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EDITORIAL

BREATH BIOPSY: A PROMISING DIAGNOSTIC TOOL

Tehseen Iqbal
RYK Medical College, Rahim Yar Khan

Breath biopsy or breath analysis is a relatively recent field of research with much promise in scientific and clinical studies. Breath contains endogenously produced volatile organic compounds (VOCs) resulting from metabolites. The principle behind breath biopsy lies in the fact that many diseases produce specific metabolic changes in the body that result in the release of unique VOCs. These compounds can be detected and quantified by using advanced analytical techniques, such as gas chromatography-mass spectrometry (GC-MS) and selected ion flow tube mass spectrometry (SIFT-MS) etc. More than 2000 VOCs have been detected in the exhaled breath. These VOCs may be related to hydrocarbons, alcohols, aldehydes, ketones, esters, ethers, carboxylic acids, heterocyclic hydrocarbons, aromatic compounds, nitriles, sulfides and terpenoids. VOCs in exhaled breath may be endogenous or exogenous. Endogenous VOCs can diagnose about twenty-five diseases including lung cancer, breast cancer, diabetes, COPD, and even COVID-19 by analysing the breath of patients.

Keywords: Breath biopsy, GC-MS, SIFT-MS, Volatile Organic Compounds
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Bad smelling breath or halitosis can result from several causes. While poor oral hygiene is the most common cause of halitosis, it is not the only one. Smoking, alcoholic drinks, diabetes, infections in nose, throat or lungs, sinusitis, tonsil stones, liver or kidney disease or head and neck cancers, may cause bad breath.[1] Exhaled breath is tested for many conditions. The police used to smell the mouth of a suspected drunkard driver and then the breath alcohol test is used to measure the amount of alcohol in the blood by testing exhaled air by an electronic device.[2] Another well known breath test is called '*H. pylori* breath test or urea breath test' that is still used to detect H. pylori infection which causes peptic ulcer.[3] Acetone breath of liver disease is well known to physicians and residents and interns since long.

Breath biopsy or breath analysis is a relatively recent field of research with much promise in scientific and clinical studies. Breath contains endogenously produced volatile organic compounds (VOCs) resulting from metabolites of ingested precursors, gut and air-passage bacteria or environmental contacts, etc. Numerous recent studies have suggested changes in breath composition during the course of many diseases, and breath analysis may lead to the diagnosis of such diseases. Identifying specific VOCs and applying them as disease-specific biomarkers to obtain accurate, reproducible, and fast disease diagnosis could serve as an alternative to traditional invasive diagnostic methods.[4] Breath biopsy or breath analysis is a non-invasive method that has gained significant attention in recent years due to its potential for early disease detection, including various types of cancers and respiratory conditions.

The principle behind breath biopsy lies in the fact that many diseases produce specific metabolic changes in the body that result in the release of unique VOCs. These compounds can be detected and quantified by using advanced analytical techniques, such as gas chromatography-mass spectrometry (GC-MS) and selected ion flow tube mass spectrometry (SIFT-MS). GC-MS combines the separation capabilities of gas chromatography with the identification power of mass spectrometry. In this method, a sample is vaporized and carried through a column where different compounds are separated based on their volatility and interaction with the column material. As compounds exit the column, they are detected and analysed by mass spectrometry, allowing for the determination of their molecular weights and structures. This technique is highly sensitive and can detect trace levels of VOCs, making it valuable in clinical diagnostics and environmental monitoring.[5]

On the other hand, SIFT-MS is a more recent technique that enables real-time analysis of VOCs. SIFT-MS utilises an extremely soft ionisation process which greatly simplifies the resulting spectra and thereby facilitates the analysis of complex mixtures of gases, such as human breath.[6] Many commercially available instruments analyse gas-phase samples. Samples need no pre-treatment. Results are in real time to pptv (parts per trillion by volume) level. Diverse chemical species are quantified simultaneously. It utilizes a flow tube where ions are generated and selected based on their mass-to-charge ratio. These ions interact with the VOCs present in the sample, leading to the production of characteristic product ions that can be quantified. SIFT-MS is particularly advantageous for its rapid analysis and the ability to provide quantitative results without extensive sample preparation. Both techniques, GC-MS and SIFT-MS, are crucial in advancing our understanding of VOCs and their implications in health and environmental science.

More than 2000 VOCs have been detected in the exhaled breath. These VOCs are related to

hydrocarbons, alcohols, aldehydes, ketones, esters, ethers, carboxylic acids, heterocyclic hydrocarbons, aromatic compounds, nitriles, sulfides and terpenoids. VOCs in exhaled breath may be endogenous or exogenous. Endogenously created VOCs comprise high vapor pressure byproducts of normal or pathophysiological metabolic pathways, as well as of microbiome metabolism. Exogenously originated VOCs are correlated with the environment and the habits of the person. Exogenous VOCs are related with cleaning fluids, personal care products, plastic-related VOCs, blazes, or air pollution due to industrial/transport gas emissions and they enter human organism through extended inhalation and are excreted via exhaled breath. Concentration differences for some exhaled VOCs could potentially be associated with an abnormal condition of the body, as the metabolic processes producing the VOCs are altered in a distinctive way by different diseases. In fact, VOC pattern or 'breathprint', consists of the signature of a specific disease, correlating with the underlying pathophysiology; this pattern should therefore be recognized so as to achieve disease diagnosis.[7]

Various volatile organic compounds (VOCs) have been identified as potential biomarkers. In breast cancer, compounds such as 2-butanone, isoprene, and toluene have been linked to malignant processes in breast tissue. Key VOCs associated with lung cancer include acetone, isoprene, and benzene. In tuberculosis, notable VOCs include 2-pentanone, isoprene, and various aldehydes. In diabetes, VOCs detected include acetone and isoprene. Acetaldehyde, isoprene, and various aldehydes are major VOCs detected in Chronic Obstructive Pulmonary Disease. A FeNO test measures the amount of nitric oxide, a byproduct of inflammation, in the breath. About twenty-five diseases including lung cancer, breast cancer, diabetes, COPD, kidney and liver diseases and even COVID-19 can be diagnosed by analysing the breath of patients.[8]

Breath biopsy is a rapid, non-invasive diagnostic tool that detects volatile organic compounds (VOCs) present in exhaled breath, which are biomarkers for various diseases. With the ongoing developments in the field and real-data analysis, breath biopsy or breath analysis holds promise to become a useful screening tool that will not only aid but also improve existing diagnoses for several diseases and disorders. Large studies in real-world screening settings, with a focus on standardizing the breath collection protocol and validation in different and independent population samples, should be carried out to accelerate the use of breath analysis for disease diagnosis.

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ORIGINAL ARTICLE

EFFECT OF BODY WEIGHT ON VITAMIN D LEVELS IN WOMEN WITH PREMENSTRUAL SYNDROME

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Background: Vitamin D deficiency and higher body weight may contribute to severity of Premenstrual Syndrome (PMS) through hormonal and inflammatory pathways. Objectives of this study were to investigate the relationship between vitamin D levels and body weight in women experiencing PMS. **Methods:** This cross-sectional study was conducted in the Gynaecology Department in collaboration with the Physiology Department, at Isra University Hospital Isra University, Hyderabad, from May to Oct 2023. A total of 200 women aged 15–45 years with a history of PMS were included in the study after consent. Demographic data, age, height were recorded and BMI was calculated, and serum vitamin D3 levels were assessed. The PMS scale was used to determine symptom severity (mild, moderate, and severe). Data were analysed using SPSS-23. **Results:** The mean age of participants was 28.97±6.49 years. Among them, 59% had vitamin D deficiency, while only 4.5% had sufficient levels of vitamin D. A significant inverse association was observed between vitamin D levels and BMI (p<0.05). Obese participants were more likely to have vitamin D deficiency, and 35% of those with severe PMS symptoms had deficient vitamin D levels. Pearson correlation analysis revealed a slight negative association between vitamin D levels and BMI (r= -0.427, p<0.001) and between PMS severity and vitamin D levels (r= -0.41, p<0.001). **Conclusion:** Vitamin D levels are inversely correlated to BMI and PMS severity suggesting that vitamin D deficiency may contribute to both obesity and worsening PMS symptoms.

Keywords: BMI, Body mass index, Obesity, PMS, Premenstrual syndrome, Vitamin D

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INTRODUCTION

Women frequently experience physical, emotional, and behavioural changes in the days leading up to menstruation. Premenstrual syndrome (PMS) is the term used when these symptoms affect a woman's regular life every month, characterized by physical (bloating, fatigue, breast tenderness), emotional (mood swings, irritability), and behavioural (social withdrawal, difficulty concentrating) changes.[1] PMS affects 8–20% of women of reproductive age worldwide, with moderate to severe symptoms impacting social relationships, work productivity, and overall quality of life.[2,3]

While the exact cause of PMS remains unclear, hormonal fluctuations, lifestyle factors, and nutritional deficiencies are believed to play significant roles.[4] Among these, vitamin D has garnered attention for its multifaceted role in reproductive health and symptom modulation in PMS. Traditionally recognized for its function in calcium metabolism and bone health, vitamin D also exerts significant effects on the immune system, neurotransmitter regulation, and hormonal balance, all of which are implicated in PMS pathophysiology.[5,6]

Vitamin D receptors (VDRs) are widely distributed in various tissues including the ovaries, endometrium, and brain, suggesting its involvement in both reproductive and neurological functions.[7] It helps regulate the synthesis of serotonin, a key neurotransmitter that influences mood, emotional well-being, and behaviour —factors commonly affected during PMS.[8] Low levels of serotonin are associated with depressive symptoms, irritability, and anxiety, which are hallmark features of PMS.[9] Vitamin D has anti-inflammatory properties that may alleviate PMS-related symptoms such as breast tenderness, bloating, and joint pain by reducing systemic inflammation and modulating the immune response.[10]

In addition to its role in PMS, vitamin D status is closely linked with body weight. Obese individuals often exhibit lower serum vitamin D levels due to several mechanisms: sequestration in adipose tissue –as a fat-soluble vitamin, vitamin D is stored in body fat, reducing its bioavailability in circulation. Decreased synthesis and metabolism –obesity may impair the conversion of vitamin D to its active form due to altered liver and kidney functions. Reduced outdoor activity –a sedentary lifestyle associated with obesity limits sunlight exposure, which is crucial for endogenous vitamin D synthesis.[11] Conversely, vitamin D deficiency may contribute to weight gain through its effects on insulin resistance, impaired lipid metabolism, and increased inflammation.[12] It has been suggested that vitamin D may influence appetite regulation and energy expenditure, both of which are critical in weight management.[13] Hormonal imbalances, such as those seen

in polycystic ovarian syndrome (PCOS), can exacerbate menstrual irregularities and PMS symptoms due to their association with obesity and vitamin D deficiency.[14,15]

Given the overlapping roles of vitamin D and body weight in PMS, this study aims to investigate the relationship between serum vitamin D levels and body mass index (BMI) among women with PMS. Understanding this relationship could provide insights into potential dietary or lifestyle interventions, such as vitamin D supplementation and weight management strategies, to alleviate PMS symptoms and improve overall well-being.

MATERIAL AND METHODS

This cross-sectional study was conducted in the Gynaecology Department in collaboration with the Physiology Department, Isra University, Hyderabad, from May to Oct 2023. A total of 200 women aged 15–45 years with a history of PMS were included in the study. The PMS was defined as the presence of recurrent physical, emotional, and behavioural symptoms occurring in the luteal phase of the menstrual cycle significantly affecting daily activities and resolved after menstruation, in the last 6 months.

A structured proforma was used to collect data, which included demographic details (age, marital status, BMI), medical history (PMS severity, vitamin D levels, co-morbid conditions), and lifestyle factors (physical activity, dietary habits, and sun exposure). The severity of PMS was assessed using the Premenstrual Syndrome Scale[16], categorizing participants into mild, moderate, and severe PMS groups. Vitamin D3 levels were measured in collaboration with a diagnostic and research facility, Jamshoro/Hyderabad. Vitamin D level below 20 ng/mL was considered deficient, 20–30 ng/mL was considered insufficient, and ≥30 ng/mL was considered sufficient.

Data were analysed using SPSS-23. Categorical variables were expressed as frequencies and percentages, while continuous variables were presented as Mean±SD. Chi square test and Pearson correlation analysis were applied to see the association between vitamin D levels and BMI, and $p<0.05$ was considered statistically significant.

RESULTS

The mean age of the participants was 28.97±6.49 years. Among the 200 participants, 127 (63.5%) were married, and 73 (36.5%) were unmarried. Regarding BMI, 75 (37.5%) had a normal BMI, while 71 (35.5%) were obese. Severe PMS symptoms were observed in 77 (38.5%) participants, while 64 (32%) had mild PMS. The majority (118, 59%) of participants, had vitamin D deficiency, whereas only 9 (4.5%) had sufficient vitamin D levels. (Table-1).

Vitamin D levels showed a significant variation across BMI categories ($p=0.001$) with a higher prevalence of deficiency in obese individuals. Among normal-weight participants, 41 (20.5%) had insufficient vitamin D levels, whereas 56 (28%) of obese participants had vitamin D deficiency. All obese participants had either vitamin D deficiency or insufficiency, with none having sufficient levels, highlighting a strong inverse relationship between obesity and serum vitamin D levels. (Table-2).

Among participants with mild PMS, 34 (17%) had insufficient vitamin D, while 70 (35%) of those with severe PMS had vitamin D deficiency. A moderate negative correlation ($r= -0.41$, $p<0.001$) was found between PMS severity and vitamin D levels. (Table-3).

Table-4 shows the correlation between vitamin D levels and BMI, indicating a statistically significant moderate negative correlation ($r= -0.427$, $p<0.001$). Similarly, a moderate negative correlation ($r= -0.41$, $p<0.001$) was found between PMS severity and vitamin D levels suggesting that lower vitamin D levels are associated with both higher BMI and increased PMS severity.

Table-1: Clinical data of the patients (n=200)

Variables	Frequency	Percentage
Marital status		
Married	127	63.5
Un-married	73	36.5
BMI		
Under-weight	17	8.5
Normal	75	37.5
Overweight	37	18.5
Obese	71	35.5
PMS score		
Mild	64	32.0
Moderate	59	29.5
Severe	77	38.5
Vitamin D level		
Deficiency	118	59.0
Insufficiency	73	36.5
Sufficiency	9	4.5

Table-2: Vitamin D levels according to BMI (n=200)

BMI	Vitamin D status			Total	*p*
	D	InS	S		
Underweight	11 (5.5)	4 (2.0)	2 (1.0)	17 (8.5)	0.001
Normal weight	23 (11.5)	41 (20.5)	11 (5.5)	75 (37.5)	
Overweight	17 (8.50	19 (9.5)	1 (0.5)	37 (18.5)	
Obesity	56 (28.0)	15 (7.5)	0 (0.0)	71 (35.5)	

Key: D=deficiency, S=Sufficiency, InS=Insufficiency

Table-3: Vitamin D levels according PMS scores

PMS score	Vitamin D status			Total	*p*
	D	InS	S		
Mild	25 (12.5)	34 (17.0)	5 (2.5)	64 (32.0)	0.001
Moderate	24 (12.0)	32 (16.0)	3 (1.5)	59 (29.5)	
Severe	70 (35.0)	4 (2.0)	3 (1.5)	77 (38.50	

Table-4: Correlation between vitamin D and BMI

	Vitamin D	BMI	PMS Severity
Vitamin D	1	-0.427	-0.41
BMI	-0.427	1	-
PMS Severity	-0.41	-	1

Correlation is significant at the 0.01 level (2-tailed)

DISCUSSION

Premenstrual syndrome is a common condition affecting women of reproductive age, often characterized by physical, emotional, and behavioural symptoms. Lack of vitamin D increases the likelihood of weight gain due to decreased intestinal absorption and impaired hydroxylation in adipose tissue.[17] A well-established correlation exists between declining vitamin D levels and increased body weight. Women suffering from PMS are particularly prone to weight gain, with obesity, sedentary lifestyles, and poor diets contributing to low calcifidiol levels. In our study, a moderate negative correlation was observed between PMS severity and vitamin D levels, suggesting that lower vitamin D levels may be associated with more severe PMS symptoms.

PMS is a common disorder among women of reproductive age, yet its precise aetiology remains unclear. Vitamin D plays a crucial role in immune regulation and may reduce inflammation during the menstrual cycle.[18] Increased adipose tissue accumulation in PMS may result from neurological, endocrine, and behavioural pathways. Several studies have demonstrated that women with PMS or recurrent menstrual symptoms are more likely to have higher body weight or be obese compared to those without PMS.[19] However, it remains uncertain whether rapid weight gain or weight cycling (fluctuations in body weight) increases the risk of PMS independent of total body fat percentage.

Husna *et al*[20] reported that obesity may influence the severity of specific menstrual symptoms. Arab *et al*[21] concluded that individuals with PMS tend to have lower levels of vitamin D, calcium, and magnesium. Vitamin D supplementation was found to alleviate PMS symptoms, emphasizing its potential role in PMS management.[21] Given the high prevalence of PMS, emphasis should be placed on improving the health and nutritional status of young women.

In the current study, vitamin D levels were significantly and inversely correlated with BMI. Overweight and obese individuals exhibited a decline in serum 25(OH)D levels as BMI increased. Previous studies have reported high prevalence of vitamin D insufficiency among overweight and obese individuals, particularly in females.[22] Adolescents with vitamin D deficiency may be at an increased risk of obesity, highlighting the importance of vitamin D supplementation in this population.

Lagowska *et al*[23] examined vitamin D levels in young women with varying body weights and their association with menstrual cycles. Among those with low vitamin D levels, 40% reported prolonged menstrual cycles, 27% had oligomenorrhea, and 13% experienced amenorrhea. In contrast, only 12% of individuals in the normal vitamin D group reported menstrual irregularities, with 6% experiencing amenorrhea and 6% reporting oligomenorrhea. Women with serum 25(OH)D levels below the recommended 30 ng/mL had a nearly five-fold increased risk of menstrual irregularities compared to those with sufficient levels.[23] Low vitamin D levels have been linked to a higher prevalence of menstrual irregularities, which may exacerbate PMS symptoms. Addressing vitamin D deficiency through supplementation could be a crucial intervention for managing PMS and promoting overall reproductive health.

Despite the insights provided by this study, several limitations should be acknowledged. The cross-sectional design limits our ability to establish a causal relationship between vitamin D levels, PMS severity, and BMI. Longitudinal studies are needed to determine whether low vitamin D levels contribute to PMS and weight gain over time. The study relied on self-reported PMS symptoms, which may introduce recall bias. Future research should incorporate standardized diagnostic criteria or clinical assessments for a more objective evaluation of PMS severity. While we identified a significant association between vitamin D and BMI, other confounding factors such as dietary intake, physical activity levels, and genetic predisposition were not extensively controlled. The sample size limits the generalisability of our findings.

CONCLUSION

There is a significant inverse relationship between vitamin D levels and BMI in women with PMS, suggesting that lower vitamin D levels may be linked to increased PMS severity and weight gain. Targeted interventions such as supplementation and dietary modifications may help improve PMS symptoms and overall well-being of the women.

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ORIGINAL ARTICLE

EFFICACY OF METRONIDAZOLE AND MINOCYCLINE GELS IN THE MANAGEMENT OF PERIODONTITIS IN DIABETIC PATIENTS: A COMPARATIVE STUDY

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Background: Diabetes and periodontitis are interlinked. Objective of this study was to evaluate and compare the effectiveness of metronidazole and minocycline gels alone and in combination, used as an adjunct to scaling and root planing (SRP) for the management of periodontitis in diabetic patients. **Methods:** This randomized single blind clinical study, randomized 380 diabetic chronic periodontitis patients presenting to Periodontology Department, Dental College HITEC-IMS HIT, Taxila, from Sep 2022 to Aug 2023, into 4 groups: SRP alone (Group-I), SRP plus metronidazole gel (Group-II), SRP plus minocycline gel (Group-III), and SRP plus both metronidazole and minocycline gels (Group-IV). The effectiveness of four treatment regimens was assessed by checking the difference in periodontal measurements, including periodontal probing depth (PPD), and bleeding on probing (BOP) at baseline, after 15 days, and 45 days of the treatment, and the percentage was measured. **Results:** Every patient showed notable progress in periodontal probing depth and bleeding on probing after the treatment. Group IV stood out in both parameters. Mean periodontal probing depth was reduced by 17.4, 32.5, 39.54, and 79.65% in Groups-I, II, III, and IV, respectively. In Groups-II, III, and IV, BOP decreased by 80%, 89.47%, and 100% respectively. **Conclusion:** Metronidazole gel has comparable efficacy to minocycline gel, and their combination has demonstrated synergistic effects in treating diabetic periodontitis patients and should be used as an adjunct to scaling and root planing for better outcomes.

Keywords: Chronic periodontitis, Diabetes mellitus, Metronidazole gel, Minocycline gel, Scaling and root planing

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INTRODUCTION

Periodontitis, a chronic inflammatory ailment of the periodontium, gradually degrades the structures supporting the teeth, culminating in tooth loss.[1] Originating as gingivitis, it evolves into chronic periodontitis.[2] It represents a global public health challenge due to its high prevalence, i.e., 20–50% of the worldwide population, laden with associated comorbidities, and economic ramifications. Notably, Pakistan bears the staggering 56.62% prevalence.[3] Beyond its oral implications, periodontitis intertwines with 60 other medical problems, such as hypertension, obesity, atherosclerosis, diabetes, and stroke.[4] Nearly 39% of individuals battling periodontitis also grappled with the challenges of diabetes mellitus.[3]

Diabetes mellitus constitutes a spectrum of metabolic disorders characterized primarily by persistent high blood sugar levels.[5] It is a chronic, non-communicable, lifelong sickness affecting approximately 9% of the global population.[6] Pakistan holds the highest diabetes prevalence globally, affecting one in four individuals (26.7%), nearly a quarter of the population.[7] Diabetes is a significant risk factor for periodontitis[8] which is considered its sixth complication resulting in increased susceptibility to periodontitis by about threefold in individuals with diabetes.[6] Not only does diabetes heighten the risk of periodontitis, but periodontitis, in turn, significantly impacts diabetes control, incidence rates, and complications.[9] Diabetes and periodontitis have a two-way relationship[10] and is primarily mediated through Inflammation.[11]

Patient compliance is the key factor for the treatment success of periodontitis. The treatment includes professional care along with patient awareness to maintain optimum oral health and reduce or eliminate the risk factors. Scaling and root planing (SRP) is the most performed procedure for the treatment of periodontitis.[12] In addition to this conventional approach, the use of various medicated agents like metronidazole, chlorhexidine, minocycline, and tetracycline can help impede the progression of the disease.[13]

Metronidazole is a synthetic compound that belongs to the nitroimidazole family. It acts by disrupting bacterial DNA, leading to the death of cells.[14] Metronidazole, in its reactive reduced form, selectively targets anaerobic bacteria, gram-negative rods and spirochetes.[12] Moreover, it aids in decreasing the influx

of inflammatory cells by suppressing cytokine production. This property enables it to protect periodontal tissue and prevent the resorption of alveolar bone. When in contact with gingival crevicular fluid, the metronidazole gel forms inverted hexagonal liquid crystals thereby preventing the gel from rapidly diffusing away from the therapeutic site within the periodontal pocket. Consequently, it effectively sustains the concentration of the drug in the subgingival area for an extended period.[13]

Minocycline, a semi-synthetic derivative of tetracycline, acts by reversibly binding to the 30S ribosomal subunit of bacteria, thus hindering the in cooperation of amino acids to developing peptides, effectively blocking protein synthesis.[2] It exhibits a broad antibacterial spectrum. Minocycline possesses several favourable characteristics, including notable substantivity, gradual-release, and excellent lipophilicity. Other than its antibacterial activity, minocycline exerts a therapeutic impact in treating periodontitis by directly preventing collagenase action. Minocycline is the antibiotic of choice for managing periodontitis, particularly when used for localized-treatment.[15]

The assessment of local administration's effectiveness in chronic periodontitis patients has been established for metronidazole and minocycline individually.

The evaluation of local metronidazole and minocycline administration's efficacy remains unexplored, particularly in the context of chronic periodontitis patients suffering from diabetes mellitus. Furthermore, the potential synergistic effects of these local administrations have not been investigated. The goal of this study was to offer tangible benefits by providing relief to diabetic periodontitis patients, enhancing patient compliance, and providing an alternative to systemic medications, thereby mitigating potential adverse effects associated with their excessive use.

The study aims to ascertain the clinical efficacy of locally administered metronidazole and minocycline in managing periodontitis among diabetic patients.

METHODOLOGY

A randomized single blind clinical study was conducted in the Periodontology Department, Dental College HITEC-IMS Taxila, Pakistan, through collaborative efforts with the Pharmacology Department, Dental College HITEC-IMS Taxila Cantt, and IIMC, Riphah International University, Rawalpindi, Pakistan. The study was approved by the Institutional Review Committee of the Islamic International Medical College (Ref. No. Riphah/IRC/22/2080) and Institutional Review Board (Ref. No. Dental/HITEC/IRB/34). The study is registered with clinicaltrials.gov (Registration No. NCT06027151). Data was collected from Sep 2022 to Aug 2023. The sample size, calculated using Cochran formula with 39% prevalence of diabetic periodontitis, Z-score, and 5% margin of error, was 380. A simple random sampling technique was used using a randomization software to generate codes for allotment of patients to groups.

The study included diagnosed diabetic patients with HbA1c 6–9% on either oral hypoglycaemics or insulin therapy, suffering from mild to moderate chronic periodontitis, visiting the Periodontology OPD of Dental College, HITEC-IMS. Each participant provided written informed consent. Pregnant or lactating women, patients with gestational diabetes, patients with history of alcoholism, smoking, recent use of anti-inflammatory or antibiotic medications within the past two months before the study, known allergy to minocycline, or metronidazole, and patients who had periodontal surgeries in the past were excluded from the study.

Initially, a comprehensive periodontal examination of the entire oral cavity was conducted and participants who met the inclusion criteria were evaluated for periodontal probing depth (PPD), and bleeding on probing (BOP) during the baseline visit (before the SRP and local drug delivery) by the periodontist. The subjects who were enrolled were allocated randomly to four groups, with each group comprising 95 patients. Group I was treated with SRP alone at baseline till a smooth, clean, and hard texture was achieved, as assumed by the examiner with the help of the ultrasonic scaler. Patients in group II were treated with SRP followed by 1 ml of 1% metronidazole benzoate gel (Revomet gel, Kaizen Pharmaceuticals) applied once subgingivally with the help of a disposable plastic tip. The tip was inserted into the base of the pocket, and then gel was gently pushed out of the syringe. The tip was slowly withdrawn from the periodontal pockets. The patients were instructed not to drink or eat for 30 minutes after gel injection. SRP followed by 1 ml of 2% minocycline hydrochloride gel (Periocline gel, Sun Star) was applied once sub gingivally in group III patients with the same method as used for group II patients. Patients in group IV received combination of treatments, starting with SRP followed by subgingival application of 1 ml of metronidazole gel and minocycline gel followed by the same procedure. The outcome was measured by evaluating PPD and BOP at the baseline visit. They were recalled for follow-ups after 15 and 45 days and PPD and BOP were rerecorded at both follow-ups.

Data was analysed using SPSS-27. Mean values of PPD at baseline visit and follow-ups were computed. The percent decrease in the mean PPD of groups I, II, III, and IV was determined. Within each group, paired *t*-tests were employed to evaluate the

efficacy of the treatment. Additionally, One-way ANOVA was used to analyse the differences in mean values among the groups. Improvement as a result of treatment in diabetic chronic periodontitis patients was determined by considering the presence or absence of BOP during the 1st, 2nd, and 3rd visit for all the groups. The difference among groups was analysed by the Chi-square test. The relationship between age and clinical parameters (PPD, BOP) was determined using the bivariate correlation and independent samples t-test. Independent samples *t*-test and Chi-square test were applied to analyse the relationship between gender and clinical parameters, i.e., PPD and BOP. A $p<0.05$ was considered statistically significant. Categorical variables were presented as counts and percentages while continuous variables were expressed as mean and standard deviation.

RESULTS

Out of 380 patients, 221 (58.2%) patients were males, and 159 (41.8%) patients were females with the mean age of 58.09±8.63. Results for BOP during three visits for all the groups are summarized in Table-1.

The effectiveness of treatment regimens within each group was assessed by estimating the mean PPD before and after treatment within the same group. The mean differences and percent decrease in PPD were subsequently evaluated. Paired *t*-test was applied before and after treatment within each group (Table-2).

In group I, the mean PPD was reduced by 17.4%, in group II by 32.5%, in group III by 39.54% and in group IV by 79.65%. One-way ANOVA was used to compare the mean values of PPD among these 4 groups, which yielded highly significant results with a $p<0.001$. Post-Hoc Tukey was applied to compare mean differences between each group. A highly significant difference was found between Group I and IV, Group II and IV, Group III and IV as well as Group I and II (Table-3).

The difference between groups for BOP was evaluated by the Chi-square test (Table-4). Patients in group IV exhibited a complete absence of BOP by the third visit. Similarly, Group II and III displayed significant effectiveness in alleviating BOP, with 80% and 89.47% of patients, respectively, experiencing recovery. Consequently, the adjunctive application of locally administered antibiotics demonstrated heightened efficacy in reducing inflammation, as quantified by reduction in BOP. This contrasts favourably with outcomes of SRP in isolation, underscoring the additional benefits conferred by the adjunctive antibiotic therapy. The highly significant $p<0.001$ and $p<0.003$ were found for the bivariate correlation and independent samples *t*-test used to determine the relationship between age and clinical parameters indicating the increase in the severity of these parameters with older age. A positive Pearson correlation of 0.192 indicates a weak positive correlation between age and PPD. No significant *p*-value was found for the independent samples *t*-test and Chi-square test used to analyse the differences between males and females in these clinical parameters representing the lack of gender-based differences.

Table-1: BOP in groups at each visit (%)

	Group I			Group II			Group III			Group IV		
Visit	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd
Yes	68.42	49.47	31.59	75.79	35.79	20.0	73.68	31.58	10.53	85.26	8.42	0
No	31.58	50.53	68.42	24.21	64.21	80.0	26.32	68.42	89.47	14.47	91.58	100

Table-2: Comparison of mean values at 1st and 3rd visit in each group (Mean±SD)

Parameter	Group I	Group II	Group III	Group IV
Mean PPD at 1st visit	3.10±1.54	3.08±1.61	3.44±1.40	3.39±1.43
Mean PPD at 3rd visit	2.56±1.50	2.08±1.49	2.08±1.37	0.69±0.87
Mean Difference	0.54±0.04	1.005±0.58	1.36±0.54	2.70±1.01
Decrease in PPD	17.4%	32.5%	39.54%	79.65%
p	<0.001	<0.001	<0.001	<0.001

(Paired *t*-test)

Table-3: Comparison of mean PPD between groups

Treatment Group	Treatment Group	Mean Difference	*p*
Group I	Group II	0.48	0.06
	Group III	0.48	0.06
	Group IV	1.87	<0.001
Group II	Group I	-0.48	0.06
	Group III	-0.005	1
	Group IV	1.38	<0.001
Group III	Group I	-0.48	0.06
	Group II	0.005	1
	Group IV	1.39	<0.001
Group IV	Group I	-1.87	<0.001
	Group II	-1.38	<0.001
	Group III	-1.38	<0.001

(Post-Hoc Tukey test)

Table-4: BOP in groups during the 3rd visit [n (%)]

Groups	Yes	No
Group I	30 (50.8)	65 (20.2)
Group II	19 (32.2)	76 (23.7)
Group III	10 (16.9)	85 (26.5)
Group IV	0 (0)	95 (29.6)

DISCUSSION

Diabetes mellitus and periodontitis are two of the most prevalent chronic inflammatory non-communicable diseases worldwide, with inflammation being the meeting point among them. They negatively impact the quality of life of the individuals suffering from them.[16]

Metronidazole and minocycline were chosen for this study because both have antibiotic as well as anti-inflammatory effects. Minocycline also has anti-collagenase properties to reduce tissue destruction and bone resorption.[17]

In research conducted, out of total 380 patients, 221 (58.2%) were males, 159 (41.8%) were females. This predisposition of chronic periodontitis in

diabetes mellitus patients towards male gender was also mentioned in another study performed in Pakistan by Fahim A *et al* where it was 55% males and 45% females.[3] However, in a study by Qureshi *et al*, in Pakistan, 44.2% were males and 55.8% were females.[10]

Within the scope of this clinical study, there was a notable reduction in PPDs, accompanied by discernible distinctions among the various treatment groups. Notably, more substantial PPD reductions were observed in groups receiving adjunct antibiotic treatments. A greater reduction in BOP was achieved, particularly in groups that underwent SRP in combination with antibiotic therapy. These clinical parameters are more severe in older patients but no gender-based differences were found. Similar outcomes were observed in a randomized clinical trial conducted by Kesarwani S *et al*, in chronic periodontitis patients. All the patients underwent full mouth SRP followed by application of 0.25% satranidazole gel in Group 1 and 1% metronidazole gel in Group 2 and clinical parameters were evaluated at baseline, after SRP, 21st day and 90th day post-treatment. They found a highly significant PPD reduction (p=0.000) with the adjuvant usage of metronidazole gel and 70% BOP was observed showing relapse after 90 days indicating a return to the original situation.[18]

The study by Qureshi *et al* documented noteworthy reductions in PPD, and BOP in both the groups subjected to the treatment involving SRP in combination with metronidazole and OHI in group 1 and SRP with OHI in group 2 which is consistent with our findings. However, in contrast to the findings of the current study, their investigation did not yield statistically significant differences among the various treatment groups.[19]

In a clinical trial focused on periodontitis patients, Hokari *et al*,[15] observed substantial improvements by employing adjunctive subgingival application of minocycline gel after SRP. Their study unveiled noteworthy improvements in PPD, and BOP, surpassing the outcomes achieved through SRP performed as an independent intervention which validates this study. Abraham *et al*, in their comparative study, observed improvements in all clinical parameters with the supplementary application of locally administered metronidazole gel and tetracycline fibers following SRP in comparison to the outcomes achieved by SRP alone. Their findings are consistent with our results.[20] In a clinical trial targeting chronic periodontitis patients, Huang *et al*, documented that the amalgamation of minocycline with tinidazole proved more efficacious in enhancing periodontal indices and alleviating discomfort compared to minocycline used alone after SRP. This validation of the current study's findings underscores the efficacy of combined treatment strategies.[21]

Miyazawa *et al*, reported inconsequential alterations in PPD within their investigation involving periodontitis patients treated with minocycline gel after SRP. This outcome contrasts with the findings of this study. Nevertheless, their study did reveal a noteworthy reduction in BOP.[22] Choi *et al*, demonstrated notable advancements in clinical parameters at both 1 and 3 months from baseline within chronic periodontitis patients who underwent treatment with SRP in conjunction with minocycline gel. This aligns with this study's findings. However, in contrast to the results of this study, they noted inconsequential disparities in comparison to the control group treated exclusively with SRP.[23] In concurrence with the investigation done, Park *et al*, also reported analogous findings. In their study involving peri-implantitis patients, a comparable antibiotic combination was administered, revealing superior reductions in PPD and BOP when contrasted with SRP intervention.[24]

In contrast to this study, Khattri *et al*, found differing results in a systematic review on systemic antimicrobials for non-surgical periodontitis treatment. Their analysis indicated that the utilization of drug combinations like metronidazole + amoxicillin or tetracyclines, for short-term interventions did not yield improvements in PPD among chronic periodontitis patients. Notably, they observed that intermediate and long-term treatments displayed superior results. However, intriguingly, short-term treatment exhibited superior outcomes in terms of reducing BOP.[25]

The improved tolerability and safety profile of these two medications make them a viable and efficient option for individuals with diabetic periodontitis. Since metronidazole is supported to be similarly effective as minocycline in periodontitis patients in this study, its use should be encouraged to avoid adverse effects caused by unnecessary use of systemic antibiotics. If the required effect is not achieved via monotherapy, a combination of minocycline and metronidazole can be used because it has shown a synergistic effect for treating diabetic periodontitis.

It was a single-centered clinical study. No clinical comparison of adverse antibiotic effects was conducted. The calculation of plaque index and the gingival index was omitted. Measuring these parameters was not feasible due to constraints on cost and time.

The potential for conducting a multi-centered study holds promise, offering broader investigation and validation opportunities. A comprehensive assessment can involve the calculation of plaque index and the gingival index. The endorsement of locally applied antibiotic gels emerges as a beneficial strategy in the chronic periodontitis treatment arsenal. Maintaining a judicious approach, it is essential to emphasize the avoidance of excessive and unwarranted systemic antibiotic utilization.

CONCLUSION

Scaling and root planing enhanced the periodontal health in diabetic patients, but superior outcomes were achieved in patients undergoing SRP and adjunct antibiotic treatment. Metronidazole gel exhibited comparable efficacy to minocycline gel for treating diabetic chronic periodontitis patients. A synergistic effect was observed between metronidazole and minocycline in treating diabetic chronic periodontitis patients.

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ORIGINAL ARTICLE

SYMPHYSIS-FUNDAL HEIGHT MEASUREMENT IN ANTENATAL CARE AT LUMHS HOSPITAL, JAMSHORO: A CROSS-SECTIONAL STUDY

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Background: Routine symphysis-fundal height (SFH) measurement during pregnancy is a widely practiced method for estimating foetal size and gestational age in antenatal care. The objective of this study was to determine the SFH values at different gestational ages among pregnant women receiving antenatal care at LUMHS Hospital, Jamshoro and to assess the relationship between SFH and gestational age. **Methods:** This cross-sectional study was conducted at LUMHS Hospital, Jamshoro, involving 50 pregnant women aged 18–40 years. SFH measurements were initiated after 28 weeks of gestation and taken at regular intervals. Statistical analysis included mean and standard deviation of SFH values, and correlation analysis. **Results:** The mean age of the participants was 27.02±3.66 years, and mean gestational age was 32.62±2.83 weeks. Mean SFH during 28–38 weeks was 30.54±2.62 Cm and increased with advancing gestational age. There was a strong correlation between SFH and gestational age (r=0.998). **Conclusion:** Symphysial-fundal height measurement showed a strong correlation with gestational age, supporting its usefulness as a supportive tool in antenatal care. However, due to potential variability from clinical and foetal factors, SFH should complement—not replace—ultrasound assessment.

Keywords: Symphysis-fundal height, gestational age, foetal growth monitoring, antenatal care

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INTRODUCTION

Routine symphysis-fundal height measurement during pregnancy has long served as a valuable tool in estimating foetal size and gestational age in antenatal care.[1] Deviations from expected fundal height may indicate pathological conditions such as intrauterine growth retardation (IUGR), small for gestational age (SGA) foetuses, or oligohydramnios.[1]

Data from various birth cohort studies and global surveys highlight the significant prevalence of term-SGA births, particularly in low-income and middle-income countries.[2,3] In 2010 alone, an estimated 32.4 million infants were born SGA in low-income and middle-income countries, with a considerable portion being born at term and low birth weight.[3] The frequency of SGA foetuses tends to be notably higher in regions like South Asia, with Pakistan reporting a substantial incidence of SGA births.[4–6]

SGA infants face heightened risks of mortality and various morbidities compared to those born appropriate for gestational age (AGA).[7] These risks include hypoglycaemia, bronchopulmonary dysplasia, respiratory distress syndrome (RDS), neurodevelopmental disabilities, and stillbirths.[8,9]

Improved antenatal detection of SGA foetuses is crucial given that foetal growth restriction often precedes adverse outcomes.[10] Symphysis-fundal height measurement using a measuring tape presents a simple, widely available, and routinely practiced method in antenatal settings worldwide, particularly in low-income settings.[10,11] However, challenges persist in accessing ultrasound for gestational age assessment, especially in resource-constrained areas, where ultrasound is costly, of suboptimal quality, and operated by undertrained personnel.[12,13] Despite the growing preference for ultrasound in detecting SGA, its limited availability underscores the continued relevance of SFH measurement as recommended by the WHO Reproductive Health Library and National Institute for Health and Clinical Excellence Guideline for Antenatal Care.[13]

SFH measurement is regarded by many healthcare providers as a scientific, objective, reproducible, and reliable method for assessing foetal growth.[13] Customized SFH charts tailored to local populations have been shown to enhance SGA detection while minimizing unnecessary referrals for further investigation.[4,13] Two observational studies conducted in the UK and Australia revealed that optimizing the utilization of screening data by plotting sequential symphysis-fundal height (SFH) and estimated foetal weight (EFW) measurements on individualized charts significantly enhanced the detection rate of foetal growth restriction (FGR) among small for gestational age (SGA) births, increasing it to over 40%.[14]

Despite its widespread use, there is limited research documenting SFH values at specific

gestational ages, particularly in Pakistan. Most studies have focused on the development of customized SFH charts or compared the accuracy of SFH measurements to ultrasound findings, leaving a gap in literature regarding baseline SFH values in clinical practice. Understanding the SFH values across different gestational ages can provide a reference for antenatal care providers, especially in settings with limited access to advanced diagnostic tools.

The objectives of this study was to determine the SFH values at different gestational ages among pregnant women receiving antenatal care at LUMHS Hospital, Jamshoro and to assess the relationship between SFH and gestational age. This population, this study aims to support clinical decision-making and enhance antenatal care practices in resource-limited settings.

MATERIAL AND METHODS

This cross-sectional study was carried out at LUMHS Hospital, Jamshoro, over six months from Jul to Dec 2024. The sample size was calculated to estimate the mean symphysis-fundal height (SFH) at 28 weeks of gestation with a 99% confidence level and a precision (margin of error) of ±0.5 Cm. Based on a previous study by Dias *et al*[15] which reported a mean SFH of 26.8±1.4 Cm in women, the minimum sample size was calculated to be 48 participants rounded to 50, selected using a non-probability consecutive sampling method.

The study included pregnant women aged 18–40 years with singleton pregnancies confirmed by an early dating ultrasound conducted between 8 and 14 weeks of gestation to ensure accurate determination of gestational age. Only primigravida within a gestational age range of 28–40 weeks at the time of symphysis-fundal height (SFH) measurement were included.

Exclusion criteria were carefully selected to minimize confounding factors and included women with known co-morbidities such as hypertension, gestational diabetes mellitus (GDM), pre-eclampsia, or renal disease. Twin pregnancies and multiple pregnancies were excluded. Participants with polyhydramnios or oligohydramnios were also excluded; for those presenting with unexpectedly high or low SFH values, growth scans were performed, and any confirmed cases of hydramnios were excluded from the study. Women with a body mass index (BMI) >35 Kg/m² were excluded to reduce variations in SFH measurements caused by increased maternal adiposity. Participants with fibroids confirmed by ultrasound were excluded.

Ethical approval was obtained from the Ethical Review Committee of Liaquat University of Medical and Health Sciences [Approval No. 2022-0057, dated 18 Jun 2024]. Written informed consent was obtained from all participants prior to enrolment.

Symphysis-fundal height measurements were initiated after 28 weeks of gestation. Participants were followed up at specific intervals, and SFH readings were recorded at 28, 32, 36, 38. SFH measurements were conducted using a flexible, non-elastic measuring tape, following a standardized protocol to ensure consistency. Each measurement was performed with the participant lying in a supine position, ensuring the bladder was empty, and the measurement was taken from the upper border of the pubic symphysis to the highest palpable point of the uterine fundus. While the primary focus of the study was on SFH measurements, data on socio-demographic and physical characteristics of participants, including age, height, weight, and BMI, were also collected.

The collected data were entered and analyzed on SPSS-19. Descriptive statistics, including mean and standard deviation, were computed for continuous variables such as height, weight, gestational age, and SFH. Correlation analysis was conducted, with SFH serving as the dependent variable and gestational age as the independent variable. Analysis of variance (ANOVA) was performed and $p \leq 0.05$ was considered as statistically significant.

RESULTS

A total of 50 pregnant women participated in the study. The mean age of the participants was 27.02±3.66 years, with a range of 18–40 years. Majority of the patients 28 (56%) were between the ages of 26 and 30 years. Patients aged 25 years or younger constituted 15 (30%) of the study population, while 7 (14%) of the participants were above the age of 30 years. The average height was 157.02±5.85 Cm, varying between 144 and 169 Cm, while the mean weight was recorded at 67.29±12.83 Kg, ranging from 36–95 Kg. The mean gestational age was 32.62±2.83 weeks, with a minimum of 28 weeks and a maximum of 38 weeks.

The mean symphysis-fundal height (SFH) between 28 to 38 weeks was 30.54±2.62 Cm. There was a progressive increase in SFH with advancing gestational age (Figure-1). Table-1 provides a detailed breakdown of the mean symphysial-fundal height (SFH) measurements across different completed gestational weeks. A gradual increase in SFH was observed as gestational age progresses from 28 to 38 weeks. For instance, the mean SFH at 28 weeks was recorded as 26.33 Cm, gradually rising to 35.50 Cm by 38 weeks. This progressive increase underscores the dynamic nature of foetal growth and development during the later stages of pregnancy.

Table-2 presents a comparative analysis of mean SFH measurements among different age groups of pregnant women. The data suggest a trend towards higher mean SFH measurements in younger age groups compared to older counterparts. Women aged ≤25 years

exhibited a slightly higher mean SFH of 31.53 Cm compared to those aged 26 to 30 years (30.39 Cm) and >30 years (29.00 Cm). While these differences did not reach statistical significance (p=0.095), they offer valuable insights into potential age-related variations in foetal growth patterns. The findings from the correlation analysis, as depicted in Figure-2, underscore the strong positive association between gestational age and SFH (r=0.998).

Table-1: Symphysial-fundal height by gestational age (n=50)

Gestational Age (Weeks)	n	Mean±SD
28	3	26.33±0.57
29	5	27.40±0.54
30	8	28.00±0.53
31	2	29.50±0.70
28 to 31	**18**	**27.72±1.02**
32	6	30.17±0.41
33	7	30.86±0.69
34	4	31.75±1.26
35	5	32.60±0.55
32 to 35	**22**	**31.23±1.15**
36	6	33.50±0.83
37	2	34.50±0.71
38	2	35.50±0.71
36 to 38	**10**	**34.10±1.10**

Table-2: Comparison of mean symphysial-fundal height (Cm) among different age groups

Age Groups (Years)	n	Mean±SD	*p*
≤ 25 Years	15	31.53±2.29	0.095
26 to 30 Years	28	30.39±2.76	
>30 Years	7	29.0±2.00	

(ANOVA test applied)

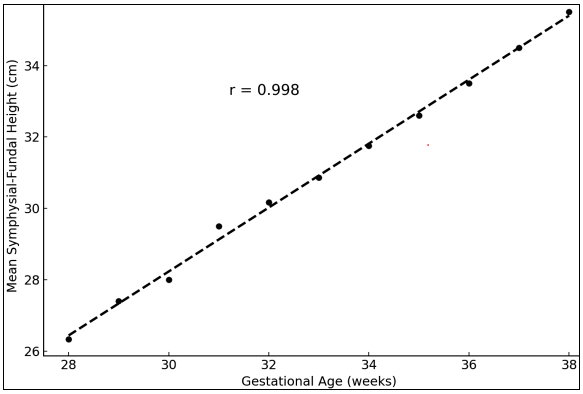

Figure-1: Correlation between gestational age and symphysial-fundal height (n=50)

DISCUSSION

Antenatal care plays a crucial role in the early detection of foetal growth restriction (FGR), a condition associated with adverse pregnancy outcomes.[16] In resource-poor settings, accurate gestational age assessment remains a challenge, primarily due to limited access to ultrasound dating.[17] Consequently, the last menstrual period (LMP) continues to be the predominant method for estimating gestational age in these settings.[18] Symphysis-pubis fundal height measurement emerges as a widely available and routinely practiced alternative for monitoring foetal growth during antenatal visits.[19] Previous studies have demonstrated the effectiveness of SFH measurement in detecting small-for-gestational-age (SGA) foetuses or intrauterine growth restriction, highlighting its utility in routine antenatal care.[20,21] Parveen *et al* concluded that symphysis-fundal height measurement is a valuable tool for estimating gestational age in most cases, showing strong correspondence with gestational age confirmed through other methods like ultrasound. They advised against relying solely on this technique without the complementary use of ultrasound for accuracy and reliability.[22]

Our findings from the correlation analysis demonstrate a strong positive association between gestational age and SFH, with Pearson correlation coefficient of r=0.998. Our results are consistent with Perveen *et al*[22] who also reported a significant positive correlation (r=0.883) between SFH and gestational age in their antenatal population. Together, these findings support the continued use of SFH monitoring in routine antenatal care, particularly in resource-limited settings where ultrasonography may not be readily available.

Our study demonstrates the potential of SFH measurement in predicting gestational age with reasonable accuracy[23]. It underscores the importance of ongoing efforts to standardize SFH measurement techniques and improve training for healthcare providers involved in antenatal care. Standardization of SFH measurement protocols can help minimize intra-observer and inter-observer variability, thereby enhancing the reliability and reproducibility of SFH assessments. Investing in training programs aimed at educating healthcare providers on the accurate measurement and interpretation of SFH can further optimize its utility as a screening tool for foetal growth assessment leading to better pregnancy outcomes and reduced perinatal morbidity and mortality.

The measurement of symphysis-fundal height is subject to intra-observer and intra-observer variability, which may introduce errors in the assessment.[24] Our study was conducted in a single healthcare setting, limiting the generalizability of the findings to other populations.

CONCLUSION

Symphysial-fundal height measurement showed a strong correlation with gestational age, supporting its usefulness as a supportive tool in antenatal care. However, due to potential variability due to clinical and foetal factors, SFH should complement—not replace—ultrasound assessment. Further large scale studies are recommended to refine its accuracy across diverse pregnancy conditions.

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MF: Data interpretation and literature review.
NM: Data analysis and interpretation
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ORIGINAL ARTICLE

OXIDATIVE CAPACITY IN POLYCYSTIC OVARY SYNDROME: EXPLORATION AND WAY FORWARD

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Background: Polycystic ovarian syndrome (PCOS) is a complex disorder characterized by elevated androgen levels, ovarian cysts, chronic anovulation, and infertility, with insulin resistance and hyperinsulinemia as key contributors. The study aims to explore the role of oxidative capacity in presentation of PCOS by estimating Total Oxidant Status (TOS), Alarin, and Fetuin in PCOS and non-PCOS infertile women. **Methods:** This cross-sectional study was conducted from Feb 2021 to Feb 2023. The study recruited 89 women with primary infertility aged 15–49 years. Participants were matched based on age and BMI, including women with a BMI ranging from normal to overweight/obese, while excluding those with secondary infertility, major health conditions, or oral contraceptive use. Healthy infertile women with regular cycles served as controls. Serum levels of TOS, Alarin, and Fetuin-A were measured using ELISA kits. Statistical analysis was performed using SPSS-23. **Results:** Forty-five (51%) of the participants had PCOS, with significantly higher BMI (p=0.002). Follicular Stimulating Hormone (FSH) and Latinizing Hormone (LH) levels were significantly elevated (p<0.001) in the PCOS group, while Anti-Mülarian Hormone (AMH) was significantly lower (p<0.001). Prolactin, vitamin D, and oxidative stress markers were comparable in both groups and were not significantly different as compared to the fertile females. The Spearman's correlation computed that Prolactin significantly and negatively correlated with Fetuin-A and TOS, while positive correlations were found between Fetuin-A, Alarin, and TOS (p<0.001). **Conclusion:** Our findings suggest a potential link between oxidative stress and PCOS, highlighting its complexity.

Keywords: Alarin, Fetuin-A, Polycystic ovarian syndrome, PCOS, Total Oxidant Status, TOS

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INTRODUCTION

Polycystic ovarian syndrome (PCOS) is marked by excessive androgen levels, bilateral cystic enlargement of the ovaries[1], chronic anovulation, and infertility. Insulin resistance (IR) and hyperinsulinemia are the primary pathogenic factors behind PCOS[2].

Fetuin-A, a 64 kDa glycoprotein secreted by the liver and adipose tissue, acts as a hepatokine and adipokine. It blocks insulin signalling and induces insulin resistance (IR) by auto-phosphorylating insulin receptors in muscle and liver. IR, often associated with PCOS, is linked to elevated Fetuin-A levels in PCOS cases, suggesting it is a potential marker for PCOS screening.[2–4] Adipose tissues secrete Fetuin-A, an adipokine with anti-inflammatory effects. Elevated Fetuin-A levels may reflect inflammation in unexplained infertility and contribute to its pathophysiology, suggesting its potential use as a biomarker for assessing infertility.[3]

Alarin, part of the 'galanin peptides' family, influences neuronal processing and female reproduction. Elevated Latinizing Hormone (LH) levels in PCOS play a key role in its onset.[1] Research endeavours are presently directed towards examining the potential correlation among serum 'Alarin' and 'PCOS' in infertile female cohorts, finding greater serum Alarin levels and a significant positive association with LH in the PCOS group afflicted with infertility. Moreover, infertile females with increased Alarin levels in serum exhibited a higher propensity for PCOS development.[5] Alarin injections in animal models increase LH secretion. Interestingly, PCOS individuals show lower Alarin levels compared to healthy controls.[5] Alarin may serve as a predictive marker for PCOS risk.[6]

Total oxidant status (TOS) reflects the body's overall oxidation state.[7] Oxidative stress results from an imbalance between oxidants and antioxidants, disrupting cellular redox. Oxidants include reactive oxygen species (ROS) and reactive nitrogen species (RNS).[8] ROS examples are hydrogen peroxide, hydroxyl radical, and superoxide, while RNS includes nitric oxide and its metabolites. ROS contributes to 95% of total oxidants.[9,10]

Multiple techniques measure individual oxidants, but assessing oxidative stress in disease requires more than measuring single oxidants. TOS measurement offers a comprehensive view of oxidative stress in disease pathophysiology.[11,12] Evaluating biomarkers like Fetuin-A, Alarin, and TOS is clinically essential. No studies have compared these biomarkers between infertile PCOS and non-PCOS women. We

hypothesize that oxidative status, as measured by TOS, Alarin, and Fetuin, is impaired in PCOS compared to non-PCOS infertile women. We aim to explore oxidative capacity in PCOS by estimating TOS, Alarin, and Fetuin levels.

MATERIAL AND METHODS

This cross-section study was carried out from Feb 2021 to Feb 2023. The sample size was calculated using OpenEpi, with infertility prevalence as 18–22%[13]. The calculated sample size was 44 participants per group, but 45 were included in the PCOS group and 44 in the non-PCOS group.

The study was vetted and approved by The Aga Khan University Ethical Review Committee (ERC# 2022-4812-20408). Informed consent was obtained from all participants.

The infertile women (n=89), aged 15–49 years, were recruited from Aga Khan University Hospital and Australian Concept Infertility Medical Centre (ACIMC) for the current study. The control group included healthy infertile women with regular menstrual cycles and no signs or ultrasound evidence of PCOS. Participants were recruited by the gynaecologist (Co-PI) and ACIMC collaborator.

The control and cases were matched based on age and BMI. Participants had primary infertility, defined by WHO as failure to conceive for over two years, and BMI classified as normal (18.5–22.9 Kg/m²) or overweight/obese (≥23 Kg/m²). PCOS diagnosis followed ASRM (American Society for Reproductive Medicine) criteria requiring 2 of 3 conditions: 1) oligo/ anovulation, 2) clinical/biochemical hyperandrogenism, and 3) ultrasound-confirmed polycystic ovaries with ≥12 follicles of 2–9 mm.[14] Women with secondary infertility, age >45, oral contraceptive use, major health conditions, congenital adrenal hyperplasia, or inherited disorders were excluded.

Ten milliliters of venous blood were collected during the proliferative phase, ideally on day two of the menstrual cycle. Sera were isolated at 4,000 rpm for 10 minutes and stored at -80°C. Serum total oxidant status, Alarin, and Fetuin-A levels were measured using commercial ELISA kits.

Statistical analysis was performed using SPSS-23. Descriptive statistics, paired *t*-tests, and the Mann-Whitney U-test compared PCOS and non-PCOS groups. Relationships between serum total oxidant status, Alarin, and Fetuin-A levels were assessed using Rapid Linear Progression and Pearson correlation tests, and $p<0.05$ was considered statistically significant.

RESULTS

There were 89 infertile female subjects in the study, out of which 45 (51%) infertile females had PCOS and 44 (49%) were without PCOS.

Table-1 contains the analysis of the descriptive variables of the study. The mean age of infertile females with PCOS was not significantly different as compared to females without PCOS. However, BMI was significantly raised in women with PCOS ($p=0.002$). The mean serum values of Follicle-stimulating hormone (FSH) and Latinizing hormone (LH) are significantly raised in women with PCOS ($p<0.001$). The Anti-Müllerian Hormone (AMH) of infertile women with PCOS was significantly decreased ($p<0.001$). Prolactin and oxidative stress markers were comparable in both groups and were not significantly different as compared to the fertile female subjects ($p=0.001$).

The Spearman's rank correlation between study variables and oxidative stress markers is shown in Table-2. A significant negative correlation was found between prolactin and Fetuin-A ($r= -0.223$, $p<0.001$) and prolactin and total oxidant status ($r= -0.223$, $p<0.001$). A significant positive correlation was found between Fetuin-A and Alarin ($r=0.434$, $p<0.001$) and Fetuin-A and TOS ($r=0.454$, $p<0.001$). A positive correlation was observed between Alarin and TOS ($r=0.423$, $p<0.001$). There was no correlation among age, BMI, FSH, LH, and AMH with oxidative stress markers.

Figure-1 describes the correlation of oxidative stress markers Fetuin-A, Alarin, and TOS. These markers have a significant positive correlation among them ($p<0.001$). Prolactin has a significant negative correlation with all OS markers ($p<0.005$). All other variables such as age, BMI, FSH, and LH do not show any correlation with oxidative stress markers.

Table-1: Demographic characteristics of females with PCOS and Non-PCOS

	PCOS (n=45)	Non-PCOS (n=44)	*p*
Age (Years)	34.29±5.51	33.27±5.01	0.482
BMI (kg/m²)	29.43±4.8	26.03±4.05	0.002
FSH (IU/ml)	12.09±15.96	6.47±2.81	<0.001
LH (IU/L)	9.28±11.07	4.78±2.12	<0.001
AMH (ng/ml))	1.65±1.19	2.94±1.42	<0.001
Prolactin (µg/L)	15.62±10.39	12.59±6.71	0.208
Fetuin-A	944.33±654.34	1188.70±1121.42	0.765
Alarin	2.01±1.63	1.79±1.6	0.164
TOS	3.81±3.49	4.14±2.99	0.135

Table-2: Correlation of study variables with oxidative stress markers

	Fetuin-A	Alarin	TOS
Age	0.041	0.018	0.142
BMI	0.030	0.043	-0.142
FSH	0.066	0.059	-0.166
LH	-0.130	-0.066	-0.174
AMH	-0.015	0.027	0.046
Prolactin	-0.223*	-0.116	-0.223*
Fetuin-A	-	0.434**	0.454**
Alarin	0.434**	-	0.423**
TOS	0.454**	0.423**	-

*$p<0.05$, **$p<0.01$

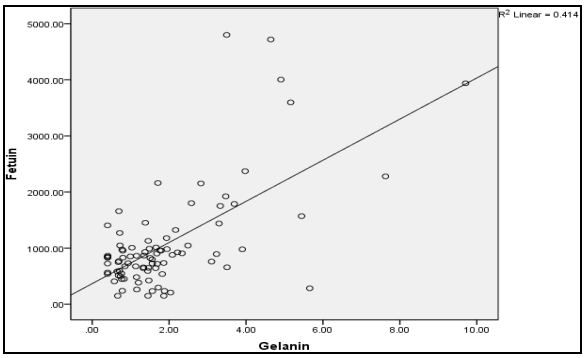

Figure-1a: Correlation of Fetuin with Gelanin in infertile women

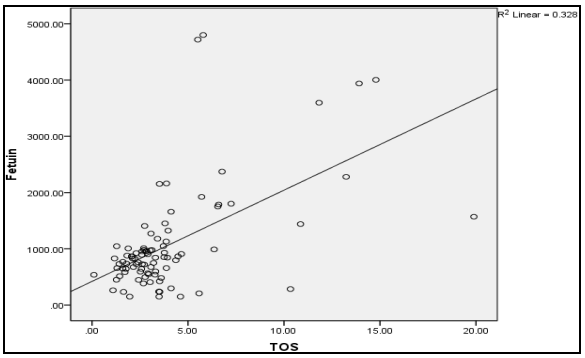

Figure-1b: Correlation of Fetuin with Total Oxidant Status in infertile women

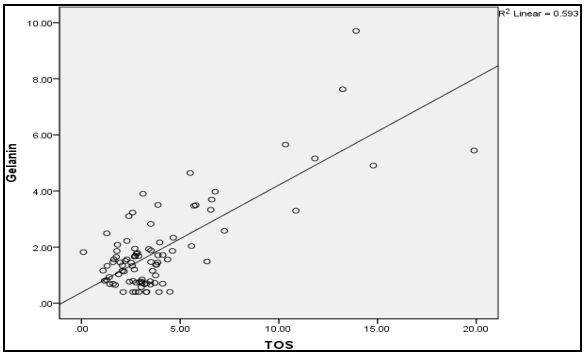

Figure-1c: Correlation of Gelanin with Total Oxidant Status in infertile women

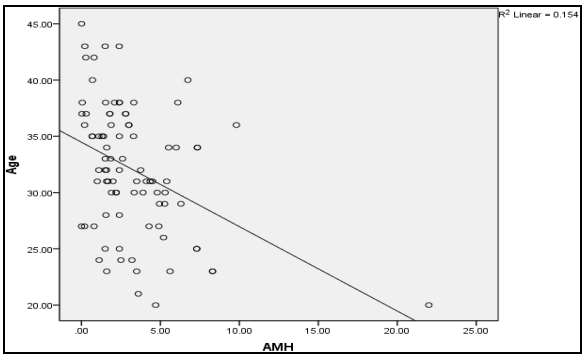

Figure-1d: Correlation of Age with Anti-Mülarian Hormone in infertile women

DISCUSSION

Polycystic ovarian syndrome is a conglomeration of metabolic disorders and hormonal disturbances resulting in female infertility.[15] PCOS is usually characterized by neuroendocrine dysfunction with deregulated gonadotropin secretion, indicating disrupted gonadotropin-releasing hormone (GnRH) secretion. It is assumed that Fetuin and Alarin have distinct causative roles for PCOS by regulating GnRH-secreting neurons.[16]

While providing demographic characteristics, the current research compared infertile women with PCOS to non-PCOS. The average age of infertile females with PCOS did not significantly differ from that of infertile females without PCOS; this indicates that age is not a distinguishing factor between the two groups according to current findings. Some studies explored that the mean age in PCOS was less than the controls. However, the research discovered that women with PCOS who are infertile exhibit a notably greater BMI compared to those without PCOS. This suggests that higher BMI may be associated with PCOS, which aligns with existing literature linking obesity to PCOS.[16] According to a meta-analysis, women classified as obese exhibited an odds ratio of 2.77 for developing PCOS in comparison to their non-obese counterparts. Another study supports the current finding and reported that the median BMI measurements exhibit markedly greater in 'PCOS' group versus controls.[17] However, in recent years, 20–50% of thin and lean women were diagnosed with PCOS.[18,19]

Our study found FSH levels to be notably increased in infertile PCOS females compared to non-PCOS counterparts. Elevated FSH levels in infertile PCOS women in current findings may reflect disturbances in ovarian function, such as anovulation. In contrast to present discoveries, the scientists mainly agreed on the point that PCOS patients have high LH and low FSH, thus raising the 'LH: FSH' ratio, linked to infertility.[19] Another study reported reduced FSH concentration in 'PCOS' patients versus control counterparts. The present investigation revealed that the serum LH concentrations are noticeably elevated in infertile females with 'PCOS' than in 'non-PCOS' counterparts. Heightened levels of LH serve as a distinctive characteristic of PCOS, correlating with hyperandrogenism and irregular menstrual patterns, thereby contributing to infertility.[18] LH release increases because of an abnormal feedback mechanism brought on by ovarian estrogen.[18] Corroborated with our findings, scientists reported that mean LH concentrations were noticeably elevated in infertile cases diagnosed with 'PCOS' than those of control counterparts.[2] Likewise, it is also noticed that in several subcategories of PCOS, serum LH and LH:FSH ratios

were observed to increase as the levels of other hormones declined.[20]

According to current findings, non-PCOS females have significantly greater concentrations of AMH compared to infertile females with PCOS. The greater AMH in the serum of non-PCOS females generally suggests a larger pool of developing follicles, which is usually considered advantageous for fertility. In contrast to the current findings, a study highlighted that median values of AMH were expressively dominant in PCOS cases.[17] In a study involving infertile women, the mean levels of AMH within the PCOS cohort exhibited notable elevation among lean individuals (BMI≤25) compared to their overweight counterparts. In a retrospective analysis encompassing a substantial cohort of young infertile women, a little but notable decline was observed in AMH concentration in serum among overweight individuals diagnosed with PCOS.[21,22]

The lower levels of Fetuin-A in the PCOS group could be explained because Fetuin-A is a proinflammatory cytokine that produce low-grade inflammation along with C-reactive protein which is usually increased in PCOS and also suppresses Fetuin-A expression in the liver.[2] This would also explain the negative association between Fetuin-A concentrations and hepatic fat. ElSirgany *et al*, signifies higher Fetuin-A levels in PCOS than the control group establishing a correlation between Fetuin-A levels and other hormones measured in infertile women with PCOS.[2] A direct relation between BMI has been found with different features of PCOS signified by research which also delineates an association between Fetuin-A and insulin resistance in the PCOS population.[23] Our results are similar to study, where insignificant differences in Fetuin-A levels were found in both groups. Alarin was found to be a significant factor in GnRH secretion and for GnRH modulated LH secretion. That study showed higher Alarin and LH levels in women with PCOS group than controls compared with our results.[5] The increased prolactin level is due to the stimulating effect of estradiol assuming that hyperprolactinemia is due to increased oestrogen secretion in PCOS females.[24] It is suggested that insulin resistance in PCOS could also lead to increased prolactin levels. We have observed higher LH levels in PCOS patients who also had high serum levels of prolactin but it is the shortcoming of our study that serum oestrogen levels were not done however, increased serum oestrogen and prolactin levels are found in PCOS patients with increased LH in a study done by Zahra Davoudi *et al*, in 2021.[24]

Various researches demonstrate elevated TOS in the serum and follicular fluid of women with PCOS.[25] Interestingly, a study pointed to lower TOS levels after both oral glucose tolerance and mixed meal tests in PCOS patients.[26] Our study demonstrates a non-significant value for TOS measured in PCOS and normal female group. But TOS shows a significant positive relation with Fetuin-A and Alarin. This demonstrates a combating/compensatory mechanism for increased low-grade inflammation associated with PCOS.

In summary, our study found decreased Fetuin-A levels, likely due to the presence of different isoforms and glycosylation defects. Alarin levels were higher in the PCOS group, consistent with previous studies. Prolactin was elevated in PCOS, likely due to increased oestrogen. TOS showed no significant difference, possibly influenced by confounding oxidants.

CONCLUSION

The study demonstrates a pronounced oxidative imbalance in the PCOS group. Oxidative stress plays a more substantial role in the pathophysiology of PCOS.

LIMITATIONS

Study limitations include small sample size, cross-sectional design, and lack of stratification of obese PCOS and control groups to assess the effects of obesity, PCOS, and insulin levels. Strengths include a homogeneous population and evaluation of two biomolecules with hormonal parameters on oxidative stress in PCOS. Larger studies comparing infertile and fertile women are needed for definitive conclusions.

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ORIGINAL ARTICLE

EFFECT OF VARIOUS FORMS OF NICOTINE CONSUMPTION ON GINGIVAL HEALTH OF DENTAL PATIENTS

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Background: Global market size of nicotine consumption has reached US$22.45 billion that will increase more in future posing burden on both economy and health status of population. Objective of this study was to investigate effect of nicotine on gingival health. **Methods:** This study was conducted at School of Dentistry, Shaheed Zulfiqar Ali Bhutto Medical University, Islamabad from 3 Feb 2023 to 4 Mar 2024. Total 300 nicotine consumer dental patients were included, i.e., Smoke-Form (S-form), Electronic cigarette-Form (E-form), and Dual-Form (D-form) users whose Gingival Index (GI) was calculated occurrence of severe gingival issues in participants. GI 0.5–1.0 was labelled as light gingival inflammation, GI 1.1–2.0 as moderate gingival inflammation and GI>2.0 as severe gingival inflammation. Chi-square test checked association of mode of nicotine usage and development of GI, and $p \leq 0.05$ was considered significant. **Results:** One-hundred and fifty-seven (52.3%) patients aged 21–30 years were D-form users compared to 97 (32.3%) middle-aged (31–40 years) and 46 (15.3%) ultra-middle-aged (≥41) participants. Chi-square test revealed that 128 (98.5%) D-form users developed severe gingivitis after 3 months, 91 (95.8%) E-form users had severe gingivitis after 6 months, and 74 (98.7%) S-form users revealed severe gingival issues after 9 months (p=0.012). **Conclusion:** Dual usage of nicotine has more detrimental impact on gingival health, followed by E-form and S-form nicotine users. General awareness programs are necessary in order to educate people regarding hazardous effects of nicotine consumption in any form.

Keywords: Gingivitis, Inflammation, Nicotine, Periodontium, Smokers

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INTRODUCTION

The global market size of nicotine consumption has reached US$22.45 billion in 2022, and shall grow @30.60% from 2023 to 2030 especially in Southeast Asian and Western countries[1] which is posing a huge burden on the economy as well as people's health worldwide. Non-contiguous diseases like malignancy, cardiovascular diseases, and diabetes occur due to nicotine consumption, either in the form of smoked cigarettes[2] or electronic cigarettes[3], which is hazardous to the general and oral health of people worldwide[4]. Nicotine-oriented non-infectious diseases are at their peak in underprivileged nations because of their low socioeconomic status and destitution.[5]

A total of about 175 million females and 942 million males belonging to different age groups in these underdeveloped, low-income countries utilize nicotine regularly.[6] WHO has reported the mortality status of about 7 million individuals globally because of nicotine consumption and its habituation, which is quite alarming.[7] This predicament has unveiled a rise of about 80% in Asian countries, especially Pakistan.[8] Nicotine consumption has been initiated in adolescence, either in Smoke-form or E-form, which then becomes a habit that might prevail throughout the life of any individual.[9] Nicotine dependency is not only responsible for enhanced mortality and morbidity to a greater extent but also for the economic, biomedical, geopolitical, cultural, and social downfall of a population.[5]

According to Food and Drug Administration (FDA), nicotine addiction is potentially injurious to oral health and its adjacent airway tissues.[4,10] A possible justification could be biofilm formation on teeth, periodontium, and other adjacent oral tissues associated with nicotine consumption.[4,11] There is a strong correlation between oral health and general well-being. Patients are enlightened about the impact of oral health on general welfare as compared to general population.[12] There is paucity of information in Pakistan regarding comparative impact of nicotine usage as Smoke form (S-form), Electronic form (E-form) and Dual form (D-form).

This study aimed to evaluate effects of nicotine on gingival health. Our findings will help Pakistani population in taking the initiative for cessation of nicotine consumption.

METHODOLOGY

This cross-sectional study was executed at the School of Dentistry, Shaheed Zulfiqar Ali Bhutto Medical University, Islamabad, from 3 Feb 2023, to 4 Mar 2024, after approval from Institutional Research Committee (SOD/ERB/2023/32-02). A total of 300 dental patients who were smokers and had gingivitis and visited School

of Dentistry were included in this study after taking their informed consent.

An analytical Gingival Index (GI) was used to investigate the association between smoking type and duration of getting severe gingivitis of these dental patients. The participants enrolled in the study were those who initiated the usage of S-form, E-form, and D-form not more than a month. Further details in the questionnaire were 1) Socio-demographic details including age, gender, and educational status, 2) Tobacco and E-cigarette usage including its frequency, and habits, 3) Oral health assessment including number of carious, missing and restored teeth, 4) Oral hygiene measures including frequency of brushing/ flossing, type of toothpaste, dental visits, and 5) Symptoms of gingivitis due to smoking. These questions were taken from 'National Adult Tobacco Survey' and 'Centre for Diseases Control'.[13]

The participants were categorized into Smoke form (S-form), Electronic form (E-form), and Dual form (D-form) users whose GI was calculated to demonstrate the association between their smoking type and duration of developing severe gingivitis. The tobacco consumption related questions and the duration of the initiation of gingival effects were also answered by participants. The GI was used to calculate the severity of gingival inflammation among these participants where GI between 0.5–1.0 displayed Light gingival inflammation, GI between 1.1–2.0 revealed Moderate gingival inflammation and GI≥2 confirmed severe gingival inflammation.[14–16]

Data were analysed on SPSS-26. Descriptive statistics as percentage and frequency were calculated as Mean±SD and SE was calculated by one way ANOVA and multiple comparisons were conducted using post hoc Tukey test. The association between smoking type and duration for severe gingivitis was calculated with Chi-square test, and $p \leq 0.05$ was considered significant.

RESULTS

A total of 300 tobacco-consuming participants participated in this study, of whom 244 (81.3%) were male and 56 (18.7%) were female. Young participants developed gingivitis more quickly in comparison to middle- and ultra-middle-aged participants. The percentage of severe gingivitis was 68 (52.3%) young, 38 (29.2%) middle-aged and 24 (18.5%) ultra-middle-aged participants after 3 months. The inflamed gingival tissues appeared after 6 months in 38 (50.7%) young, 27 (36.0%) middle-aged and 10 (13.3%) ultra-middle-aged participants. On the other hand, severe gingivitis appeared after 9 months in 51 (53.7%) young, 32 (33.7%) middle-aged and 12 (12.6%) ultra-middle-aged participants. (Table-1 and Table-2).

Table-1: Gender stratification of dental patients for mode of nicotine usage and time for occurrence of severe gingivitis [n (%)]

Variables	Males	Females
S-form users	57 (76.0)	18 (24.0)
E-form users	77 (82.8)	16 (17.2)
D-form users	110 (83.3)	22 (16.7)
Duration of severe gingivitis occurrence		
After 3 months	108 (83.1)	22 (16.9)
After 6 months	58 (77.3)	17 (22.7)
After 9 months	78 (82.1)	17 (17.9)

Table-2: Age stratification of dental patients for mode of nicotine usage and time for occurrence of severe gingivitis [n (%)]

	Young (21–30 Yrs)	Middle aged (31–40 Yrs)	Ultra middle aged (≥41 Yrs)
S-form users	39 (52.0)	26 (34.7)	10 (13.3)
E-form users	48 (51.6)	33 (35.5)	12 (12.9)
D-form users	70 (53.0)	38 (28.8)	24 (18.2)
Duration of severe gingival inflammation			
After 3 months	68 (52.3)	38 (29.2)	24 (18.5)
After 6 months	38 (50.7)	27 (36.0)	10 (13.3)
After 9 months	51 (53 .7)	32 (33.7)	12 (12.6)

The mean Gingival inflammation index (GI) in D-form users, E-form users and S-form users was calculated to be 2.51±1.07, 2.88±1.11 and 3.06±1.13. This GI got severe in D-form user participants more quickly after 3 months, followed by E-form users. It became severe after 6 months compared to the S-form users that displayed its severity after 9 months (p=0.001). The mean GI was found to be moderate in D-form users, E-form users, and S-form users both at baseline p=0.014 and after one month p=0.011. The mean GI was calculated to be severe in D-form users but moderate in E-form users and S-form users after 3 months p=0.017. The mean GI got severe in E-form users also along with D-form users but remained moderate in S-form users after 6 months (p=0.019). The mean GI got severe in S-form users also after 9 months (p=0.012). Thus, it confirmed the quickest onset of severe gingival inflammation in D-form users after 3 months in comparison to E-form users and S-form users where this onset was observed after 6 months and 9 months respectively. (Table-3).

Table-3: Comparison of mean GI among different mode nicotine users during certain time duration

Duration	Mean gingival inflammation index			
	S-fom	E-form	D-form	*p*
Baseline	1.17	1.39	1.61	0.014
1 month	1.41	1.63	1.97	0.011
3 months	1.72	1.99	2.51	0.017
6 months	1.95	2.32	2.88	0.019
9 months	2.46	2.61	3.06	0.012

The Chi-square test revealed that 128 (98.5%) D-form users developed severe gingivitis after 3 months as compared to 91 (95.8%) E-form users who developed severe gingivitis after 6 months. On the other hand, 74

(98.7%) S-form users developed severe gingival issues after 9 months (p=0.001). (Table-4).

Table-4: Association of mode of nicotine usage and time for developing severe GI among dental patients

Association	Pearson χ^2 Value	Likelihood ratio	Linear-by-linear association	p
Mode of nicotine usage and time for developing severe GI	560.15	575.29	93.39	0.001

DISCUSSION

Tobacco intoxication is a malediction on mankind. It has preponderantly transfigured the modern era with its untoward consequences for oral and general well-being. Nicotine, an addictive substance, chiefly accredits tobacco dependency. Tobacco is strongly linked to oral malignancy, mucous lesions, gingival and periodontal diseases. *Porphyromonas gingivalis* is a potential pathogen associated with the initiation and propagation of gingival and periodontal diseases. It is chiefly found in higher proportions in smokers than non-smokers. Tobacco consumption is the leading cause of gingivitis in smokers.[17] The key findings in the current study elaborated that young participants are more inclined towards nicotine habituation in comparison to middle-aged participants and ultra-middle-aged participants.

The results of the current study regarding the increased tobacco dependency in younger participants agree with the literature where a study[18] reported the same upsurge in tobacco consumption among the younger population in several other countries. Tobacco use in middle-aged and ultra-middle-aged users was not evaluated in aforementioned research[18] which became significant parameter investigated in current study.

Certain stresses arising due to cultural, social, political, geographical, and economical differences might be responsible for the escalating trends towards tobacco consumption and its habituation.[5] D-form users reported development of gingivitis at the earliest just after 3 months followed by E-form users at a moderate rate after 6 months, and S-form users who displayed severe gingivitis at the slowest rate after 9 months. These results were not per other studies because the association between development of severe gingivitis and smoking type in participants was not scrutinized before this study. Previous studies reported evident correlation between gingivitis and socioeconomic demographics where low education, diabetes, increased plaque and old age were the main factors considered responsible for enhancing gingivitis.[16]

The GI index is considered authentic tool used to estimate the clinical implication and severity of inflammation in the gingival tissues where Light GI ranging between 0.5–1.0, Moderate GI between 1.1–2.0 and severe GI>2.0.[19] The GI got severe in D-form users after 3 months, followed by E-form users where it became severe after 6 months compared to the S-form users who developed its severity after 9 months (p=0.001). That might have become possible because of the enhanced dual absorption and adsorption of the multiple toxic chemicals including nicotine in the oral mucosa of the D-form users as compared to the E-form and S-form users. According to WHO, nicotine habituation has a damaging effect on the health, environment, social and economic status of the global population.[20] Although tobacco smoke contains more than 700 dangerous toxins that might become cancerous[21], a few components in E-cigarettes including acrolein, aldehyde and formaldehyde are more hazardous[22]. These chemicals might infiltrate the lungs, and in turn adversely affecting the health of the people using the various forms of nicotine.[23] These toxins could pose more disastrous effects in the oral cavity before hitting the airway passage and lungs.

The outcomes of the current study are not in collaboration with the prior studies because the effect of various forms of nicotine habituation on gingival tissues has not been addressed previously. However, the available literature has provided the association between smoking and other socioeconomic demographics especially in children.[24,25] The strong association between tobacco consumption and oral diseases including periodontal diseases, gingivitis, dental caries, halitosis, soft tissue lesions, oral candidiasis and xerostomia has been discerned irrespective of the tobacco form and its type being consumed.[4,11] Numerous other studies also reported the interrelationship between nicotine consumption and oral diseases such as dental caries[25], compromised oral health, general welfare, black hairy tongue, xerostomia[4,11] and oral-mucous lesions[4,19]. Still, nicotine consumption in E-form is more detrimental as compared to the S-cigarettes respectively. This is since a single E-form cigarette comprises high levels of nicotine that equals the amount present in 20 conventional S-form cigarettes.[26,27]

Recently, it has been reported that tobacco consumption in Pakistan is at its peak[28] due to its handiness and accessibility without any impediment.[29] Therefore, influential awareness campaigns and strict implementation actions are required to preclude the easy accessibility of this fatal material.[30]

CONCLUSION

Young participants are more indulged in nicotine consumption compared to middle-aged and ultra-middle-aged participants. Tobacco consumed in D-form is reported to precipitate severe gingivitis earlier than E-form and S-form. Dual usage of tobacco is more deleterious towards oral health.

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ORIGINAL ARTICLE

RECTUS SHEATH SLING PROCEDURE: A NEW HORIZON IN UTEROVAGINAL PROLAPSE TREATMENT

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Background: Abdominal sacrocolpopexy offers a durable solution for pelvic organ prolapse through mesh suspension techniques. This study assessed the outcome of rectus fascial sling procedure. **Methods:** This single-arm interventional study was done on 50 patients with uterovaginal prolapse, in Department of Obstetrics and Gynaecology, DG Khan Medical College, DG Khan and Nishtar Medical University, Multan. PFDI-20 (Pelvic Floor Disability Index) was measured preoperatively to assess severity of symptoms. Rectus sheath sling procedure was performed. Surgical outcome was measured one year after surgery. Difference in mean PFDI-20 score before and after surgery was calculated. **Results:** Mean age of the patients was 27.9±11.5 years; 46 (92%) women had 2nd degree uterovaginal descent and 4 (8%) women had 1st degree prolapse. Mean time of operation was <1 hour in 46 (92%) and >1 hour in 4 (8%) of patients. Sixteen (32%) women had hospital stay of 1–2 days after the surgery and 34 (68%) women stayed for 3–4 days in the hospital. Surgical outcome was measured post operatively one year after surgery with PFDI-20. Ninety-two percent women reported no symptoms post procedure. Mean PFDI-20 score after surgery was 11.6±5.61. ΔMean PFDI-20 score was 43.4±9.4 showing substantial improvement in the symptoms after surgery. PFDI-20 score after surgery was found to be significantly associated with age (p<0.001). **Conclusion:** Rectus sheath sling operation for uterovaginal descent is effective, minimally invasive, and less time consuming. It can be recommended for women desiring to preserve their fertility.

Keywords: Rectus sheath sling, Uterovaginal descent, Uterovaginal prolapse

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INTRODUCTION

The most frequent concerns among women who visit outpatient gynaecology clinics, particularly in developing nations, is uterovaginal prolapse.[1] Pelvic organ prolapse (POP) is a bulge of a pelvic organ that protrudes beyond the introitus.[2] It is reported in around 10.3% women in Pakistan.[3] Malnutrition, low income level, and deliveries by inexperienced birth attendants are the primary drivers of this condition's widespread prevalence in developing countries.[4] Pelvic organ prolapse causes a variety of symptoms, including frequent micturition, urinary incontinence, sexual dysfunction, voiding difficulty, faecal incontinence, pelvic pain, low backache, and pelvic heaviness.[5] Purandare[6] derived a newer technique for the management of uterovaginal prolapse in young women in 1965. The prolapsed uterus was held up through a rectus fascial strip, which was attached to the cervix anteriorly at isthmus level. Later on several modifications of the procedure were done. Autologous fascia is frequently used during pelvic reconstructive surgeries and for stress urinary incontinence. It is a safe material for POP repair.[7]

Women with pelvic organ prolapse have a variety of treatment choices, but subjective symptoms are crucial because the patients' suffering determines the course of treatment rather than the severity of physical examination.[8] Basic connective tissue dysfunction is most likely the main underlying condition that predisposes women to uterine prolapse. The possibility of inherited weakness in connective tissue strength as an aetiological factor is suggested by racial differences in incidence, the familial tendency for pelvic relaxation and the frequent concurrent finding of hiatal hernia in prolapse patients.[8]

The traditional wisdom regarding the most suitable surgical repair has changed over the last three decades, moving from native tissue regeneration to grafts, to synthetic materials, and back again.[7] In young women, the aim is not only to treat the prolapsed but also conserve the fertility.[9] Uterus preserving surgeries are getting common day by day. They are typically less intrusive, resulting in less problems, a quicker recovery, and minimal blood loss. Some uterus-sparing procedures are sacrospinous ligament hysteropexy, uterosacral ligament hysteropexy, rectus fascia hysteropexy and Manchester repair. The best surgical technique is still to be proven.[5]

Most of the research on abdominal suspension procedures focuses on mesh-based methods, and there is little data regarding the results of autologous rectus fascial slings.

This study aims to evaluate the surgical results, complications, and quality of life in women who have undergone rectus fascial sling surgery for uterovaginal descent. This prospective study will give important information regarding the effectiveness of autologous fascial sling suspension for uterovaginal descent. It will help in creation of evidence-based guidelines and surgical breakthroughs.

METHODOLOGY

This prospective single-arm interventional study was done simultaneously in two centres: Gynaecology Department, DG Khan Medical College, DG Khan and Nishtar Medical University, Multan. Approval from ethical committee was taken vide ERB No. 252/DME/DGKMC, dated 1 Aug 2023. A total of 50 participants were included, 25 from each centre during one year from 1 Sep 2023 to 1 Sep 2024. Sample size was calculated with formula $n=[(Z\ \alpha/2+Z\ 1-\beta)^2 \times \sigma^2]/\Delta^2$, where $\alpha=0.05$, $1-\beta=0.8$, Z $\alpha/2$ (Z score corresponding to significance level $\alpha=1.96$, Z $1-\beta$ (Z score corresponding to power $1-\beta$) $=0.842$, σ=standard deviation of the outcome variable (13.4%) (standard deviation of surgical duration of uterine conservative surgery of prolapsed)[5] and Δ (minimum detectable change from baseline outcome)=3.83%.

Women aged 18–50 years diagnosed with uterovaginal prolapsed were included. Written informed consent was taken. Prolapse was classified in degree according to Beecham classification. Women having pelvic floor surgery previously, history of medical morbidities like diabetes and chronic obstructive lung disease were excluded. Data was collected from patient health records for demographic variables, surgical notes and pelvic floor disability index (PFDI-20) before surgery to check the severity of condition. Demographic data included age, body mass index and parity of women. Detailed history was taken to review the risk factors.

Surgery was performed after written informed consent and preoperative preparation. Through a pfannenstiel incision, the abdomen was opened till rectus sheath. A horizontal incision, about 15 Cm long, was made in the rectus sheath. A 2 Cm broad sleeve was retrieved. Incision was extended laterally up to the rectus muscle's lateral border, exposing internal inguinal rings on both sides. Both strips were clamped separately. The peritoneal cavity was opened. Slings were tied to the cervix posteriorly, medial to the uterosacral ligaments, using a Prolene No. 1 suture. The uterus was pulled upward following sling attachment. The rectus sheath was thoroughly sutured, focusing on the inguinal rings. Abdomen was closed in layers in reverse order. (Figure-1).

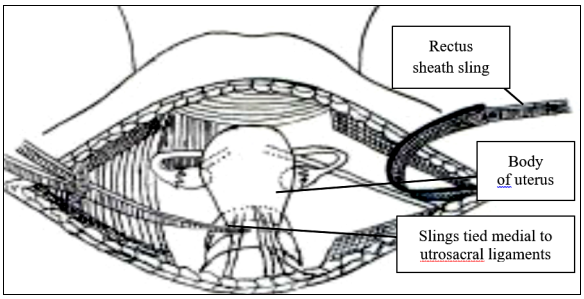

Figure-1: Diagrammatic representation of rectus sheath sling procedure (Rahat-un-Nisa and Parveen Z)[10]

Postoperative coughing and constipation were avoided. Duration of surgery, mean hospital stay, mean blood loss and postoperative complications were recorded. Primary outcome was improvement in symptoms of uterovaginal prolapse. It was measured as PFDI-20 score. The questions were asked if woman had certain symptoms related to bowel, bladder or pelvic organ prolapse and if there were no symptoms, score was 0 and how much symptoms were bothering her on a response scale from 1 to 4; 1= not bothering at all, 2= somewhat bothering, 3= moderately and 4= quite a bit. Score 21–40 was considered as mild, 41–60 as moderate, 61–80 as severe, and 81–100 as very severe.

Patients were called back again 4 times for follow-up after the surgery, 1st at 2nd week, 2nd at 3 months, 3rd follow-up at 6 months, and 4th at one year. Surgical outcome was measured postoperatively one year after surgery in terms of PFDI-20. Difference in mean (Δ Mean) PFDI-20 score was calculated. Δ Mean PFDI-20 score >10 means patients had substantial improvements in their symptoms after surgery.

The data was analysed using SPSS-27. Frequencies and percentages were calculated for categorical variables. Effect modifiers like age, and parity was controlled by stratification and Chi-square test was applied to see their effect on outcome and $p\leq0.05$ was taken as statistically significant.

RESULTS

Fifty patients were included in our research. Mean age was 27.9±11.5 years; 22 (44%) were <30 years, 22 (44%) were 30–40 years and 6 (12%) were aged 41–50 years. Four (8%) women were nulliparous, 30 (60%) were having less than 3 children and 16 (32%) were having 3 or more children. Difference in means (Δ Mean PFDI-20) was 43.4±9.4. Outcome after surgery in terms of PFDI-20 was significantly associated with age ($p<0.001$). (Table-1).

Twenty (40%) women had no rectocoele and 30 (60%) had mild rectocoele. Thirty (60%) women had mild cystocoele and 20 (40%) had moderate cystocoele. Thirteen (26%) women had chronic

cough, 13 (26%) had chronic constipation, 15 (30%) had history of difficult delivery, and 9 (18%) women had history of heavy weight lifting.

Operations were completed in <1 hour in 46 (92%) and >1 hour in 4 (8%) patients. Sixteen (32%) women stayed in hospital for 1–2 days after the surgery and 34 (68%) women stayed for 3–4 days in the hospital. Postoperative complications like fever, urinary tract infection, wound infection and voiding dysfunction were documented. Most common complication was urinary tract infection in 10 (20%) women. (Table-2).

Mean PFDI-20 score before surgery was 55±15.01. It was 11.6±5.61 after surgery. Forty-six (92%) patients reported no symptoms (PFDI score 0–20) and 4 (8%) had mild symptoms (PFDI score 21–40) after surgery. (Table-3).

The cervix was noticed at or above the level of the ischial spines on examination at the time of discharge in all the patients. At one year follow up, 46 (92%) patients were symptom free. Only 4 (8%) were having mild symptoms. Eight patients conceived during the subsequent one year follow-up.

Table-1: Demographics and their association with postoperative success rate measured as PFDI-20 after surgery (n=50)

Variables	PFDI-20 score		Total	
Age Groups	**0–20**	**21–40**	**50**	***p***
<30 years	22	0	22 (44%)	**<0.001**
30–40 years	21	1	22 (44%)	
41–50 years	3	3	6 (12%)	
Parity				
Nulliparous	4	0	4 (8%)	**0.37**
<3	27	3	30 (60%)	
≥3	15	1	16 (32%)	
Body mass index (Kg/m^2)				
18.5–24.9	3	1	4 (8%)	**0.34**
25–29.9	40	0	40 (80%)	
≥30	3	3	6 (12%)	
Prolapse classification				
1st degree	3	1	4 (8%)	**0.19**
2nd degree	43	3	46 (92%)	

Table-2: Postoperative complications (n=50)

Complications	Frequency	Percentage
No complications observed	33	66
Urinary tract infection	10	20
Wound infection	3	6
Fever	2	4
Voiding dysfunction	1	2
Recurrence of prolapse	1	2

Table-3: Pelvic floor disability index score (PFDI-20) among participants

PFDI-20	Before surgery	After surgery
Mean Score	55±15.01	11.6±5.61
No Symptoms	0	46 (92%)
Score 21–40 (mild symptoms)	8 (16%)	4 (8%)
Score 41–60 (moderate symptoms)	22 (44%)	0
Score 61–80 (severe symptoms)	19 (38%)	0
Score 81–100 (very severe symptoms)	1 (2%)	0

DISCUSSION

The technique hasn't been widely reported in the literature. Some studies exist in which rectus sheath slings were utilized for urinary incontinence and utero-vaginal prolapse. A study by Rahat-un-Nisa and Zahida Perveen[10] from Abbottabad also revealed comparable outcomes. Some studies showed 95% success rate with abdominal sacrohysteropexy, however, in many cases, women would undergo reoperations within the first year following the treatment.[11–13] Mesh erosion and infection were also reported as complications. We used the posterior approach to attach of the sling, and the advantages of this technique are as following:

- It is a simple approach
- It is less time-consuming, takes ~40 minutes
- It causes minimal blood loss and poses no danger of bladder injury

Patients who have had previous pelvic surgery, such as a Caesarean section or anterior and posterior vaginal repair, anterior approach will be more challenging. In these circumstances, a posterior strategy seems to be suitable. We observed that modified sling method yields favourable outcomes with fewer problems. We have used PFDI-20 score for assessment and this distinguishes our study from previous work.

In our study 44% women were under 30 years of age and 44% women fell in 30–40 years age group. Zulfiqar S *et al*[4] showed that 55% women were under 30 years of age and 45% of women were under 40 years of age in their study. Mean age of our patients was 27.9±11.5 years in our study. Khan N *et al*[14] have reported mean age of their patients as 43.23±8.29 years in their patients, and in Abid S *et al*[15] study, the mean age of the patients was 30±4.12 years showing quite wide range of the patients' age.

Presentation of prolapse, its severity and degree correlate with parity of the women along with other factors. Sixty percent of our patients had less than 3 children while in study done by Zulfiqar S *et al*[4], 55% women were having less than 3 children. In the study by Khan N *et al*[14] 8.5% were nulliparous, 20% were para 1 while 71.4% were multiparous. In our study, 92% women had 2nd degree uterovaginal prolapse while in Khan N *et al*[14] study 51.4% women had 2nd degree prolapse, and 45% women had 2nd degree prolapsed in study by Zulfiqar S *et al*[4]. Abid S *et al*[15] reported 1st degree prolapse in 60% patients while 2nd degree prolapse was observed in 40% patients. We observed that 92% of women were asymptomatic at 1 year post-surgery. Other 8% of our patient reported mild symptoms. Success rate was reported as 100% by Abid S *et al*[15], and 95% by Zulfiqar S *et al*[4]. The difference in success rate may be due to large sample size in our study. Zulfiqar *et al*[4] included only 20 women while Abid S *et al*[15], included 30 women.

Postoperatively, fever was reported in 4% of women and recurrence was reported in 2% of women. In the study by Zulfiqar *et al*[4], postoperative fever was reported in 5% and recurrence reported in 5% of patients while postoperative fever and recurrence were noted in 2 (6.67%) and 2 (6.67%) patients respectively in study done by Abid S *et al*[15].

CONCLUSION

Rectus sheath sling operation is highly effective, minimally invasive and has fewer complications. These complications can be avoided with judicious use of antibiotics and proper wound care. It is an excellent choice for women wishing to preserve their fertility and for those who cannot endure prolonged anaesthesia. Use of PFDI-20 score can help to understand the true benefits of the procedure in terms of symptomatic improvement and quality of life.

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ORIGINAL ARTICLE

ROLE OF ACTIVATED CHARCOAL IN MITIGATING LEAD-INDUCED HEPATOTOXICITY IN ALBINO WISTAR RATS

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Background: Lead is a pervasive environmental toxin known for its severe impact on human health, particularly targeting vital organs like the liver through mechanisms involving oxidative stress and inflammation. This study was conducted to evaluate the hepatoprotective effects of activated charcoal against lead-induced hepatotoxicity in albino Wistar rats. **Methods:** This quasi-experimental study was conducted from Feb to Aug 2024. Thirty-six male albino Wistar rats were divided into 3 groups (n=12): Group A (control group); Group B (lead-acetate group); and Group C (lead-acetate+activated charcoal group). Blood samples were collected by cardiac puncture and analysed for liver function tests. Liver tissues were harvested for histopathological analysis under high power after H&E staining. **Results:** Group B showed significant increases in AST (79.31±9.79 U/L), ALT (38.28±2.29 U/L), ALP (209.09±8.49 U/L), bilirubin (0.98±0.43 mg/dL), and reductions in albumin (2.88±0.28 g/dL) and total proteins (6.92±0.22 g/dL) (p<0.001). Group C had intermediate values between Groups A and B for these parameters. Glutathione peroxidase and superoxide dismutase levels were highest in Group A, lower in Group B, and intermediate in Group C (p<0.001). Histological examination revealed significant vacuolar degeneration and lymphocyte infiltration in Group B, while Group C exhibited milder histological changes with fewer infiltrations. **Conclusion:** Activated charcoal significantly reduces lead-induced hepatotoxicity in albino Wistar rats, evident from biochemical and histological improvements.

Keywords: Charcoal, Heavy metals, Lead, Lead-acetate, Oxidative Stress

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INTRODUCTION

Lead, a commonly occurring and non-biodegradable heavy metal, causes several physiological as well as biochemical alterations in the body and is a major cause of toxicity in the environment.[1] Over 140,000 deaths occur each year from lead exposure, which is still a serious public health concern in both industrialized and developing nations. Lead is ranked among the top 10 most deadly compounds by the WHO.[2,3]

Lead exposure primarily occurs via the gastrointestinal tract and the respiratory systems, after which it is metabolized in liver and distributed to other organs of the body such as the brain, kidneys, testes, etc.[2] Owing to its metabolic role and its importance in the detoxification of toxins, liver is the first organ which gets affected when lead is either ingested through the gastrointestinal tract or inhaled via the respiratory system.[4] Chronic lead exposure can lead to liver dysfunction and has been implicated in the development of chronic liver disease (CLD) as it causes oxidative stress, inflammation, and fibrosis within liver tissues.[5,6]

Like all heavy metals such as cadmium, nickel, chromium, etc., lead-induced toxicity is attributed to its potential to disrupt the body's homeostatic mechanisms and cause oxidative stress.[4] Lead causes inhibition of the antioxidant enzymatic defences of the body leading to a build-up of free radicals.[2]

Chelation therapy, the administration of substances that find and stick to metals in the bloodstream (ethylenediaminetetraacetic acid, EDTA), dimercapto succinic acid (DMSA), etc., is the treatment of choice for heavy metal poisoning, especially lead.[7]

Activated charcoal, a black, finely split powder, is made by pyrolyzing carbonaceous materials like wood, coconut shells, or petroleum and then oxidizing the substance at high temperatures of 600–900 °C with either air or steam.[8] It can adsorb a wide array of substances, ranging from heavy metals like lead to various industrial pollutants, including dyes and harmful organisms.[8,9] Activated charcoal mitigates lead toxicity primarily through adsorption in the gastrointestinal tract, preventing systemic absorption and interrupting enterohepatic recirculation of lead. Although it does not chelate substances like EDTA or DMSA, its non-specific binding reduces lead bioavailability and indirectly lowers oxidative stress by limiting toxin-induced reactive oxygen species (ROS) production.[10]

Since most antidotes of lead poisoning are biological products, their limited availability and efficacy can be attributed to factors such as their cost, production processes, immunogenic response potential, generation time, and instability issues, which makes it difficult to provide prompt treatment. Therefore, research into easily accessible and natural antidotes is necessary for treating lead poisoning.

The objective of this study was to assess the hepatoprotective effects of activated charcoal against lead-induced hepatotoxicity in albino Wistar rats.

MATERIAL AND METHODS

This quasi-experimental study was conducted from Feb to Aug 2024, in Departments of Pharmacology and Physiology, Isra University Hyderabad. Following approval by Ethical Review Board, 36 male albino Wistar rats, aged 8–10 weeks, weighing 200–250 grams were included. All animals were procured from the Agriculture University of Tando Jam. The standard power analysis method for animal studies was employed for sample size calculation.[11–14]

The experimental animals were allowed to acclimate for two weeks under regulated conditions (temperature maintained at 22±2 °C, relative humidity 50–60%, and a 12-hour light/dark cycle). During this period, they were provided standard chow diet and clean water *ad libetum*. The animals were assigned to 3 groups, each consisting of 12 rats. Group A (control group), Group B (lead-acetate group), and Group C (lead-acetate+activated charcoal group). The doses of lead-acetate (60 mg/Kg by oral gavage) and activated charcoal (1 gm/Kg by oral gavage) were based on previous studies.[8,15] Lead-acetate (GRM756) and activated charcoal powder were procured from local resources. The body weights of all animals were noted before the commencement of the experiment.

After 30 days, all experimental animals were weighed and sacrificed by cervical dislocation following anaesthesia using chloroform-soaked cotton in a container. Blood samples were collected via cardiac puncture and transferred to gel tubes, where they were left to stand for 30 minutes before being centrifuged to separate the serum, which was then stored for analysis of liver enzymes (ALT, ALP, AST). In addition, total serum bilirubin, serum total proteins, and serum Albumin were also assessed using an automated analyser. The liver was excised and, after rinsing with saline, was preserved in 10% formalin. Using a microtome, hepatic tissue slices were created, and then they were stained with haematoxylin and eosin (H&E) for histological examination.[16]

Data were analysed using SPSS-25. The results are presented as Mean±SD. One-way ANOVA followed by post hoc Tukey's test were applied with a significance threshold of $p \leq 0.05$.

RESULTS

The pre-experimental body weights of groups A, B, and C were 240.11±8.3 gm, 242.58±6.7 gm, and 239.93±7.2 gm respectively. A significant difference was observed in the post-experimental body weight across all three groups. Animals in the control group A showed increased body weight (247.32±8.8 g), while those in lead-treated group B experienced a significant decrease (201.58±11.31 g). A similar decline was observed in group C (213.49±10.76 g), though the weight loss in group C was less severe compared to group B (Table-1).

There was a significant increase ($p<0.001$) in the levels of liver function markers, AST, ALT, ALP, and bilirubin in lead-treated animals (group B) when compared with control group A. These liver function markers decreased significantly ($p<0.001$) in group C. The serum albumin levels and total proteins were markedly lowered in group B compared to control group A ($p<0.001$). However, no significant difference was observed between group A and group C (Table-1).

There was a significant ($p<0.001$) decline in the serum levels of antioxidants including Glutathione peroxidase and Superoxide dismutase in group B when compared with control group A. similar decline was also observed in group C, though it was less severe compared to group B (Table-2).

The histological evaluation showed normal hepatic architecture in the control group A, i.e., intact hepatocytes surrounding normal central veins. However, there were marked alterations seen in group B which showed vacuolar degeneration of peripheral hepatocytes with lymphocyte infiltration in the peripheral areas. Group C showed significantly reduced histological alterations with slight infiltration of inflammatory cells around the portal areas.

Table-1: Distribution of body weight and liver function markers across experimental groups

	Group A	Group B	Group C	*p*
Post-experimental body weight (gm)	247.32±8.8^{B}	201.58±11.31^{A,C}	213.49±10.76^{B}	<0.001
AST (U/L)	56.84±1.84^{B}	79.31±9.79^{A,C}	51.06±6.12^{B}	<0.001
ALT (U/L)	17.55±3.71^{B}	38.28±2.29^{A,C}	20.67±5.48^{B}	<0.001
ALP (U/L)	178.45±4.21^{B}	209.09±8.49^{A,C}	181.64±12.68^{B}	<0.001
Bilirubin (mg/dL)	0.29±0.15^{B}	0.98±0.43^{A,C}	0.41±0.58^{B}	<0.001
Albumins (g/dL)	4.19±0.27^{B,C}	2.88±0.28^{A,C}	3.83±0.39^{A,B}	<0.001
Total proteins (g/dL)	8.32±0.16^{B,C}	6.92±0.22^{A,C}	7.83±0.61^{A,B}	<0.001

Note: Different superscript letters (A, B, C) indicate statistically significant differences ($p<0.001$) between these groups

Table-2: Distribution of oxidative stress markers across experimental groups

	Group A	Group B	Group C	*p*
Glutathione peroxidase (U/L)	461.37±34.81^{B,C}	258.25±67.14^{A,C}	349.89±84.21^{A,B}	<0.001
Superoxide dismutase (U/mL)	138.16±8.32^{B}	118.48±6.81^{A,C}	135.68±3.39^{B}	<0.001

Note: Different superscript letters (A, B, C) indicate statistically significant differences ($p<0.001$) between these groups

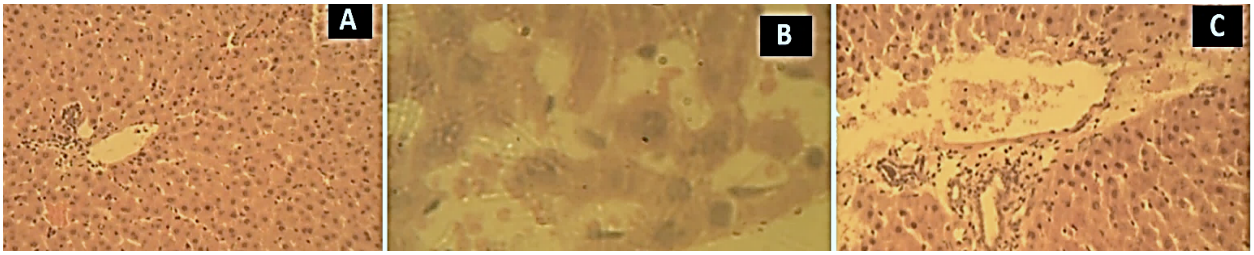

Figure-1: Photomicrographs of liver tissue of experimental groups stained with H&E

DISCUSSION

Lead is one of the most dangerous heavy metals that significantly impacts public health. Lead exposure commonly occurs through industrial emissions, contaminated water supplies, ingestion of lead-based paints or contaminated food, and occupational contact in battery manufacturing, mining, and smelting industries.[17] Contrary to popular belief, encountering a small amount of heavy metals like lead may play a far larger role in the development of chronic illnesses like diabetes, liver dysfunction, renal disease, cancer, and male infertility.[18] Chelators, the only antidote for lead exposure that is currently on the market, have several negative side effects, including gastrointestinal problems, and discomfort.[19] Certain chelators can be difficult to administer but also costly and difficult to find. Furthermore, the Centers for Disease Control advises against using chelation therapy unless necessary and the blood lead concentration is more than 45 ug/dL.[20]

The administration of lead-acetate to rats in this research resulted in a notable elevation of blood AST, ALT, ALP, and bilirubin, accompanied by a decrease in albumin and overall protein levels which is consistent with the findings of Abubakar *et al.* and Abdel Fattah *et al*.[2,21] Elevations in liver enzymes in rats administered lead-acetate indicates damage to the liver's underlying structure. This damage occurs due to hepatocyte necrosis, leading to the release of these enzymes into the bloodstream. Lead exposure disrupts the lipid composition of cellular membranes, increasing their fluidity and compromising structural integrity. This alteration facilitates the leakage of intracellular enzymes such as AST and ALT into the bloodstream. Simultaneously, lead interferes with antioxidant defence systems, promoting excessive generation of ROS. These ROS initiate lipid peroxidation, protein oxidation, and DNA damage, ultimately leading to cellular dysfunction and hepatocyte necrosis.[22] Asiwe *et al*, and Abdelhamid *et al*, also reported similar findings where lead administration was associated with marked liver dysfunction as reflected by an elevation of plasma ALP, AST, and ALT levels.[23,24]

However, these changes were reversed by administering activated charcoal in this study. This aligns with the findings of Offor *et al*[8], who reported that charcoal administration significantly reduced the elevated plasma levels of liver enzymes caused by lead toxicity. This is attributed to the ability of activated charcoal to absorb potentially harmful substances including drugs, plant-based toxic substances, and poisonous chemicals onto its surface, and in doing so preventing their absorption from the gastrointestinal tract into the bloodstream. It also interrupts enterohepatic circulation, which acts as a secondary decontamination mechanism.[25] This is also consistent with the findings of Zhao *et al*, who studied the hepatoprotective effects of charcoal-processed products and observed that all doses reduced the ALT and AST levels, especially the high dose.[26]

Lead administration was associated with marked oxidative stress in the present study as reflected by a significant ($p<0.05$) depletion of oxidative markers in group B. This is due to the underlying fact that free radical generation is one of the most important mechanisms involved in lead-induced toxicity. In doing so, lead exposure disrupts the pro-oxidant and anti-oxidant balance of the body, leading to excessive ROS production, cellular antioxidant depletion, and, consequently, DNA and protein damage, mitochondrial impairment, and ultimately apoptosis.[24] Albasher *et al*. reported a significant decline in the levels of markers of oxidative stress (SOD, GPX, etc.) in lead-treated animals, which is consistent with the findings of the present study.[27] Charcoal administration, however, was associated with significant ($p<0.05$) less oxidative stress in group C. As mentioned above, activated charcoal has the inherent ability to adsorb potentially harmful substances onto its surface, preventing their absorption from the gastrointestinal tract into the bloodstream. By binding to these substances, activated charcoal indirectly helps reduce oxidative stress by limiting the availability of toxins that can generate ROS within the body.[25,28] Offor *et al*. also reported in their study that the treatment of rats with activated charcoal caused a significant increase in Glutathione Peroxidase and Superoxide Dismutase levels as compared to lead-treated animals, which is consistent with our findings.[8]

Lead administration was also observed to cause significant alterations in hepatic histological architecture in the present study. This is similar to the findings of Abubakar *et al*, who noted that lead

administration was associated with congestion, infiltration of inflammatory cells, and activation of resident hepatic macrophages, which aligns with our findings.[2] Zou *et al*, also reported similar findings, noting that lead administration resulted in cells exhibiting degenerative and necrotic features indicative of microvesicular steatosis.[29] In contrast, charcoal administration was associated with a marked decrease in these hepatic alterations in the current study. This conforms with Offor *et al*, who observed that activated charcoal administration significantly reduced histological markers of hepatic dysfunction, such as inflammatory cell infiltration, congestion, and vacuolar degeneration.[8]

This study highlights the potential hepatoprotective effects of activated charcoal in mitigating lead-induced hepatotoxicity. These findings underscore the potential of activated charcoal as a cost-effective and accessible therapeutic agent for mitigating lead-induced hepatotoxicity. Future research should explore its protective effects across other organ systems and evaluate its efficacy in long-term exposure models. However, certain limitations should be acknowledged, including the lack of assessment of oxidative stress and inflammation markers, which could have provided further insight into the potential anti-inflammatory and antioxidant effects of charcoal. Additionally, the study focused solely on the hepatotoxic effects of lead and the hepatoprotective effects of charcoal, without evaluating other organ systems, such as renal, nervous, and reproductive systems.

CONCLUSION

Activated charcoal exerts a potent hepatoprotective effect against lead-induced hepatotoxicity in albino Wistar rats. This hepatoprotective effect can be attributed to the anti-oxidant properties of activated charcoal. In cases of persistent lead-induced liver deterioration, activated charcoal might be helpful.

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ORIGINAL ARTICLE

A PROSPECTIVE COMPARISON OF NON-OPERATIVE AND OPERATIVE MANAGEMENT OF HUMERUS MID-SHAFT FRACTURES

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Background: Humerus shaft fractures are common long bone fractures and the treatment options range from conventional plaster application to plate osteosynthesis. This study aimed to determine outcomes of non-operative *vs* operative techniques in managing mid-shaft humerus fractures. **Methods:** This randomized control trial was conducted in Department of Orthopaedics, Ayub Teaching Hospital, Abbottabad, from 10 Oct 2022 to 9 Apr 2023. Patients aged 20–70 years with unilateral, displaced, and closed humeral shaft fracture were included using non-probability consecutive sampling, after approval of hospital ethical committee and written informed consent of patients. Patients were randomly divided into two groups. Patients in group A were treated with operative technique and patients in group B were treated with non-operative technique. Outcomes were observed on 12 weeks' follow-up. Data was entered and analyzed using SPSS-22. **Results:** One-hundred-fifty patients were enrolled, 75 patients in each group. Mean age was 43.75±14.7 years in group A and 40.64±14.9 years in group B. There were 58.7% males in group A and 61.3% in group B. Females were 41.3% in group A and 38.7% in group B. In group A non-union was present in 5.3%, malunion in 10%, and radial nerve injury in 5.3% patients. In group B non-union was present in 17.3%, malunion in 26.7% and radial nerve injury in 21.3% patients. All these showed statistically significant difference with *p*-value 0.020, 0.041 and 0.004 respectively. **Conclusion:** Operative technique is better than non-operative technique in management of mid-shaft humerus fracture.

Keywords: High energy trauma, Humerus fracture, Internal fixation, Non-operative technique, Open reduction

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INTRODUCTION

Humeral shaft fractures account for 1–3% of all fractures.[1] Globally reported annual incidence is 12 per 100,000 adults.[2] They are usually caused by simple falls, motor vehicle crashes, and sports injuries.[3] Humeral shaft fractures have a bimodal age distribution with the first peak seen in men aged 21 to 30 years following high-energy trauma, commonly resulting in comminuted fractures with associated soft tissue injuries. The second peak is witnessed in women aged 60 to 80 years, typically following low-energy trauma.[4] Fractures caused by low-energy trauma, such as fall from standing height, should raise the suspicion of poor bone quality associated with osteoporosis or oncologic disease. Patients present with pain, disability, a swollen upper extremity and visible deformity.[4] The skin must be carefully inspected to rule out an open wound. The initial neurological examination is then carried out, specifically focusing on radial nerve function. It is critical to establish and record the baseline function of the radial nerve. The first examination can be limited by pain and therefore it is essential to repeat this after pain control has been achieved. It is also mandatory to reassess the radial nerve function before and after any manipulation and/or treatment (cast, surgery, etc.).[4]

The treatment of humeral shaft fractures is still controversial. In most of these fractures, treatment is nonsurgical.[5] Controversies still exist whether surgical intervention is needed for humeral shaft fractures. Surgical treatment is generally recommended for fractures with large displacement angles, multiple fractures, comminuted fractures, and fractures complicated with vascular and nerve injury.[6,7]

In a study comparing non-operative technique with operative technique, main outcome measures were evaluated retrospectively and included non-union, mal-union and incidence of radial nerve palsy. The occurrence of non-union and malunion was statistically significant and more common in the non-operative group.[8]

Mid-shaft humerus fractures are very common in our population[1] and its treatment is mainly non-operatively as described in previous studies.[6,7] But risk of radial nerve injury make it necessary to review our treatment plan.[8] The objective of this study was to determine and compare outcome of non-operative and operative techniques in managing mid-shaft humerus fracture. Our results will help development of best practice guidelines for management of mid-shaft humerus fractures.

MATERIAL AND METHODS

A randomized control study was carried out at Department of Orthopaedics, A Ward, Ayub Teaching

Hospital Abbottabad from 10 Oct 2022 to 9 Apr 2023. Non-probability consecutive sampling technique was used. Sample size was calculated using WHO Sample Size Calculator keeping 80% power of study, 10% absolute precision, keeping mal-union in non-operative as 12.7% and in operative group as 1.3%.[8] Sample size was 75 patients in each group.

Inclusion criteria was patients aged 20–70 years of either gender who presented with unilateral, displaced, and closed humeral shaft fracture, as seen on X-ray. Exclusion criteria included pathological fractures and other concomitant injuries affecting the same upper limb. The patients with internal organ, brachial plexus, or vascular injury requiring surgery and poly-trauma patients were excluded.

After approval from hospital ethical board, patients fulfilling the inclusion criteria were enrolled from surgical emergency of Ayub Teaching Hospital, Abbottabad. A written informed consent was taken after explaining the purpose of study. Demographic data including age, gender and duration of injury was recorded. Complete history was taken and physical examination was done.

Patients were randomly divided into 2 groups using block randomization. Patients in group A were treated surgically. A standard open reduction and internal fixation was carried out with plate and screws (Figure-1). The surgery was performed within 14 days of the fracture. Patients were allowed to move their arm immediately postoperatively but were instructed to avoid weight-bearing until 6 weeks after surgery. In group B, patients were treated non-operatively. First, hanging slap was advised for 7 to 10 days; that was converted to functional brace after pain control. Trained plaster technician applied a functional brace that covered the arm from shoulder to elbow but left the motion of both these joints free. Patients were given instructions on how to cope with the brace and how to tighten it as the swelling subsided and were instructed to wear the brace until fracture union. Active non-weight-bearing exercises of the elbow and hand and pendulum exercises of the shoulder was allowed immediately, followed by assisted exercises of the shoulder at 3 weeks, and finally, gradual weight-bearing at 6 weeks.

At the end of 12 weeks patients were assessed in out-patient department for union, malunion and radial nerve injury. Data was entered in specially designed proforma. Non-union was defined as absence of bridging fracture callus in 3 of the 4 cortices on digital X-ray humerus (AP and lateral view). Mal-union was defined as varus angulation of humerus at the fracture site. Radial nerve injury meant presence of wrist drop and loss of sensations in lateral aspect of arm.

Data was entered and analysed using SPSS-22. Mean and standard deviations were calculated for quantitative variables like age and duration of injury. Frequency and percentage were calculated for categorical variables. Outcome was compared in both groups using Chi-square test, and $p \leq 0.05$ was taken as statistically significant.

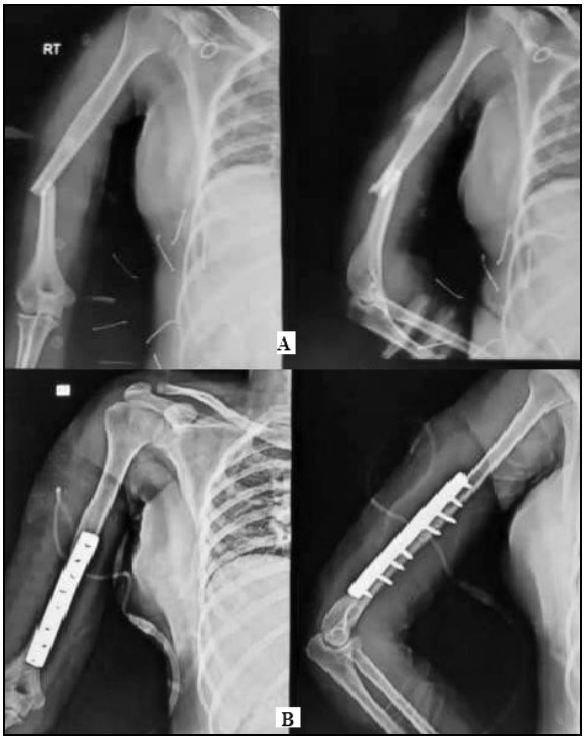

Figure-1: (a) X-ray of mid-shaft humerus; (b) Postop X-ray of same patient after ORIF with broad DCP

RESULTS

Total 150 patients were enrolled in the study, 75 patients in each group. Mean age of the patients was 43.75±14.7 years in group A and 40.64±14.9 years in group B. There were 58.7% males in group A and 61.3% in group B, females were 41.3% in group A and 38.7% in group B. Mean duration of injury was 5.65±2.2 days in group A and 5.5±2 days in group B. none of these preoperative measurements differed significantly among the study groups. (Table-1).

In group A non-union was present in 5.3%, which was significantly lower than the non-union in the group B (p=0.020). Malunion rate was significantly lower in group A, i.e., 10% compared to rate in non-operative group (26.7%), (p=0.041). Radial nerve injuries were less common in operative group, i.e., 5.3% in comparison with 21.3% in the non-operative group (p=0.004). (Table-2).

Table-1: Comparison of preoperative variables among the two management groups

Variable	Group A	Group B	*p*
Males	44	46	0.739
Females	31	29	
Mean Age (Years)	43.72±14.79	40.64±14.91	0.206
Duration of injury (Days)	5.65±2.26	5.56±2.06	0.792

Table-2: Comparison of outcome variables among management groups

Variable	Group A	Group B	*p*
Non-union (Yes/No)	4/71	13/62	0.020
Malunion (Yes/No)	10/65	20/55	0.041
Radial nerve injury (Yes/No)	4/71	16/59	0.004

DISCUSSION

Humeral shaft fracture typically occurs following high-energy trauma, commonly resulting in comminuted fractures with associated soft tissue injuries. The second peak is witnessed in women aged 60 to 80 years, typically following low-energy trauma.[4] Several options are possible for the management of HSF: conservative management, open reduction and internal fixation (ORIF) with a plate, or closed reduction and intramedullary nailing (IMN). An external fixator is also an option, however rarely indicated. Un-displaced or minimally displaced HSF are routinely treated conservatively. In fact, anterior angulation of 20°, a varus or valgus of 30°, 15° of mal-rotation and 3 Cm of shortening have been shown to adequately maintain the upper limb function.[9] For this reason, fractures that are displaced within these values following immobilization are good candidates for non-operative technique. Union rate with this technique ranges between 77.4% and 100%.[10] In a review including almost 2000 patients, Papasoulis *et al*[10], reported a union rate of 94.5% and a mean time to union of 10.7 weeks. Ali *et al*[11], observed an overall union rate of 83% on a series of 138 fractures of the humeral mid-shaft fracture treated with functional bracing. Papasoulis *et al*[10] reported a decrease in clinical scores with a residual deformity of less than 10° of varus in more than 85% of their patients. We compare outcomes of operative versus non-operative management of mid-shaft humerus fracture.

In a study[8] non-operative technique was compared with operative technique. Main outcome measures were evaluated retrospectively and included non-union, malunion and incidence of radial nerve palsy (RNP). The occurrence of non-union (20.6% *vs* 8.7%, p=0.013) and malunion (12.7% *vs* 1.3%, p=0.001) was statistically significant and more common in the non-operative group.[8] Union rate after plating ranges from 87% to 96%, with an average time to union of 12 weeks.[12] The complication rate ranges from 5% to 25%,[13] most commonly found to be non-specific complications such as infection, non-union and malunion. Iatrogenic RNP is a risk with most approaches to the humeral shaft, and Streufert *et al*[14] reviewed 261 HSF treated with ORIF, iatrogenic RNP occurred in 7.1% of anterolateral, 11.7% of triceps-splitting and 17.9% of triceps-sparing approaches. It is vital that the radial nerve is identified and protected in all open dissections.

In a meta-analysis it was found that non-operative management resulted in a significantly higher non-union rate of 17.6% compared to 6.3% with fixation. Operative management had a significantly higher iatrogenic nerve injury rate of 3.4% and infection rate of 3.7%. All non-unions within the included studies went on to union after plate fixation. There were no significant differences in delayed union or patient-reported outcome measures. There was a significantly increased risk of malunion with non-operative treatment, however, this did not correlate with the outcome.[15]

STUDY LIMITATIONS

It was a single-centre study with limited number of cases. A multi-centre study with larger study group will provide higher level of evidence that can be used for formulation of best management guidelines.

CONCLUSION

Operative management of mid-shaft fracture of humerus is associated with decreased non-union, malunion and radial nerve injuries compared to conservative management.

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ORIGINAL ARTICLE

A NOVEL DEPSIDE SEKIKAIC ACID IS PROTECTIVE AGAINST CYCLOPHOSPHAMIDE INDUCED CARDIOTOXICITY IN RATS

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Background: Cyclophosphamide is a widely prescribed chemotherapeutic agent. Its toxic metabolites induce oxidative stress which burdens the cardiac tissue. With recent advancements in gaining deeper insight in natural antioxidants, Sekikaic acid (SA) is gaining popularity as a novel depside compound with significant organ protective potential. This study seeks to explore and assess the cardio-protective and antioxidant effects of sekikaic acid against anti-cancer drug toxicity. **Methods:** Experimental animals were divided into three groups: normal untreated control, cyclophosphamide toxic control, and cyclophosphamide with sekikaic acid group where sekikaic acid was administered for 14 continuous days followed by a single dose of cyclophosphamide. For assessing myocardial injury serum cardiac biomarkers (creatine kinase, lactate dehydrogenase, troponin I, C-reactive proteins) and tissue oxidative stress markers (glutathione, super-oxide dismutases and malondialdehyde) were evaluated. **Results:** The control group demonstrated normal physiologic values of serum and tissue biomarkers. The toxic control demonstrated signs of cardiac injury with a single dose of cyclophosphamide, with raised levels of serum and tissue oxidative stress markers alongside receding natural antioxidant pools of glutathione and super-oxide dismutases. On the contrary the oxidative burden on myocardium was significantly reduced ($p \leq 0.05$), and cellular antioxidants were preserved with co-administration of sekikaic acid validating its potential as a cardioprotective agent. **Conclusion:** The antioxidant potential of sekikaic acid can effectively ameliorate cyclophosphamide induced cardiotoxicity and can be incorporated in cyclophosphamide containing chemotherapeutic and immunosuppressive regimens.

Keywords: Cardiotoxicity, Cyclophosphamide (CP), Oxidative stress, Sekikaic acid (SA)

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INTRODUCTION

Cyclophosphamide (CP), is a classic alkylating agent with a global utilization as an immunosuppressant and cancer chemotherapeutic. It is a prodrug which undergoes biotransformation by CYP450 isozymes. Its therapeutic end-product is phosphoramide mustard which produces its cytotoxic action by formation of deoxyribonucleic acid (DNA), adducts and immunosuppression by activation of T-lymphocytes and myeloid derived suppressor cells. The dose-limiting factor is the concurrent production of the toxic byproduct acrolein.[1]

Acrolein can cause cardiac damage by inducing nitrative and oxidative stress raising concern about rocketing prevalence of post chemotherapy cardiovascular events including myocardial ischemia/ infarction; ventricular arrythmias and hypertension. The various aetiological pathways identified so far include direct injury to vascular endothelium, lipid peroxidation raising serum malondialdehyde levels, exhaustion of nicotinamide dinucleotide (NAD^+) and adenosine triphosphate (ATP) leading to altered mitochondrial respiration and electron transport chain, and formation of acrolein-protein ducts with glutathione pools.[2]

Sekikaic acid (SA) gained its explorative potential after Ramalina species became renowned for their biopharmaceutical potential. SA is a carbonyl compound extracted from lichens particularly Ramalina species.[3] Sekikaic acid was discovered around 2007 as a bioactive compound.[4] It has a complex depside structure with benzoic acid derived polyphenolic aromatic rings bonded with ester linkages. This unique structure has the capability to scavenge and neutralize reactive oxygen species (ROS) including superoxide anions (O_2^-), nitric oxide (NO), peroxyl radicals (ROO•), and hydroxyl radicals (OH•) by donation of a hydrogen atom from its hydroxyl groups. It further inactivates lipid peroxidation helping conserve the integrity of cellular and organelle membrane.[5]

Sekikaic acid and its analogues are under research regarding their anti-oxidant potential. While there is no direct evidence linking SA to cardioprotective effects, its antioxidant and enzyme-inhibitory properties suggest it could have a role in reducing cardiovascular risk factors. Since the cardiotoxicity by CP attains its pathology via oxidative stress we have tried to explore the protective potential of SA against CP since the scavenging ability of this compound against 2,2-diphenyl-1-picrylhydrazyl (DPPH) which is one of the most crucial oxidative biomarkers involved in cardiac injury was established by fellow researchers.[6–8]

This study, using a mouse model, aimed at assessing the cardio-protective and antioxidant effects of Sekikaic acid against anti-cancer drug toxicity. The longitudinal objective was to optimize the CP containing anticancer and immunosuppressants regimen.

MATERIAL AND METHODS

This laboratory-based experimental study was conducted in the Department of Pharmacology, Army Medical College, Rawalpindi, after obtaining approval from the Ethical Review Committee (ERC ID No. 11/2024/435).

Commercial preparations of injectable CP were procured from Pharmedic Laboratories, Pakistan. SA was collected and further processed via extraction by Soxhelt method by Global Pharmaceuticals Islamabad on request.[7] One mg/mL solution was formulated with 0.5% dimethyl sulfoxide (DMSO) to ensure safe oral consumption.

Homogeneous population of 36 Sprague Dawley rats, aged 6–8 week and weighing 200–250 grams were included via non-probability convenient sampling. Pregnant, diseased and younger animals were excluded. The rats were kept under standard housing conditions. The animals were divided into three equal groups, (n=12 each) via simple randomization technique. Each group received following respective protocol for 14 days.[6] (Table-1).

Table-1: Experimental groups and treatment protocol

Group	Description	Treatment	Time point of Sample Collection
Control Group	Normal baseline group	No Treatment	14th Day of study
CP Group	Toxic Control	Single dose of 200 mg/Kg CP (IP) on 14th day	24 hours post CP administration on 15th day of study
CP+SA Group	Treatment group, receives SA and CP	2 mg/Kg SA was administered orally once daily for consecutive 14 days. One hour after the last dose of SA on day 14, a single injection of 200 mg/Kg of CP was administered IP	24 hours post CP administration on 15th day of study

The procedure for sample collection was performed in a bio-safety cabinet ensuring standard safety protocols. Euthanasia was done with drop jar method using 30% v/v isoflurane in propylene glycol. The blood was drawn through cardiac puncture and exsanguination for facilitating tissue sampling via mid-line sternotomy. The serum was separated by centrifugation. The tissue homogenate was prepared in isotonic phosphate saline buffer and centrifuged to obtain the supernatants.

Biochemical markers including creatine kinase-myocardial band [CK-MB], lactate dehydrogenase (LDH) and C-Reactive Proteins (CRP) were calculated by automated high-throughput chemistry analyzer (Selectra XL) effectively within 1–1.5 mL of the blood sample and Troponin-I was estimated by with commercial Cardiac Troponin I (cTnI) Rapid Test Kit utilizing a blood volume of 75–90 µL. Markers of oxidative stress including glutathione [GSH], malondialdehyde [MDA] and superoxide dismutases (SOD) were assessed by Sandwich Enzyme-Linked Immunosorbent Assay (ELISA) with commercial kits.

The data were recorded on Microsoft Excel Sheets as Mean±Standard Error of Mean and analysed using SPSS-22. Quantitative variables were compared through ANOVA followed by Post Hoc Tuckey test, and $p \leq 0.05$ was considered significant.

RESULTS

To assess the cardiotoxicity each parameter with CP alone was compared with baselines. There was high statistical significance as CP markedly increased the tissue injury indicators, i.e., CK-MB (765.33±39.89 IU/L), LDH (2790.83±89.3 IU/L), Troponin-I (21.54±3.44 pg/mL), CRP (72.8±6.28 mg/mL) and MDA (2.87±0.006 U/mL) ($p<0.05$). Simultaneously there was reduction in endogenous antioxidant GSH (0.5±0.03 ng/mL) and SOD (100.43±5.22 U/mL) ($p<0.05$ and $p<0.01$ respectively).

The SA+CP group helped to gauge antioxidant potential of SA by reducing the deranged CK-MB (365.66±15.39 IU/L), LDH (1,529±10.23 IU/L), Troponin-I (15.43±0.97 pg/mL), CRP (54.48±4.99 mg/mL) with a remarkable statistical significance ($p<0.001$). Our values validated the potential of SA against cardiotoxicity caused by CP. SA actively reduced the deranged values of MDA (2.02±0.05 U/mL) ($p<0.001$) and restoration of SOD (122±3.11 U/mL) reserves ($p<0.01$). The glutathione levels with SA were numerically but not significantly raised compared to toxic controls ($p=0.12$). (Table-2).

Table-2: Comparison of quantitative values of serum and tissue oxidative stress markers indicative of cardiac injury

Parameters (Units)	Control Group	CP Group	SA+CP Group	*p*
CK-MB (IU/L)	75.16±19.69	765.33±39.89	365.66±15.39	<0.001
LDH (IU/L)	900.89±8.24	2790.83±89.3	1529±10.23	<0.001
Troponin-I (pg/mL)	5.96±0.92	21.54±3.44	15.43±0.97	<0.001
CRP (mg/mL)	20.09±0.9	72.8±6.28	54.48±4.99	<0.05
GSH (ng/mL)	0.98±0.12	0.5±0.03	0.847±0.32	0.12
MDA (U/mL)	1.75±0.02	2.87±0.006	2.02±0.05	<0.001
SOD (U/mL)	152.58±2.32	100.43±5.22	122±3.11	<0.01

ANOVA and post Hoc Tuckey Test applied

DISCUSSION

Cyclophosphamide (CP) has a dose-dependent cardiotoxicity mediated by its metabolite acrolein. Acrolein's affinity for thiol groups leads to glutathione depletion, lipid peroxidation, and irreversible damage to cellular membranes.[1] Recent data suggests upregulation of inflammatory cytokines (e.g., TNF-α, IL-6), activation of NF-κB, and disruption of mitochondrial membrane potential, releasing cytochrome c and activating caspase-mediated apoptosis.[9] This cascade is also substantiated in our study with a significant rise in serum CRP, aligning our findings with the pro-inflammatory milieu reported earlier.[1,2,9] This suggests that CP toxicity model not only validates the sustained oxidative stress burden but also shows its translational potential in mimicking the clinical signs of CP induced cardiotoxicity.

These lethal effects warrant the pursuit of compounds with dual antioxidant and anti-inflammatory potential. In the present study, Sekikaic acid (SA), a lichen-derived polyphenolic depside demonstrated a significant amelioration of CP-induced cardiotoxicity. To our knowledge, this is the first *in vivo* report exploring the cardioprotective effects of SA in a CP-induced toxicity model. The novel findings highlight the candidature of SA as an adjuvant therapy in CP based chemotherapeutic regimens.

The observed improvements in serum biomarkers, i.e., CK-MB, LDH, Troponin-I, and CRP alongside favourable regulation in oxidative stress markers such as GSH, SOD, and MDA, strongly support the hypothesis that SA confers tissue protection via redox modulation, likely by scavenging reactive oxygen species (ROS) and up-regulating endogenous defence systems. SA is structurally rich in functional hydroxyl and carbonyl groups, which are ideal for electron donation and proton transfer, the rudimentary mechanics behind radical scavenging.[8] Various natural antioxidants quercetin, resveratrol, and curcumin, among others, have shown protective effects against CP or doxorubicin-induced cardiomyopathy through similar redox-sensitive pathways.[8] SA shares structural and functional resemblance to these compounds, yet its utility remains underexplored in cardiovascular settings. Importantly, the significant decrease in MDA and increase in SOD and GSH levels in our treatment group is consistent with other antioxidant intervention studies involving N-acetylcysteine, melatonin, and ellagic acid.[10,11]

Research has indicated that SA exhibits inhibitory effects on key metabolic enzymes α-glucosidase, which indirectly contributes to cardiovascular health by reducing hyperglycaemia induced cardiac stress.[12] SA exhibits potent antidiabetic effects in streptozotocin-induced diabetic rats, by significant reduction of blood glucose levels and promoting pancreatic β-cell regeneration, attributed to its inhibition of carbohydrate digestive enzymes.[5,13] SA is reported to have hypolipidaemic affects by reducing LDL and total triglycerides and cholesterol levels. Strengthening its cardiometabolic protective role.[13]

SA may also exert cardioprotection by modulating inflammatory and apoptotic pathways. Several *in-silico* docking studies and *in vitro* enzyme assays suggest that SA interacts with cyclooxygenase-2 (COX-2) and 5-lipoxygenase, inhibiting pro-inflammatory mediators. It also inhibits key regulatory cardiotoxic enzymes including matrix metalloproteinases (MMPs) and nitric oxide synthase (NOS).[14]

While not directly studied for SA, related compounds like caffeic acid have demonstrated vasorelaxant effects through the endothelial nitric oxide (NO) pathway. This involves the activation of endothelial NOS, leading to increased NO production, which is crucial for vascular relaxation and overall cardiovascular health.[4] It is plausible that SA may exert similar effects given its structural properties. Some studies on related compounds suggest that they may interact with adrenergic receptors, which play a significant role in regulating heart rate and vascular tone. The potential for SA to influence these receptors remains an area for further exploration.[4,14]

SA reduced the C-reactive protein levels to a significant extent when administered with CP. This C-reactive protein reduction was also demonstrated by the compound and its analogue in an arthritic murine model where CRP levels were reduced significantly in murine model of rheumatoid arthritis.[15] The significant reduction in CRP levels observed in our study aligns with murine models of arthritis and colitis treated with SA, where it dampened systemic inflammation and modulated immune responses. This dual antioxidant and anti-inflammatory capacity makes it an ideal candidate for further exploration in cardio-oncology.

These findings encourage incorporation of SA as a complementary agent in CP-containing regimens, especially considering the lack of approved drugs specifically targeting CP-induced cardiotoxicity. As in comparison to the synthetic agents like dexrazoxane, which interferes with anticancer efficacy or have own toxicities, natural agents like SA offer a safer multi-oriented approach.[16]

While SA shows potential through its antioxidant and enzyme-inhibitory activities, more targeted research is needed to fully understand its cardioprotective properties and mechanisms of action. This study opens avenues to evaluate the molecular mechanisms of SA in myocardial tissue, particularly its impact on Nrf2/ARE, NF-κB, and PI3K/Akt signalling pathways.[8,10] Studies for evaluation of its efficacy with other established antioxidants in CP cardiotoxicity models can bring validation to its potential.

CONCLUSION

The study highlights the protective role of SA against CP induced cardiotoxicity by its antioxidant, anti-inflammatory and enzyme inhibitory pathways. Sekikaic acid successfully restored both serum and tissue oxidative stress markers against CP induced oxidative burden. While these results are encouraging, they warrant further research towards exploration of molecular landscapes and clinical trials to explore the feasibility of adding SA in existing chemotherapeutic regimens.

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ORIGINAL ARTICLE

MANAGEMENT OF INFANTILE HYPERTOPHIC PYLORIC STENOSIS WITH OPEN PYLOROMYOTOMY

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Background: Infantile hypertrophic pyloric stenosis (IHPS) is a common cause of gastric outlet obstruction in early infancy, primarily affecting males. Prompt diagnosis and surgical intervention are essential to prevent complications and ensure favourable outcomes. Objective of this study was to evaluate the characteristics and outcomes of surgical management of IHPS using open pyloromyotomy. **Methods:** A case-series conducted at the Paediatric Surgery Department, Bahawal Victoria Hospital, Bahawalpur from 31 Jan to 30 Jun 2023. Forty-seven children aged 1 day to 3 months diagnosed with IHPS and scheduled for open pyloromyotomy were included. Diagnosis was based on non-bilious projectile vomiting and/or palpable pyloric mass, confirmed on ultrasonography. All underwent surgery under general anaesthesia following standard protocols. **Results:** Of 47 infants, 32 (68.1%) were boys and 15 (31.9%) girls. Mean age was 5.1±3.2 weeks; 34 (72.3%) were aged 2–6 weeks. Preterm birth and Caesarean delivery were recorded in 28 (59.6%) each. Common symptoms included projectile vomiting (100%), weight loss (55.3%), and visible gastric peristalsis (51.1%). Mean surgery duration was 37.3±7.4 minutes. Postoperative vomiting occurred in 16 (34.0%), surgical site infection in 4 (8.5%), and sepsis in 3 (6.4%). Mortality was 6.4%. **Conclusion:** IHPS predominantly affects boys and presents with projectile vomiting. Open pyloromyotomy resulted in favourable outcomes, though 6.4% mortality was observed.

Keywords: Infantile hypertrophic pyloric stenosis, Mortality, Pyloromyotomy, Vomiting, Weight loss

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INTRODUCTION

Infantile hypertrophic pyloric stenosis (IHPS) is characterized as hyperplasia of smooth muscle fibres of the pylorus that leads to narrowing of the pyloric canal and gastric outlet obstruction among infants.[1] The incidence of IHPS ranges between 1 to 4 per 1,000 live-births while male gender and preterm are found to be more affected.[2–4] Literature also reports family history of IHPS to be a major risk factor for IHPS.[5]

Surgical approach employing pyloromyotomy is considered to be the standard treatment for IHPS.[6] In recent years laparoscopic pyloromyotomy has shown popularity among paediatric surgeons as it has advantages like being minimally invasive, shorter hospitalization period and improved cosmetic outcomes.[7,8] In developing countries, data is still unsatisfactory exploring complications related with laparoscopic pyloromyotomy. Controversy also exists whether open pyloromyotomy or laparoscopic pyloromyotomy is better treating IHPS.[7,9]

Not much data is on view from Pakistan exploring surgical outcomes of IHPS so this study was planned. At our setting, open pyloromyotomy is the standard approach for the treatment of IHPS. The present study aimed to evaluate the characteristics and outcome of surgical management of IHPS with open pyloromyotomy.

METHODOLOGY

This prospective, descriptive, case-series was conducted at the Department of Paediatric Surgery, Bahawal Victoria Hospital, Quaid-e-Azam Medical College, Bahawalpur from 31 Jan to 30 Jun 2023. Approval from Institutional Review Board was acquired vide No. 2234/DME/QAMC Bahawalpur. Informed written consents were sought from parents/caregivers of all infants. No specific sample size calculations were performed as all cases of IHPS undergoing open pyloromyotomy during the study period were included.

Children of both genders aged 1 day to 3 months with diagnosis of IHPS and planned to undergo open pyloromyotomy were included. Children who had other intraoperative diagnosis than IHPS or those who had any previous history of surgical procedures were excluded. The IHPS was labelled on clinical assessment as non-bilious projectile vomiting and/or palpable pyloric olive mass, confirmed by ultrasonography.

General anaesthesia was given in all cases undergoing open pyloromyotomy. Standard preparation and surgical protocols were adopted for all infants undergoing IHPS. Outcomes such as post-surgery complications, mortality and duration of hospitalization were documented. Data were recorded on a special proforma and analysed using SPSS-26. Categorical data were tabulated as frequency and percentage whereas numeric variables were represented as Mean±SD.

RESULTS

A total of 47 infants with IHPS underwent open pyloromyotomy. There were 32 (68.1%) boys and 15 (31.9%) girls representing boys to girls ratio of 2.1:1. The mean age was 5.1±3.2 weeks (range: 1–12 weeks), and 34 (72.3%) infants were aged 2–6 weeks. There were 28 (59.6%) infants who were born preterm. Mode of delivery was lower segment Caesarean section in 28 (59.6%) cases. (Table-1).

Projectile vomiting, weight loss and evident gastric peristalsis were the most common form of presentation noted in 47 (100%), 26 (55.3%) and 24 (51.1%) infants respectively. Table-2 is showing frequency of clinical features and presenting complaints recorded among infants with IHPS.

The mean duration of pre-surgery hospital duration was 3.1±2.8 days. Any fluid or electrolyte disturbances, dehydration or anaemia were corrected during pre-surgery hospitalization. The mean duration of surgery was 37.3±7.4 minutes ranging between 23 to 44 minutes. Test feeds were initiated within 12 hours following surgery in most infants (32, 68.1%). There were 3 infants (6.4%) who had intra-operative perforation and were kept nil per os for more than a day.

Post-surgery, vomiting was observed in 16 (34.0%) cases, surgical site infection 4 (8.5%) and sepsis 3 (6.4%). Mortality was reported in 3 (6.4%) owing to post-surgery sepsis in 1 case and intra-operative perforation in 2 cases. The mean duration of post-surgery hospitalization was 5.6±2.8 days (4–14 days). (Table-3).

Table-1: Baseline characteristics of infants with IHPS (n=47)

Characteristics		Frequency (%)
Gender	Boys	32 (68.1)
	Girls	15 (31.9)
Age (weeks)	<2	4 (8.5)
	2–6	34 (72.3)
	>6–12	9 (19.1)
Gestational Age	Preterm	28 (59.6)
	Term	19 (40.4)
Mode of Delivery	Lower segment C-section	28 (59.6)
	Normal vaginal delivery	19 (40.4)
Birth Weight	Low	30 (63.8)
	Normal	17 (36.2)
Feeding Types	Exclusive breastfeeding	13 (27.7)
	Bottle feeding	10 (21.3)
	Mixed	24 (51.1)

Table-2: Frequency of clinical features/ presentation among infants with IHPS (n=47)

Clinical feature/presentation	Frequency	Percentage
Projectile vomiting	47	100
Weight loss	26	55.3
Evident gastric peristalsis	24	51.1
Dehydration	22	46.8
Palpable olive epigastric mass	19	40.4
Constipation	14	29.8

Table-3: Post-surgery outcomes (n=47)

Post-surgery outcomes		Frequency	Percentage
Complications	Vomiting	16	34.0
	Surgical site infection	4	8.5
	Sepsis	3	6.4
Mortality		3	6.4

DISCUSSION

The IHPS is known to be the most frequent cause of gastric outlet obstruction among infants, requiring surgical intervention.[10] Open pyloromyotomy has been considered to be the mainstay approach for the treatment of IHPS but in recent decades surgeons have been opting for laparoscopic pyloromyotomy and yielding good outcomes.[11] Although, there is no consensus that which surgical approach is better for treating IHPS as both have their own advantages and disadvantages, open surgical technique is still the most common approach when treating infants with IHPS.[12,13]

In this study, 68.1% infants were boys representing a boy to girl ratio of 2.1:1. A study[2] reports male to female ratio 4–6:1 which correlates well with the present findings. Another study representing 4-year experience from Cameroon described a male to female ratio of 4.3:1.[14] In present study, the mean age of infants with IHPS was 5.1±3.2 weeks. A Cameroon study[14] reported mean age of children treated for IHPS as 5.2±1.2 weeks which is very similar to what we noted.

Projectile vomiting was reported to be the most common presenting complaint. This is quite similar to what has been described in literature.[15,16] The classical presentation from IHPS is stated as an olive mass in the epigastric region confirmed by the palpation but we noted that in only 40.4% infants meaning that not all cases present with these features.

We noted that the mean duration of surgery was 37.3±7.4 minutes. Post-surgery, test feeds were initiated within 12 hours following surgery in most infants (68.1%). Binet *et al*[8] noted that the operative time significantly affected the time needed to reach full feeding ($p<0.01$). Ismail *et al*[17] reported that laparoscopic pyloromyotomy group had relatively shorter time to reach full feeding, less post-operative vomiting, and shorter duration of hospitalization.[17] Some other studies have shown that there are no statistically significant differences in terms of reaching to full feeds in between different surgical approaches treating IHPS.[9,18]

In this study, mortality was reported in 6.4% infants undergoing open pyloromyotomy. A study analysing 28 IHPS cases over 4 years undergoing surgical interventions reported post-surgical mortality as 9.5%.[14] The literature reports mortality following pyloromyotomy around 1%[19] but data from developing countries have shown that the mortality following abdominal surgeries soars up to 7 times when compared to developed countries.[20] The differences could be

because of late presentation at healthcare settings, late diagnosis, insufficient healthcare facilities, or differences in competency of the surgeons.

This study reported post-surgery, surgical site infection rate of 8.5%, and sepsis in 6.4% of cases, with mortality at 6.4%. Ismail *et al*[17] found no significant difference in complications between laparoscopic and open pyloromyotomy.[17] Muse *et al*[21] reported 17.1% unfavourable outcomes, identifying delayed presentation, dehydration, and electrolyte imbalances as major risk factors for complications, which this study addressed preoperatively. Miyata *et al*[22] found higher perioperative complications (11.5%), with prolonged hospitalization (6 *vs* 1 day, $p<0.001$), suggesting that underlying conditions contribute to a more complicated post-surgical course.[22] The relatively higher infection rate in this study may warrant a review of perioperative antibiotic protocols and surgical asepsis.

Being a single centre study with a relatively small sample size were some of the inherent limitations of this study. Long-term post-surgery outcomes of IHPS were not assessed which warrants further work.

CONCLUSION

The infantile hypertrophic pyloric stenosis is more common among boys while most common presenting complaint is projectile vomiting. Open pyloromyotomy yielded good outcomes in terms of post-surgical complications, relatively shorter hospitalization stay, and mortality.

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ORIGINAL ARTICLE

EVALUATION OF PALPABLE BREAST LUMP BY FINE NEEDLE ASPIRATION CYTOLOGY AT ALLAMA IQBAL TEACHING HOSPITAL, DERA GHAZI KHAN

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Background: Fine needle aspiration cytology (FNAC) is an important part of triple assessment in the diagnosis of a breast lump. It can differentiate the breast lesions in to benign, suspicious and malignant lesions. It can also identify the residual disease after treatment. This study aims to evaluate the frequency and distribution of various palpable breast lesions with FNAC. **Methods:** This cross-sectional descriptive study on palpable breast lump in female patients was carried out from Nov 2022 to Dec 2023 at the Pathology Department of DG Khan Medical College, Dera Ghazi Khan. All patients with history of breast lump were included. FNAC was done by standard procedures, smears prepared and stained with hematoxylin and eosin (H&E) and examined under microscope. **Results:** A total of one hundred cases of breast lump were evaluated, out of which 70% were benign (C2), 1% atypical/suspicious but probably benign (C3), 2% suspicious but probably malignant (C4), and 27% cases were malignant (C5). Among benign breast lesions Fibroadenoma was the most common benign breast lesion (48%) followed by inflammatory breast lesions (10%). Fibrocystic disease of breast (7%) was third in frequency among the benign breast lesions. **Conclusions:** Fibroadenoma is the most common benign breast lesion among the young patients and malignant breast lesions are more common in old age patients.

Keywords: Benign, Breast lesions, Breast lump, Fine needle aspiration cytology, Malignant

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INTRODUCTION

Breast lump in women may be benign or malignant and is a cause of anxiety to the patients as well as to their family members. Carcinoma of the breast is the most common malignant neoplasm in women and is the leading cause of death from cancer in women. The incidence of breast carcinoma is more than one million cases occurring worldwide annually.[1,2] Pakistan is ranked 5th in Asia with women suffering from breast cancer and the number of patients is increasing each year.[3] Pakistan also has the highest death rates (25.2/100,000) due to breast cancer.[4] Patients are reported in advanced stage of the disease due to poverty, existing social circumstances, lack of education and lack of screening facilities in rural and remote areas. Lack of female doctors in rural and remote areas is also a contributing factor as women hesitate to be examined by a male doctor for breast disease.[5] Though histopathological diagnosis is universally accepted confirmatory method of diagnosis and follow-up of breast lump. Globally triple assessment is done for the investigation of the breast lump which includes clinical examination, imaging and FNAC.[6]

In poor resources, FNAC is useful for its obvious advantage that it is cheap, fast, and reliable diagnostic method. It also reduces the frequency of open biopsies. Scope of FNAC has now been extended into identifying the sub types of benign lesions, malignant lesions, and residual diseases, and for the purpose of planning treatment and eventual follow-up.[7] The aim of this study was to evaluate the frequency distribution of palpable breast lesions in patients seeking advice at Allama Iqbal Teaching Hospital, DG Khan, Pakistan.

MATERIAL AND METHODS

This cross-sectional descriptive study was carried out in female patients visiting for investigation of breast lump at the Department of Pathology, DG Khan Medical College, Dera Ghazi Khan from Nov 2022 to Dec 2023.

A total 100 cases of female patients of age group 12 to 65 years with palpable breast lump were included in this study. Patients having history of previous breast trauma, with recurrent malignancy, and on chemotherapy/ radiotherapy were excluded from the study. Written consent was taken from each patient. A detailed history, general physical and clinical examination was carried out. FNAC was performed using 10 cc disposable syringes. The procedure was repeated twice or thrice depending upon the size, growth and appearance of the lump. For each patient, 6–10 smears on slides were prepared. The prepared smears were air dried and stained with hematoxylin and eosin (H&E). The stained smears were examined under light microscope with high power objective lens.

Cytological findings were grouped into 5 categories laid down by International Academy of

Cytology[8], i.e., C1: Inadequate/insufficient material, C2: Benign, C3: Atypical/suspicious, probably benign, C4: suspicious/probably malignant, and C5: Malignant.

RESULTS

A total of 100 female patients presenting with breast lump were evaluated in this study. The spectrum of breast lesions on cytomorphological interpretation was 70% benign (C2), 1% atypical/probably benign (C3), 2% suspicious/probably malignant (C4), and 27% cases were malignant (C5). (Table-1)

Table-1: Cytological spectrum of breast lesions on FNAC (n=100)

Cytological Diagnosis	No.	%
C1 (Inadequate)	0	0
C2 (Benign)	70	70
C3 (Atypical probably benign)	1	1
C4 (Suspicious probably malignant)	2	2
C5 (Malignant)	27	27

Among the 70 benign (C2) cases, fibroadenoma was the most common benign lesion (68.60%) followed by inflammatory benign breast lesions (14.3%). Fibrocystic disease of breast was 3rd in frequency among the benign breast lesions (10.0%). Among the inflammatory benign breast lesions (8.6%) cases were of acute pyogenic mastitis and (5.7%) cases were of chronic tuberculous mastitis. Other benign lesions were galactocele 4.3%, benign phyllodes 1.4%, and lipoma 1.4. (Table-2).

Table-2: Distribution of benign breast lesions (n=70)

Cytological Diagnosis	No.	%
Fibroadenoma	48	68.6
Acute pyogenic mastitis	6	8.6
Chronic tuberculous mastitis	4	5.7
Fibrocystic disease of breast	7	10.0
Galactocele	3	4.3
Benign Phyllodes	1	1.4
Lipoma	1	1.4

The age of the patients in the present study varied from 12–65 years. The maximum number of patients in this study were 23 in the 4th decade (31–40 years) followed by 22 in 3rd decade (21–30 years) and 18 in the second decade (12–20 years) respectively. The maximum number of cases in this study was benign and common in young age group of 12–40 years. Malignant breast lesions were seen predominately in old age group. The maximum number of malignant cases was observed in age group of 31–65 years.

Table-3: Distribution of benign and malignant breast lesions according to age

Age Group (Years)	Benign		Malignant		Total
	N	%	N	%	
12–20	18	25.71	0	0	18
21–30	22	31.42	0	0	22
31–40	18	25.71	5	18.51	23
41–51	7	10.00	8	29.62	15
51–60	4	5.71	9	33.33	13
61–65	1	1.42	5	18.51	6

DISCUSSION

Fine needle aspiration cytology (FNAC) is a rapid and effective method for the primary categorization of a pulpable breast lump into benign, atypical/suspicious and malignant categories FNAC is a useful tool in the pre operative evaluation of breast lumps. The most significant advantage of FNAC is, its high degree of accuracy, rapid results and a less invasive procedure than a tissue biopsy.[9] In this study the spectrum of breast lesions on cytomorphological interpretations was, benign (C2) 70%, atypical probably benign (C3) 1%, suspicious probably malignant (C4) 2% and malignant (C5) 27%.

In this study benign lesion were most common (70%) followed by malignant lesion (27%). Yeoh *et al*[10], studied 1533 breast lump cases on FNAC and found that 70.4% cases were benign. Malik *et al*[11], studied 1724 cases over a period of 20 years and reported 72.9% benign lesions and 27% malignant lesions in his study which very much co-relates with this study. These findings were also justified by similar findings stated by Panjvani *et al*[12], who reported 68.18% benign lesions and 31.08% malignant lesions in his study. Similar findings were also observed by Gorasiya *et al*[13], who reported 69.38% benign and 30.62% malignant lesions Ahmad *et al*[14], and Bukhari *et al*[9], reported 30.5% and 31% cases of malignancy in their studies respectively which also much co-relates with this study. Our study is also similar to study of Ahmed H *et al*[15], and Sarfraz *et al*[16], who reported 31.6% and 30% of malignant lesions of breast lump in their studies respectively.

In this study the most common benign breast lesion was fibroadenoma comprising 48% cases Poudel *et al*[17], evaluated 61 cases of breast lump and found fibroadenoma the most common benign breast lesion (47.54%) in his study. Aslam *et al*[18], also documented fibroadenoma the most common benign lesion in his study. The literature shows that among benign breast diseases the most common lesion is fibroadenoma.[19,20] Shah *et al*[21], showed 42.4% incidence of fibroadenoma in their studies.

Regarding the other benign breast lesions like inflammatory breast lesions, fibrocystic disease of breast, phyllodes tumour galactocele and lipoma, our findings are supported by Pandey *et al*[22]. Three (3%) cases of galactocele revealed milk during aspiration and patients were under the reproductive age (20–30 years), having history of breast feeding. Unlike our study Khanam *et al*[23], studied 50 cases out of which 43 (86%) were benign and 7 (14%) were malignant. The incidence of fibroadenoma, fibrocystic disease of breast and inflammatory breast lesions were 40%, 27% and 18% respectively in their study.

The age of the patients in our study was from 12 to 65 years. The maximum number of benign lesions

was seen in 12–40 years of age, similar findings were also documented by Khemka *et al*[24], and Rocha *et al*[25] who reported maximum number of benign lesions in the age group of 15–44 years and 14–40 years respectively. In this study the most common age group presenting with breast lump was 4th decade (31–40 years) in which the number of cases was 23 followed by 3rd decade (21–30 years) in which number of cases was 22. Hussain *et al*[26], and Khemka *et al*[24] studied 50 patients and found majority of the patients in age group of 31–40 years.

In our study malignant breast lesions were found in the age group of 31–65 years. Khemka *et al*[24] observed that benign breast lesions were more commonly seen in young age group. Omoniyi-Esan G *et al*[27] reported majority of the patients with malignant breast lesions in the fourth to seventh decade of life. All these findings are consistent with our study.

CONCLUSION

Fibroadenoma is the most common benign breast lesion seen mainly in the young age group, while the malignant breast lesions are common in old age group. The incidence of malignancy increases with age. FNAC can be used as a screening tool for determining nature of the breast lump.

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ORIGINAL ARTICLE

THE SPECTRUM OF SNAKEBITE INJURIES FROM WOUND INFECTION TO ACUTE RESPIRATORY DISTRESS SYNDROME

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Background: Snakebite envenoming is a medical emergency recognized as category A neglected disease in tropical areas of Pakistan. Objective of this study was to determine the minor injury to major damage in snake envenomed patients. **Methods:** This cross-sectional study was conducted in Emergency, Medical, Surgical, and Intensive Care Units of Ayub Teaching Hospital, Abbottabad from Jan 2022 to Sep 2024. The non-probability convenient sampling technique was applied for data collection. The snake envenomed patients aged 16–60 year were included. The data was analysed on SPSS-21 for characteristics of snakebites and complications, and $p<0.05$ was taken as significant. **Results:** A total of 52 patients were seen, 23 (44%) belonged to District Batagram with the mean age of 35.6±15.4 Years. Skin oedema was observed in 30 (58%), skin ulceration in 6 (11%), while 16 (31%) had normal skin appearance. Five (10%) patients had paresthesias, 3 (6%) had nerve palsies. Nine (18%) patients had ARDS complicated by septic shock and needed ventilator support. Fasciotomy was done in 8 (15%) patients and DIC alone was found in 6 (12%) patients. All patients complicated by ARDS and multiple organ failure died during admission with mortality of 12%. **Conclusion:** Systemic complications and end-organ damage secondary to snakebites is not rare. ARDS is most deadly complication of snakebites. The mortality of snakebites is double as compared to previously reported.

Keywords: Acute kidney injury, Acute respiratory distress syndrome, ARDS, Envenomation, Snakebite

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INTRODUCTION

Snakebites are the major risk to the health of 5.8 billion individuals around the world.[1] In Pakistan, prevalence of snakebites is typically predominant in rural areas. Every year thousands of cases occur with many fatalities due to limited access to medical emergency and anti-venom. Particularly affected regions are Sindh, Punjab and Balochistan.[2] Snakebites carry a high financial burden for the people who are affected and often cannot be met and drives families further into poverty and causing considerable fear and anxiety in other family members. In June 2017 snakebite envenoming was recognized in the highest category (A) of Neglected Tropical Diseases (NTD) and approved that the mortality and morbidity associated with this disease chiefly in tropical and subtropical regions have been underrated and hence a coordinated response is needed internationally.[3]

It is difficult to approximate morbidity, disability and mortality as a result of snakebite due to a number of reasons, for example, snakebite is most prevalent in underprivileged agricultural communities who have poor access to health care where specific data is not collected. In 1954, WHO attempted to enumerate deaths as a result of snakebite envenoming based on inadequate data and reached at 30,000 to 40,000 deaths per annum.[1] More recent attempts (better but still incomplete data) provide broad approximation of 81,000 to 138,000 deaths resulting from 1.8 to 2.7 million cases of envenoming and up to 5.4 million snakebites.[4] Institute for Health Metrics and Evaluation estimated that there were 79,000 deaths due to bites from venomous animals in year 2016, with an uncertainty range of 56,800 to 89,400[5], which is less than that in other studies.[1,6,7]

Snake venoms are mixture of toxins. As a defence strategy, the toxin is injected through snakebite or is sprayed into eyes or on mucosal surfaces to kill the prey by affecting various cell receptors. Snakebite envenoming can result in multi organ or multi system disorders. It can cause local tissue damage including swelling, oedema and necrosis, damage to eyes leading to blindness, damage to nerves and neuromuscular transmission blockage resulting in muscle paralysis including muscles vital to breathing. Indirect effects can be on multiple organs and systems. Toxins may affect pregnant mother and foetus due to bleeding and placental dysfunction. The effects of snakebite envenoming on body depends on specie of snake and types of toxins in the venom. There is a wide variety of snake species, some are venomous and some are not.

Snake venom are complex natural secretions that contains many chemicals including more than 100 enzymes, proteins, peptides and inorganic cations like potassium, calcium, sodium, zinc, iron and magnesium. Among them phospholipase A_2 or lecithinase plays an

important role in pathogenesis of systemic envenomation.[8,9] The composition of snake venom depends upon different factors like snakes' specie, sex, habitat, external temperature, season and age of snake. In Pakistan the risk of bites is high in season of Monsoon due to alteration in external environment and habitat. A recent study claimed that 17% snakebite injuries get complicated by severe systemic illness and end-organ damage.[10]

The present study aimed to estimate various injuries, complications resulted from envenomation of snake bites, and their outcome.

MATERIAL AND METHODS

This prospective cross-sectional study was conducted in Ayub Teaching Hospital, Abbottabad. The duration of study was from Jan 2022 to Sep 2024. Non-probability convenient sampling technique was used for sample collection. The sample size was calculated with WHO calculator with estimated frequency of 17% complications in snakebites and absolute precision of 10% at 95% confidence interval. The estimated sample size was 50.

The ethical approval was obtained from Hospital Ethical Committee. The data was collected on pre-designed written questionnaire. Patients aged 16–60 years, presenting with complaints of snakebites were included in the study. The patient with snakebite were admitted in medical on-call department for anti-snake venom (ASV), clinical monitoring and lab investigations at least for 24 hours. Patients who were shifted to other departments (Surgical and ICU) for procedures like fasciotomy or ventilatory support were followed till discharge. The patient who refused inclusion, or age <14 years were excluded from the study.

The demographic variables, wound characteristics, systemic signs and end-organ assessment were recorded. To access the mental status Glasgow Coma Scale was used. Berlin Criteria was used to classify acute respiratory distress syndrome (ARDS), i.e., Pao_2/FiO_2 ≤300 mm of Hg or CPAP ≥5 Cm of H_2O.

Data was analysed on SPSS-21. The categorical data was presented as Mean±SD, while qualitative data was presented in frequency and percentages. Chi-square and Fischer exact test were applied, and $p<0.05$ was taken as statistically significant.

RESULTS

A total of 52 patients were seen of whom majority 23 (44%) belonged to District Batagram. Most of the patients were in the age range of 16–65 years with the mean age of 35.6±15.4. Rural and urban population was affected almost equally (52% *vs* 48%). Most (60%) of the patients presented within initial 24 hours of the incidence. Fang marks were identified clinically in 46 (88%) patients. The common time of bites noted was morning (27%) and (29%) evening. The most common places of incidence were streets (44%) followed by fields (31%). The most common snake identified by patients was viper (14%) while 38% of snakes were not identified. (Table-1).

Out of 52 patients 30 (58%) had skin oedema, 6 (11%) had skin ulceration while 16 (31%) had normal skin examination. Among neurological symptoms 5 (10%) patients had paresthesias, 5 (10%) had nerve palsies, 32 (62.7%) had normal mental status, 12 (23.5%) were anxious, 5 (10%) were drowsy, and 2 (4%) were comatose.

Out of 52 patients 31 (61%) had muscle pain and 22 (43%) had deranged muscle creatinine kinase level with mean range of 853 U/L (176–4,000). Deranged Alanine Transaminase level was found in 6 (17%) patients with mean value of 257 U/L and acute kidney injury in 8 (15%) patients with mean creatinine level of 1.48 mg/dL. Nine (18%) patients had acute respiratory distress syndrome complicated by septic shock and ventilator support in medical ICU. Fasciotomy was done in 8 (15%) patients and DIC alone was found in 6 (12%) patients. All patients complicated by ARDS and multiple organ failure died during admission in medical ICU. (Table-2).

Table-1: Demographic and clinical characteristic features of patients presented with snake bites (n=52)

Variables		Frequencies (%)	Variables		Frequencies (%)	Variables		Frequencies (%)
Address	**Abbottabad**	7 (13)	**WBC**	**Deranged**	24 (47)	**Haemorrhagic Syndrome***	**Gum bleed**	4 (8)
	Batagram	23 (44)		**Normal**	28 (53)		**Petechia**	7 (13)
	Haripur	4 (8)	**Prothrombin time**	**Deranged**	34 (65)		**Haematuria**	12 (23)
	Manshera	16 (31)		**Normal**	18 (35)		**Unknown**	29 (56)
	Kohistan	2 (4)	**Creatinine**	**Deranged**	11 (21)	**Steroid received**	**Yes**	12 (24)
ALT	**Deranged**	6 (12)		**Normal**	41 (79)		**No**	40 (76)
Area	**Rural**	27 (52)	**Muscle CK**	**Deranged**	22 (43)	**Paresthesias**	**Yes**	5 (10)
	Urban	25 (48)		**Normal**	30 (57)		**No**	47 (90)
Patient mental Status	**Alert**	33 (63)	**Total ASV ampule**	**Zero**	6 (11)	**Discharge status**	**Died**	6 (12)
	Anxious	12 (23.5)		**5–10**	27 (52)		**Improved**	30 (60)
	Comatose	2 (4)		**11–20**	17 (33)		**Referred**	2 (4)
	Drowsy	5 (10)		**>21**	2 (4)		**Treated**	14 (24)

Table-2: Comparisons of complications in patients of snake bites

Parameters		ARDS (n=1)	AKI (n=6)	DIC (n=2)	DIC+AKI (n=4)	Fasciotomy +AKI (n=2)	ARDS+AKI+DIC+ Haemorrhagic syndrome (n=8)	*p*
Address	**Abbottabad**	-	-	-	-	-	-	0.09
	Batagram	1	4	-	-	-	8	
	Haripur	-	-	2	-	-	-	
	Mansehra	-	2	-	4	2	-	
	Kohistan	-	-	-	-	-	-	
Total ASV ampoules	**5–10**	-	2	2	2	-	2	0.008
	11–20	1	4	-	2	2	4	
	≥21	-	-	-	-	-	2	
Haemorrhagic Syndrome	**Gum bleed**	-	-	2	-	-	2	0.000
	Petechiae	1	4	-	-		2	
	Haematuria	-	2	-	4	2	4	
Discharge status	**Died**	-	-	-	-	-	6	0.000
	Improved	1	4	-	2	-	1	
	Referred	-	2	-	-	-	1	
	Treated	-	-	2	2	2	-	

DISCUSSION

In the present study the mean age of sampled population was recorded as 35.6 years while Zafar J *et al*[9] from Islamabad, Pakistan reported the mean age of their patients as 30 years. This can be due to difference in geographical areas and difference in urban and rural population. In the current study 52% population was rural while Zafar J *et al*[9] reported 78%.

We observed haemorrhagic syndrome in 44% of cases in form of petechial rashes, haematuria and gum bleeding while 15% of cases got complications of severe haemorrhagic syndrome accompanied by ARDS. Zafar J *et al*[9] observed haemorrhagic syndrome in 58% of their patients. The difference in frequency and degree is probably due to late presentation from far flung areas, as majority of our population was from Batagram (44%), and may be due to nature of snake toxin. We observed that 10% population developed neurotoxicity while Zafar *et al*[9] reported neurological signs in 13% and nerve palsies in 7.5%. Many patients of Zafar *et al*[9] belonged to Kashmir while our study had no patients from Kashmir. Common snakes found in Kashmir valley are Levantine fame with local name of *Gunas* which causes haemotoxicity more than neurotoxicity.[11] An older study done in Thar region also reported complication haemorrhage in 68% of cases while bleeding diasthasis in 27% of cases; despite severe complications only 0.5% mortality was reported.[12] We observed the total mortality of 12% while Zafar J *et al*[9] recorded 5% mortality. The big difference in mortality could be due to end-organ involvement in our patients because all patients who died developed acute respiratory distress, haemorrhagic syndrome and multiple organ involvement. A recent study from India observed that snakebites with acute kidney injury have greater mortality.[13] This justifies the reason of high mortality in our study as 21% of sampled population in our study had AKI. A study by Saleem K *et al*[10] reported 2.7% mortality and highlighted that all cases of mortality were due to vasculotoxic snake bites. An older study done in Ayub Teaching Hospital reported 8% mortality.[14]

We recorded the complication secondary to snakebite in detail but certain parameters still need further evaluation and elaboration. All variables are not possible to be compared with the local literature due to scarcity of data on snakebite complications.

CONCLUSION

Systemic complications and end-organ damage secondary to snakebites is not rare. Almost both rural and urban population are equally affected by snake envenomation. ASV is relatively safer as no adverse effects were recorded. More than half of sample population encountered the complications which were preventable by immediate care. Haemorrhagic syndrome with ARDS is the commonest complication. Respiratory distress syndrome is deadly complication of snakebite. The mortality of snakebites in our setup is almost double compared to previously reported.

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ORIGINAL ARTICLE

EFFECT OF MONTELUKAST SODIUM ADMINISTRATION ON AIRWAY HYPERSENSITIVITY OF TANNERY WORKERS

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Background: Tannery workers are prone to develop Bronchial Hypersensitivity. Objective of this study was to see the role of leukotriene receptor antagonist Monteleukast Sodium (MKS) on airway hypersensitivity in tannery workers. **Methods:** One-hundred and fifty asymptomatic tannery workers were divided into 3 groups based on their duration of exposure to tannery chemicals, i.e., 6 months to one year Group I, 1–2 years Group II, and 2–5 years Group III, with 50 subjects in each group. After baseline Pulmonary Function Tests (PFTs), subjects were categorized into those with Forced Expiratory Volume in First Second (FEV_1) <80%, and those who had FEV_1 >80%. Histamine inhalation challenge was given to all subjects with FEV_1 >80% via provocative cumulative dose of methacholine causing a 20% fall in FEV (PD_{20}). Later, MKS was given to subjects for six weeks and PFTs were repeated again. **Results:** Out of 150 subjects, 34 had FEV_1 <80% and 116 had FEV_1 >80%. Hyper-responsiveness of airways was affected in 6% subjects in Group-I, 24% in Group-II, and 38% subjects in Group-III. PD_{20} within subjects of all groups before giving MKS at day 0 and 42 was non-significantly different. After giving MKS, the PD_{20} at day 42 was significantly improved in all experimental groups as compared to day 0 (p=0.05, p=0.05, and p=0.05 respectively). **Conclusion:** Tannery workers have high histamine sensitivity and reduced pulmonary efficacy which improves with administration of MKS.

Keywords: Bronchial hyper-responsiveness, Histamine diphosphate, Histamine Inhalation Challenge, HIC, Motelukast Sodium, MKS, Occupational Hazards, Pulmonary function tests

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INTRODUCTION

Exposure to tannery pollutants such as chromium, oxides of nitrogen (NOX), oxides of sulphur (SOX), particulate matter (PM10), volatile organic compounds (VOCs), and hydrogen disulfide (H_2S) has been linked to an increased prevalence of allergic diseases, bronchial asthma, and enhanced airway responsiveness to inhaled allergens.[1] This finding has highlighted the role of these pollutants in triggering inflammatory and immune responses in exposed individuals. Chromium salts, specifically, bound with epidermal proteins to form complex antigens that stimulate elevated IgE antibody levels, ultimately contributing to the development of bronchial asthma.[2]

The initiation of occupational asthma (OA) among tannery workers is believed to result from histamine surges experienced in response to inhalation of tannery-related chemicals.[3] Experimental studies have demonstrated that histamine exposure in otherwise healthy individuals leads to non-significant reduction in Forced Expiratory Volume in 1st second (FEV_1), an effect that is exacerbated in individuals with prior exposure to asthma-inducing pollutants.[4] In workers with co-existence of asthmatic tendencies, histamine surges are often associated with further declines in Forced Vital Capacity (FVC) in addition to reduction in FEV_1, indicating progressive increase in airway obstruction.[5]

Among various drug classes available for managing asthma, leukotriene receptor antagonists like montelukast sodium (MKS) have gained increasing attention. Recent work confirms that montelukast effectively antagonizes leukotriene D_4 (LTD_4) at the cysteinyl leukotriene receptor in human airways, thereby preventing airway oedema, smooth muscle contraction, and excessive mucus secretion.[6] Despite its established efficacy in managing asthma and allergic rhinitis[7], limited data exist regarding its comparative superiority over corticosteroids or antihistamines, particularly within occupational asthma cases.[8]

Since limited data is available regarding airway hypersensitivity and/or asthma within tannery workers, and since the amount of data regarding the efficacy, supremacy and toxicity of Montelukast within this population is limited, this study was conducted with the aim to explore these facts in depth. This data, in turn was aimed to be utilized to create awareness regarding the occupational asthma within the society and for effective prophylactic use of MKS.

MATERIAL AND METHODS

A cohort study was conducted through the collaboration of the Departments of Physiology of Multan Medical and Dental College, Multan, and Nishtar Medical University, Multan. Approval from the Institution Review Board and Ethical Committee vide letter No. C-92-1046 dated 1-1-2024 was obtained. The sample size of 150 for study was approached by convenient random selection and calculated utilizing WHO Sample Size Calculator[9].

The protocol of histamine inhalation was standardized according to American Academy of Allergy and Immunology.[10] Histamine inhalation test, now called bronchial provocation test (BPT) and PD_{20} is defined as the dose of histamine diphosphate in µmoles, which produces a 20% decrease in baseline FEV_1.[9–11]

The tannery workers were divided into 3 groups of 50 each depending upon their duration of exposure: Group I= 6 months to 1 year, Group II= 2–5 year, Group III= 6–20 year. Within each exposure group, subjects were further divided based on FEV_1 <80 or >80%. Histamine inhalation challenge (HIC) was performed on FEV_1 >80% group.[12] The test was performed to find the state of airways whether they are hypersensitive to inhale histamine diphosphate or not. The test was performed as standardized by Yan *et al*[11] by a standardized protocol.

Half of the subjects in each subgroup received MKS, while the other half served as controls. The dose protocol started from 0.5 µmol up to maximum of 8.0 µmol, the inhalation protocol was as standardized as Yan *et al*[11]. Spirometry and HIC were performed accordingly to evaluate lung function and airway responsiveness. The uniform design allowed comparisons across time and within subgroups to minimize the cofounders.

Kruskal-Wallis Test was applied to compare the means and standard deviations of group at a confidence interval 95%. Mann Whitney U-Test was applied to compare the means in subgroups as their ultimate size was smaller, and $p<0.05$ was considered statistically significant.

RESULTS

Table-1 shows frequency distribution of the study population, categorizing subjects into three groups based on the duration of exposure to environmental factors, airway sensitivity (HIC+ or HIC-), and MKS administration (MKS+ or MKS-). The table includes groups with varying exposure periods ranging from 0.5 to 5 years, distinguishing between subjects who are hyperresponsive to inhaled histamine (HIC+) and those who are not (HIC-). It also highlights whether the subjects received MKS treatment or not.

Table-2 presents the comparison of PD_{20} values in HIC+ subjects after 42 days of MKS administration among three different duration groups. The data indicate a significant improvement in airway sensitivity, even when PD_{20} values increased to the maximum level of 8 µmole for all groups ($p<0.05$).

Table-3 shows comparison of PD_{20} values of HIC positive subjects who did not receive MKS treatment. In these groups, no significant changes in airway sensitivity were observed after 42 days. The PD_{20} values remained relatively unchanged or slightly altered, ($p>0.05$).

Table-1: Frequency distribution of study population

	Baseline Airway Sensitivity		MKS Administration	
Group	**FEV_1 Status**	**N (%)**	**-**	**+**
Group-1 n=50	**<80%**	3 (6)	1	2
	>80%	47 (94)	-	-
Group-2 n=50	**<80%**	12 (24)	7	5
	>80%	38 (76)	-	-
Group-3 n=50	**<80%**	19 (38)	9	10
	>80%	31 (62)	-	-
	Airway sensitivity after HIC		**MKS Administration**	
Group	**HIC Status**	**N (%)**	**-**	**+**
Group-1 n=50	**HIC +**	15 (30)	8	7
	HIC -	32 (64)	17	15
Group-2 n=50	**HIC +**	16 (32)	9	7
	HIC -	22 (44)	10	12
Group-3 n=50	**HIC +**	16 (32)	7	9
	HIC -	15 (30)	7	8

KEY: MKS(-) Montelukast Sodium not given; **MKS(+)** Montelukast Sodium given. **HIC(+)** Hyper responsive to inhaled histamine (PD_{20}<8 µmole); **HIC(-)** Non-responsive to inhaled histamine (PD_{20}>8 µmole)

Table-2: Comparison of PD_{20} of HIC+ subjects after 42 days of MKS administration

Groups	(Duration of exposure)	N	PD_{20} (µmole) At 0 Day	PD_{20} (µmole) After 42 Days	*p*
Groups 1	**0.5–1 Year**	7	3.11±0.90	8±0	0.01**
Groups 2	**1–2 Year**	7	4.88±0.30	8±0	0.01**
Groups 3	**2 – 5 Year**	9	4.91±0.31	8±0	0.01**

*Statistically significant

Table-3: Comparison of PD_{20} of HIC+ subjects after 42 Days without MKS administration

Groups	(Duration of exposure)	N	PD_{20} (µmole) At 0 day	PD_{20} (µmole) After 42 Days	*p*
Groups 1	(0.5–1 Year)	8	3.80±0.19	4.11±0.19	0.92
Groups 2	(1–2 Year)	9	5.15±0.27	3.71±0.10	0.81
Groups 3	(2–5 Year)	7	3.81±0.12	4.99±0.10	0.70

DISCUSSION

The study investigated the impact of montelukast sodium (MKS) on histamine hypersensitivity and airway responsiveness among tannery workers exposed to occupational irritants. Subjects receiving MKS demonstrated significant improvements in PD_{20} values, as evidenced by the increase from baseline levels across all exposure durations. This finding has also been reported elsewhere.[13]

It could be explained on the basis of logical reasoning provided by contemporary research which suggests that leukotriene antagonists reduce airway hyperresponsiveness by blocking leukotriene D_4 receptors, thereby justifying bronchoconstriction and inflammation.[14]

In Group 1, PD_{20} increased from 3.11±0.90 µmol to 8.00 µmol in MKS-treated subjects ($p<0.001$). Subjects in Group 2 exhibited an improvement from 4.88±0.30 µmol to 8.00 µmol, and group 3 exhibited an increase from 4.91±0.31 µmol to 8.00 µmol. These outcomes suggest that montelukast sodium effectively alleviates histamine-induced airway constriction regardless of the duration of exposure. This is in agreement with Chen *et al*[15].

This finding has been reported in literature and can be attributed to montelukast's capacity to decrease leukotriene-mediated inflammation, as suggested by research on airway re-modelling mechanisms.[16]

Subjects who did not receive MKS showed no significant improvement in PD_{20} values after 42 days, with baseline levels remaining relatively unchanged. For instance, in the 0.5–1 year group, the PD_{20} improved only marginally from 3.80±0.19 µmol to 4.11±0.19 µmol, and *p* across all groups exceeded 0.05. This finding has been observed in similar studies and could be explained by evidence indicating that natural resolution of histamine sensitivity without pharmacological intervention is minimal[17], as histamine-mediated airway inflammation tends to persist without targeted treatment.[18]

Notably, the consistency of montelukast's efficacy across exposure durations underscores its value as a preventive treatment in occupational settings. Workers exposed for up to 5 years showed comparable improvements in PD_{20} to those with shorter exposure durations, suggesting the drug's potential to address both acute and chronic airway hypersensitivity. This observation aligns with Gauvreau *et al*[19] and could be explained by the sustained effect of leukotriene receptor antagonists in reducing chronic airway inflammation and hyperresponsiveness, as indicated in recent mechanistic studies.[20] Another study has also reported this and could be explained on the basis of logical reasoning given by contemporary research.[21]

Our findings suggest that leukotriene antagonists prevent bronchoconstriction and inflammation, the key mechanisms in occupational asthma and airway hyperresponsiveness[22].

CONCLUSION

This study reinforces the role of montelukast sodium in managing histamine hypersensitivity and preventing airway hyperresponsiveness. By improving PD_{20} significantly, MKS can be an effective intervention for tannery workers exposed to chemical irritants. Further studies could explore its long-term benefits and applicability to other occupational environments.

OUTCOME AND UTILIZATION

This study advocates to the medical practitioners to consider administration of montelukast sodium to all those who are exposed to the tannery environment so that if they get afflicted by an exacerbating factor like COVID-19 or smog, the emergence of acute or chronic asthmatic attacks could be prevented. This in turn will considerably reduce burden of respiratory emergencies as well as chronic morbidities.

LIMITATION & RECOMMENDATIONS

This was a single-centre study, hence the results cannot be generalized. Further work is recommended to generalize our findings.

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ORIGINAL ARTICLE

ONLINE TEACHING: READINESS AND WILLINGNESS AMONG 1ST AND 2ND YEAR MBBS STUDENTS DURING COVID-19 PANDEMIC —EFFECTS ON PROFESSIONAL EXAMINATION RESULTS

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Background: Online education despite various challenges emerged as the best possible solution to prevent academic loss during COVID-19 pandemic. This study evaluated online teaching for students' readiness and willingness and its impact on overall performance during COVID-19. **Methods:** A cross-sectional study was conducted at Gujranwala Medical College, Gujranwala in May-Jul 2020. All students of first and second year MBBS at Gujranwala Medical College were given a voluntary opportunity to fill an online questionnaire regarding their readiness and willing to start online teaching. One hundred and fifty-one (151) students filled and returned the questionnaire. It included basic demographics and questions related to access, knowledge, willingness and problems regarding online teaching. **Results:** Less than 50% (49.4%) of students were willing to start online teaching; 91.8% students had readily available internet facility at their homes, and 8.2% students were deficient in this facility. Most students (50.6%) had broadband as their internet source. Personal smart phone was the most common (67.1%) gadget available with students likely to be used to take online classes; 73.4% students were aware of some media used for online teaching, whereas 26.6% were not familiar with any one of them. The results of professional examination conducted during pandemic were comparable to subsequent years. **Conclusion:** In spite of partial willingness of students and limited resources, the learning objectives could be achieved well through online teaching which is evident from examination results. Online teaching is a suitable option during any unpredictable and untoward circumstances.

Keywords: COVID-19, Online teaching, Readiness, Willingness

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INTRODUCTION

The first suspected case of COVID-19 was reported to World Health Organization (WHO) from Wuhan City of People's Republic of China in the last month of 2019.[1] Initially no one could imagine that this newly discovered virus could result in a life-threatening pandemic that would disrupt all daily life affairs and social distancing would become indispensable.[2] At the end of January 2020, COVID-19 was labelled as Public Health Emergency by WHO and was declared pandemic on 11 March 2020.[3] Pakistan reported its first case of COVID-19 in February 2020, almost two months after it emerged in China. This disease was contracted by a traveller coming from Iran.[4]

This pandemic evoked as a challenge for the whole world. Among the various challenges of health, business and economy, one of the major challenges was the continuance of teaching and learning. To limit disease spread, gathering had to be avoided and classroom teaching was unachievable in this scenario. The possible solution to continue teaching outside classroom was to bring in online teaching.[5]

It was not an easy task and all countries did not possess adequate resources to deal with this unexpected situation. This disease was highly infectious with a striking mortality rate and each country dealt this situation in its own way.[6] This necessitated evolution of a mechanism in which the disease dissemination could be limited and educational deprivation could be minimized. The valuable time of students had to be employed in a beneficial way.[7]

The modern technology evolved as a game changer during this condition in various ways. It provided an opportunity to convert classroom teaching to remote teaching which could also be applied in future for convenient learning.[8] However, this is not as simple as it seems to be. To be efficacious it needs knowledge, resources and willingness to adapt this mode of teaching. Many difficulties can be encountered while using this methodology that includes administrative issues, technical in expertise, lack of motivation and financial constraints.[9] For smooth running of online classes, internet and gadget should be available and the user should have knowledge how to use it. Adequate space should be available to user for undistracted learning. The internet connection should be efficient to avoid any audio-visual disparity, voice lagging and interrupted streaming of videos.[10] The primary focus of medical education is found to be optimization of online education for high income countries. Low and middle-income countries however remain underprivileged due to limited resources, connectivity and electricity

outrages, lack of experience and institutional difficulties.[11] Comparison of higher education across various countries during early COVID-19 pandemic revealed that developed countries smoothly shifted to online teaching, whereas developing countries closed educational institute for a certain period of time.[12] In Pakistan also, the measure taken in educational sector was closure of schools, colleges and universities to create social distancing. This was done in mid of March 2020.[13] This unexpected situation created anxiety and unrest in students of all disciplines. Medical students were also worried and concerned about continuity of their academic session.

This study evaluated online teaching for readiness and willingness of 1st and 2nd Year MBBS students at Gujranwala Medical College, and its impact on their overall performance in university examinations during COVID-19.

METHODOLOGY

This was a questionnaire-based study conducted during May–Jul 2020 among first and second year medical students at Gujranwala Medical College, Gujranwala. The study started after approval from Institutional Review Board vide No. Admin.373/GMC.

Total population sampling was done by enrolling all students of first and second year MBBS in the study who were asked to fill the electronic Google form-based questionnaire on voluntary basis. All questions were mandatory and responses were obtained with the student's consent. A structured, pre-designed questionnaire was used to assess students' readiness and willingness for online teaching. The questionnaire included sections on demographics, internet accessibility, device availability, and familiarity with online teaching platforms.

The questionnaire was divided into two parts. In the first part, participants' basic demographic data, such as gender, age, residing city, level of medical education etc. was asked and in the second part questions related to their access, knowledge and problems regarding online teaching programme were asked. Their willingness to start online classes was also asked.

The data was analysed using SPSS-21. Frequencies of qualitative variables were expressed as percentages. Correlation of various factors with willingness to take online classes was computed using Pearson's test and $p \leq 0.05$ was considered statistically significant. Pearson's correlation test was applied to assess the relationship between different factors and willingness to start online teaching.

RESULTS

The majority (91.8%) of students in our study had internet access whereas only a small portion (8.2%) of students was lacking this facility. The internet sources were broadband (50.6%), mobile 4G (37.3%), and fibre/cable (7%). The majority (67.1%) of students had personal smartphones, followed by laptop (31%), desktops (1.3%), and/or tablets (0.6%) for the online learning activities. (Table-1).

Out of 158 students, 42 students (26.6%) had never heard/were unaware of different media used for online teaching, 94 (59.5%) students had heard about Zoom, 14 (8.9%) students had heard about Google Classroom and 5 (3.2%) students had heard about Google Meet. The students were least familiar with Microsoft Teams. Only 3 (1.9%) students had heard about this medium. Approximately half (49.4%) of the students were willing to start online teaching, whereas 50.6% of the students were not willing to start online learning. (Table-2).

Pearson's correlation values show that none of the correlations between various factors required for online classes and willingness for online teaching were significant ($p>0.05$). Positive and negative signs represent positive and negative correlation respectively. (Table-3).

The results of University/Professional examinations conducted after online teaching during the pandemic were comparable, rather better, to those in subsequent years. The pass percentage of 1st year MBBS was 96% in 2020 and it was 92% in 2022. The pass percentage of 2nd year MBBS was 92% in 2020 and it was 85% in 2022. (Table-4).

Table-1: Frequency and percentage of availability of technological resources

Resources	Responses	Frequency	Percentage
Internet access	Yes	150	94.9
	No	8	5.1
Internet type	No internet	8	5.1
	Broadband	80	50.6
	Fiber	11	7.0
	Mobile 4G/LTE	59	37.3
Device type	Desktop	2	1.3
	Laptop	49	31.0
	Tablet	1	0.6
	Smartphone	106	67.1

Table-2: Tool awareness and willingness of students

Student knowledge and attitudes	Responses	Frequency	Percentage
Tool Awareness	No awareness	42	26.6
	Zoom	94	59.5
	Google Classroom	14	8.9
	Google Meet	5	3.2
	Microsoft Teams	3	1.9
Willingness	Yes	78	49.4
	No	80	50.6

Table-3: Correlation of various factors with the willingness to start online teaching program

Factors	Pearson correlation R	*p*
Internet access	0.065	0.415
Internet type	0.028	0.724
Device type	-0.108	0.178
Tools awareness	0.086	0.285

Table-4: Professional examination results of 1st and 2nd Year MBBS during COVID-19 pandemic (2020) and 2 years later (2022)

	1st Year MBBS					2nd Year MBBS				
			Subject-wise Failures (%)					Subject-wise Failures (%)		
Examination	Total	Pass %	Anatomy	Physiology	Biochemistry	Total	Pass %	Anatomy	Physiology	Biochemistry
2020	109	96	4	1	1	103	98	2	0	0
2022	125	92	8	6	6	117	85	12	7	5

DISCUSSION

This study was conducted at the time when all educational institutions across the country were closed to deal with the challenging situation of COVID-19. It was the time that required innovative thinking regarding learning mandatory techniques and expertise to cope up with accessible means of online education. However, it was also the same time that students were frustrated, anxious and distressed about their academics. The present study was conducted among students of first two years of Gujranwala Medical College, Gujranwala. The study aimed at evaluating the basic resources available to these students and their willingness to start online teaching. Inadequate skills for using electronic devices with superadded drawback of restricted internet access and other resources due to financial and social constraints create hindrance in web-based learning.[14]

Research on willingness of students for online education has shown variable results across the globe. The basic factors that determine willingness were found to be level of knowledge and availability of resources. In a study by Alsoufi *et al*[15], 90% of students were highly efficient and familiar with technology but their high reluctance (78.3%) to shift to online mode of teaching was due to civil war and financial catastrophe in country. Medical students from various medical schools of Egypt were assessed for their attitudes towards online education; 51% students were in favour of face to face teaching as compared to online teaching. Technological familiarity was not an issue but poor internet connectivity was a major hindrance in those students.[16] Qazi A *et al*[17] conducted a comparison study between underdeveloped and developed countries in coping with pandemic regarding education and found that in Pakistan people were less to moderately satisfied with online resources and had financial limitations.

In our study 67.1% students had smartphones and 31% had laptops and no significant correlation was found between type of device and willingness for online teaching. Results of the study conducted by Al-Araibi *et al*[18] manifested a positive correlation between hardware accessibility and readiness for online learning. Their study recommended that better standard of resources enhances the willingness for online teaching.[18] In our study, 26.6% of students had no awareness about online teaching tools, 59.5% had heard about Zoom, 8.9% had heard about Google Classroom, 3.2% had heard about Google Meet and only 1.9% had heard about Microsoft Teams. The level of awareness was generally low among the students. The results of our study are consistent with the results of Carvalho *et al*[19] where public sector university students were found deficient in knowledge about e-learning due to scarcity of resources.

Willingness for online education in medical students in India has disclosed variable results. The study conducted by Sud R *et al*[20] in undergraduate medical students showed that majority of students were cognizant of various features of e-learning. These students had a previous exposure to online learning resources even before the pandemic, so they were comfortable using this medium. In an extensive study conducted by Singh *et al*[10] among students of 200 medical and nursing colleges across India, only 45% of students were in favour of online teaching to save their time during pandemic. This was due to lack of preparedness for this unexpected situation in all fields especially education sector.

In Pakistan also, the willingness of students did not show the same pattern across the country. The results of the study conducted by Anwar *et al*[21] among 283 medicine and dentistry students at a private medical institution were quite different from our study, where majority of students were ready for online education. This may be attributed to their strong socioeconomic background with easy access to online resources and a better technical knowledge.

The results of a study conducted by Abbasi S *et al*[22] in a private medical college of Pakistan showed lack of readiness for online learning among medical and dental student. Seventy-seven percent students had a negative attitude toward e-learning. Despite technological advancement and easy access, students still preferred face-to-face teaching over online teaching as they were used to classroom teaching and felt comfortable in that environment.

The better result of professional exam during COVID-19 pandemic can be attributed to students focus on study realizing unpredictable circumstances and thier responsibility of self-study as well as lenient behaviour of examiners during marking of theory and practical exams being compassionate with students.

The results of present study were utilized in proposing, devising, and carrying out such e-learning sessions that could enhance student and faculty satisfaction and improve quality of learning. Improved IT support and training of students and faculty were suggested to college administration for a better outcome.

STRENGTH OF THE STUDY

The study provides valuable insights into the readiness and willingness of medical students for online education during an unprecedented situation. It captures a complete sample of first and second-year students at an institution. Study assessed the relationship between various factors and willingness for online learning.

WEAKNESSES OF THE STUDY

The study is limited to a single medical college, making it difficult to generalize findings to other institutions. The self-reported nature of the questionnaire may introduce response bias. Study does not explore long-term adaptation and effectiveness of online teaching beyond students' initial willingness.

CONCLUSION

In spite of partial willingness of students and limited resources, the learning objectives could be achieved well through online teaching which is evident from results of University/Professional examinations. Online teaching is a suitable option during any unpredictable and untoward circumstances. The study highlights significant gaps in technological resources and awareness among students regarding online teaching. Addressing these gaps through improved training, institutional support, and enhanced digital infrastructure is crucial to ensure the successful integration of online education.

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