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EDITORIAL

Liquid Biopsy: Opportunities and Expectations

Rizwan Hashim

Introduction

Tumors have an abnormal rate of cell growth and cell division. Tumor cells release circulating tumor DNA (ctDNA), cell free DNA (cfDNA), mRNA and microRNA,[1] in the blood and body fluids which are the by-product of tumor cell lysis.[2] Both ctDNA and cfDNA provide valuable information regarding the cancer related mutations, genetic aberrations and presence of cell free Nucleic acid (cfNA).[3] The ctDNA provides information about the primary tumor and its secondaries / metastases. It is these ctDNA that anchor into novel locations and start dividing to develop secondaries of the tumours.[2]

Tumor biopsy is an invasive procedure in which tissue is excised from a growth and examined under a microscope. However, "Liquid Biopsy" is a relatively newer technique to find and evaluate cancer cells or their products in blood and body fluids that circulate after tumor cell lysis.

Historical Aspect for the Use of Liquid Biopsy:

The basis of liquid biopsy was the observation made by Ashworth[4] in 1869, where the circulating tumor cells were detected in a patient with tumor secondaries. The metastatic sites also shed tumor cells in the blood stream that could be detected and analyzed. It was after a long gap when scientists realized that the cell free DNA (cf DNA) could be detected, analyzed, and quantified. It was in 1948 when cell free DNA and free RNA was first detected and quantified.[5] This was done both in healthy individuals and those who had cancer. Progressing forward, it was in 1966 the researchers detected large volumes of cell free DNA in patients who had lupus. By 1980 the cell free DNA was also detected among oncology patients. In 1994 the scientists started detecting specific mutations from the cell free DNA present in the blood of oncology patients and by 2000 Veridex introduced CELL SEARCH® CTC test, for liquid biopsy assay as a first commercially available test for liquid biopsy, however CAPP Seq: cancer Personalized profiling by deep sequencing is another method for quantifying ctDNA analysis that is being used.[6] The Cobas® EGFR was the first liquid biopsy test that was approved by FDA in 2016. This test was for EGFR gene mutation to be detected in blood drawn from cancer patients.[7] Nowadays, ctDNA and cfDNA can be analyzed commercially by Mag Max Cell Free DNA Isolation kits and Cell Free Nucleic Acid Isolation Kits used for liquid biopsy specimens.[1]

Applications and Advantages of the Liquid Biopsy Technique:

When blood/body fluids are drawn as liquid biopsy for ctDNA and cfDNA, they also contain membrane bound lipid globules called Exosomes.[8,9] They contain tumor proteins, lipids, DNA fragments and micro RNA. The tumor related material in the exosomes can be analyzed to provide information about the mechanism involved in signals between the tumor cells, especially between primary tumors and metastatic sites. This is one of the many novel features of liquid biopsy and is not achieved by conventional tissue biopsy. Liquid biopsy provides a window of opportunity to understand tumor cell signaling that can be manipulated by various treatment modalities for cancer management. As cancer is a complex problem with systemic effects, liquid biopsy with frequent sampling provides a unique chance for mutation characterization and exosomal analysis.[10,11,12,13]

Together, these analytes have the strength to give the details of the tumors genetics, its metastasis and various stages of tumor progression.[14,15] The information provided is used for: genomics, epigenomics, transcriptomics, proteomics, metabolomics and information regarding minimal residual disease.[16] The major oncology domains where liquid biopsies have been successfully used are: colorectal,[17] breast and lung cancers, mainly to predict therapy responses and to monitor the

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patients for relapse. The developed assays are sensitive to detect these organ related mutations.[18,19]

Among other promising capabilities of liquid biopsy are the analysis of heterogeneity of tumor genetics, detection of very early treatment related resistance, detection of residual disease affecting prognosis, recurrence and follow up.[17,20,21,22]

Among the common applications for use of liquid biopsy technique is, early detection of cancer related DNA. This would help to plan the treatment, to review how well the patient is responding to treatment and to detect the recurrence of cancer. As liquid biopsy usually involves detection of cancer cells/DNA by drawing of blood, the same can be done multiple times. This helps the oncologists to monitor the molecular changes taking place in the tumor during treatment.[23] Liquid biopsies also provide a method to trace the tumor genetic variations sequentially that is not possible by using traditional tissue biopsy.[22]

Hence, liquid biopsy provides a non-invasive substitute for traditional tissue biopsies [25] and has become a popular field, with features for improved diagnoses for oncology and other types of diseases like Down Syndrome screening and detection of fetal DNA in maternal circulation.[2, 25, 26] Recently, liquid biopsy has made a place in Precision Medicine that manages patient with targeted therapies with improved detection of various genetic aberrations.[27]

Limitations of Liquid Biopsy Technique:

Despite many advantages there remain many challenges; from timing of sample, [28, 29] collection [30, 31] with relation to the stage of disease [32, 33, 34] adequate volume of sample collected, proper storage of sample, DNA isolation, sequencing and detection of relevant mutation, careful analysis with clinical validation of mutation analysis procedures.[35]

Summary and Conclusion:

It appears that the future of liquid biopsy is an ambitious endeavor and entails technological advancements but this procedure is gaining worldwide acceptance for early cancer detection, genetic evolution and monitoring of treatment resistance.[36] Moreover, with quantum leaps in technology and computation of data we can achieve much with the use of liquid biopsy and also save many precious lives through early revelation and analysis. [37,38,39,40]

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ORIGINAL ARTICLE

Clinical Spectrum, Laboratory Profile and Antibiotic Susceptibility Pattern of Children with Enteric Fever at a Tertiary Care Hospital of Karachi

Misbah Anjum, Shazia Soomro, Shazia Kulsoom, Safia Bibi, Sadaf Asim, Maira Riaz

ABSTRACT

Objective: To describe the clinical, laboratory and antimicrobial susceptibility profile of enteric fever patients hospitalized at a tertiary care children hospital of Karachi.

Study Design: Cross Sectional Study.

Place and Duration of Study: The study was conducted in three medical units of National institute of child health (NICH) Karachi from September 01, 2018 till February 28, 2019.

Materials and Methods: Patients of enteric fever diagnosed by positive blood culture for *Salmonella enterica Serovar Typhi,* hospitalized at National Institute of Child Health (NICH) Karachi were included. Clinical history and physical examination findings were recorded in Performa and routine laboratory tests and treatment was done according to hospital protocol. Outcome of patients was recorded in terms of alive or died. Data entry and analysis was done in SPSS 24.0. Descriptive statistics were applied for statistical analysis.

Results: Total 76 patients with culture proven typhoid were enrolled in the study including 39(51.3%) males and 37(48.7%) females. Mean age of children was 5.7± 2.81 years. Mean duration of fever in these patients was 19.04±8.39 days. Common symptoms were abdominal pain (64.5%), diarrhea (43.4%), vomiting (48.7%) and decreased appetite (67.1%). Antibiotic resistance profile showed 98.7% to chloramphenicol and trimethoprim-sulfamethoxazole, 96% resistance to ampicilline, 94.7% to cefixime and ceftriaxone, 82.2% to ciprofloxacin, 2.63% to azithromycine and no resistance to meropenam. All children were discharged with mean duration of hospital stay 8.296±3.33 days.

Conclusion: There are high rates of resistance against commonly used antibiotics for the treatment of enteric fever, including fluoroquinolones and cephalosporins leaving only extremely limited and costly options for treatment and prolonged length of hospital stay due to need of intravenous therapy.

Key Words: *Antibiotic Resistance, Cephalosporins, Enteric Fever, Salmonella Typhi.*

Introduction

Enteric fever is one of the important cause of morbidity and mortality in countries with middle to low income especially in south east Asia.[1-3] In Global Burden of Disease 2010, enteric fever was included which accounted to cause 12.2 million illnesses and 190200 deaths.[3,4] In 2015, it was estimated 17 million cases of typhoid and paratyphoid fever with highest incidence seen in South Asia and other areas like Southeast Asia and Sub-Saharan Africa.[5,6,7]

The clinical features of enteric fever are variable and may mimic any other febrile illness like influenza, malaria or dengue fever, which may cause delay in the diagnosis and start of appropriate therapy.[1] However most persistent feature is high grade continuous fever.[1]

Although a number of serological tests are available for diagnosis of typhoid fever but gold standard for the diagnosis is blood culture.[6]

Resistance to antimicrobials has been emerged in Salmonella species. In 1970, main stay of treatment was chloramphenicol.[3] First epidemic of enteric fever which was resistant to chloramphenicol was reported in 1972.[8,9] By late 1980, multiple drug resistance (MDR) enteric fever defined as resistance to the traditionally used three first line antimicrobial drugs (chloramphenicol, ampicillin, trimethoprim and sulfamethoxazole) was reported from many countries.[9] This led to the use of fluororuinolones (ciprofloxacin) as recommended first line therapy.[8] By early 2000, the increasing non susceptibility to

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flouroquinolones including intermediate and full resistance occurred and in 2006 , 55% cases of salmonella were resistant to ciprofloxacine.[10,11]This led to the use of third generation cephalosporins (ceftriaxone) as first line recommended therapy.[1,5] Most recently, an epidemic of extensively drug resistant (XDR) typhoid strain has been reported from Sindh, Pakistan, where resistance has been found to first line three antimicrobial drugs (chloramphenicol, ampicillin , trimethoprim and sulfamethoxazole), along with resistance to fluoroquinolone and also to 3rd generation cephalosporins.[10]In case where MDR and fluoroquinolone resistance is present, azithromycine has shown good efficacy by multiple trials, but by its widespread use in suspected cases of enteric fever, there is risk of emergence of resistance to it as well.[9]

Mortality due to enteric fever in pre-antibiotic era was 10-30%, but with the use of effective antibiotics, it has been reduced to <1%.[2] Reported mortality rate from Pakistan is less than 2 % from enteric fever.[3]

Considering high burden of enteric fever in developing countries like Pakistan and recent epidemic of XDR typhoid, we conducted this study at a tertiary care hospital of Karachi (Sindh, Pakistan) to assess clinical, laboratory profile and to check for antibiotic resistance pattern of *S. typhi* so that local data may be generated about recent trends on antimicrobial resistance in enteric fever cases and to modify local empirical treatment strategies and research priorities regarding typhoid fever.

Materials and Methods

This cross-sectional study was conducted in three medical units of National Institute of Child Health (NICH) Karachi over the period of 6 months, from 1st September 2018 till 28th February 2019. The study was conducted in compliance with ethical standards on human subject research, Informed consent was obtained from all patients' attendants. The study was approved by Institutional ethical review board (IERB) of NICH.

National Institute of Child Health is one of the largest tertiary care children hospitals of Pakistan that provides health care service to pediatric patients mainly from Sindh, Balochistan and South Punjab. Patients hospitalized in NICH during the study period with enteric fever proven by a positive blood culture for *Salmonella typhi* were included. Children with clinical suspicion of enteric fever but negative blood culture were excluded. Similarly, patients who left hospital against medical advice before completion of treatment were also excluded.

After explaining about the nature of the study informed consent was taken from parents or guardians. Patients' history and physical examination's findings were recorded in a semi-structured performa by principal investigator and co-investigators from their respective wards. Results of routine laboratory tests including Complete Blood Picture, Serum Urea, Creatinine, Electrolytes and Liver Function Tests were also recorded in Performa.

Blood Culture and sensitivity testing was performed at the microbiology laboratory of NICH. Five ml blood sample of each child suspected for enteric fever was drawn and inoculated in blood culture bottle aseptically and sent to laboratory. At microbiology laboratory blood culture bottles were sub-cultured after every 18-24 hours for 3-days, Identification of *S. typhi* was based on conventional biochemical tests and serotyping. Sensitivity testing was performed using Kirby Bauer disk diffusion method as per CLSI guidelines.

Other laboratory tests as needed according to the condition of child were also sent to laboratory as per NICH protocol. Antibiotic treatment started according to the hospital protocol and was recorded. Outcome of patient along with total duration of hospital stay was recorded. Outcome was recorded in terms of either recovered or died.

All data was entered and analyzed through statistical package for social sciences (SPSS) version 24. Descriptive statistics were applied. Frequency and percentages were calculated for qualitative variables including clinical signs and symptoms, previous history, drug sensitivity pattern etc. Mean± S.D was calculated for quantitative variables including age, fever history, duration of hospitalization, duration of antibiotic treatment, Biochemical Test Results Etc.

Results

Total 76 patients with culture proven enteric fever were enrolled in the study including 39(51.3%) males and 37(48.7%) females. Mean age of children was 5.7± 2.81 (range 0.83-12) years. Only 5 (6.5%) patients were from interior Sindh while 71 children (93.5%) were from Karachi.

Mean duration of fever in these patients was

19.04±8.39 days with minimum 4 days and maximum 45 days history of fever. Thirty-six (47.37%) had fever duration of >20 days. Other common symptoms among these patients were abdominal pain, diarrhea, vomiting and decreased appetite. Frequency of different clinical symptoms along with mean duration at the time of hospitalization are summarized in table I.

History of treatment before hospitalization with duration is presented in table II, which shows most of the patients already had a history of using cephalosporins, fluoroquinolones and some had also taken antimalarial treatment.

Hematological and biochemical findings of patients are presented in table III. Ultrasound (USG) abdomen was done in 63(82.9%) cases, out of which, USG was normal in 50 cases (79.4%). Major finding on sonography was hepatosplenomegaly and lymphadenopathy found in 11(17.5%) cases, 1(1.6%) had cholecystitis, and 1(1.6%) had ascites with hepatosplenomegaly.

All 76 children's blood culture was positive for *Salmonella enterica Serovar Typhi*. Antibiotic sensitivity profile of isolates is shown in figure 1 which showed high rates of resistance against all commonly used antibiotics for typhoid including fluoroquinolones and cephalosporins. However, none of the isolates showed resistance against meropenem.

In the hospital, antibiotics were started according to culture and sensitivity. Most of the patients were treated with intravenous meropenem with mean duration of 7.95±2.79 days out of which <7 days treatment was given in 10(25.6%) patients, 7-10 days treatment was received by 27(69.2%) patients while 2(5.1%) required treatment for >10 days to become afebrile. Thirty-three (43.4%) children were treated with azithromycin (mean duration of 6.23±1.38 days).

Enteric fever related complications observed among study participants included bone marrow suppression in 45(59.2%) with 35(46.1%) patients having moderate anemia (Hb<9.0g%), 14(18.4%) having leucopenia (TLC<4.5x10^3/UL), while thrombocytopenia (plt<150x10^3/UL) was present in 31(40.8%). Clinical jaundice was present in 4(5.3%), while subclinical hepatitis with raised SGPT (>40 IU) was present in 25(32.9%). Acute kidney injury (based on deranged renal function) was present in 2(2.6%). Hyponatremia (serum sodium <130meq) was seen in 7(9.2%), hypokalemia (serum potassium<3.5 meq) was seen in 9(11.8%). Other complications included cholecystitis 1(1.3%), ascites 1(1.3%), meningitis 1(1.3%), sepsis 1(1.3%), hepatomegaly 29(38.2%) and abdominal distension 19(25%).

Outcome in term of mortality was nil and all children (100%) were discharged. Mean duration of hospital stay was 8.296±3.33 days with range of 1-17 days. Hospital stay of <5 days was found in 7.4%, 5-7 days in 37%, 8-10 days in 35.2% and >10 days in 20.37%.

Table I: Clinical Features of Typhoid Patients Hospitalized at NICH.

Clinical Signs/ Symptoms	Number (%) of Patients	Mean Duration in Days (Min-Max)
Decreased appetite	51(67.1%)	6.25±1.5 (4-7)
Abdominal pain	49 (64.5)	4.75±3.05 (2-14)
Vomiting	37(48.7)	4.8±3.04 (2-13)
Diarrhea	33 (43.4)	6.33±5.82 (02-30)
Chest symptoms	3(3.9%)	
Clinical anemia	43(56.6%)	
Hepatomegaly	29(38.2%)	
Abdominal distension	19(25.0%)	
Splenomegaly	16(21.1%)	
Juandice	2(2.6%)	
Bruises	1(1.3%)	
Lymphadenopathy	1(1.3%)	
Edema	1(1.3%)	

Table II: Treatment History Before Culture and Sensitivity

Drug name	Number (%)	Mean Duration± SD (Range) days	Duration ≥7days N (%)
Ceftriaxone	39(51.3)	5.38±1.84(1-10)	14(18.4)
Cefixime	37(48.7)	5.97±2.48(7-14)	20(54)
Artemether and lumefantrine	20(26.3)	3	-
Ciprofloxacine	16(21.1)	8.25±4.16(3-17)	11(14.47)
Ampicillin Amoxicillin-clavulanate	10(13.2)	5.1±1.79(1-7)	3(3.9)

Discussion

In Pakistan enteric fever is amongst the leading causes of febrile illnesses due to bacterial pathogens.[12] There are alarming reports of

Table III: Hematological and Biochemical Features of Typhoid Patients Hospitalized at NICH

Laboratory Parameters	Mean± SD (range)
Hb (g/dl)	8.97±2.023 (4.1-13.9)
WBC (X10^3/UL)	7.19±3.55(0.5-19.6)
Platelets t(X10^3/UL)	230.5±218.2 (13.00-1060.0)
Urea (mg/dl)	21.77±15.54 (4-82)
Creatinine (mg/dl)	21.77±15.54 (4-82)
Sodium (meq/L)	135±6.4 (120-155)
Potassium(meq/L)	4.0±0.767 (2.3-5.3)
Chloride (meq/L)	96.99±17.03 (97-115)
Bilirubin (mg/dl)	0.99+0.992 (0.2-3.6)
ALT (IU/L)	70.4+64.54 (10-325)
Alkaline phosphatase (IU/L)	273.5+162.6 (64-620)

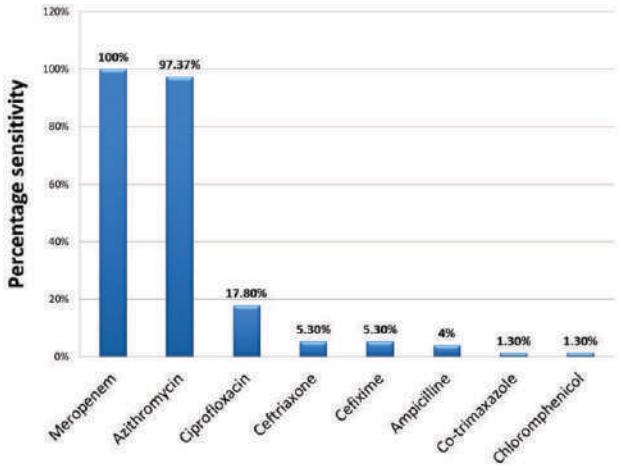

Fig 1: Antibiotic Sensitivity Profile of *S. Typhi* Isolates

antimicrobial resistance from Pakistan with need of careful selection of antimicrobials in children with enteric fever.[13]

In present study, mean duration of fever was19 days, therefore most of the patients were already in third week of illness where complications of enteric fever are expected. This suggests that there is need of early suspicion and early diagnosis in order to reduce complications and reduce mortality. Common associated problems were decreased appetite, abdominal pain, clinical anemia, vomiting, diarrhea, hepatosplenomegaly, abdominal distension and chest congestion. *Thompsom et al* also found the associated symptoms of anorexia, diarrhea and cough along with high grade fever in children of enteric fever.[13]

In the present study results are alarming with 97.7% of MDR isolates, resistance to third generation cephalosporins as 93.4% and resistance to ciprofloxacin in 82.2% leading to serious limitation in therapeutic options, leaving only behind options of carbapenam and azithromycin. *Klemm* et al in 2018 has reported the emergence of extensively drug resistant *S. typhi* showing resistance to flouroquinolones and third generation cephalosporin along with resistance to three first line drugs in Sindh Pakistan.[14] Gene sequencing of MDR *S. typhi* revealed that it is haplotype H58, encoded by plasmid having additional resistant elements including extended spectrum beta lactamase and exhibited high identity to the plasmids of other enteric bacteria found worldwide highlighting ability of *S. typhi* to convert from MDR to XDR by plasmid acquisition from other enteric bacteria.[14]

In the past there have been few sporadic reports of resistance to ceftriaxone where Indian Network for Surveillance of Antimicrobial Resistance Group has reported 3% resistance to third generation cephalosporin and *Jain et al* has reported 2% resistance to it.[11,15] Similar proportions of resistance has also been reported by *Abdullah et al* from Karachi Pakistan.[16] In previous study from Pakistan, in 2014, fluoroquinolone resistance was 82% and ceftriaxone resistance was not reported.[17]

Azithromycin is another oral option which has been reported as safe and effective against *S. typhi* but it can only be use d in uncomplicated early cases of enteric fever. Resistance to azithromycin has been found in 2(2.63%) cases. Resistance to azithromycin has also been reported by *Jain et al* in New Delhi where 7.3% isolates of salmonella has shown resistance to azithromycin.[11]

Most of the patients in present study were receiving cefixime (48.7%), ceftriaxone (51.3%) and ciprofloxacin (21.1%), indicating these medications may have been started on clinical grounds by suspicion of simple MDR *S. typhi*. Many patients had a treatment history suggestive of their condition being diagnosed as upper respiratory tract infection and malaria as antimalarial was taken in 26.3% cases, and amoxicillin/amoxicillin+co-amoxiclav was given in 13.2% cases. These figures are indicating high rates of empirical antibiotic treatment without culture reports which may be a factor towards harboring of

XDR *S. typhi* and resistance to other pathogens, as we can look; intravenous ceftriaxone was received by 51.3% cases before culture reports.

Most common complications were bone marrow suppression was found in 59.2% and subclinical hepatitis in 32.9% and similar results have been reported by Iftikhar et al.[18] We didn't find any case of intestinal perforation. One of its reasons may be that we only enrolled patients admitted in all medical units while enteric fever patients with perforation are usually admitted in pediatric surgical units.

The limitations of the study are that it included only hospitalized patients. Secondly, study enrolled only blood culture proven cases, so enteric fever patients treated with negative blood culture might have been missed.

Conclusion

There are high rates of resistance against all commonly used antibiotics for typhoid including fluoroquinolones and cephalosporins. There is shift of antimicrobial susceptibility leaving only extremely limited and costly options for treatment and increases length of hospital stay due to need of intravenous therapy resulting in increased financial burden over families as well as government.

Recommendation

In settings of poor sanitation, lack of vaccination coverage and the emergence of XDR *S. typhi* suggest the appropriate policies for antimicrobial usage, need for nationwide vaccination campaigns, and Govt. based policies for provision of safe drinking water. There is also needed to revise guidelines for treatment of enteric fever in both empirical and in culture proven cases. Treatment of enteric fever patients with two or more sensitive drugs may also be considered as this policy has been implemented in cases of malaria and tuberculosis to prevent further emergence of resistance.

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ORIGINAL ARTICLE

The Serum Electrolyte Imbalance in Children with Severe Acute Malnutrition

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ABSTRACT

Objective: To analyze the serum electrolyte imbalance in children with severe acute malnutrition (SAM) aged 1-59 months.

Study Design: It was a cross sectional study.

Place and Duration of Study: Six months from 1st March 2018 to 30th August 2018 at Nutrition Stabilization Centre of The Children's Hospital and Institute of Child Health Multan.

Materials and Methods: All the children between age group 1-59 months and admitted in Stabilization Centre of The Hospital and Institute of Child Health Multan with diagnosis of severe acute malnutrition during the study duration were included in this study. After inclusion criteria fulfilled sample was collected from 100 different patients and sent to Pathology department of Children's Hospital and Institute of Child Health Multan for biochemical assessment. The serum was analyzed for Mg^{2+}, Ca^{2+}, K^{+} and Na^{+} by spectrophotometric method. All the data was entered and analyzed by using SPSS version 21. The percentages were calculated through SPSS.

Results: Total 100 children with severe acute malnutrition were included in current study. 60 (60%) were male and 40 (40%) were female, mean age reported was 23.65 months. There were 66% cases presented with diarrhea. Hypokalemia was found in 23 (23%) children and hyponatremia was present in 28 (28%) children. Mean of sodium & potassium were 138.96 (8.692) and 3.06 ($\pm$1.7517) respectively. Mean (S.D) of calcium and magnesium were 8.51 (1.58) and 2.23 (2.38) respectively.

Conclusion: Electrolyte imbalance is common in children with severe acute malnutrition with or without diarrhea. Determination of electrolyte profile of children with severe acute malnutrition admitted at stabilization centers in Pakistan should be ensured to reduce the mortality and morbidity.

Key Words: *Diarrhea, Electrolytes Imbalance, Severe Acute Malnutrition (SAM).*

Introduction

Malnutrition is the big contributor of illness in children worldwide.[1] Malnutrition especially under nutrition is the cause of mortality and morbidity and results in 45% of child mortality (under 5 years of age) worldwide.[2] Pakistan is the fifth country having higher cases of Severe Acute Malnutrition.[3]

Severe acute malnutrition leads to major complications in the body along with electrolyte imbalances which may worsen with diarrhea.[4] In a healthy child, sodium potassium pump exists, which maintains the potassium concentration in the cell by pumping sodium out and potassium in to the cell. When a child is suffering from Severe Acute Malnutrition, the sodium potassium movement become impaired. The level of sodium inside the cell became high and potassium is lost. When the child is treated, Na+, K+ levels are maintained. Therefore, feeding must be very slow otherwise it will cause fluid overload, metabolic disturbances and system will be disturbed. Magnesium is essential for potassium to enter the cell. Potassium and magnesium are recommended according to World Health Organization (WHO) 3-4mmol/kg/day and 0.4-0.6mmol/kg/day respectively.[5]

The children with Severe Acute Malnutrition occasionally suffer from diarrhea which leads to the electrolyte imbalance.[6] The children suffering from Severe Acute Malnutrition may have low serum level of potassium (hypokalemia), high level of sodium (hypernatremia) and altered level of calcium. Rehydration Solution for Severely Malnourished Children (ReSoMal) is recommended which is less in

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sodium and more in potassium. Serum electrolyte imbalances in SAM children can be due to numerous reasons but are more evident during diarrhea.[7]

The malnourished children are more susceptible to morbidities and mortalities related to electrolyte balance. The findings of this study will help to determine the extent of electrolyte imbalance in children suffering from SAM and further researches can be conducted for detailed analysis. The purpose of current study was to analyze the serum electrolyte imbalance in children with severe acute malnutrition (SAM) aged 1-59 months admitted to The Children's Hospital and Institute of Child Health Multan.

Materials and Methods

The current study was cross sectional study, conducted in Nutrition Stabilization Centre of The Children's Hospital and Institute of Child Health Multan from March 2018 to August 2018. Simple random sampling technique was used. All the children between age 1-59 months and admitted in Stabilization Centre of The Children's Hospital and Institute of Child Health Multan during the duration of study period and diagnosed with severe acute malnutrition were included in this study. A total of 110 children were admitted during the study period. Severe Acute Malnutrition was defined as children having mid upper arm circumference less than 11.5cm, weight-for-height/length ratio less than -3SD and bilateral edema or anyone out of above. All Children admitted with severe acute malnutrition aged between 1-59 months with or without diarrhea were included in this study. The study was started after getting permission from IRB committee of the hospital. Written consent was taken from the parents/guardians of the admitted patients. Out of 110 patients only 100 gave consent and were thus enrolled for the study. The children whose parents refused to give consent were excluded from this study.

A self-made questionnaire was designed by the principal researcher and filled by the trained nursing staff of stabilization center. The demographic data (Age, Gender, and Name) was collected. Anthropometric assessment (Weight, height, MUAC, Weight/Height or Length Ratio) was done and noted by the nurses. About 2ml blood sample was collected from 100 patients admitted in Stabilization Centre ward and sent to Pathology department of Children's Hospital and Institute of Child Health Multan for biochemical assessment. Sodium and potassium analysis were performed by using patient's serum on a fully automated Electrolyte Analyzer Diestro 103 AP, that work on the principle of ion selective electrode (ISE) while serum calcium and magnesium levels were measured on a fully automated chemistry analyzer Beckman Coulter AU-680, that works on the principle of Spectrophotometry. The biochemical assessment was performed by lab technicians of the pathology department and results were recorded on the questionnaire by the nurses. The data collected was nonparametric.

All data was computed and analyzed by using SPSS version 21. Descriptive cross-sectional analysis of complete data was analyzed, and results are reported as frequencies and percentages. Mean SD and averages were calculated where required.

Results

Total 100 children with severe acute malnutrition were included in current study. 60 (60%) were male and the mean age in months was 23.65. there were 66% cases presented with diarrhea. Mostly children 93 (93%) belong to poor socio-economic status (Table I).

The serum level of potassium was low in 23 (23%) and high in 12 (12%) patients. The serum level of potassium was normal in 65 (65%) children.

The serum level of sodium was low in 28 (28%) and high in 15 (15%). The serum level of potassium was normal in 57 (57%) children.

The serum level of magnesium was low in 16 (16%). The serum level of Mg^{2+} was normal in 73 (73%) children.

The serum level of calcium was low in 28(28%) and high in 3 (3%). The serum level of potassium was normal in 69 (69%) children (Table II). The mean serum potassium and sodium was 3.06 ± 1.75 mmol/L and 138.96 ± mmol/L respectively. (Table III)

Discussion

Current study was intended to explain the electrolyte profile with severe acute malnutrition, admitted in Stabilization Centre of The Children's Hospital and Institute of Child Health Multan and their age were 1-59 months. This age group is very critical group because weaning is initiated, and children have higher risk of developing severe acute malnutrition. According to the findings of this study, in severe

Table I: Gender, Age, Socio-Economic Status of Children with SAM (N= 100)

Characteristics	Total Count (n=100)	Percentage (%)
Gender		
Male	60	60
Female	40	40
Age		
Less than 6 months	34	34
Greater than 6 months	66	66
Socio Economic Status		
Poor	93	93
Middle	7	7
High	Nil	Nil

Table II: Serum Electrolyte Profile of Children with Severe Acute Malnutrition

Serum Electrolytes	Reference Range	Count (n=100)	Percentage (%)
Potassium			
Normal	3.5-5.8 mmol/L	65	65
Hypokalemia	<3.5 mmol/L	23	23
Hyperkalemia	>5.8 mmol/L	12	12
Sodium			
Normal	136-146 mmol/L	57	57
hyponatremia	<136 mmol/L	28	28
Hypernatremia	>146 mmol/L	15	15
ASU			
Magnesium			
Normal	1.5-2.5 mg/dL	73	73
Hypomagnesaemia	<1.5 mg/dL	16	16
Calcium			
Normal	8.1-10.4 mg/dL	69	69
Hypocalcaemia	<8.1 mg/dL	28	28
Hypercalcaemia	>10.4 mg/dL	3	3

Table III: Mean Serum Electrolyte Profile of Children with Severe Acute Malnutrition

Serum electrolyte	Mean ±SD
Potassium	3.06 ± 1.75 mmol/L
Sodium	138.96 ± 8.6 mmol/L
Calcium	8.51 ± 1.58 mg/dL
Magnesium	2.23 ± 2.38 mg/dL

acute malnutrition, hypokalemia and hyponatremia were significant risk factor for acute diarrhea. Serum electrolyte balance along with diarrhea may results in increased mortality and morbidity in SAM children.

In current study, 86 (86%) children were of age 25-59 months. This study included 60% males and 40% females. Current results were comparable with the previous study conducted in India [8]. It may also indicate that despite gender discrimination, male children were at more risk of severe acute malnutrition than female child or perhaps more male children were admitted to hospitals for treatment than female child.

There were 66% cases presented with diarrhea and 34% cases without diarrhea which is comparable with a study conducted in Zambia (64% cases presented with diarrhea).[9] According to previous studies, diarrhea is directly linked to severe acute malnutrition and have a major impact on serum electrolyte balance as well.[10] These findings were also confirmed by the current study. Chronic and persistent diarrhea leads to severe acute malnutrition and SAM children were more prone to diarrhea due to low immunity and prolonged infections.[11]

In this study almost one third of cases had hyponatremia and these results were supported by another study conducted by Zulqarnain et al. which reported hyponatremia in 31.1% cases and 22.6% cases were reported by Bilal at al.[5,12] Our study included serum sodium level less than 136mmol/L as standard hyponatremia but Negussie at al. included serum sodium level less than 130mmol/L as hyponatremia.[13]

There were 23% cases of hypokalemia in current study, but previous studies included 55% and 13.7% cases of hypokalemia.[5,12] In current study, 12% and 15% cases were hyperkalemia and hypernatremia respectively. Current results included higher cases of hyperkalemia and hypernatremia as compared to previous studies.[12,14] The cases of hypocalcemia in current study were much higher than the previous studies.[14] This also indicated that the samples enrolled in our study were at much higher risk for complications regarding hypocalcemia.[15]

This research, however, is subject to limitations, the primary limitation was short time duration of six months, due to which random sampling technique was used. All the patients registered at the hospital during those six months, fulfilling the inclusion criteria were recruited. A study done for longer

period of time may include more samples and provide more concrete results to strengthen the findings of this study. According to the findings of this study, in severe acute malnutrition, hypokalemia and hyponatremia were significant risk factor for acute diarrhea.

Conclusion

Electrolyte imbalance is common in children with severe acute malnutrition with or without diarrhea. Determination of electrolyte profile of children with severe acute malnutrition admitted at stabilization centers in Pakistan should be ensured to reduce the mortality and morbidity.

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ORIGINAL ARTICLE

HbA1c Levels in Diabetic Patients with Chronic Liver Disease

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ABSTRACT

Objective: To investigate the accuracy of HbA1c in diabetic patients with chronic liver disease (CLD).

Study Design: Cross-sectional/ observational study.

Place and Duration of Study: Fauji Foundation Hospital, Rawalpindi (FFH), from July 2019-July 2020.

Materials and Methods: This study was carried out on 100 subjects divided in two groups i.e. Group A and Group B. Group A included diabetics with CLD (chronic hepatitis C) and Group B included diabetics without CLD. Each group consisted of 50 known type 2 diabetes mellitus (T2DM) participants, who were randomly selected from liver and medical OPD of FFH, Rawalpindi. Blood samples of the participants were analyzed for HCV, HbA1c and liver enzymes. Chemical analysis was carried out at the department of Pathology FFH, Rawalpindi. For statistical analysis version 21 of SPSS was used.

Results: Our study showed that HbA1c levels were low in group A (6.6±1.10% vs. 9.58±2.09% $p < 0.05$) when compared to group B. Group A showed significantly higher levels of alanine aminotransferase (ALT) than group B (74.65±21.84 U/L vs. 38.44±23.79 U/L $p<0.05$). Serum albumin was also lower in group A in comparison to group B (29.52±2.21g/L vs. 36.24±3.99 g/L $p<0.05$). HbA1c levels showed significantly negative association with ALT in group A (r-0.418 $p<0.05$) while in group B the negative correlation was not significant statistically (r-0.197 $p=0.171$). A significant negative association of HbA1c with Albumin was also seen in group A (r-0.391 $p<0.05$) Regression analyses showed a significant relationship between HbA1c and ALT in group A.

Conclusion: Our study concludes that HbA1c levels are significantly decreased in diabetic patients with CLD (chronic hepatitis C) than diabetics without CLD. Therefore, HbA1c is not a reliable predictor for long-term glycemic monitoring in diabetic patients having CLD.

Key Words: *Chronic Liver Disease, Chronic Hepatitis C, Cirrhosis, Diabetes Mellitus, HbA1c.*

Introduction

From a decade or so liver disease been recognized as a major complication of type 2 diabetes.[1,2] Chronic liver disease (CLD) generally takes the clinical form of chronic hepatitis, its long term complications include cirrhosis and hepatocellular carcinoma (HCC).[3] Chronic liver disease is accompanied by significantly impaired glucose homeostasis. In CLD, around 80% of patients show glucose intolerance, while in 30–60% of patients there is presence of frank diabetes. Hepatic glucose metabolism is significantly affected by CLD.[4,6]

There are many causes for CLD, of which Hepatitis C Virus (HCV) is most common.[7] HCV is one of the most alarming health problems globally, with an incidence of 200 million (3.3%) of the world's population.[8] "Till the year 2017 around 4 million individuals were infected with HCV in the United States and 2.7 million of them were careers."[9] Nearly 30,000 new cases of HCV have been diagnosed annually.[10] The HCV infected people in Pakistan is approximated to be more than 10 million.[11] The organ primarily affected by HCV is the liver which then progresses to cirrhosis, chronic liver failure and hepatocellular carcinoma.[12] Ghani ur Rehman et al. in 2017 revealed that almost one-third of the HCV patients were diabetic. Our data records showed that 26.42% of the HCV infected patients were found to have T2DM.[13] The high prevalence of hepatitis C in T2DM has been shown by multiple epidemiological studies, and have also postulated the progression of hepatitis C into development of DM. HCV infection & age as

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documented by Mason et al. were independent predictors for DM.[14,19]

The American Diabetes Association (ADA) and World Health Organization (WHO), both, regard HbA1c as the most reliable chemical tool for diagnosis as well as prognosis of glycemic control in T2DM.[20] The use of HbA1c for the diagnosis of T2DM is supported by plenty of literature.[21] The turnover of erythrocytes is increased in CLD patients, while there is a decrease in serum albumin level.[22] Therefore, glycated albumin (GA) and HbA1c should not be utilized as tools in CLD diabetic patients for monitoring of chronic plasma glucose control.[23]

Literature search showed that many studies[24,29] have been carried out to see the reliability of HbA1c in diabetics with CLD in respect to cirrhosis but hardly any studies have been done to see accuracy of HbA1c in diabetic population having chronic hepatitis C infection and non-such study has been found to be carried out in Pakistan so far. Our study aimed to determine HbA1c accuracy in T2DM patients with chronic hepatitis C which is first stage of CLD to timely avoid complications like cirrhosis, hepatocellular carcinoma along with the subsequent death rate.

The objective of the present study was to provide information on how reliable is HbA1c in patients having diabetes with chronic hepatitis C and what alternative parameters can be utilized to check the previous 3-4 months control of diabetes in such patients.

Materials and Methods

This was a cross sectional/observational study. It was carried out at the pathology department FFH, Rawalpindi from July 2019 to July 2020 after approval from the institutional ethical committee. The inclusion criteria of the study were hundred known diabetic patients with and without CLD fulfilling criteria of chronic hepatitis C (Lasting > 6 months). The exclusion criteria included those who were free of illness like acute liver disease, renal disease/failure, hepatitis B or any other virus, pregnant females and those who did not want to be included in the study. The study subjects were split in two groups (group A & group B). Group A included diabetics with CLD (chronic hepatitis C) and Group B included diabetics without CLD. Each group consisted of 50 known diabetic participants.

Patients were randomly selected from liver and medical OPD of FFH, Rawalpindi. Proper consent was taken after explaining the aims and objectives of the study to all participants.

Blood sample was collected from all participants under aseptic conditions. Centrifugation of blood for 5 minutes at 4000rpm was carried out to separate the serum for the evaluation of liver function tests including ALT**,** ALP, albumin, and bilirubin. These parameters were measured using chemistry auto-analyzer Dimensions RxL. HbA1c was analyzed using fully automated analyzer Beckman Coulter.

Statistical analysis was carried out using version 21 of SPSS. Quantitative data was expressed as mean± Standard deviation (SD). Comparisons between the two groups were made using independent t-test. To show relationship of the variables with HbA1c Pearson's correlation co-efficient was used. Regression analysis was used to show the relationship between the dependent variables and the independent variables. Results were considered as significant with $p< 0.05$ and highly significant with $p<0.001$.

Results

Data was stated as mean± SD. Comparison of the two groups was done using independent t-test with considering $p < 0.05$ as statistically significant. The present study included 100 participants divided into group A and B. Group A, diabetics with chronic hepatitis C, included 50 participants with mean age of 61.32±10.70 years while group B, diabetics without chronic hepatitis C, also consisted of 50 participants with mean age of 57.44±11.08 years.

Low levels of HbA1c were seen in group A than group B (6.6±1.10% vs. 9.58±2.09% $p<0.05$).

Group A reported significantly higher level of serum ALT as compared to group B (74.65±21.84U/L vs. 38.44±23.79U/L $p<0.05$).

Group A also showed lower serum albumin as compared to group B (29.52±2.21g/L vs. 36.24±3.99g/L $p<0.05$). The comparison between the groups is summarized in Table I.The association of HbA1c with different parameters is shown in Table II. HbA1c levels were negatively associated with ALT in group A (r-0.418 $p<0.05$) while in group B the negative correlation was not significant statistically (r-0.197 $p=0.171$). Similarly, Albumin showed a significant negative correlation with HbA1c in group

A (r-.391 p<0.05), while negative correlation of ALP and bilirubin were not significant in both the groups. The correlation of ALT with HbA1c in group A is shown in Figure 1 with (r^2=0.175 p<0.05)) calculated by linear regression analysis while group B results are plotted in Figure 2 (r^2=0.039 p=0.171) The ALT results are plotted on x-axis and HbA1c on y-axis

Table I: Comparison of Demographic, Clinical and Biochemical Characteristics of the Study Groups

Variable	Diabetics with CLD	Diabetics without CLD	*P* value
Age (years)	61.32±10.70	57.44±11.08	0.078
ALT (U/L)	74.68±21.84	38.44±23.79	<0.05***
ALP (U/L)	159.96±36.10	172.08±55.56	0.199
Albumin (g/L)	29.52±2.21	36.24±3.99	<0.05***
Bilirubin (µmol/L)	10.64±5.37	9.92±5.90	0.525
HbA1c (%)	6.60±1.10	9.58±2.09	<0.05***

Note: *Significance at p <0.05, **Significance at p <0.01, ***Significance at p< 0.001

Table II: Correlation of HbA1c (%) with Different Parameters in the Study Groups

Variables	Group A (DM+CLD)		Group B (DM)	
	R	*P*	R	*P*
ALT (U/L)	-0.418	<0.05**	-0.197	0.171
ALP (U/L)	-0.048	0.740	-0.125	0.386
Albumin (g/L)	-0.391	<0.05*	-0.264	0.064
Bilirubin (µmol/L)	-0.160	0.268	-0.189	0.189

r is coefficient of correlation, * Significance at p<0.05, ** Significance at p<0.01, *** Significance at p<0.001

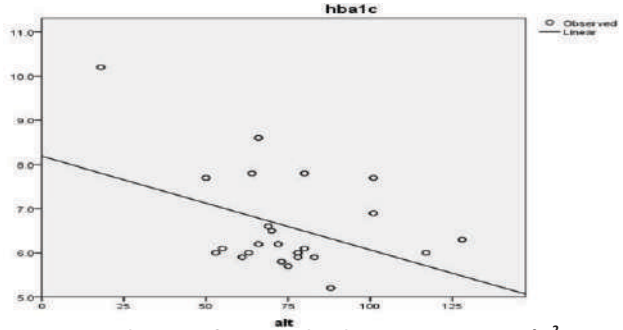

Fig 1: Correlation of ALT with HbA1c In Group A (R^2=.175 P=.003) Calculated by Linear Regression Analysis

Discussion

Chronic liver disease and diabetes are two major chronic illnesses afflicting a major segment of Pakistan's population.

The cost in terms of number of work hours lost due to illness is enormous. Moreover, both diseases require long term management and treatment. The average Pakistani can ill afford to bear the costs of such expensive treatments. Hence there was dire need to correlate HbA1c with CLD and see if the results of HbA1c in CLD depicted the actual levels or not.

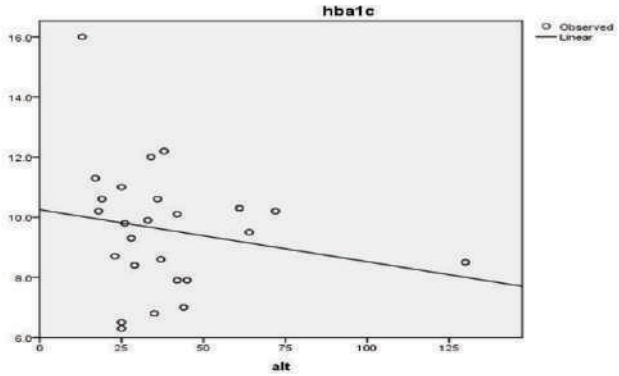

Fig 2: Correlation of ALT with HbA1c Group B (R^2=.039 P=.171) Calculated by Linear Regression Analysis

Multiple studies have been carried out to see the reliability of HbA1c in diabetics with CLD in respect to cirrhosis but hardly any studies have been done to see accuracy of HbA1c in diabetic population having chronic hepatitis C infection. Our study is aimed from this aspect as diabetes and chronic hepatitis C, account for major cause of chronic disease in Pakistan.

In our study it was observed that HbA1c level was significantly lesser in the diabetic patients with CLD in comparison to diabetic patients without CLD. ALT level was significantly more in the diabetics having CLD while serum albumin was seen to be significantly lower in this group, which may be sign of progression of the disease to cirrhosis.

Koga et al. in their study observed that in CLD patients the measured HbA1c levels were lower than estimated HbA1c levels.[23]

Lahousen et al. in their work measured HbA1c for the evaluation of long-term plasma glucose control in chronic hepatitis patients, with compensated cirrhosis and in ribavirin treated chronic hepatitis patients. The levels of HbA1c in all cases were seen to be below the diabetic range.[30]

The results of all these studies are in concordance with this study.

Nadelson et al. performed their study in 2016, in which they observed that HbA1c was not a reliable biomarker of glycemic index in cirrhotic patients with HbA1c levels ranging between 5-6%.[25]

MF Bashir et al. in their study showed that ALT was significantly higher in diabetics with hepatitis C (HCV) but they showed HbA1c higher in HCV plus diabetics. This could be because their patients did not fulfill the chronic hepatitis C criteria.[31]

Our study also showed a significant negative correlation of HbA1c with ALT and albumin in the diabetics with CLD as compared to diabetics without CLD. Similar results were shown by Christman et al. who observed that low HbA1c was associated with elevated liver enzymes (ALT) and low albumin levels.[32]

The limitation of our study includes a small sample size. It is suggested that HbA1c should be used with caution & fasting plasma glucose levels should be relied upon more along with liver function tests and red blood cell indices when prolonged glycemic control in type 2 diabetic patients with CLD is monitored. Studies to investigate newer options for monitoring glycemic levels are required in these patients.

Conclusion

We conclude that HbA1c is not a reliable tool for the long-term glycemic control in CLD patients with diabetes mellitus and clinicians should be aware of limitations of HbA1c as a marker of glycemic control in patients with CLD. For accurate monitoring of long-term glycemic control of such patients, HbA1c should only be evaluated in context with fasting plasma and post prandial glucose levels along with liver function tests.

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ORIGINAL ARTICLE

Bisphenol A: A Testicular Teratogen in Developing Rats During Prenatal and Early Postnatal Life

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ABSTRACT

Objective: To study the teratogenic effects of Bisphenol A on the testicular histogenesis of developing rat pups during prenatal and early postnatal life.

Study Design: Laboratory-Based Randomized Control Trial.

Place and Duration of Study: The present study was carried out at Anatomy Department of Islamic International Medical College Rawalpindi in association with National Institute of Health (NIH) Islamabad from September 2018 to September 2019.

Materials and Methods: Eight weeks old 10 pregnant female rats were divided into 2 groups each containing 5 pregnant female rats. Pups were delivered by spontaneous vaginal delivery. Control group A was composed of 15 male rat pups from 5 pregnant female rats fed on the standard diet during pregnancy and lactation till day 21. Experimental group B was composed of 15 male rat pups from 5 pregnant female rats which were given 250µg/kg/day Bisphenol A subcutaneously during pregnancy and lactation till day 21.

Results: Deterioration of histological parameters was significantly seen in group B. Tubule differentiation index(TDI) in group B was 69.22%; significantly reduced as compared to control group A. The modified Johnsen's criteria in group B was score 3 (in 33.3% rats) and score 1 (in 26.6% rats) as compared to score 7 (in 100% rats) in control group A. 66.6% rats in group B showed severe (grade 4) and 26.6% rats showed moderate (grade 3) epithelial degeneration as compared to control group A. 46.6% rats in group B showed severe (grade 4) and 40% had moderate (grade3) congestion as compared to control group A.46.6% rats in group B showed severe (grade 4) vacuolization and 33.3% had moderate (grade3) vacuolization as compared to control group.

Conclusion: The teratogenic effect of BPA on developing rat testes is evident when given during prenatal and early postnatal life. The teratogenicity of BPA may adversely affect spermatogenesis and male fertility.

Key Words: *Bisphenol A, Developing Rats, Early Postnatal Testicular Teratogen, Prenatal.*

Introduction

Bisphenol A was manufactured first by A. P. Dianin in 1891 and investigated in 1930s during the hunt for synthetic estrogens and is thus an important endocrine disrupting chemical.[1,2] BPA is a xenoestrogen which mimics estrogens and is able to bind estrogen receptors.[3] Edward Charles Dodd first identified the estrogenic characteristics of BPA when he was in search of an estrogen.[4] A study on BPA reported that humans are exposed to 10µg/day of bisphenol A.[5] The European Food Safety Authority (EFSA) has determined the temporary tolerable daily intake of BPA which is 4µg/kg/day.[6] According to the data published by EFSA in 2015; BPA exposure for men and women is 1.01 to 1.06µg/kg/day.[7]

Bisphenol A is a ubiquitous endocrine disrupting chemicals (EDC) which is used in synthesis of epoxy resins and polycarbonate plastics, food cans, water pipes, receipt papers, baby feeding bottles and currency notes.[3] Reproductive system is one of the main systems targeted by this endocrine disruptor.[8] Exposure to such chemicals in humans is a probable cause of male infertility.[9] Developing testis is fundamental component of male reproductive system which is susceptible to teratogens. BPA has deteriorating effects in pregnancy when exposed during critical period of embryonic testicular development.[10] Animal studies revealed that BPA has a potent teratogenic potential.[11] In rats during prenatal testicular development (gestational days:

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13.5-16.5), there is migration of primordial germ cells towards genital ridge along with sertoli cells differentiation, formation of seminiferous cords and leydig cells differentiation. Postnatal testicular development includes neonatal period (3–7 post natal days), early infantile period (8–14 days), late infantile period (15–20 days), juvenile period (21–32 days) and peri-pubertal period (32–55 days).[12] Oxidative stress induced by BPA during the testes development is the key factor in aetiology of male infertility.[11] Exposure of pre- implantation embryos to BPA impairs testes development and disrupts testosterone synthesis.[6] A study of BPA exposed rats revealed abnormal spermatids, interstitial congestion, scanty cellular components and empty tubular lumina with significant decrease in Leydig cell count, germinal epithelium height and tubular diameter.[5]

Numerous phenol derivatives were detected in water bodies of river Indus in Pakistan. All samples were adulterated with Phenol,2,4-bis(1,1-dimethylethyl).[13] To the best of researcher's knowledge, in Pakistan where significant quantity of BPA is leached into water, such a study was needed to be conducted in pregnant rats to see teratogenic role of BPA during prenatal and early postnatal life. With exposure to BPA, this study could highlight the probable reason of increasing male infertility in Pakistan by identifying the problem in progression of spermatogenesis in rat pups with the help of seminiferous tubule differentiation and histological scoring.

Materials and Methods

The experimental study was carried out by randomized control trial in animal house of National institute of health, Islamabad (NIH) from September 2018 to September 2019 after approval of Ethics Review Committee. Randomization was by simple random sampling. Eight weeks old 10 female and 5 male albino Sprague Dawley rats were kept under standard temperature at 22 ± 0.5˚C in stainless steel cages in 12-hour light dark cycle with 50% humidity. Adult female rats were caged with adult male rats (2 females/ male). They were given food and water ad libidum for 7-days to acclimatize. They mated under normal conditions in cage. Female rats with presence of vaginal plugs were considered at day 0 of pregnancy. Pregnant rats were divided into 2 groups. Each group comprised of 5 pregnant rats. One group was fed on normal diet and other group was given BPA (Table I). Pups were delivered by spontaneous vaginal delivery at time of birth. Male rat pups which were 21 days old were included in the study. Male pups with obvious pathology or male pups older than 21 days old were rejected. Delivered male pups were divided into two groups as in Table I. This study constituted a sample size of 30 male rat pups.

Table I: Grouping of Animals (N=30)

Control Group (Group A), n=15	Experimental Group (Group B), n=15
15 male rat pups from 5 pregnant female rats. Their mothers were kept on standard diet during pregnancy and lactation till day 21.	15 male rat pups from 5 pregnant female rats. They were given standard diet orally. BPA 250µg/kg/day was given to their mothers via subcutaneous route during pregnancy and lactation till day 21.

After 42 days of experiment, 21 days old male rat pups were euthanized and dissected. Testes were fixed in Bouin's fixative, stained with Eosin and Hematoxylin and examined under X10 and X40 power of light microscope and parametric data was collected by the principal researcher. Microscopic parameters included tubule differentiation index (% of tubules with more than 3 layers of differentiated germinal epithelial cells) and assessment of spermatogenesis with modified Johnsen's crirteria from 1-7 with 1 being the lowest (absence of germinal epithelium and cells) and 7 being the highest (presence of rounded spermatids). In between 7 and 1 spermatogonia, spermatocytes and sertoli cells were seen[14]. These two parameters were assessed by independent samples T test. The parameters of epithelial degeneration, congestion and vacuolization were classified according to International Harmonization of Toxicologic Pathology Nomenclature.[15] These three parameters were assessed by Chi square test. The data was analysed by SPSS version 21. A *p-value* of less than 0.05 was considered significant.

Results

Tubule differentiation index (TDI) and modified Johnsen's criteria were reduced in group B significantly as compared to control group A (Figure 1, Table II). Severe and moderate grades of epithelial

degeneration, interstitial congestion and vacuolization were seen in rats of group B as compared to control group A. (Figure 2, Table III, Figure3, Table III, Figure 4, Table III respectively).

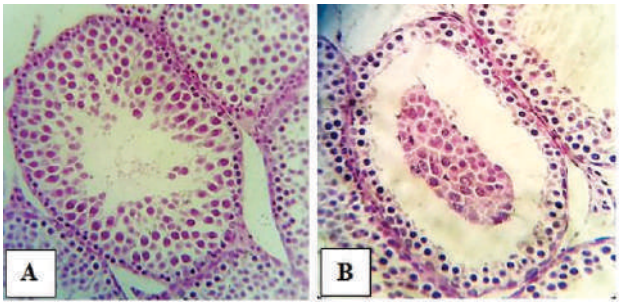

Fig 1: Figure 19 TDI and Assessment of Spermatogenesis with Modified Johnsen's Criteria in Testes of Albino Rat Showing Score 7 in Control Group A and Score 3 in Experimental Group B. (A: Control Group A, B: Experimental Group B) (H&E, X40)

Table II: Group Wise Distribution of Tubule Differentiation Index (ANOVA) and Assessment of Spermatogenesis with Modified Johnsen's Criteria (Chi square test) of Seminiferous Tubules of Testes among the Control and the Experimental Groups in Albino Rats (N=30).

Tubule differentiation index (%)				Assessment of spermatogenesis with modified Johnsen's criteria (N)							
Groups	Mean	S.D	SEM	Groups	1	2	3	4	5	6	7
A	100	0	0	A	0	0	0	0	0	0	15
B	69.22	3.97	1.03	B	4	0	5	2	1	2	1
p value	0.002			0.000*							

p-value ≤ 0.05

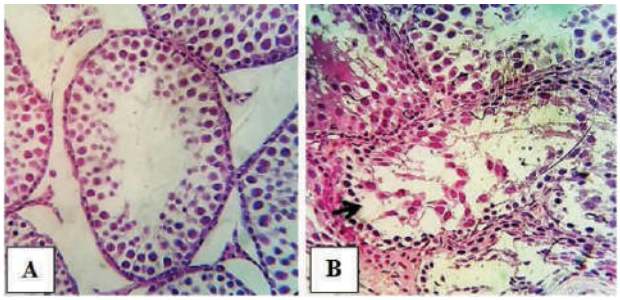

Fig 2: Germinal Epithelial Degeneration (Severe) in Testes of Albino Rat (Arrow) Shown in Experimental Group B. Negligible Degeneration is Seen in Control Group A (A: Control Group A, B: Experimental Group B) (H&E, X40)

Discussion

Infertility is defined as failure to conceive after unprotected and frequent sexual intercourse for more than a year which affects 15% couples

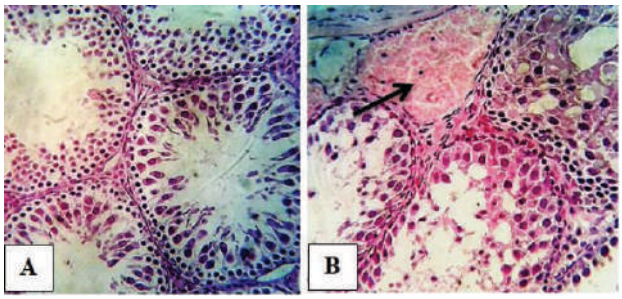

Fig 3: Interstitial Congestion (Severe) in Testes of Albino Rat (Arrow) Shown in Experimental Group B. Negligible Congestion is Seen in Control Group A (A: Control Group A, B: Experimental Group B) (H&E, X40)

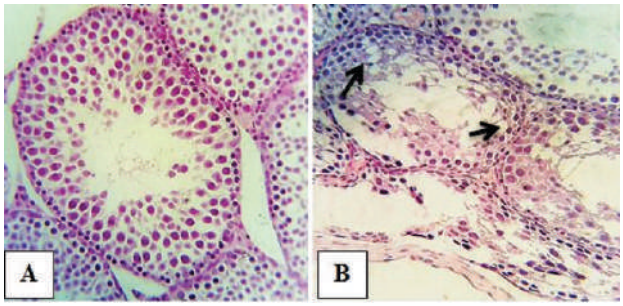

Fig 4: Vacuolization of Germinal Epithelium (Severe) in Testes of Albino Rat (Arrows) Shown in Experimental Group B. Negligible Vacuolization is Seen in Control Group A (A: Control Group A, B: Experimental Group B) (H&E, X40)

Table III: Group wise Distribution of Tubular Degeneration, Interstitial Congestion and Germinal Epithelial Vacuolization (Chi Square Test) In Testes of Albino Rats among the Control and the Experimental Groups

Parameters	Tubular Degeneration (N, %)		Congestion (N, %)		Vacuolization (N, %)	
Study Groups	A	B	A	B	A	B
Grades						
Negligible (Grade 0)	15(100%)	0 (0%)	15(100%)	0 (0%)	12 (80%)	0 (0%)
Minimal (Grade1)	0 (0%)	0 (0%)	0 (0%)	1 (6.6%)	3 (20%)	1 (6.6%)
Mild (Grade2)	0 (0%)	1 (6.6%)	0 (0%)	1 (6.6%)	0 (0%)	2 (13.3%)
Moderate (Grade3)	0 (0%)	4 (26.6%)	0 (0%)	6 (40%)	0 (0%)	5 (33.3%)
Severe (Grade 4)	0 (0%)	10 (66.6%)	0 (0%)	7 (46.6%)	0 (0%)	7 (46.6%)
p-value	0.000*		0.000*		0.000*	

p-value ≤ 0.05

worldwide. Almost half of these cases are attributed to male partner. A study by Majid et al (2019) depicted that oxidative stress due to EDCs is one of major mechanisms involved in causing male infertility.[16] Programming of masculinization occurs during the gestation days 15–18 in laboratory albino

rats and in humans between weeks 8–14 of gestation.[17] Animal studies have shown that pregnant females are at risk from BPA exposure since BPA is a known teratogen as well.[18,19] In humans, BPA has been detected in maternal and fetal plasma, amniotic fluid, placental tissue and in milk of lactating mothers.[20]

In present study, BPA was administered during pregnancy and 21 days after delivery during lactation. Testicular histogenesis was assessed by quantitative and qualitative parameters. BPA adversely affected its development owing to its teratogenicity. TDI and modified Johnsen's criteria have been evaluated in this study both prenatally and in early postnatal period to see teratogenic potential of BPA and its role in development of subsequent male infertility.

Differentiation of spermatogenic epithelium is indicated by tubule differentiation index. In present study, TDI in group B was significantly reduced as compared to control group which was due to disruption of germinal epithelial cells lipid membranes which occurred by generation of ROS by BPA. A study on diabetic rat has shown reduction in TDI in testes showing tubular damage.[21]

Modified Johnsen's criteria manifests progression of stages of spermatogenesis. The reduced score in this study with BPA indicated spermatogenic arrest due to Leydig cells disruption which caused testosterone levels to fall. Since testosterone is principally implicated in progression of spermatogenesis so the score fell. Along with tubular degeneration there was loss of cells of spermatogenic lineage. This has been supported by a study in which BPA with dose of 100mg/kg/day was used.[22]

In a study, BPA has induced mitochondria-mediated apoptosis due to activation of caspases in cytosol of hepatic cells.[23] In present study group B, significant degeneration of germinal epithelium was seen. Loss of normal cellular array and architecture of spermatogenic lineage was clearly evident in group B. As a result, sloughing of germinal epithelium from basement membranes occurred due to increased movement of smooth muscle cells as a result of ongoing inflammation by the released ROS. This result is in line with a study in which BPA-induced oxidative damage is seen in kidney, testis and liver of rats with degenerative changes.[24,25]

In present study, testicular interstitium showed significant congestion in BPA group. This was due to ongoing inflammation by free radicals which led to recruitment of inflammatory mediators in testicular blood vessels. This caused increased blood flow and hyperemia by focal dilation of blood vessels evident as congestion.[26] This result has been supported by various studies on rat testes when exposed to BPA.[27]

Vacuolization is due to disturbance of fluid balance in spermatogenic and Sertoli cells. Uncharged lipophilic bases and phospholipids enter cells by diffusion which become positively charged in organelles. Accumulation of charged form of weak bases increases intraorganellar osmotic pressure which attracts water from extracellular compartment and thus vacuoles appear.[28] In present study, severe vacuolization of spermatogenic cells and Sertoli cells was seen in group B rats as compared to control group. This result is in accordance with other studies.[29]

Being a rodent based developmental study it has offered constraints in inducing pregnancy in all the rats at the same time. Lactation was an important event during the current study in which mothers and newborn pups showed individual variations.

This study recommends future investigation into the imperative male infertility markers like semen analysis, sperm morphology and immunohistochemistry.

Conclusion

The present study indicates that BPA has detrimental effects on histology of developing testes owing to its teratogenic potential. Thus, spermatogenesis and male fertility may be adversely affected by BPA especially when exposed to pregnant and nursing mothers.

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ORIGINAL ARTICLE

Metabolic Syndrome Frequency in Patients with Acne Vulgaris

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ABSTRACT

Objectives: To determine the frequency of metabolic syndrome in patients with *Acne Vulgaris* at unit of Dermatology Liaquat Medical University Hospital Hyderabad.

Study Design: Case Control Study.

Place and Duration of Study: The Dermatology Unit Liaquat Medical University Hospital Hyderabad from 1st August 2018 to 31st December 2019.

Materials and Methods: A total of 75 patients with acne vulgaris were included in this study. The demographic characteristics of patients have been carried out. After a detailed history and a full clinical review, the patients underwent relevant investigations, which were established earlier, for which overnight fasting blood sample of each patient was sent to the institutional pathology laboratory where the Pathology Consultant prepared each report and the data were collected on pre-designed proforma. Confounders and effect modifiers including age, gender, BMI, occupation and socio-economic status were addressed through stratification and post stratification. Statistical test conducted was Chi-square.

Results: Metabolic syndrome association in patients with acne vulgaris was observed in 13.33% (10/75) cases. Stratification was performed and observed that, in patients with acne vulgaris, incidence of metabolic syndrome was not statistically significant with respect to age groups, BMI, duration of acne vulgaris, occupation, socio economic status and gender.

Conclusion: The frequency of metabolic syndrome in patients with acne vulgaris showed a weak positive association.

Key Words: *Acne Vulgaris, Chronic Inflammatory Disease, Insulin Resistance, Metabolic Syndrome.*

Introduction

Acne is a chronic inflammatory condition that occurs in age groups of teenagers. There is variety of misconceptions and misperceptions, in patients and also healthcare professionals, on the causes and treatment of acne.[1] Acne is a common pilosebaceous unit multifactorial disease. The functions of sebaceous gland androgenic stimulation, Hyperkeratosis of inflammation of the follicular infra infundibulum, and classically well-known etiological factors in acne pathogenesis are increased colonization of propionic bacterium acnes.[2] Abnormalities in metabolism of androgenic steroids, resistance to insulin, different clinical symptoms can result in cell signaling and uncontrolled inflammation that includes Acne Vulgaris.[3] It affects around 85% of adolescent patients and about 3% of the world's adult patients aged 35 and under about 44.[4] It is not disease that threatens life, depression, low self-esteem and low living standards are associated with it. Besides these results, it can also be associated with systemic disorders including chronic syndromes of hypovitaminosis, metabolic syndrome, and Behcet's syndrome.[5] Metabolic syndrome (MS) defined as an increased waist circumference with ethnicity-specific values plus any of the following four factors: triglyceride levels of at least 150 mg/dL; HDL cholesterol levels of at least 40 mg/dL for males and below 50 mg/dL for females; systolic BP levels of at least 130 mm Hg or diastolic BP levels of at least 85 mm Hg; and fasting plasma glucose levels of at least 100 mg/dL. It is a collection of predisposing factors, raising the risk of type 2 diabetes mellitus and heart disease.[6] The main risk factors are elevated dyslipidemia, blood pressure, central obesity and hyperglycemia.[7] Although

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metabolic syndrome in itself is not a dermatological diagnosis, many skin indications are a clinical predictor, increasing metabolic syndrome facilitating early diagnosis.[8]

The rationale for the study was small national and international data, and therefore the findings of international data are not relevant to our population as we are genetically and geographically distinct. This research was carried out in our community to determine the frequency of metabolic syndrome in patients with *Acne Vulgaris* at unit of Dermatology, Liaquat Medical University Hospital Hyderabad.

Materials and Methods

It was a case control study, conducted at Unit of Dermatology, Liaquat Medical University Hospital Hyderabad from 1st August 2018 to 31st December 2019. The sample size calculated for this study was 75. A non-probability consecutive sampling technique was used to select the patients. Ethical approval was received from the Research Ethics Committee of the College of Physicians and Surgeons of Pakistan (CPSP) before the study was performed.

Inclusion criteria were confirmed cases of acne vulgaris for at least one year in both genders aged 18 to 30 years. The exclusion criteria were considered to be other skin disorders associated with metabolic syndrome such as Psoriasis, patients already on medications that cause hyperglycemia, dyslipidemia or hypertension, and patients who were not willing to participate in the study.

All the diagnosed cases of acne vulgaris presented in outdoor patients were enrolled in the study by the principal investigator. Demographic characteristics like age, BMI, abdominal circumference in centimeter, blood pressure, weight, height, total cholesterol and fasting blood sugar of the patients were obtained on pre-designed proforma. After an extensive history and complete clinical examination (local and general), the patients undertook the requisite investigations described before, for that overnight, the fasting blood sample of each patient was sent to the institutional pathology laboratory where the pathology consultant drafted every sample. All patients received informed consent to participate in the study. An examination of metabolic syndrome in patients with acne vulgaris was the primary outcome variable. Using SPSS (Registered) Version 22, the data was analyzed. For qualitative variables such metabolic syndrome, gender, occupation, socio economic status frequencies and %ages were calculated. Quantitative data like age, weight, height, BMI and abdominal circumferences were presented as mean ± standard deviation. Confounders and effect modifiers including age, gender, BMI, occupation and socio-economic status were addressed through stratification and post stratification. Statistical test conducted was Chi-square. Confidence interval was set at 95% and probability value ≤0.05, as statistically significant.

Results

This research included a total of 75 patients with Acne Vulgaris. Table-I indicates the age distribution of the patients. The average age of the patients was 24.45±2.90 years. Similarly, the other demographic characteristic of the patients is also shown in Table I. There were 80% male and 20% female. Most of the patients 56 (74.67%) were working in inside office and 19 (25.33%) doing field work. Socio economic status of the patients was 26 (34.67%) were earning >30,000 and 49 (65.33%) were 15,000 to 30,000. Average of abdominal circumference, serum triglyceride, HDL, SBP, DBP and blood fasting sugar are also reported in Table II.

Metabolic syndrome frequency in patients with acne vulgaris was observed in 13.33% (10/75) cases. Stratification was performed and observed that, in patients with acne vulgaris, incidence of metabolic syndrome was not statistically significant with respect to age groups, BMI, duration of acne vulgaris, occupation, socio economic status and gender shown in Table III.

Table I: Descriptive Characteristics of Patient (n=75)

Variables	Mean	95% Confidence Interval for Mean		Std. Deviation
		Lower Bound	Upper Bound	
Age (Years)	24.45	23.78	25.12	2.90
Weight (kg)	74.34	70.32	78.37	17.48
Height (cm)	161.11	158.93	163.28	9.45
BMI (kg/m^2)	28.67	27.08	30.26	6.91
Duration of Acne Vulgaris (Months)	16.99	15.92	18.05	4.62

Table II: Clinical Characteristics of Patients (n=75).

Variables	Mean	Std. Deviation	95% Confidence Interval for Mean	
			Lower Bound	Upper Bound
Abdominal Circumference (cm)	89.67	9.67	87.44	91.89
Serum Triglyceride(mg/dl)	145.89	42.27	136.17	155.62
HDL (mg/dl)	42.69	13.02	39.70	45.69
SBP (mmHg)	129.13	9.84	126.87	131.4
DBP (mmHg)	78.73	7.21	77.07	80.39
Blood Fasting Sugar (mg/dl)	86.20	11.19	83.62	88.78

Table III: Frequency of Metabolic Syndrome in Patients with Acne Vulgaris In Male and Female (n =75)

Gender	Metabolic Syndrome		Total	P-Value
	Yes	No		
Male	7(11.7%)	53(88.3%)	60	0.396
Female	3(20%)	12(80%)	15	

Chi-Square= 0.721

Discussion

In present study, most of the patients were under the age of 25 years, with a mean age of 24.45±2.90 years. A total of 80% male and 20% female were part of the study sample. The mean duration of Acne Vulgaris was 16.99 months and the mean BMI was found to be 28.67 kg/m^2. Although, 25.3% of field workers have been identified with it. The prevalence of office work as an occupational effect on acne vulgaris is shown by 74.67% of patients. In this study, a prevalence rate of 20.7% was found in the 26-30 years age group. The occurrence of metabolic syndrome was observed in 13.33% (10/75) cases.

In their community-based research, Alshammrie et al[9] recorded the age of respondents in a range of five years. Much of the respondents were between 21 and 25 years of age. In comparison to our study, most of the respondents (84%) were female. Thirty-eight percent had a body mass index (BMI) equal to more than 30%. Most of the respondents were Saudis (98%) and Hail City residents (93%).

Acne is seen in 85% of adolescents in the U.S.[4] An overall prevalence of 60.7% was observed among Turkey's female secondary students.[10] Whereas another community-based research conducted in China found a prevalence of acne of 38% among women aged 15-19 years.[11] According to research conducted in Saudi Arabia, the overall prevalence of female secondary school students was 14.3 per cent. Most of these studies were carried out in middle school or high school students and very few in college or university.[12]

In similarity to our study, Neupane et al. stated that, according to BMI, 65.5% were average, 12 % were overweight, 20.5 % were underweight, and 2 % were obese. Much of the patients had Grade 2 acne (52.6%). Just 4.8% of the population had grade 4 acne. Grade 2 and 3 acne were more prevalent in females, while grade 3 and 4 acne were more prevalent in males.

Agreeing to current study, Babar et al.[13] reported that approximately half of the participants reported that their clothing was not affected by their skin. Social and recreational habits of 60% of participants were influenced by acne at 9.7%, 14.5% a lot, 35.8% a little, and 40% not at all. The majority of participants 67.3% were not affected by their acne in sports. In addition, 73.9% of participants were not affected by acne when studying and working, and more than half of them did not have an acne impact on their relationship with their partners and close friends.

In comparison to our research, the prevalence of metabolic syndrome was 39.0% in the Indonesian population and 29.2% in the Dutch population. The sex-stratified prevalence was 28.0% and 46.2% for men and women in Indonesia and 36.2% and 23.8% for men and women in the Netherlands. Males had a higher prevalence than women of four out of five metabolic syndrome components in the Netherlands population, with the exception of abdominal obesity reported by Sigit et al.[14]The higher prevalence can be explained by the age of the studied populations in the present study: one study included elderly people and the other younger.[15,16]

Acne vulgaris is one of the world's most common skin disorders and is usually seen in teenagers. Androgens have been identified as raising the size of the sebaceous glands, enhancing sebum production, inducing keratinocyte proliferation, which is what triggers acne.[13,17,18]

The limitation of research is a small sample size and minimal study setting. By incorporating more

research variables and wider study settings, it is recommended to design studies on a larger scale.

Conclusion

In our research, the frequency of metabolic syndrome in patients with acne vulgaris showed a positive association in cases.

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ORIGINAL ARTICLE

Dental Anxiety in Patients in Lahore Medical and Dental College: A Cross Sectional Study

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ABSTRACT

Objective: To assess the prevalence and level of dental anxiety in patients reporting to the Outpatient Department in Lahore Medical and Dental College.

Study Design: A Cross Sectional Comparative Study.

Place and Duration of Study: The Outpatient Department of Lahore Medical and Dental College during the months of August and September 2019.

Materials and Methods: A total of 300 patients were assessed for dental anxiety utilizing Corah's Dental Anxiety Scale. The qualitative variables were described as frequencies and percentages and the quantitative variables were expressed as mean ± standard deviation. Data was subjected to descriptive statistical analysis and significance testing between the variables was done using Independent samples ∓ *t* test and Analysis of variance (Anova). Level of significance was set at 0.05.

Results: Dental anxiety was prevalent in 29.7% patients, mostly children with a mean dental anxiety score of 7.23 (±3.78). The incidence of dental anxiety among the patients was 70.3% non-anxious, 15.3 % moderate anxiety, 6.7 % high anxiety and 7.7 % severe anxiety. Females recorded a higher mean Dental Anxiety Score, 7.89 (±4.07) as compared to males 6.44 (±3.39). There was a highly significant ($p<0.05$) difference in Dental Anxiety scores based on age, gender, and past dental experiences.

Conclusion: The Dental anxiety levels reported in the study population were low and the high level of anxiety was seen mostly in children. This highlights the importance of developing a comfortable dentist-patient relationship to help anxious patients and support them during the course of the treatment.

Key Words: *Corahs Dental Anxiety Scale, Dental Anxiety, Dental Fear, Dental Survey, Past Dental Experience.*

Introduction

Dental anxiety refers to a state of fear in regard to something bad happening during dental treatment, whereas dental fear is the normal response to a particular threatening stimulus in a dental setting.[1] Dental Anxiety (DA) is considered a major factor which results in patients avoiding treatment ultimately leading to an increase in the severity of the disease.[2] Such patients may be more uncooperative during the visit and may respond to pain stimuli differently.[3]

It is imperative for dentists to address dental anxiety in patients so that they can develop empathy for them and make a treatment plan specifically for such patients.[4] According to epidemiological data, between 3% and 20% people worldwide suffer from dental anxiety which is a cause for concern.[5] Around 10-20% of the population in the United States suffers from dental anxiety.[1] Dental anxiety has also been linked to deteriorating oral health and lack of timely treatment.[5] It has been found that people suffering from dental anxiety have more decayed or missing teeth as compared to those in non-anxious patients.[2] Although advancements in dental technology have been made but no change in dental scores has been observed over the years.[1]

Previously, limited studies have been done on the prevalence of dental anxiety in patients in Pakistan and there was a need for a study in our population.[3,6] The objective was to relate dental anxiety scores with age, gender and past dental experiences. This could help dentists in anticipating the responses of their patients when they report to the clinic. The Corah's Dental Anxiety Scale (DAS) was used to create a 20-point questionnaire. DAS is the most

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commonly and widely used scale for measuring dental anxiety.[7] If the level of anxiety experienced by patients is known, then the dentist can be better prepared to deal with such patients. The aim of our study was to determine the prevalence and level of dental anxiety in patients reporting to the Outpatient Department (OPD) in Lahore Medical and Dental College.

Materials and Methods

This cross-sectional comparative study was done at Lahore Medical and Dental College (LMDC), Lahore, Pakistan starting from 1st August to 30th September 2019. A formal permission was obtained from the Ethical Review Board of the college to conduct the study. All consecutive patients who came for treatment in the OPD of the college were enrolled in the study. The patients were asked to fill a questionnaire. Those patients who refused to participate were excluded from the study. Informed consent as a standard procedure was taken and confidentiality and anonymity of their responses was ensured. The study sample consisted of 300 patients. The sample population comprised of non-probability convenience sampling. All the patients belonged to a low socioeconomic background. The data was collected using the following method.

A questionnaire was prepared to measure the level of anxiety of the patients before a dental visit. A bilingual translator translated the questionnaire into Urdu language. The first part of the questionnaire consisted of questions regarding age, gender, and previous dental experience. The second part consisted of questions based on Corah's Dental Anxiety Scale. This is a 20-point scale where each response is given a set score. It is based on 4 questions each having 5 options. Each question may have a total maximum score of 20 or a minimum score of 4. This is calculated as a sum of all the scores of the five multiple choice items. A score of 9-12 was considered moderate anxiety, 13-14 was high anxiety, 15-20 as severe anxiety or phobia. 4 was the lowest score indicating no anxiety.

The data was collected, and Statistical Package for Social Sciences (SPSS) version 21.0 was used to do the statistical analysis. The qualitative variables were described as frequencies and percentages. Data was subjected to descriptive statistical analysis and presented as mean ± standard deviation. The Independent student T test was used to compare the mean DAS score in both the genders and groups with previous dental experiences. Analysis of variance (Anova) and post hoc analysis was done to compare the DAS scores in the different age groups. Level of significance was set at 0.05.

Results

A total of 300 (170 females and 130 males) patients were involved in the study out of which 56.7% were females and 43.3% were males. The ages of the patients ranged from 4 years to 73 years with a mean age of 28.2 (±12.27). The total number of patients in each group and mean ±standard deviation (SD) of age, gender, previous dental experience, and dental anxiety scale are given in Table I. Each variable with its statistical significance to dental anxiety is also given in Table I.

Table I: Bivariate Relationship Between Dental Anxiety and Sociodemographic Variables

Variables		n	DAS score (Mean±Standard deviation)	Significance
Age	≤ 10 years	23	12.47 ±3.85	p<0.001
	11-20 years	51	9.25 ±4.64	
	21-30 years	110	6.50 ±3.14	
	31-40 years	83	6.14 ±2.83	
	41-50 years	23	6.34 ± 3.03	
	≥ 51 years	10	5.00 ±2.00	
Gender	Female	170	7.89 ±4.07	0.001
	Male	130	6.44 ±3.39	
Previous Dental Experience	Pleasant	258	6.46 ±3.24	p<0.001
	Unpleasant	42	12.16 ±3.77	

The prevalence of dental anxiety among the patients was 70.3% non-anxious, 15.3 % moderate anxiety, 6.7 % high anxiety and 7.7 % severe anxiety. Females recorded a higher Dental Anxiety Score (DAS) 7.89 (±4.07) as compared to males 6.44 (±3.39). The bar chart in Figure 1 shows the dental anxiety level for each of the items of the questionnaire. There was a highly significant ($p<0.05$) difference in DAS scores based on age, gender, and past dental experiences.

Discussion

This cross-sectional study was done to find the prevalence and dental anxiety levels in patients reporting to the OPD of Lahore Medical and Dental College, Lahore. The prevalence of dental anxiety was found to be 29.7% with a mean dental anxiety score of 7.23 (±3.78). Around 70.3% population was classified as non-anxious. Association of demographic variables with dental anxiety were

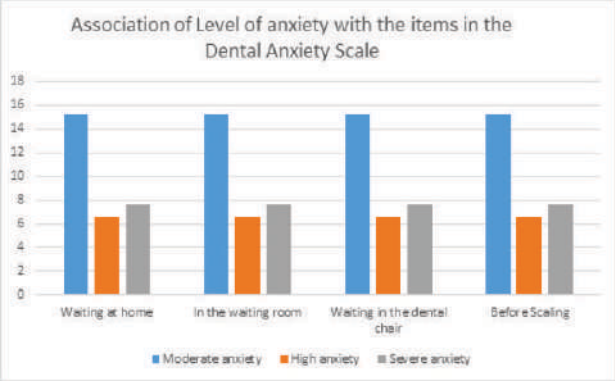

Fig 1: Association of Level of Anxiety with the Items in the Dental Anxiety Scale

identified using bivariate analysis.

These findings are comparable to other studies done for the same region like Shimla, India where incidence of dental anxiety was found to be 29.2% and mean dental anxiety score was 9.22 (±4.5).[8] Similarly in an Australian population, the DAS score recorded was 9.04 (± 3.45).[9] In a Nigerian study the dental anxiety score was similar to our study, 7.33 (± 3.20) and 92.5% of the population was found to be non-anxious.[10] An Iranian study showed higher DAS score, 12.34 (±4.74) where prevalence of dental anxiety was 58.8%.[2] In a similar study done in a teaching hospital in Karachi, the mean DAS score recorded was much lower, 2.73.[5]

There was a highly significant difference ($p \leq 0.05$) between the anxiety levels based on age, gender, and past dental experiences. Females had a higher dental anxiety score than males (7.89 and 6.44 respectively) and similar association has been extensively documented in other studies too.[2,5,9,12] It was found that younger female patients with past unpleasant experiences had a greater chance of developing anxiety before a dental procedure.[13] There are multiple reasons reported for this difference mainly because of a difference in the genetic makeup as females have a more developed fight and flight response due to presence of progesterone and estrogen.[5] In addition females may also find it easier to express their emotions and fears openly.[14]

It has been reported that as the age of the patient becomes more, the anxiety experienced by them before dental treatment also becomes less.[14] The reason could be a level of maturity that comes with age and the result of forming a good rapport with the dentist over time that ensures more confidence. This was also true for our study as higher DAS score (12.47 ±3.85) was seen in children as compared to adults' ≥ 51 years of age (5.00 ±2.00) ($p<0.001$). It has been extensively documented that children have more anxiety before a dental procedure and higher DAS scores have been reported in many studies.[15,17] In a study in Iran, no difference in DAS score based on age was found.[2] In an Australian study, highest DAS score was found in the 35-44 years age group.[9] This anxiety could be due to diverse factors like the sociocultural background, child's age, personality and nature of the parent's dental experiences.[16] Anxiety in young children can lead to an increased incidence of decayed teeth and more oral health related problems in life.[18]

Our study also showed that there was a highly significant association between DAS scores and past unpleasant experiences of the patients. Higher DAS score was observed in patients previously having a bad experience with the dentist (12.16 ±3.77) than the other group (6.46 ±3.24). This trend is expected and underlines the importance of a healthy patient and dentist relationship. The dentist's positive attitude and reassurance can make a patient feel safe and secure. Similar findings have also been highlighted in a study in Iran, where a statistically significant association was seen between the two variables thus further highlighting the role of the dental care givers in reassuring the patient.[2]

Limitations of our study were due to the small sample size confined to one hospital only where patients belonging to a low socioeconomic and educational background reported. It was felt by the authors that the avoidance of dental treatment despite low DAS score was due to low dental awareness rather than anxiety. However, further data is needed from diverse populations and people from all kinds of backgrounds to fully understand the factors responsible for dental anxiety and fear.

Conclusion

It is concluded that most patients are non-anxious and the high level of anxiety that is seen is mostly in children. Dental anxiety is prevalent in a small percentage of people and dental anxiety scores recorded are low. Female patients are more anxious and those patients with previous unpleasant dental experiences have higher levels of anxiety. This highlights the importance of developing a

comfortable dentist-patient relationship in order to help anxious patients and support them during the course of the treatment. Adequate measures must be taken to help children overcome their fear and anxiety so that they are not scarred for life.

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ORIGINAL ARTICLE

Measurement of Vulnerability Markers for Depression: A Study on Translation, and Validation of DAS-A

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ABSTRACT

Objective: The objective of current research was to establish the validity and reliability of Dysfunctional Attitude Scale-Form A in Pakistani population.

Study Design: The present study incorporates the cross-sectional design based on confirmatory factor analysis.

Place and Duration of Study: The study was conducted in twin cities of Rawalpindi and Islamabad from March 2016 to February 2017.

Materials and Methods: The data was collected through purposive convenient sampling. The Dysfunctional Attitude Scale- Form A (DAS-A) was translated in Urdu language and construct validity of the instrument was tested by the aid of confirmatory factor analysis. It is a 40-items self-report instrument measuring both the dysfunctional and the adaptive aspects of attitudes. The study included a total sample of N=641 (324 male & 317 female) young adults aged above 18 years (M= 19.56; SD = 1.79) and selected from the normal population.

Results: The findings revealed significant alpha coefficients, homogeneity, and stability of the DAS-A. Meanwhile, the confirmatory factor analysis provided strong support for a two-dimensional model. Using the sample variance-covariance matrix as input and a maximum likelihood solution, the overall chi-square was statistically non-significant *(CMIN/df=1.24; p > .05)*, the Tucker-Lewis Index was .90, the incremental fit index was .96, the Normed Fit Index was .97, the Comparative Fit Index was .96 and the Root Mean Square Residual / Error (RMSE) for the predicted minus observed correlation matrices was .03. All these values suggest good model fit for the DAS-A.

Conclusion: Subsequently, findings suggest that DAS-A (Urdu) is an efficient, reliable, and valid instrument for the assessment of dysfunctional attitudes which predispose to depression among young adults. Meanwhile, this research laid the foundation for further research in clinically depressed population.

Key Words: *Depression, Dysfunctional Attitudes, Reliability, Validity*.

Introduction

Dysfunctional attitudes are an important risk factor in the onset and maintenance of depression. Beck[1] proposed that dysfunctional attitudes are vulnerability factors that play a causal role in the onset of depression. In Beck's theory, negative self-schemas that include irrational or dysfunctional beliefs are the core of vulnerability to depression. Childhood experiences along with a negative stressor increase the risk of depression in those individuals with maladaptive cognitive patterns.[2] Accordingly, these dysfunctional attitudes are considered as predisposing risk factors for depressive episodes or indirectly as a factor of vulnerability under stressful conditions.[3] Previous studies revealed that dysfunctional attitudes are related to the incidence of depression [4,5] and that there was a correlation between high levels of dysfunctional attitude and the longer duration of episode[5,6,7] as well as shorter remissions in depression.[8]

Taken together, a reliable and valid measure is necessary for understanding depression.[9] Thus, dysfunctional attitudes are measured with the Dysfunctional Attitudes Scale (DAS)[2, 10] which has been one of the leading cognitive instruments in clinical research and diagnosis for measuring levels of cognitive vulnerability to depression [11,12,13] for more than 30 years.[14] DAS is one of the cognitive assessments that have a direct link or direction to symptoms of depression.[15] It is a self-report inventory designed to measure attitudes that can predispose a person to depression. DAS was originally a 100-item scale developed using a college student population. It was divided into two parallel

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forms, 40-item forms A and B. The DAS Form A (DAS-A) has been widely used in depression research, particularly in testing the cognitive theory of depression.[16] Items were rated on a seven-point scale, ranging from totally agree (1) to totally disagree (7). Total scores can ranges from 40 to 280, with higher scores indicating greater grace of negative beliefs. The original English version of this scale was found to have satisfactory reliability coefficients and could discriminate significantly between depressed and non-depressed groups. Additional studies have further supported the adequacy of this scale in terms of internal consistency and validity among college students.[1,2,4,17,22] Also, some researchers supported its adequacy in terms of internal consistency and validity in the general population.[23]

The factor structure of the DAS has been studied with models consisting of two factors [18, 24-27] three factors[28,29], and four factors.[30,31] Meanwhile, in Pakistan previous research had explored the four-factor model [32,34] and unidimensional model of DAS-A English version, while the existing research is still scarce on adaptive attitudes. To date, no study has been designed to assess adaptive aspects of the instrument, although the ten items in the instrument are designed in a functional way.[1,35] Consequently, valid and reliable cognitive measures are important for two reasons; first, to establish the adequacy of this instrument specially designed to assess depressive cognitions in a Pakistani context and second, to further investigate the theoretical and empirical validity of the cognitive-behavioral approach in Pakistan to measure both adaptive and dysfunctional attitudes. Therefore, considering the current status of the DAS, the objective of current research was to establish the validity and reliability of Dysfunctional Attitude Scale-Form A in Pakistani population, so that the DAS could be used with confidence to assess the vulnerability markers of depression.

Materials and Methods

The present study is a cross-sectional research which was being approved by the ethics committee of the National Institute of Psychology, Quaid-i-Azam University, Islamabad, Pakistan. Data was collected by researcher from 641 community individuals of Rawalpindi and Islamabad through a purposive convenient sampling technique from March 04, 2016 to February 16, 2017. Materials needed for the administration include the 40 items Dysfunctional Attitude Scale- Form A. The subject must be adequately motivated and in good physical and mental condition. The scale is administered without a time limit after all the necessary materials and adequate environmental conditions are provided. The 40 items of DAS are phrased as statements usually underlying depressive idiosyncratic thinking. Each item elicits information on the individual's dysfunctional beliefs, which act as schemas used to construct the world. [1,36-37.] These beliefs include approval, love, achievement, perfectionism, entitlement, omnipotence, and autonomy. Subjects assess each statement, considering the way they usually think, by using a 7-point Likert scale, where:1 = Totally agree, 2 = Agree very much, 3 = Agree slightly, 4 = Neutral, 5 = Disagree slightly, 6 = Disagree very much, 7 = Totally disagree. Meanwhile, the 10 items i.e., items 2, 6, 12, 17, 24, 29, 30, 35, 37, 40, were designed in an adaptive way. Afterwards, the data was assessed to test the assumptions of normality, and it was found that the data was essentially suitable for parametric testing. Thus, the first step to meet the study objectives was to translate the scale from English to Urdu. After taking permission from the authors, the scale was translated into Urdu by following Beaton's [38] translation method. Another essential phase in the translation of the scale was to establish that potential respondents could understand statements of the translated items. With this aim, the final translated scale was administered on 25 volunteer young adults. Respondents of this pilot-testing confirmed that translated items were suitable and simple to comprehend. After translation, the instrument was validated into Urdu language. The process of cross-language validation tries to produce equivalency between source and target language based on content. During this step 40 adults (13 male & 27 female) were tested. Accordingly, the scales were administered twice to two researcher's identified groups of bilingual Pakistani adults in English-Urdu, and Urdu-English sequences. The administration of the test was carried out individually on one to one basis. Participants were randomly assigned to the two conditions in first

administration i.e., English test and Urdu retest; Urdu test and English retest, and these groups were made to control the experiences of learning that may take place due to the administration of Urdu and English tests with two weeks apart retesting. Afterward, the factor structure of the translated instrument was confirmed through a confirmatory factor analysis. The findings helped in determining the structure of factors for young adults and examined whether the construct has the same structure, or they depict a new pattern for the Pakistani sample. CFA basically depends on multiple statistical tests to assess the acceptability of model fit to the data. CFA aimed to confirm to what extent the existing factor structure fits the present study data. [39-42] In the current study, the researcher has considered widely used model fit indices i.e., CMIN/df, CFI, NFI, TLI, and RMESA[43,44] and factor loading (.30 and above) as criteria to test the validity of items.[45] The data was analyzed through AMOS 24[46] and the Statistical Package for Social Sciences 25.0 for Windows.[47]

Results

Table I, II, and III show the findings of test-retest reliability, establishment of alpha coefficients reliability, and the confirmatory factor analysis.

In order to determine cross-language validity and test-retest reliability of the scales (Table I), moderate correlation coefficient ranging from .61 ($p < .01$) to .72 ($p < .01$) were found. Afterward, the instrument was tested on a diverse group to confirm the factor structure in the Pakistani population.

Using the whole sample, internal consistency, and validity analysis was conducted. The reliability of the two factors i.e., dysfunctional attitude and adaptive attitude was evaluated. Table II shows the internal consistency and descriptive values of the DAS-Urdu. Using Cronbach's alpha to estimate the reliability coefficient, a high to moderate alpha was obtained for the dysfunctional attitude (.86), and the adaptive attitude (.62). CFA was subsequently utilized to examine the construct validity of the two-correlated factor model. The findings of CFA are presented in table III. Given these results, using Maximum Likelihood procedures to estimate the model, most of the indices indicated a good fit ($x^2 = 357.60$, df = 289; *CMIN/df*=1.24, CFI=.96, IFI=.96, TLI=.90, and RMESA=.03 which is indicating a good model fit. The CFA has confirmed the factor structure. These results depict that the translated version of the Dysfunctional Attitude Scale is statistically valid for measuring the respective attitudes across Pakistan. The inspection of non-standardized regression weights indicated significant loadings for all items that were above .30.

Table I: Retest Reliabilities of Urdu and English version of the 2-Factors of Dysfunctional Attitude Scale (DAS) (N=40)

	UE	EU
Dysfunctional Attitude	.72**	.72**
Adaptive Attitude	.61**	.67**

Note. UE = Urdu English, EU = English Urdu
**$p < .01$, *$p < .05$

Table II: Descriptive of Subscales of Dysfunctional Attitude Scale among Young Adults (N=641)

					Range	
Variables	**Item**	**α**	**M**	***SD***	**Potential**	**Actual**
Dysfunctional Attitude	30	.86	3.5	.72	1-7	1.9-5.7
Adaptive Attitude	10	.62	3.2	.73	1-7	1.3-5.4

Table III: Confirmatory Factor Analysis (indices of model fit) for Dysfunctional Attitude Scale (DAS) (N=641)

Indices	CMIN	Df	CFI	IFI	TLI	NFI	RMESA
	357.60	289	.96	.96	.90	.97	.03

Note. CFI=Comparative Fit Index, NFI= *Normed fit index,* RMSEA=Root Mean Square Error of approximation, TLI =Tucker-Lewis Index

Discussion

The purpose of the present research is to assess the factor structure, reliability, and validity of the DAS-Urdu among the Pakistani population. This paper shows that the Dysfunctional Attitudes are a hallmarks of depression. Despite a central role for dysfunctional attitudes in cognitive theories of depression and the widespread use of the Dysfunctional Attitude Scale, form-A,[1] the psychometric advancement of the DAS-A has been limited. Few studies have been published which report its use as a measurement tool of the presence and intensity of dysfunctional attitude among the depressive individuals. It is a self-report instrument measuring both the dysfunctional and the functional (adaptive) aspects of attitudes. The present study

establish that DAS-A is reliable and can assess the dysfunctional as well as adaptive attitudes. Meanwhile, it is consistent with the previous literature[1.34-37] which asserted that 10 items are phrased in functional way.

The strength of our study is that this tool is translated and validated in Urdu to access dysfunctional and adaptive attitudes in the Pakistani population, addressing a gap in the literature. One limitation of the study is geographical, as the data were collected from only two cities due to convenience sampling. In addition, our sample in this study was based on a higher educated population than the mean education level in the country. Furthermore, in view of these limitations, further studies on diverse samples are required to achieve a higher level of validation of the Urdu version of the DAS-A. Accordingly, it was established that the DAS-A (Urdu) is a reliable instrument for assessing the vulnerability markers of depression in Pakistani population. It is a valid scale, appropriate to be used in clinical and research settings. It is a concise yet two-dimensional measure with solid psychometric properties to facilitate screening in both clinical and research settings. However, future research is strongly recommended to continue to assess the scale reliability and validity in clinically depressed population.

Conclusion

Taken together, it is concluded that the DAS-A (Urdu) is an efficient, reliable, and valid instrument for the assessment of dysfunctional attitudes which predispose to depression among young adults. Meanwhile, this research laid the foundation for further research in clinically depressed population.

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ORIGINAL ARTICLE

Effectiveness of Faculty Development Workshop on Introduction and Development of Student's Portfolio: A Pilot Project

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ABSTRACT

Objective: To evaluate the effectiveness of faculty development workshop on "Introduction & development of student's portfolio" using first two levels of Kirkpatrick (KP) model.

Study Design: A quantitative evaluation study based on evaluation of one day workshop, conducted on 15th November 2019.

Place and Duration of Study: The study was conducted at Islamic International Medical College at Department of Medical Education.

Materials and Methods: The study aimed to evaluate the efficacy of faculty development workshop on Kirkpatrick's model of program evaluation. A 4-hours workshop was carried at Islamic International Medical College on 15th November 2019. Sampling technique was purposive (n=21). The reaction of the study participants about the training experience was evaluated through a feedback evaluation proforma. The assessment of knowledge was done through MCQs and reflective writing skill was assessed through a checklist based upon framework of Gibbs reflective cycle. Both knowledge and skill were assessed before and after the workshop. The content of pre-test and post-test was same.

Results: The knowledge of the participants about portfolio was remarkably increased, from 3.95 ± 1.35 (median=4) in pre-test to 6.23 ±1.17 (median=6) in post-test. The improvement in reflective writing skill was also significant 2.14 ± 0.727 (median=2) in pre-workshop reflection to 4.23 ± 0.70 (median=4) in post-workshop reflection. The Wilcoxon signed rank test showed a significant increase in both knowledge and skill (p-value <0.000). The reaction of the participants showed high percentages regarding content delivery (95%), learning environment (100%) and increase in knowledge (95%) measured on 5-point Likert scale.

Conclusion: Faculty development workshop on student's portfolio was highly valued by faculty members. Most of the participants were convinced and ready to start portfolio for learning and assessment of students in the institution.

Key Words: *Faculty Development, Kirkpatrick Model, Portfolio, Reflective Writing, Students Learning.*

Introduction

Portfolio is one of the most useful cognitive learning and assessment tool. It stimulates deeper learning from experience by the process of reflection.[1] The reflection is a metacognitive process that improves future course of action by creating deeper understanding about one's self and situation. It makes students strategic life-long learners.[2]

The reflective writing is a fundamental skill required for development of portfolio which is not necessarily inherent for teachers and learners. Hence the medical educators need to devise the strategies necessary to develop this skill among medical teachers.[3] Thus teachers training on reflective writing is directly related to portfolio development by the students.

For faculty development different activities can be arranged, among which workshops are most popular and desired ones. This may be due to an interactive ambiance in a small group, an ease of communication and flexible environment.[4]

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After the successful implementation of any workshop the next challenge is to measure its impact on the learning of the participants. The most suitable framework to evaluate the workshop training programs is the Kirkpatrick's model. The evaluation of reaction, learning, behavior and results through different levels of the Kirkpatrick model-KP is an invaluable tool to assess the achievement of an outcome.[5]

The literature review has shown that the trainings on the development of reflective writing capacity are lacking in Pakistan and this is the reason due to which the institutions are unable to use the effective tool of portfolio both for students learning and assessment.[6] Portfolio is a regular part of undergraduate curriculum in majority of medical schools worldwide and the teachers are formally trained to achieve excellence in the skill of reflective writing through series of ongoing training programs. The purpose of current study is to evaluate the effectiveness of faculty development workshop on introduction and development of student's portfolio. The article will provide guidelines on faculty training in context of reflective writing in developing countries like Pakistan. It will also be helpful to start with portfolio as an effective tool of learning and assessment in a curricular program.

Materials and Methods

It was a quantitative evaluation study carried at the department of Medical Education, Islamic International medical college, Rawalpindi on 15th November 2019. It was 4 hours workshop on **Introduction and Development of Student's Portfolio**

The workshop evaluation was based on KP model. The workshop was attended by 21 participants and the sampling technique was purposive sampling. Approval from the institutional ethical review committee was taken and informed written consent was obtained from all participants. The study participants were both from basic and clinical sciences, including professors, associate professors, assistant professors and senior registrars with certificate or master's degree in medical education. Newly inducted faculty members and those without any background of medical education were not included in the workshop.

The content delivered in the workshop was about the introduction of student's portfolio, the process of developing a sample portfolio and its importance in learning and student's assessment. The pre-existing knowledge of the participants about portfolio was assessed through a pretest consisting of 10 MCQs. It was followed by a reflective writing activity by the participants on any of the incident from their academic life experience. This was to assess the reflection writing skill of the participants before attending the workshop. The evaluation of learning of knowledge was done through MCQs and the reflective writing skill was assessed through a checklist based on Gibbs reflective cycle (table I). Both knowledge and skill were assessed by the workshop facilitator. The MCQs & reflective writing assessment checklist were approved from the expert in medical education. After the workshop, MCQs and reflective writing activity was repeated as post-test. MCQs and reflective writing activity were kept same in both pre-test and post-test. Both the pre- and post-test MCQs and reflective writing scores were compared to see any change in the learning of participants. The reaction of the participants to workshop was collected on a pre-designed feedback evaluation proforma, given to the participants at the end of workshop. The feedback (reaction) was assessed on 5-point Likert scale, where 1=strongly disagree (SD), 2=disagree (D), 3=do not know (DK) 4=agree (A) and 5=strongly agree (SA). For analysis of data, SPSS version 20 was used. The frequencies & percentages were calculated for the feedback evaluation of participants while mean & median scores were calculated for MCQs & reflective writing. For comparison of pre-test and post-test scores, Wilcoxon signed-rank test was used as the data was non-parametric.

Table I: Checklist for Evaluation of Reflective Writing Skill

S.No	Parameters	Assigned score
1	Description	2
2	Feelings	2
3	Evaluation	2
4	Conclusion	2
5	Action Plan	2
	Total score	10

Results

The workshop was attended by 21 participants. There were 3 (14%) males & 18 (86%) female

participants, all appeared in pre & post-test. The reaction of the participants assessed through feedback evaluation proforma is given in figure 1. Except for one domain "appropriate time allocation", all the responses fall into the categories of 4 (A) & 5 (SA) on Likert scale. Both category 4 and 5 were considered positive response and sum of the percentages in each of these categories show a high satisfaction of the participants to the workshop regarding content delivery (95%), learning environment (100%), teaching strategy (90%) and increase in knowledge (95%).

The knowledge of the participants about portfolio was remarkably increased, from 3.95 ± 1.35 (median=4) in pre-test to 6.23 ±1.17 (median=6) in post-test. The improvement in reflective writing skill was also significant 2.14 ± 0.727 (median=2) in pre-workshop reflection to 4.23 ± 0.70 (median=4) in post-workshop reflection figure 2. The Shapiro-Wilk test showed the non-normal distribution of the scores and Wilcoxon signed rank test confirmed remarkable improvement (p=0.000) in the post-workshop knowledge and reflective writing skill of the participants about student's portfolio (table-II).

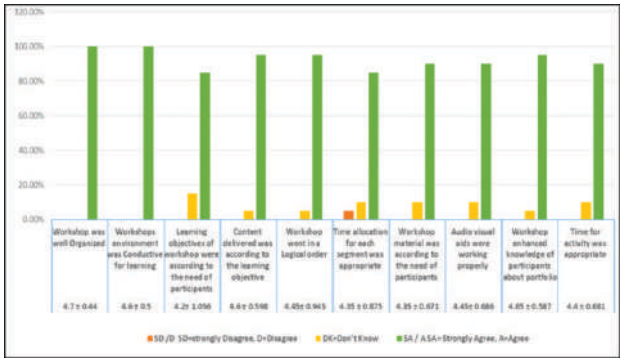
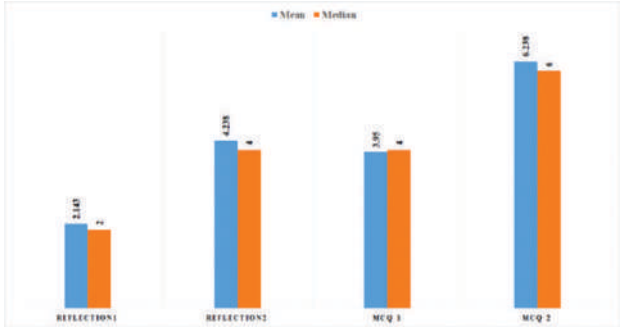


Fig 1: Reaction of The Participants to the Workshop on 5-Point Likert Scale



1=Pre-test, 2=post-test

Fig 2: Descriptive Statistics of Pre and Post Workshop Knowledge and Skill Scores

Table II: Improvement in Knowledge and Reflective Writing Skill

Variable	Wilcoxon Signed Rank Statistic ±SE	Standardized Test Statistic	P -value	Net gain (Mean ± SD)
MCQs	210±26.589	3.949	0.000	2.28+1.34
Reflective Writing	210± 26.053	4.030	0.000	2.1+0.64

Discussion

The knowledge of participants about portfolio and skill of reflective writing were remarkably improved after attending the workshop. The highly satisfactory response of the study participants further proved the effectiveness of workshop. Such activities are necessary to develop the reflective writing skill of the medical teachers which is fundamental to the process of portfolio development.[7]

The biggest challenge for health educators is the pedagogical tool to help students to write reflectively. Portfolio is one such tool to develop reflective capacity of students to foster their critical thinking and problem-solving skills to prepare them for informed clinical judgments.[8] It helps them to perform better in written assessments and to develop decision making skills as a clinician. The implementation of any new strategy either for teaching or assessment of students is always very challenging. One of the biggest challenges is faculty resistance. The people do not feel comfortable to carry out the new responsibilities either due to inadequate exposure or lack of training. Hence it is imperative to do capacity building of teachers at first.[9] The current study has implications for many institutions across the country, planning to begin with portfolio as an integral part of students teaching and assessment.

There is not enough literature on the reflective capacity building of teachers in Pakistan. The concept is relatively new in teachers training programmed on our set up. In a study conducted by M Ilyas, Shawana Fazal & M Amin, the comparison of perceptions of students and university teachers in Pakistan and UK showed that majority of teachers and students here were not even well aware about the notion of reflection.[10] Teachers represent one of the key resources for the academic success and investment in their training results in unceasing success despite all challenges.[11]Teachers training

always results in improvement of skills, motivation, and performance. It helps to boosts up one's self-confidence and brings positive behavioral changes among teachers for educating students.

In this study, an outcome-based workshop was conducted as apart of faculty development program, with targeted audience. The workshop was evaluated to assess the outcome at level I and level II of Kirkpatrick's model for reaction, improvement of knowledge and reflective writing skill of the participants. The study showed statistically significant increase in knowledge and reflective writing skill of all the participants after the workshop on student's portfolio. The pre workshop reflection was very ordinary, there was no framework followed by the participants, the coherence and flow of events was missing. After the workshop, all reflective writings improved significantly. The participants were trained to reflect using Gibbs cycle of reflection and assessment was done on a checklist based on it. Gibbs reflective cycle is an effective model for teaching of reflection.[12] After workshop, there was good reflection of description of situation along with feelings, critical analysis of the context and action plan. The evaluation of reaction of participants also showed good response with 95% satisfaction with content delivery and increase in knowledge.

The participants valued and enjoyed all teaching and learning activities in the workshop on student's portfolio with 100% satisfaction on learning environment. They acknowledged the activities were interesting, informative, and contextual with less lecturing and more hands-on work. These findings highlighted the effective planning of the workshop. The pro-active measures in relation to expectations of the participants are of utmost importance for an effective workshop.[13]

There are various other tools which can be used as valuable avenues to promote reflective writing. In another study conducted by Karkabi K et al., the use of abstract paintings and narrative literature was used to develop reflective capacity in a multinational faculty development workshop.[14] The participants found this arts-narrative based workshop very useful to facilitate their reflective writing in context of educator-learner relationship. Qualitative analysis of participants feedback was done. The sample size was small and no objective quantitative evaluation of the net gain in knowledge and skill about reflective writing of the participants before and after the workshop. Also change in behavior was not evaluated.

In another study by Donald Boudreau J et al., a workshop on reflective writing for clinical teachers, the evaluation of outcome was limited only to level I of Kirkpatrick's model, quantitative data focused on patient satisfaction with qualitative analysis of their narrative writings.[15] Narrative methods had been used successfully to nurture self-reflection through a half day interactive workshop. In comparison to current study, objective assessment of pre- and post-workshop reflective writings was lacking. In this study, the quantitative data interpretation was done before and after the workshop for both knowledge and reflective skills of participants along with their feedback evaluation. Use of multiple tools like MCQs and reflective writing skill evaluation in a 4-hours workshop is another strength of this study.

Present study strongly emphasizes to initiate and continue these workshops for teachers training to promote their reflective writing skill. This is not only essential for portfolio development, but it is the need of hour to help our students to become critical thinkers and reflective life-long practitioners for better health care services.[16]

Conclusion

The workshop on student's portfolio development was highly valued by the participants. There was remarkable improvement in knowledge and reflective writing skill of the participants required to develop a portfolio. Such workshops are immensely needed for building reflective capacity of teachers which is one of the core competencies all health care professionals.

Limitations

The results are generalized from the single workshop, which is main limitation of the study. Further research is recommended with use of different validated qualitative and quantitative tools.

Disclaimer

This manuscript has not been previously presented or published in any conference and it is not a part of research, PhD or thesis project.

Conflict of interest

It is declared that there are no personal, financial, and professional interests involved.

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REVIEW ARTICLE

Role of Exercise and Positioning in Acute Respiratory Complications in COVID-19 A Review

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ABSTRACT

Viral infections are major cause of respiratory diseases. The number of cases of viral infections are higher in older adults and children. The symptoms associated with viral infections are fever, coughing , sneezing, runny and stuffy nose with body aches. There is no specific unified antiviral treatment suggested for COVID-19, and no vaccine is presently available. The treatment is symptomatic, and oxygen therapy signifies the major treatment intervention for patients with severe respiratory issues due to viral infection. The prevalence of acute respiratory distress syndrome (ARDS) in patients with COVID-19 is 8.2 %. Commonly used supportive interventions are Range of Motion exercises, progressive mobilization, strengthening exercises and Passive exercise to build up balance and coordination for the better body functioning and decrease respiratory complications. The patient management algorithm is divided into three categories according to the severity for suspected cases and confirmed patients in acute care setting. The physiotherapy intervention's goals during respiratory complications is to reduce the risk associated with prolonged recumbent position and to facilitate oxygenation. Prone positioning can reduce the mortality rate in ARDS and also increases the $PaO_{2/}FiO_2$. Mobilization should be done, as cough is an aerosol generating procedure and lead to the mucus secretions and expectoration which can be secreted out using Mobilization methods . These methods improves cardiopulmonary endurance and oxygen saturation among patients having respiratory distress due to viral infections. Further, exercise program increases the resistance to upper respiratory tract infections. Before application of such methods the staff members should be properly equipped with personal protective strategies for all the suspected or confirmed cases and the droplet precautions must be executed.

Key Words: *COVID-19, Exercise ,Patient Positioning ,Protection Strategies, Viral Infections.*

Introduction

Globally, viral infections are the major cause of respiratory diseases.[1] The relatively common viruses include corona , influenza, meta pneumovirus and para influenza as well as. The number of cases of viral infections is higher in older adults and children.[2] As per WHO viral infections are considered as major public health issue. Various viral epidemics have been witnessed in the past two decades including severe acute respiratory syndrome coronavirus (SARS-Cov) and Influenza (H_1N_1).The Middle East respiratory syndrome (MERS-Cov) was first noted in 2012 in the Kingdom of Saudia.[3] Coronavirus 2 (SARS-Cov 2) appeared in 2019.[4] The novel corona virus is highly contagious and led to the current pandemic. The COVID-19 is a major viral attack emerged as outbreaks of respiratory complications.[3] The mode of transmission is from one person to another by touching hands or having contact to a contaminated surface and then touching the mouth, nose or eyes. During coughing and sneezing the infected airborne particles (aerosols) are produced which remain alive for up to three hours in the air.[5] These infected airborne particles can be inhaled by a person or might be present at the mucus membranes of eyes. The viral respiratory infections are communicable and most commonly affect the upper and lower respiratory tract. The symptoms associated with viral infections are fever, coughing or sneezing, runny or stuffy nose and the body aches.[6]

The severity of disease ranges from no symptoms to mild illness of the upper respiratory tract, while severe symptoms may lead to respiratory failure and death.[7] The common sign and symptoms related to coronavirus are fatigue fever and dry cough. A few

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numbers of patients can develop sign and symptoms such as sore throat, nasal congestion, runny nose and diarrhea. Few patients can have low grade fever, light fatigue and no occurrence of pneumonia. In severe cases of infection shortness of breath, difficulty in breathing and pneumonia can be present. Severe cases then progress to septic shock, acute respiratory distress syndrome (ARDS), metabolic acidosis and can lead to death.[8] According to WHO, globally till 19th May 2020, there have been 4,731,458 confirmed cases of COVID-19, including the 316,169 deaths.[9,10]

Viral Attacks and Respiratory Illness

Until now there is no exact unified antiviral treatment for COVID-19, and no vaccine is presently available. The treatment depends on symptoms, and oxygen therapy remains the major treatment for patients who are severely infected. Mechanical ventilation may be required in cases of respiratory failure non-compliant to O_2 therapy, although hemodynamic support is important for dealing with the septic shock.[3]

Due to COVID-19 the prevalence of acute respiratory distress syndrome in Wuhan China is 8.2 %.[11] Studies suggest that in moderate to severe ARDS , prone positioning (PP) can reduce the death rate and also increases the PaO_2/FiO_2. According to an observational cohort study during an acute respiratory distress syndrome the early administration of high flow nasal cannula (HFNC) and prone positioning can help to avoid the need of intubation. The patients with severe acute respiratory distress syndrome were not suitable for the HFNC/NIV (non-Invasive Ventilation) with prone positioning.[12] The patients remained in long term prone position treatment sessions results in decreased mortality rate in those patient.[9] The mechanism of prone positioning for improvement in ARDS patients is by improving the tidal volume, decrease of alveolar shunt, increase in end expiratory lung volume, increase in elasticity of chest wall, and affecting recruitment in dorsal lung regions.[13] However the effectiveness of prone positioning depends on accurate selection of patients and application of appropriate treatment protocol. According to a meta-analysis by Munshi et al, e prone positioning applied for at least 12 hours per day, resulted in decreased mortality in ARDS patients. [14]Another meta-analysis revealed that prone position reduces the mortality rate only in cases where patients with severe hypoxia are getting ventilation with low tidal volume, and the treatment is started in the initial 48 hours. If Prone position is administered in the initial hours, for longer period of time and for the patients with severe impaired oxygenation, it reduces mortality rate [15] Recently in an observational study Guerin et al. revealed that among the ARDS patients only 13.7% have been positioned in prone position. Even in severe ARDS patients the prone positioning was 32.9%. There were two main reasons behind the reluctance of physicians about this treatment: one was that Physicians judgment in mostly cases that hypoxemia is not severe enough to justify the usage of prone position and still suggest prone position as safe/rescue method and second was that most patients with ARDS have hemodynamic instability which prevents the decision for the use of the prone position.[16]

Physiotherapy Management

In patients with critical illness, medications and supportive role of physiotherapy techniques is to reduce the complications in such cases. These can be associated with prolonged static position, due to respiratory complications and after effects of prolonged lying in same position. The physiotherapy management includes exercises with passive Range of Motion and positioning.[17] Mobilization techniques include mobility in bed, sitting out of the bed, and balance during sitting, from sitting to stand, walking, tilt table, ergometry of upper and lower limb and other exercise programs. It is advisable to initiate the early mobilization.[15] To prescribe the exercise and mobilization program there should be proper consideration of the patient condition (stable with stable hemodynamic and respiratory function).[18]

Management Categories

The patient management is divided depending on the severity of the illness of the patient. Patients are managed with recommended classification in three management categories given by a Medical Corporation in Qatar.[28]These includes category A , for sedative or ventilated patients , while Category B includes Ventilated and minimally sedative and category C is further divided in to Category C-1, C-2 and C-3 , bed bound or less conscious patients ,active

but conscious and dependent ones ,conscious and functionally independent patients respectively (Table I and II). In ICU survivors the early exercise program increase recovery and functional status.[19] Prolonged extensive exercise results in immune suppression, whereas the moderate intensity exercises enhance immune function and minimize the severity and possibility of respiratory tract viral infections.[20]

Qin Sun and coworkers used prone position during management of Novel coronavirus for pneumonia and reported an improvement in oxygenation and pulmonary heterogeneity. [17] In a systemic review to assess the effectiveness of exercise in the occurrence, duration and severity of acute respiratory infections, the authors analyzed 14 trials of participants aged from 18 to 85 years. The key results of study showed that there is no difference between the exercise group and no exercise group while comparing the number of episodes per person of acute respiratory infections.[21] A narrative review highlighted the evidence that exercise during the lifespan increases the confrontation to upper respiratory tract infections, while the immune system is suppressed by the regular strenuous exercise. [22] A nested cross-sectional study described that after critical illness the inflammation occurs which is associated with poor physical recovery throughout the first 3 months post-intensive care unit (ICU) discharge. During the sedentary period of hospitalization the muscular adaptation and skeletal function are extra benefits of regular exercise. One systematic review of exercise-based rehabilitation trials following intensive care unit (ICU) discharge quoted lack of methodological strength through studies and observed no noticeable effect on functional capacity or health-related quality of life (HRQOL) after exercise interventions that were commenced during the post-ICU period.[23]

These studies state that there should be further trials related to respiratory complications in patients undergoing rehabilitation in intensive care units .These not only help in early rehabilitation of patients but also help the health departments to design policies. There can be specific trainings and short courses to deal with such pandemic situations.

Precautions During Respiratory Interventions

1)When interventions related to rehabilitation are indicated, plan it with team and properly arranged execution.

2) Ensure the use of minimum number of staff members to perform the activities.[18]

3) Make sure that all equipment is available and working properly in reachable range.

4) All equipment should be decontaminated.

5) If there is need of equipment to be shared between patients, properly disinfect it.[24]

6) Interventions of respiration like breathing exercises, postural drainage, and clearance of secretions techniques are basically aerosol producing techniques. That's why these interventions should be avoided.

7) Before initiating any procedure, the physiotherapist must compare the benefits with the risks. PPE (Personal protective equipment) such as N-95 mask, head covers, protective eye covers, shoe-covers, face shields and disposable gowns should be used

8) If possible the physiotherapist should follow the posterior approach while performing the mobilizations and position themselves to a distance of ≥2 m to be out of the line of cough.(standing on the foot side of table) Dyspnea may be present in the case of acute hypoxemia despite the administration of O_2 >10-12 L per minute with a reservoir mask.[25] In this case during the physiotherapy procedures the use of non-invasive ventilation (NIV) or continuous positive airways pressure (CPAP) or the high flow nasal oxygen (HFNO) can be helpful.[26]

9) Face mask should be preferred than the nasal cannula when the patient has to be mobilized. If the nasal cannula is the single option, fix it properly in the nostrils and then cover it with the surgical mask.[27]

10) When the patient is on non-invasive ventilation make sure there is no air leakage while starting the physiotherapy sessions.

11) For open mouth breath patients to facilitate the O_2 saturation a non-invasive mask can be used which is connected by a T tube to circuit.

12) For the purpose of improving the oxygenation the patient should be in sitting or semi sitting position. Lateral decubitus position's alternations,

prone or half prone positions can be helpful.

13) To minimize the muscle activity and to improve the ventilation these positions preferred to be passive.

14) The patients who are on non-mechanical ventilation must wear a face mask during the session.

15) Do not share the same equipment for different patients without sterilization. [21]

Need of Protection In Acute Phase

1)It is necessary not to perform the procedures which cause extra stress on the breathing because it can put the patient to a higher risk of the respiratory distress.[28]

2)The procedures that are fewer recommended during the acute phase of COVID-19. includes: Pursed lip breathing exercises, Diaphragmatic and deep breathing exercises.

3) Lungs re-expansion techniques such as cough machines, PEP bottle or bronchial hygiene Rib cage stretching/manual mobilizations/ respiratory training of muscles.[29]

Personal Protection Strategies

1) For droplets prevention hair covers fluid resistant shoe covers and should be used.

2) Repeated shoe cover usage is avoided because recurrent removal can increase the risk of contamination in the staff.

3) During the patient care the PPE especially face mask should not be adjusted.

4) Local guidelines should be followed about the information of laundering and wearing uniforms. Staff should be encouraged to change their uniform before leaving the work place and should be carried to home in plastic bag for the washing.

5) Before entry to the clinical areas all personal used items should be removed such as mobile phones, jewelry, watches, pens etc.

6) There should be limited use of stethoscope. If necessary usage of dedicated stethoscopes within the isolation areas can be done.

7) If the PPE items are reusable such as goggles, these items must be properly disinfected and cleaned before re-use.

8) If there is expected large amount fluid exposure an extra plastic apron should be wear.[10]

9)All the staff members should be trained properly for the wearing (donning) and removing (doffing) of the personal protective equipment. Including the N-95 mask by fit checking. A list of staff has to be maintained who has completed their personal protective equipment training and education.[30]

10) To ensure the good fitting of mask the staff

Table No I: Respiratory Status and Physiotherapy Management of COVID-19 Patients

	Status	Management Strategies
Category A	**Sedative or Paralyzed/ Ventilated Patients**	It includes patients who are critically ill and they may be in prone position.
		The patients who are on ECMO (Extra Corporeal Membrane Oxygenation).
		The physiotherapy intervention's goals on this stage are to reduce the risk associated with prolonged recumbent position and to facilitate oxygenation.
		Physiotherapy management might consist of therapeutic positioning and Passive joint ROM exercises.
		The decision of continuing the physiotherapy treatment must depend on the potential capacity of patient and further considering the risk vs. benefits comparison.
		Regarding physical therapy management there should be regular communication with the multidisciplinary team.
Category : B	**Ventilated and Minimally Sedative Patients.**	Depending on the patient's level of consciousness and level of cooperation, develop an individual treatment plan.
		The main aim of physiotherapy management at this stage is to reduce the risk of longtime recumbent position.
		To facilitate the process of oxygenation and to improve the functional independence.
		Physiotherapy management at this stage may include the Range of Motion exercises, therapeutic positioning and progressive mobilizations.
		Aerosol generating processes should be limit or minimized. Before initiating any procedure the physiotherapist should

members with beard should be motivated to shave their facial hairs. For all the suspected or confirmed cases the precautions regarding droplets must be implemented. The staff members must have to wear items including, surgical mask, long sleeve fluid resistant gowns, face shields or goggles and gloves.[31]

Table No: II Respiratory Status and Physiotherapy Management of COVID-19

Category C	**Status**	**Patients on Non-Mechanical Ventilation**	
Outcomes		Depending on the level of consciousness and functional independence the patients in this category are Further divided into three categories.	**The Overall Aims of the Interventions are :** To minimize the work load of breathing To smooth the process of oxygenation. To improve the capacity of lungs. To improve the functional capacity.
		Interventions	
Category C-1	**Less conscious , bed bound patients**	Physical therapy interventions may consist of positioning and passive range of motion exercises. Further progression of treatment and number of session depends on the rehabilitation potential of the patients. Regular basis meeting with MDT to make sure the patient's adherence to the exercise plan.	
Category C-2	**Active, Conscious and Dependent, Patients**	Interventions in this category include range of motion exercises, progressive mobilization, strengthening exercises and exercise plan to build up balance and coordination. Based on the patient's dependency of oxygen, functional independence and muscle power, an individual plan is developed. Before starting the mobilization technique, make sure the availability of all necessary equipment. Mobilization would be done as it is an aerosol generating procedure and lead to the cough and expectoration. These techniques should be performed with maximum precaution and care. Use of mobility aids should be available in the isolation area for the usage of coronavirus patients only. All the mobility aids should be kept in the room of patient, and if there is a need to be reused, the equipment should be disinfected and cleaned properly. Large equipment should be avoided as much as possible .The use of properly disinfected if being used for confirmed cases/ patients. Strictly follow the precautionary measures while performing the mobilizations.	
Category C-3	**Patients are active, conscious and functionally independent.**	Physiotherapy treatment includes breathing exercises, ROM exercises, and progressive ambulation. Develop an individual exercise plan according to the endurance of patient. Limit exposure by counseling the patient and encourage them to continue the plan independently. Regular meeting with the multidisciplinary team about the patient's performance.[32]	

Conclusion

Early ICU rehabilitation including positioning and exercises can reduce the complications associated with acute respiratory distress syndrome.

Limitation

1)This review was written about the role of exercise and positioning only, other physical therapy interventions were not included.

2)This review has discussed conservative management not the medication, which is first line symptomatic administration.

Recommendations

This review suggest that the further trials based on patient conditions and severity of respiratory complications ,should be conducted. Such trails will not only beneficial for the patients ,but also will help the practitioners ,physical therapists and respiratory therapists to work in collaboration with health care providers in such critical situations. This is responsibility of healthcare providers, research scholars, epidemiologists and health care department to play their role to fight against any outbreaks.

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REVIEW ARTICLE

Periodontal Plastic Surgery as a Support to Regenerative Therapies

Muhammad Faisal Rana

ABSTRACT

Aim: To review the present literature, on the basic principles of periodontal plastic surgery and its integral role in improving the outcome of periodontal regeneration.

Methodology: Using the MESH term an electronic search was done using the PubMed data base. The obtained articles were screened on the bases of selection criteria. Systematic reviews and case series were included. The review is divided in to three main components: a) Clinical criteria of selection b) Biological principals to promote regeneration c) Selection of the regenerative materials.

Result: Clinical criteria of selection and post-operative care is a fundamental element to active optimal results. Not only understanding the size, type , nature and the basic knowledge of anatomy around the defect guides the operator to the selections of flap design and regeneration material but also plays an important role in predicting the outcomes .Surgical site preparation , space maintains, and blood clot stability leads to primary intension healing.

Conclusion: Space maintenance and blood clot stability are fundamental biological principles to success. Periodontal regeneration provides long term high success rates for these clinical procedures, provided that good oral hygiene and infection control is maintained.

Key Words: *Bone Defect, Bone Regeneration, Periodontal Plastic Surgery.*

Introduction

Gingival Recession, an oral condition is defined as the "displacement of the marginal tissue apical to the Cementoenamel Junction"[1]. This occurs due to a constant mechanical force or any periodontal disease leading to any inflammation. This causes the loss of alveolar bone and periodontal connective tissue leading to root exposure. Severe cases require surgical intervention for the root coverage treatment. Surgical procedures aim to increase the soft tissue coverage and to regenerate the bone to effectively deal with recession.[2] The main treatments for gingival recession which are effectively in use are periodontal plastic surgery and periodontal regeneration surgeries. The term periodontal surgery was first defined by miller in 1993 as "surgical procedures performed to prevent or correct anatomic, developmental, traumatic or disease induced defects of the gingival, alveolar mucosa or bone" [3]. To obtain the desired results from a surgical procedure fundamental protocols have to be followed. It is very important to choose the patient carefully evaluating closely that the oral hygiene maintenance will be strictly followed during the treatment phase.

Methodology

An electronic search of the articles published with in the last 10 years were screened on the bases of set inclusion and exclusion criteria. The search was done with the help of Booleans (AND OR NOT) used with the 'keywords. PubMed (MEDLINE) was the database used to generate the relevant articles for this study, in-addition to manual search of the papers cited in relevant studies found in the electronic database. Relevant publication's bibliography was screened for articles to be included relevant to the research question. All the obtained Articles were screened on the bases of inclusion and exclusion criteria. Studies reporting radio-graphical and clinical outcome (gain in bone height and width), Studies from Jan 2010 to Jan 2020 (10 years) and finally studies published in English language only were considered. All the reviews involving medically compromised patients, studies not clearly reporting numerical outcomes, studies reporting cast measurement to assess the dimensional changes of the post extraction socket

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and studies without graft intervention were excluded from the selection criteria. A total of 52 articles were obtained in initial search. After passing through three staged screening protocol only 5 articles were selected (Fig 1).

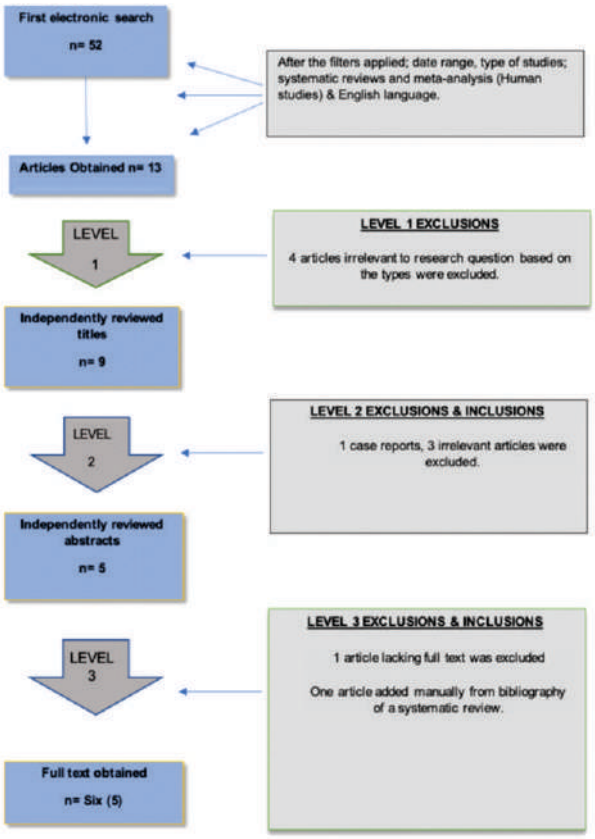

Fig 1: Screening protocol

MESH:

("bone regeneration"[MeSH Terms] OR ("bone"[All Fields] AND "regeneration"[All Fields] OR "bone regeneration"[All Fields] AND (periodontal[All Fields] AND ("surgery, plastic"[MeSH Terms] OR ("surgery"[All Fields] AND "plastic"[All Fields] OR "plastic surgery"[All Fields] OR ("plastic"[All Fields] AND "surgery"[All Fields] AND (("bone and bones"[MeSH Terms] OR ("bone"[All Fields] AND "bones"[All Fields] OR "bone and bones"[All Fields] OR "bone"[All Fields] AND defect[All Fields]

Clinical criteria of selection

The clinical criteria are divided in to, site specific and patient specific.

Patient specific

Poor oral hygiene is one of the top factor which should be considered before any surgical intervention, it can severely affect the optimal outcomes of the periodontal surgery.[5] The periodontal status should be routinely checked before the surgery by assessing the plaque accumulation[6] and the presence of BOP.[7] Good oral hygiene provide three major benefits , (a) healthy tissue improve handling and closure of the mucosal flap[8] (b) prevent wound infection[9] (c) good wound healing.[10] Secondly behavioral aspect such as smoking also have negative influence on the treatment result [11] .The disease status of the patient should be evaluated before, because of their interaction with the wound healing. For the achievement of optimum results, the Full Mouth Plaque Score (FMPS) and Full Mouth Bleeding Score (FMBS) should be less than 15%.

Site Specific

In regard to the site-specific factors, the most important is to understand the aim of the surgery, when aiming to gain more keratinized tissue and remove recession, perioplastic surgery is preferred. The purpose of periodontal regenerative surgery is the regeneration of the pocket defect.[13,14] A 4 year longitudinal study was done by Pini prato whose evidence supports the above mentioned statement [15] (Fig.2) The extent of recession is an important prerequisite which determines the type of surgery to be performed, Mucogingival surgery was seen to provide good results of root coverage for recessions shallower than 4.98 mm while for the recessions deeper than 4.98 mm GTR was found to be more effective14,15 (Fig.3) in the periodontal regeneration surgery the amount of soft tissue gain after surgery is positively predisposed by the deeper defect but negatively affected by the width.16 The number of remaining walls also affect the outcome of the surgery. 1 wall defect responds less favorably to regeneration while 3 wall defect have a high tendency to regenerate11 The neo-connective tissue attachment depends on the adhesive forces between the clot and the root.17 This adhesion can be effected by the mechanical force such as muscular and frenum insertions, so these factors have to be considered. 18

Most important of all factors is the interproximal tissue support (papilla). As the papilla have large number of blood vessels and one of the main controller of the blood supply, thus the vascularity and stability of the flap and the retention will be

severely compromised if the papillary height is reduced.19 In periodontal plastic surgery the root coverage outcome is totally dependent upon the height and level of the tooth supported inter proximal structure. Similarly, the regeneration results are limited in class 3 and class 4 recession defects due to limited periodontal cells.

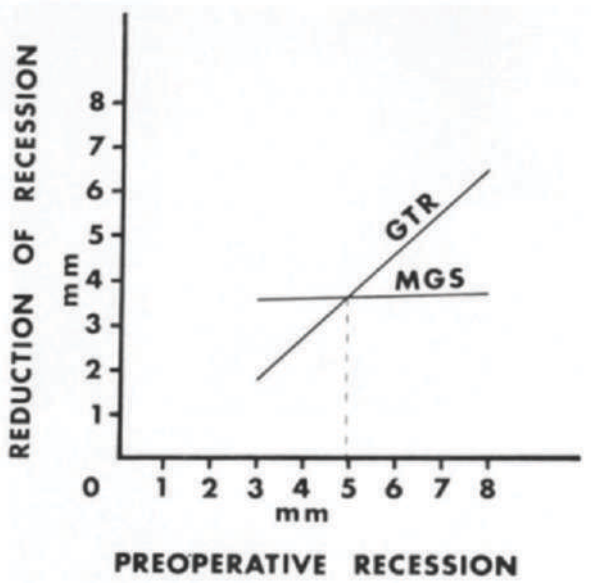

Fig 2: Mean Values of All Periodontal Parameters in Each Group at Each Time Point: Baseline, 18 Months And 4 Years

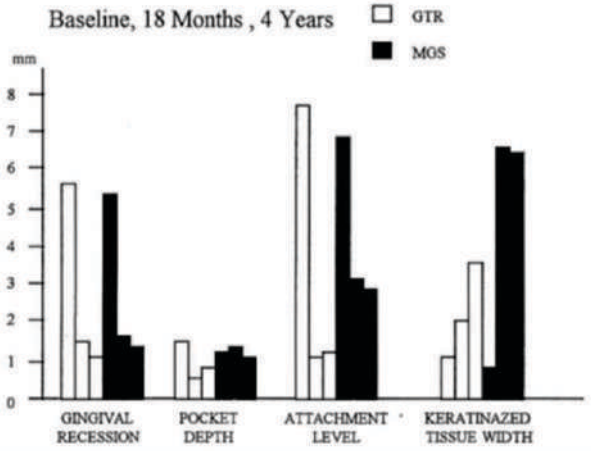

Fig 3: If the Preoperative Recession Is Less Than Value, The Mucogingival Surgery Is Expected to Achieve A Better Result. On the Contrary, If the Recession Is Deeper Than 4.98 Mm, The Membrane Procedure Is Expected to Yield A Better Result Biological Principals to Promote Regeneration

Considering the biological principals, it is really important to understand the mechanism of wound healing. It is a complex process which involves cell migration or proliferation, and extra cellular matrix formation or remodeling. 21 Periodontal wound healing is basically to provide wound stability, provision of space and healing by primary intention22 (Fig . 4) .

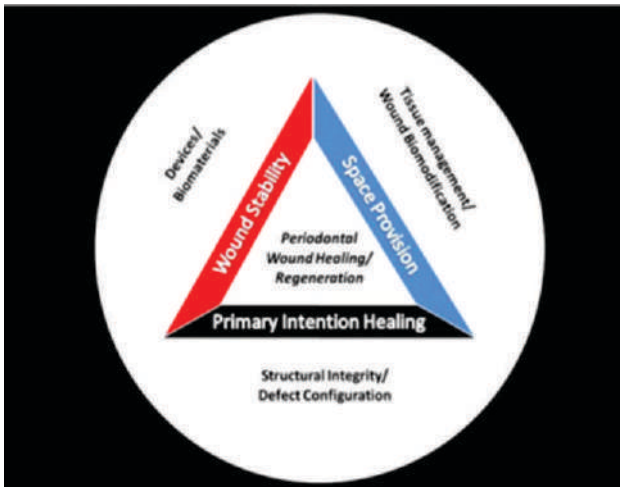

Fig 4: Bio Clinical Principles for Periodontal Wound Healing / Regeneration Surgical Site Preparation

The preservation of soft and hard tissue are important for regenerative periodontal therapy[23] following this minimal invasive surgery , preservation techniques and microsurgical techniques are introduced thus reducing surgical trauma for good periodontal wound healing and regeneration[24,26]. Retzepi compared the minimal invasive procedures to the traditional surgical techniques. A significant return of the blood flow (day 4) was observed, as compared to when a standard procedure was done (day 7)[27]. Burkhardt & Lang assessed the impact of microsurgery on the vascularity of connective tissue. The vascularity restored faster as compared to other procedures.[28] A key component to promote regeneration and healing is cleansing, detoxification or use of biomodifications agents. Many air polishing, manual, ultrasonic instruments, and lasers have been used to remove the biofilm. The root surface conditioning agents such as EDTA (Ethylene Di amine Tetra acetic Acid, and Tetracycline HCL are generally used. The idea is to remove the smear layer and promote adsorption / adhesion of fibrin clot.[29,30] Thus, it can be stated that the choice of surgical technique, removal of granulation tissue minimizing the trauma and securing the vascularity is the key to success of the treatment.

Cell Occlusion and Space Provision

Space provision plays an important role in

periodontal and bone regeneration.[31] A significant correlation between space provision with a membrane and the regeneration outcomes has been reported in various studies.[32] A human study was done in which the infrabony defects were treated with GTR & a resorbable membrane was placed. The histological evaluation of the study significantly showed the synthesis of a long and healthy junctional epithelium (JE). Below the JE, healthy cementum and periodontal ligament was also newly formed.[33] Recently a high-density PTFE (Poly tetra fluoro ethylene membrane) has been tested, because of its smaller pore size in comparison to e-PTFE (microporous membranes). The d-PTFE (high density membrane) has shown better combating results, as it significantly withstands exposure to bacteria from the oral cavity.[34,35] Clinical attachment gain and the bone fill attracts the clinicians due to the misapprehension of clinical improvement provided by the fill of the periodontal defects, but cautious interpretation should be done because limited amount of evidence is available for the regeneration effect of the biomaterials.[36]

Flap Design

The utmost important outcome of any regenerative procedure is, wound stability and primary intention closure of surgical flaps.[37] A closely, adapted flap with the root is the prerequisite to a healthy periodontal healing process.[38] Preserving flap thickness and including healthy periosteum in the flap is of utmost importance, in order to achieve improved recession reduction results at the end of year 1. This results in improved patient based outcomes with a greater reduction in the hypersensitivity at the end of year 1 and decreased **VAS** discomfort reported at seven days.[39] Selection of flap is based upon surgical access and intrabony defect of an individual (Fig 4). Three different approaches to surgical intervention have been described in literature. The first is the simplified papilla preservation flap **(SPPF).**[40] The second technique described by Cortenelli et al is the modified papilla preservation technique.[41] Cortenelli et al also describe a third technique, which is the crestal incision technique.[42] A cohort study reported the assessment of 26 patients, all of whom had intrabony defects and were treated with papilla preservation techniques. A primary closure was achieved at the barrier in 100% of the defects. When followed over time, the closure was successfully maintained in 92.3% of the cases.[43]

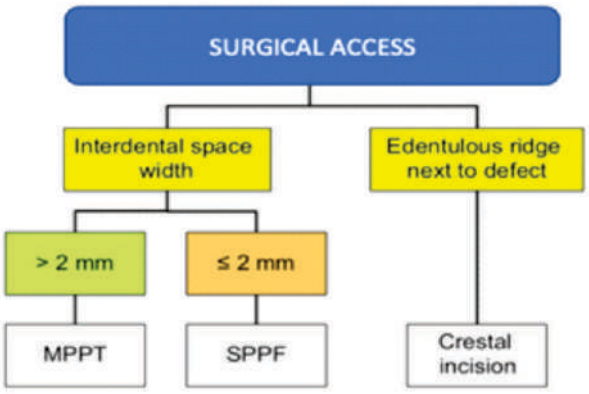

Fig 5: If the Interdental Space width is Greater than 2mm Modified Papilla Preservation Technique is Recommended MPPT, If the Space is Less than 2 mm than Simplified Papilla Preservation Flap SPPT is Recommended

Primary Intention Healing

Periodontal healing and regeneration primarily depend on the wound closure by primary intention healing. It is an exigent situation because of the location, rigidity and the mineralized, vascular nature of root.[32] Suturing technique is of prime importance as primary intension healing mainly depends on suture , care should be taken not to constrict the vascular support and the flap's integrity.[39] Having an adequate amount of tensile strength is a basic requirement of the suture during the healing process as it reduces reduction. It is observed that the increased tensile strengths usually occurs after 10 days of wound healing.[44] Bioresorbable natural and synthetic-fiber sutures (e.g., polyglycolic acid) are widely recommended as they are easy to use. Nevertheless, the clinicians should be aware of the tensile strength of the suture to be used as the tensile strength of these suture materials decreases considerably faster than their resorption rate.[44] The suturing approach should be selected in accordance to the type of regenerative strategy in function[36] (Fig .6) .When aiming for a regeneration, sutures should not be tightened excessively when done at the apical region because of the space provision and it would thicken the clot, When approaching the coronal part, the sutures should be tight & the flap should be coronal in order to gain a good adaptation.[45]

Blood Clot Stability

Blood clot stability plays an important role in the

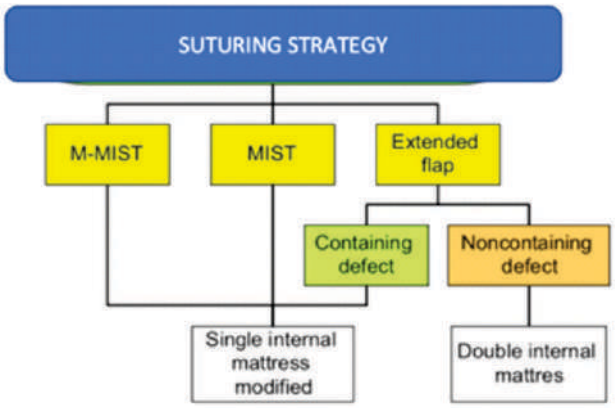

Fig 6: Suturing Strategy MIST, Minimally Invasive Surgical Technique; M-MIST, Modified Minimally Invasive Surgical Technique

process of regeneration. Therefore, the anatomic defects need to be supported by the further use of biomaterials. To achieve this clinical outcome. the anatomic deficiencies of the defects have to be supplemented by the additional use of biomaterials. Another away to achieve increased stability is to adopt particular surgical strategies, which require minimal elevation of tissues.[46] Tooth mobility specifically effects the stability of the blood-clot. Teeth having mobility of Miller's grade II or III should be splinted in order to prevent any blood clot disruption at the earlier stages of healing.[47] The regeneration area should be protected by surgical interventions that are specifically designed for its protection.

Selecting Materials for Regeneration

Selecting the appropriate materials for regeneration should be based on a few factors. Firstly, the anatomical defect should be kept into consideration. Secondly, flap designs which is appropriate for exposure of the defect should also be considered. If it is decided to opt for the modified minimally invasive technique, then the material of choice would be either no regenerative materials or amelogenins.[48] For minimally invasive surgical techniques, amelogenins may be used either in isolation for containing defects. However, for non-containing defects, fillers should also be used along with amelogenings. (Fig 7).[46] In case of elevating a larger flap, the stability of area should be secured effectively by using either barriers or fillers; combinations of fillers with barriers, or even combinations of fillers along with amelogenins or growth factors. In a well-supported two wall or three-wall morphological defect, amelogenins should be used in isolation.

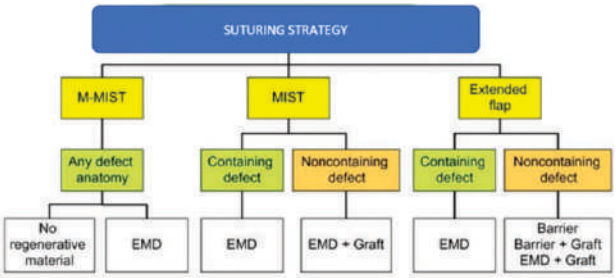

Fig 7: Regenerative Strategy. EMD, Enamel Matrix Derivative; MIST, Minimally Invasive Surgical Technique; M-MIST, Modified Minimally Invasive Surgical Technique

Conclusion

Periodontal plastic surgery / regenerative surgery may be described as a process which is completed in multiple phases. Initially, factors related to the patient are catered for, such as low levels of plaque, high compliance and absence of smoking, stress and systemic diseases. Few of the local factors such as infection, endo issues should be cleared before doing any regenerative procedures. All of the above-mentioned factors lead to the success of regenerative surgery. Selection of flap and its design plays a vital part in the outcome of surgery. Selection of the procedure mainly depends on the width of interdental papilla and shape of the defect. The preservation of the papilla and its location helps in providing vascularity and a limit for the regeneration and recession coverage procedures. Primary healing is a key to success and it totally depends on the suture's strength and their adaptation. This prevents the exposure of membrane and contamination. Space maintenance and blood clot stability are the fundamental biological principle to success. Selection of the right principal leads to clinical success. Even though all of the proposed regenerative techniques showed significant variations in clinical outcomes in terms of increase in clinical attachment level, not a single technique showed the capability to solve all the varying and exclusive presentations of patient defects. So, it is vital to select the right material and technique for each individual. New techniques such as tissue wall techniques proved batter in CAL (Clinical Attachment Level) gain. Good oral hygiene and infection control are mandatory to achieve long term, stable clinical

outcomes in periodontal regeneration procedures.

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JOURNAL OF ISLAMIC INTERNATIONAL MEDICAL COLLEGE (JIIMC)

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