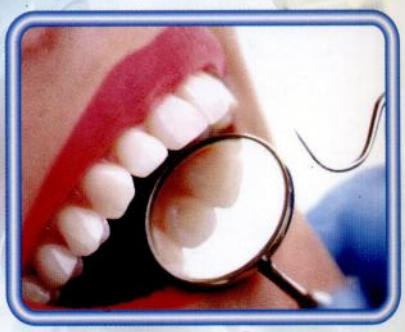

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