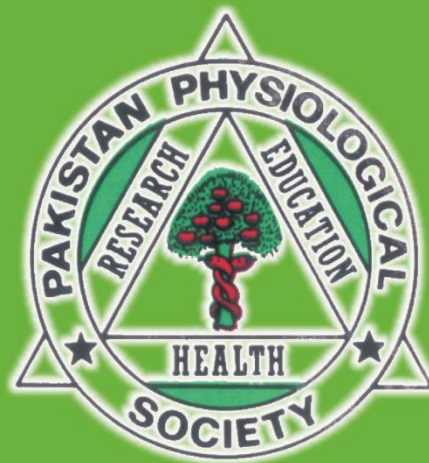


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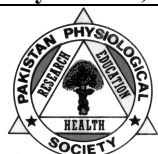
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EDITORIAL

NETWORK PHYSIOLOGY: HISTORY, ROLE, AND SCOPE

Ahmed Badar

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Network physiology is a contemporary concept that connects established systems physiology with modern interdisciplinary methodologies. This field examines the network-like interactions of physiological systems, utilizing computational and mathematical analyses of physiological signals to elucidate health and disease. Network physiology represents a significant shift in the study of human physiology. By emphasizing the interconnected nature of physiological systems, it surpasses traditional limitations and offers new understandings of health, disease, and human performance. The interdisciplinary nature and technological basis of this evolving field suggest substantial opportunities for future medical innovation and scientific discovery.

Keywords: Network Physiology, Human Physiome, Interdisciplinary Physiology

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The human body operates as an intricate system where various physiological systems synchronize to maintain balance. While individual systems like the cardiovascular, nervous, and endocrine systems have been extensively studied in isolation, the connections between them are equally crucial. Network physiology, a developing interdisciplinary area, offers a new perspective to examine these interactions, focusing on the network structure underlying physiological functions. By investigating the interplay among these systems, this field has the potential to revolutionize our understanding of health and disease.

Historically, physiology has often employed reductionism, a method that analyzes systems by dissecting them into individual components for separate study. Although this approach has yielded significant knowledge, it often neglected the interdependencies between systems. The origins of network physiology can be traced back to advancements in systems biology and computational physiology. In the 20th century, researchers began using computational models to simulate interactions between physiological components. The emergence of technological innovations such as big data analytics and machine learning enabled scientists to analyze physiological signals across multiple systems.

The ‘Human Physiome’ refers to a comprehensive framework or model that maps the interactions and interdependencies among various physiological systems in the human body. It aims to provide a holistic understanding of how different organ systems, such as the cardiovascular, nervous, respiratory, and endocrine systems, work together to maintain homeostasis and respond to changes in health and disease. By studying the human physiome, researchers can gain insights into the complex dynamics of these systems, identify patterns of dysfunction, and develop more effective diagnostic and therapeutic strategies.¹

Network physiology gained formal recognition as a distinct field in the early 21st century, spurred by significant studies linking network theory to physiological phenomena.² Recent research has underscored the importance of network physiology in understanding complex physiological interactions, such as how different systems like the cardiovascular and respiratory systems synchronize to maintain homeostasis. Studies on heart rate variability highlighted how cardiac function depends not only on the heart itself but also on the central nervous system, respiratory dynamics, and endocrine responses. These findings emphasized the necessity of a network-based approach to physiology. Research has explored the application of adaptive networks in modelling physiological systems, demonstrating how dynamic interactions between subsystems contribute to emergent behaviours critical for physiological regulation, such as synchronization and phase transitions.³ The practical applications of network physiology have also been a focus, with research on adaptive networks providing insights into how physiological systems reorganize in response to stress or disease states. This has implications for critical care, where real-time monitoring of network dynamics can inform interventions.² The integration of wearable biosensors and machine learning has become a significant area of focus, enabling continuous monitoring of physiological signals and offering new opportunities for personalized medicine and predictive modelling.

Network physiology is based on several core principles including network topology, interdisciplinarity, dynamical systems, and a health and disease framework. Network topology, the arrangement and interaction of nodes (physiological components) and edges (connections between them), is central to this discipline. Network physiology exemplifies interdisciplinary collaboration by integrating insights from physiology, computational biology, mathematics, and physics. Physiological systems are inherently

dynamic, and network physiology incorporates nonlinear dynamics to study how system states change over time. Similarly, by comparing healthy and diseased states, this field clarifies how disruptions in network interactions contribute to disease.

Network physiology offers significant insights into the mechanisms of health and disease. Unlike traditional methods that may focus on individual organs, this field examines interactions within and across systems. For example, by analyzing heart rate variability alongside respiratory and autonomic signals, researchers have identified network disruptions as early indicators of cardiovascular pathologies. In epilepsy, network analysis of brain regions has revealed pathways that propagate seizures. Similarly, studies in neurodegenerative diseases like Parkinson's have identified 'altered communication' between motor and autonomic systems. Beyond clinical settings, this field has applications in areas such as sports science, aging, and stress research.

The potential applications of network physiology are extensive. Integrating network analyses into clinical diagnostics could facilitate personalized treatment plans that account for system-wide interactions. Advances in wearable biosensors provide continuous physiological data streams, supporting real-time network analysis. Machine learning algorithms are well-suited to identify patterns in complex physiological networks, enhancing predictive modelling. Network physiology could also be applied to epidemiological studies, examining how population-level factors influence physiological networks.

Professor Plamen Ch. Ivanov (Boston University, US) formally introduced the field, explaining how health and disease could be redefined by focusing on the complex coordination and dynamic interactions of diverse physiological systems, networks,

and organs, rather than studying each in isolation. Professor Plamen Ch. Ivanov maintains a blog on network physiology on 'The Physiological Society UK' website. It introduces this emerging field and provides links to related webinars.⁴

'Frontiers in Network Physiology' is currently the only dedicated journal for network physiology with its first issue published in 2021. The journal's website introduces its scope as 'It focuses on the emerging field of network physiology, aiming to understand the dynamic interactions and integration of diverse physiological systems and sub-systems in health and disease.' The website also lists topics of interest and specifies that 'The journal encourages research focused on the development of novel biomedical device platforms for synchronized high-frequency recordings and the creation of the Human Physiome, a comprehensive dataset containing large-scale signals from multiple physiological systems.'⁵

Looking ahead, challenges such as standardizing methodologies, ensuring data privacy, and fostering interdisciplinary collaboration need to be addressed to fully realize the potential of this field.

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ORIGINAL ARTICLE

ANTIOXIDANT QUERCETIN LEVELS IN SUB-FERTILE MEN
AND THEIR ASSOCIATION WITH OXIDATIVE STRESSQurat ul Ain Fatima, Sadia Rehman*, Faizania Shabbir**, Tallat Naureen,
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Background: Oxidative stress contributes significantly to male sub-fertility, underscoring the importance of antioxidants in improving semen quality. The primary objective of this study was to evaluate *in vivo* levels of antioxidant quercetin in sub-fertile men. **Methods:** This cross-sectional study was conducted over a period of 6 months at Imran Idrees Teaching Hospital Sialkot, Pakistan. The study population consisted of 44 males, divided into two groups: 11 healthy fertile controls and 33 sub-fertile men. The study group was further subdivided into those diagnosed with varicocele (n=11) and those without varicocele (n=22). Semen samples were collected following 3–4 days of abstinence in a sterile environment through masturbation. Semen analysis was performed according to WHO criteria, and sperm morphology was assessed using Kruger's strict criteria. Quercetin levels in semen were measured using High-Performance Liquid Chromatography (HPLC). Data analysis was conducted using SPSS, with One-way ANOVA employed for variable comparisons and Scheffe's test for correlation studies. **Results:** Healthy controls had higher sperm counts (80.09 ± 5.40 million/mL) and motility ($77.73 \pm 3.72\%$) than sub-fertile men with varicocele (14.00 ± 4.02 million/mL, $30.18 \pm 7.13\%$) or without varicocele (23.55 ± 3.17 million/mL, $34.77 \pm 6.51\%$) ($p < 0.001$). Quercetin levels were significantly higher in controls (865.84 ± 227 µg/mL) compared to study group ($p < 0.05$). **Conclusion:** This study confirms the presence of quercetin in semen and demonstrates its positive correlation with sperm parameters related to fertility. Diet rich in the antioxidant quercetin may enhance male fertility, particularly in men seeking treatment for sub-fertility.

Keywords: Antioxidant, Oxidative Stress, Quercetin, Sub-fertile, Infertility

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INTRODUCTION

Infertility problem is a serious concern in our society. Approximately, 8–12% couples are affected out of which half can be due to either partner. Infertility has been defined as an incapability of a couple to conceive after having a regular unprotected intercourse for one year.¹

Male sub-fertility is due to disruption in spermatogenesis progression normally. It is presently known that sub-fertile men have numerous structural and functional defects of spermatozoa. Thermal harm to the proteins and nuclear deoxyribonucleic acid (DNA) of spermatozoa is possibly the reason for change in spermatogenesis. Additional factors, for instance, cigarette smoking, progressed fatherly age, infections and inflammation of genital tract, drugs, hormones, raised scrotal temperature and varicocele, and intra-testicular components, for example, adjusted spermatozoal chromatin bundling, exorbitant reactive oxygen species (ROS) in seminal discharge and pre-ejaculatory abortive apoptosis subsidize to aetiology of spermatozoal chromatin defect.²

Varicocele is abnormally convoluted and enlarged spermatic veins in pampiniform rete having receding heme flux in internal spermatic vein. Varicocele is the commonest cause of sub-fertility

among males, seen in 15% of healthy fertile male and 40% in sub-fertile men. Varicocele leads to defective spermatogenesis due to increased level of oxidative stress among sub-fertile males.³

Under aerobic conditions, human spermatozoa can produce ROS. Abnormal spermatozoa or seminal leukocytes are the main sources of excess ROS production endogenously. The loss of counterbalance among ROS generation and antioxidants defensive action to detoxify ROS by neutralization and removal leads to oxidative stress (OS). Relatively 25% of the subfertile males present with increased ROS levels and decreased antioxidant capacity of semen.⁴

Antioxidants are present in dietary substances and are also available as supplements. Both enzymatic and low molecular weight non-enzymatic form of antioxidants such as flavonoids are present in seminal plasma. Anti-oxidants prevent DNA fragmentation and damage. Standard antioxidants in semen incorporate vitamin C, vitamin E, glutathione, superoxide dismutase, and thioredoxin. These antioxidants kill free radical movement and shield sperm from ROS. Sub-fertile men have reduced anti-oxidant levels of semen and large amounts of ROS distinguished from fertile men, proposing an association between total antioxidant

capacity and male infertility. Among anti-oxidants, our focus of research was Quercetin.⁵

Quercetin is world's most broadly utilized dietary flavonoid. It is a reddish pigment found in apple, tea, vegetables, onion, and red wine. Quercetin is a strong anti-oxidant having cytoprotective properties in repressing oxidative damage and apoptosis caused by ROS and other forms of free radicals.⁶

Quercetin has been reported to mitigate effects of heavy metal toxicity on male reproduction. By scavenging free radicals Quercetin protects against reperfusion ischemic tissue injury. The role of quercetin as defensive agent has been reported on laboratory male animals' reproductive system and *in vitro* investigations on human semen samples so far.⁷ Reproductive function in man has been emphasized to be conserved by quercetin. Quercetin has been demonstrated to enhance the functional and morphological integrity of the male reproductive system, as evidenced by increased sperm count, heightened tubular epithelial cells, augmented proliferative capacity and enhanced antioxidant enzyme activity and diminished levels of oxidative stress-related compounds. In rats, the higher concentration of Quercetin is found in testes and lungs and lowest in brain among all organs.⁸ There are numerous reported approaches for the apprehension of quercetin quantitatively. The most common techniques for the quantifiable revelation of quercetin are High-Performance Liquid Chromatography (HPLC) and, High Performance Thin Layer Chromatography (HPTLC). The preferred method for the immediate determination of quercetin in food, plant extracts, body fluids and pharmaceutical products is HPLC.⁹

Despite the growing body of research on antioxidants and male fertility, limited studies specifically address the role of quercetin as a biomarker for oxidative stress. Recent advances in fertility research emphasize the importance of antioxidant supplementation, but their direct correlations with seminal parameters remain under explored. This study aims to bridge this gap by assessing the association of quercetin levels with male sub-fertility.

MATERIAL AND METHODS

The study was approved by the Ethics Committee of Imran Idrees Teaching Hospital, Sialkot, Pakistan. Subjects in the study provided written informed permission after being explained the overall purpose and process of the investigation. The sample size of 44 was calculated using OpenEpi software at a 95% confidence interval, with an effect size adequate for detecting significant differences in quercetin levels. The study population was divided into three categories, healthy fertile males as the control group (n=11), sub-fertile men without varicocele (n=22) and sub-fertile men with varicocele (n=11). Healthy samples were taken from

general population and volunteers who were married males having children or their wives were pregnant at the time of study, and the sub-fertile group was categorized after obtaining a detailed history, clinical examination, and investigations of men married for more than one year going to the Infertility Clinic of the Hospital for their fertility assessment and therapy for lack of conception. Men with azoospermia and female factors responsible for sub-fertility or infertility were excluded from our study. Semen samples were collected in the vicinity of lab after an abstinence period of 3–4 days by masturbation in a sterile environment.

Sperm concentration, percentage motility, and sperm morphology were identified after liquefaction at 37 °C according to WHO criteria 2010 and sperm morphology by Kruger's strict criteria. Reliability of HPLC was ensured through calibration with standards from Sigma-Aldrich and validation using spiked semen samples with known quercetin concentrations. Reproducibility and accuracy were assessed with an intra-assay coefficient of variation of <5%.

Test sample (300 µL) was taken in a screwed test tube and 2 ml of 1:1 water and chloroform mixture were added. Then it was sonicated for 5 minutes with shaking. Chloroform layer was extracted in a separate screwed test tube. Aqueous layer was extracted twice with 1 ml portions of chloroform. Organic layers were combined and evaporated. Residue was dissolved in 0.5 ml ethanol and 50 µL of this solution was used for quercetin quantification via HPLC.

Hitachi Primaide HPLC system was used for quantification of quercetin in semen samples at a wavelength of 370 nm. HPLC/UV analysis were performed on a HITACHI Primaide Organizer System (Tokyo, Japan).

Data were entered and analysed on SPSS-21. Mean±SEM were used to represent data. One way ANOVA was applied, and $p < 0.05$ was considered statistically significant.

RESULTS

Fertile men had an average age of 29.27 ± 1.83 years, whereas the sub-fertile group without a varicocele had a mean age of 32.73 ± 1.29 years and the sub-fertile varicocele group had a mean age of 34.64 ± 2.24 years.

Table-1 shows a significant ($p < 0.001$) increase in sperm count of healthy controls as compared to subjects with or without varicocele. The percentage of spermatozoal motility shows a significant ($p < 0.001$) decrease in subjects with or without varicocele as compared to healthy fertile controls. In accordance to the Tygerberg strict criteria, mean percentage normal morphology of spermatozoa was non-significant ($p > 0.05$) in varicocele-positive and varicocele-negative men in comparison to healthy controls.

Table-1: Seminal parameters according to WHO 2010 Criteria (Mean±SEM)

Sperm Parameters	Controls (n=11)	Sub-fertile males without varicocele (n=22)	Sub-fertile males with varicocele (n=11)	p
Semen volume (mL)	3.01±0.31	2.66±0.11	2.36±0.24	0.140
Sperm count (million/mL)	80.09±5.40	23.55±3.17	14.00±4.02	0.000*
Sperm motility (%)	77.73±3.72	34.77±6.51	30.18±7.13	0.000*
Normal sperm morphology (%)	15.82±1.63	14.59±2.06	10.27±2.64	0.276
Leukocyte count (million/mL)	0.53±0.04	1.42±0.49	0.40±0.19	0.177

*Highly significant using ANOVA

Figure-1 shows low levels of anti-oxidant Quercetin ($p<0.01$) in sub-fertile groups with or without varicocele as compared to healthy fertile controls.

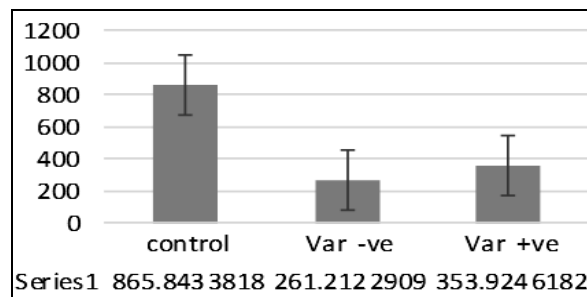


Figure-1: Levels of quercetin in control and subfertile men (µg/mL)

Var -ve: subfertile without varicocele, Var +ve: subfertile with varicocele

DISCUSSION

This study aimed to evaluate seminal parameters and quercetin levels in fertile and sub-fertile males, with a focus on the impact of varicocele. The results indicated significant differences in sperm count, motility, and quercetin levels between fertile and sub-fertile males with or without varicocele.

The observed reduction in sperm count and motility in sub-fertile males with varicocele aligns with findings from several studies. A study by El-Khawagah *et al*¹⁰ demonstrated that quercetin supplementation in semen extenders significantly improved sperm motility and viability in buffalo bulls after cryopreservation. Our study found that quercetin levels were significantly lower in subfertile groups, suggesting a potential deficiency in natural antioxidant defences, which could exacerbate oxidative stress and further impair sperm function.

In our study, sub-fertile males without varicocele showed a significant reduction in total sperm count and motility compared to healthy controls. This finding is consistent with the work of Avdatek *et al*¹¹ which reported that oxidative stress markers are elevated in infertile males, leading to impaired sperm quality. Our findings reinforce the notion that oxidative stress plays a crucial role in male infertility, particularly

in the presence of varicocele, where the stress levels are likely to be higher due to impaired blood flow and increased testicular temperature.

The non-significant differences in sperm morphology between the groups observed in our study contrast with the findings of other studies, such as that by Silva *et al*¹² who reported significant improvements in sperm morphology with antioxidant supplementation, including quercetin. This discrepancy might be attributed to differences in study populations, methodologies, or the specific antioxidants evaluated.

Quercetin levels were significantly lower in subfertile groups compared to healthy controls, a finding that is consistent with other studies emphasizing the role of antioxidants in preserving sperm quality. Tvrdá *et al*¹³ highlighted the protective effects of quercetin against oxidative damage in bovine spermatozoa, particularly in maintaining mitochondrial function, which is crucial for sperm motility. This parallels our observation that reduced quercetin levels are associated with diminished sperm motility in sub-fertile males.

The findings of this study align with existing literature emphasizing the role of oxidative stress in male sub-fertility and the potential therapeutic value of antioxidants like quercetin. By demonstrating significantly reduced quercetin levels in sub-fertile men, particularly those with varicocele, our results highlight the need for targeted dietary or therapeutic interventions. Comparisons with broader studies on antioxidant profiles underscore the importance of integrating multiple biomarkers to better understand oxidative stress and its impact on fertility. Our study highlights significant differences in seminal parameters and quercetin levels between fertile and sub-fertile males, with varicocele exacerbating these differences. The results are consistent with the general research on the significance of oxidative stress and anti-oxidants in male fertility, notably the potential therapeutic effects of quercetin supplements in treating varicocele-related infertility. However, further study is needed to determine the best quercetin dosage techniques and to investigate its impact on other sperm characteristics, in other populations.

Antioxidant activity in biological systems is influenced by a complex interplay of various enzymatic and non-enzymatic factors. While quercetin was the primary focus of this study due to its established role as a potent dietary antioxidant, we recognize that it is only one component of the antioxidant defence system. Comprehensive assessment of antioxidant activity would ideally include a broader range of biomarkers, such as glutathione, superoxide dismutase, catalase, and total antioxidant capacity. These biomarkers could provide a more holistic view of the oxidative stress status and its implications on male fertility.

The decision to focus on quercetin was based on resource limitations and its emerging significance in fertility research. However, we acknowledge this as a limitation and propose that future studies expand their scope to include additional markers. By integrating these parameters, subsequent research can further elucidate the potential of antioxidants in mitigating oxidative damage and improving reproductive outcomes. One limitation of this study is the exclusion of body mass index (BMI) as a variable. BMI is a known factor influencing oxidative stress and could have provided additional insights into the relationship between oxidative stress, quercetin levels, and sperm quality. Including BMI as a parameter would have strengthened the findings and offered a more nuanced understanding of its role in male fertility. The cross-sectional design of this study limits its ability to establish causation between quercetin levels and sub-fertility-related oxidative stress. While the findings provide valuable insights into potential associations, a longitudinal or interventional study would better elucidate causal mechanisms. Future studies should overcome these limitations by involving bigger, more varied groups and studying the implications of quercetin supplementation over extended periods.

CONCLUSION

Sub-fertile males, particularly those with varicocele, exhibit significantly reduced sperm count, motility, and quercetin levels compared to fertile males. The findings highlight the role of oxidative stress in male infertility and suggest that lower quercetin levels may contribute to diminished sperm quality.

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ORIGINAL ARTICLE

EFFECT OF DEMOGRAPHIC AND PHYSICAL FACTORS ON NERVE CONDUCTION IN MEDIAN AND ULNAR NERVES IN HEALTHY SUBJECTS

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Background: Nerve conduction study (NCS) is an important method to assess the integrity of peripheral nervous system. It helps in diagnosis, planning of treatment, and prognosis of diseases of peripheral nerves. NCS parameters are affected by several different physical factors like temperature, demography, and ethnicity. It can also be affected by age, gender, height, weight, BMI, etc. The present study was targeted at finding out the changes in median and ulnar nerve conduction related to demographic parameters. **Methods:** Seventy-five healthy subjects aged 40–70 years undertook NCS using standard protocol. It was a cross-sectional study conducted at Institute of Basic Medical Sciences, Khyber Medical University, Peshawar and Combined Military Hospital, Abbottabad. The NCS were performed on Nion Kohden Neuropack MI EP/EMG measuring system. Various physical and demographic factors, i.e., age height, weight BMI were correlated to NCS parameters using Pearson's correlation. **Results:** A total of 75 people 32 (42.66%) females and 43 (57.33%) males were recruited for study after qualifying the inclusion criteria. A decline in all NCS parameters was observed with advancing age. Height affects sensory and motor latencies (negative correlation). Weight and BMI don't have prominent effects on NCS parameters. **Conclusion:** The sensory NCS characteristics (amplitude, latency, and Nerve Conduction Velocity (NCV) and motor NCS parameters (amplitude and NCV) of the median and ulnar nerves significantly deteriorate with age. Except for ulnar sensory amplitude and latency, there was no discernible variation in NCS characteristics between males and females.

Keywords: Nerve Conduction, Median Nerve, Ulnar Nerve, MyelinPak J Physiol 2025;21(1):7–9, DOI: <https://doi.org/10.69656/pjp.v21i1.1762>

INTRODUCTION

An important technique for the diagnosis and assessment of neuromuscular disorders is the electro diagnostic (EDX) study.¹ Neural conduction studies (NCS) and needle electromyography (EMG) are two of these studies. The electro diagnostic examinations are crucial for localizing lesions and can assist in determining the kind and location of the lesion such as neuropathy, myopathy, neuromuscular junction lesion, or central nervous system problem. They play a vital role in localizing neuropathic diseases.²

The source of a nerve lesion can be investigated at multiple levels, including the neuron, nerve, nerve plexus, or nerve root. Conditions such as mononeuropathy (affecting a single nerve), mononeuropathy multiplex (involving several individual nerves), and polyneuropathy (impacting all nerves) warrant further exploration. If needed, additional research can determine the type of fibres involved in neuropathic disorders, whether sensory, motor, or mixed. This investigation can also clarify whether axon loss or demyelination is the underlying cause of the condition.³ This approach can measure the extent of demyelination or axonal loss, providing insights into the

prognosis and severity of the disease, as well as the duration of acute, sub-acute, or chronic neuropathy. It offers valuable information on the distribution of the condition (proximal, bulbar, or generalized), the underlying pathology (such as presynaptic conditions like botulism or postsynaptic conditions like myasthenia gravis), and the aetiology (whether genetic or acquired) related to neuromuscular disorders.⁴ The fundamental method for studying nerve conduction involves applying an external electrical pulse that generates a propagated nerve action potential (NAP), which can be recorded at a distal site along the same nerve.² It also generates a compound muscular action potential (CMAP), which is the result of a particular muscle fibre activation. Surface electrodes or needle EMG can be used to capture both of these potentials.⁵ Nearly half of debility after the age of 65 is attributed to neurological diseases, which are particularly prevalent in old age.⁶ Alzheimer's disease and dementia are age-related illnesses. Between 65 and 85 years of age, the incidence of Alzheimer's disease doubles every five years.⁷ The structure and function of peripheral nerves are significantly impacted by aging. Because the blood-brain-barrier shields the CNS from external stressors, it is less susceptible than the

peripheral nervous system to trauma, damage, and infections. The nerve fibres consist of myelin sheaths and axons. Therefore, these two factors are primarily responsible for the loss in nerve function. Thus, although there may be other contributing factors as well, these two elements are also primarily responsible for the loss of nerve function.⁸ Decreases in nerve conduction velocity are caused by changes in the myelin sheath. The amplitude of CMAP or sensory nerve action potential (SNAP) may decrease with age due to a possible decrease in the number of functioning axons. As people age, their non-neural cells may undergo additional alterations, and deficiencies in vitamins B₁₂ and E which might cause damage to their nerves.⁹ The present study was targeted at finding out the changes in median and ulnar nerve conduction related to demographic parameters.

METHODOLOGY

The study was conducted at the Institute of Basic Medical Sciences at KMU Peshawar and CMH Abbottabad, with approval from the ASRB and the KMU ethical board. Using STAT (UBA), the sample size was determined with a power of 0.80 and α value of 0.05. Seventy-five people made up the sample size for this cross-sectional study, which was chosen via convenient sampling. Those without a history of upper limb injury, myopathy, or neuropathy, as well as those with normal fasting blood sugar (FBS), urea, creatinine, liver function tests (LFTs) and ESR were included in the study.

The nerve conduction studies were performed on Nihon Kohden Neuropack M1 EP/EMG measuring system MEB-9200/MEB-9300. For motor studies the nerves were studied orthodromically. For sensory studies the nerves were studied antidromically. All electrodes were placed on specific and measured distances from each other according to the standard technique given by AANEM.¹⁰ All the parameters, e.g., demographic parameters, blood parameters, and NCS parameters were entered into Microsoft Excel worksheet. All these parameters were then organized, and coding was done to import them into SPSS-22. The nominal variables were looked for frequencies and percentages, while numerical variables were analyzed for Mean $\pm$ SD. Different groups were compared with each other using Pearson's correlation for multiple group comparisons, and $p \leq 0.05$ was considered statistically significant.

RESULTS

A total of 75 people between the ages of 40–70 were recruited for the study after qualifying the inclusion criteria, out of which 32 (42.66%) were females and 43 (57.33%) were males. A decline in all NCS parameters was observed with advancing age (decreased amplitude,

increased latency, and decreased NCV significantly in sensory assessments). Height has a negative correlation with sensory and motor latencies. Weight and BMI do not have significant effects on NCS parameters.

The mean age of the subjects was 53.83 $\pm$ 8.33 Years, height was 162.67 $\pm$ 7.91 Cm, weight was 72.59 $\pm$ 11.38 Kg, and BMI was calculated as 27.32 $\pm$ 3.68 Kg/m².

With advancing age, median motor latency increased ($\rho=0.475$) while median motor amplitude decreased ($\rho=-0.419$). Height had effect on median motor latency which increases with increase in height ($\rho=0.388$). Weight is affecting ulnar motor amplitude which is increasing with increasing weight ($\rho=0.229$). Increased BMI increases median motor amplitude ($\rho=0.298$). (Table-1).

With advancing age, median sensory latency increased ($\rho=0.511$), median sensory amplitude decreases ($\rho=-0.457$), ulnar sensory latency increases ($\rho=0.297$) whereas ulnar sensory amplitude decreases ($\rho=-0.330$). Height has a significant effect only on ulnar sensory latency which increases with increasing height ($\rho=0.412$). However, weight and BMI don't have any significant effect on median and ulnar sensory parameters. (Table-2).

Table-1: Correlation of motor NCS parameters with various factors

Parameter	Median Nerve			Ulnar Nerve		
	Latency	Amplitude	NCV	Latency	Amplitude	NCV
Age	0.475*	-0.419*	-0.131	0.163	-0.137	-0.148
Height	0.388*	0.207	-0.080	0.462*	0.235*	-0.078
Weight	0.260	0.055	-0.076	0.136	0.229*	0.008
BMI	-0.079	0.298*	-0.022	-0.197	0.109	0.052

*Correlation is significant at the 0.01 level

Table-2: Correlation of sensory NCS parameters with various factors

Parameter	Median Nerve			Ulnar Nerve		
	Latency	Amplitude	NCV	Latency	Amplitude	NCV
Age	0.511*	-0.465*	-0.457*	0.297*	-0.330*	-0.110
Height	0.113	-0.097	0.097	0.412*	-0.154	-0.215
Weight	0.037	-0.028	0.101	0.050	0.003	0.002
BMI	-0.020	0.027	0.024	-0.260*	0.111	0.182

*Correlation is significant at the 0.01 level

DISCUSSION

An essential and sensitive method for assessing neuronal diseases is nerve conduction study.¹¹ In addition to other variables (height, gender, and BMI), age and ethnicity have a significant impact on nerve conduction parameters.¹² As one ages, there is a decrease in NCV, an increase in latency, and a decrease in the amplitude of the action potential; however, there is no specific age at which this decline starts.¹³ There is a decrease in the number of nerve fibres, reduced nerve diameter, and change in fibre membrane with aging.¹⁴ The reduced diameter of nerve fibre is either due to a reduction in myelin content or axon loss in nerve fibres.

We observed that as people aged, the amplitude of their CMAP and SNAP decreased. Werner *et al*¹⁵ reported similar results, observing a drop in median and ulnar peak sensory amplitudes throughout the course of a four-year follow-up investigation. Since amplitude is a measure of all the active axons in a nerve fibre, a fall in amplitude with age can be the cause of decline in axon function or number. Hennessey *et al*¹⁶ observed that as people age, the median nerve's CMAP amplitude decreases. They stated that the average drop in sensory amplitude was 10% per decade, but the average decline in motor amplitude was 5% per decade.¹⁶ In our subjects there was an increase in the sensory and motor latency of the ulnar and median nerves as people aged, indicating that nerve impulses are transmitted to the nerve fibres more slowly as people age. Numerous reasons, including changes in the myelin sheath (myelin figures/myelin balloons), the loss of nerve fibres, and a decrease in neurotransmitters, could be the cause of this.¹⁷ After 4 years, the median and ulnar sensory latencies rises from their baseline values.¹⁵ The current study also showed a reduction in median and ulnar nerve conduction velocities with increasing age. Since NCV indicates the degree of myelination of peripheral nerve fibres, the loss of myelinated nerve fibres may be the cause of the decline in NCV with aging.¹⁸ The NCV reduction starts from age 30 to 40.^{13,19}

CONCLUSION

The sensory NCS characteristics (amplitude, latency, and NCV) and motor NCS parameters (amplitude and NCV) of the median and ulnar nerves significantly deteriorate with age. The median nerve showed more pronounced age-related alterations than the ulnar nerve. A larger sample size and continued follow-up with the same research population would have produced more reliable results.

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ORIGINAL ARTICLE

DIAGNOSTIC SIGNIFICANCE OF BONE MARROW TREPHINE BIOPSY
IN DIFFERENT TYPES OF LYMPHOMAMaham Arshad, Aiza Saadia, Sunila Tashfeen, Saima Bashir,
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Background: Bone marrow trephine biopsy is a critical diagnostic tool for detecting lymphoma. This study was conducted to evaluate the usefulness of bone marrow trephine (BMT) biopsy of lymphomas followed by immunohistochemistry. **Methodology:** This cross-sectional retrospective study was done at Department of Pathology (Haematology), Pak Emirates Military Hospital, Rawalpindi from Apr to Jun 2023. Through non-probability consecutive sampling, bone marrow aspiration and trephine biopsy of 43 patients were assessed to see the morphology of cells and pattern of involvement of bone marrow. Immunohistochemistry was also applied on the trephine to confirm the diagnosis and know the origin of these cells. Statistical analysis was done using SPSS-22. Quantitative data was presented as Mean $\pm$ SD and qualitative data was presented as frequency and percentage. Chi-square test was applied and $p \leq 0.05$ was taken as statistically significant. **Results:** There were 32 (74.4%) male, and 11 (25.6%) female patients. On bone marrow aspiration 16 (37.2%) had non-Hodgkin's Lymphoma, 11 (25.6%) had Chronic Lymphocytic Leukaemia, 2 (4.7%) had diffuse Large B-Cell Lymphoma, 2 (4.7%) had Acute Lymphoblastic Leukaemia, 1 (2.3%) had T-Cell, and 2 (4.7%) showed others diagnosis like Myelodysplastic syndrome. Six (14.0%) showed inconclusive results and 3 (7.0%) had reactive bone marrow. Thirteen (30.2%) patients showed non-Hodgkin's Lymphoma B-Cell in both aspirate and trephine, 3 (7.0%) had Chronic Lymphocytic Leukaemia, and 1 (2.3%) showed T-Cell, ($p < 0.001$). **Conclusion:** Bone marrow trephine biopsy plays a pivotal role in the diagnosis of various types of lymphoma and enhances precision of diagnosis.

Keywords: Immunohistochemistry, lymphoma, Trephine biopsy

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INTRODUCTION

Lymphomas are heterogeneous group of malignancies that arise from the clonal proliferation of B cell, T cell and natural killer (NK) cell, subsets of lymphocytes at different stages of maturation. Lymphoma represents approximately 5% of malignancies.¹ Lymphomas are broadly classified into Hodgkin lymphoma and non-Hodgkin lymphoma.² Different diagnostic modalities are used for the diagnosis of lymphoma in bone marrow including bone marrow aspiration and bone marrow trephine biopsy augmented by immunohistochemistry.³

Lymphoid infiltrates in bone marrow trephine biopsies are most commonly encountered as part of diagnostic or staging work up for leukaemias/lymphomas or as an incidental finding in a sample taken for neoplastic or non-neoplastic bone marrow pathology.⁴ Bone marrow trephine biopsy is invasive but well-tolerated and should be considered the first-line diagnostic test in patients with suspected lymphoproliferative disorders.⁵ The findings of bone marrow trephine biopsy can be confirmed by application of specific immunohistochemistry markers on the biopsy specimen. Further staging can be done with incisional and excisional biopsy of the relevant enlarged lymph nodes. The involvement of bone marrow by lymphomas, predicts the disease evolution and prognosis.⁶

The clinical presentation of lymphomas shows vast variation, it can be indolent or highly aggressive. Lymphadenopathy in supra diaphragmatic region is mostly Hodgkin lymphoma but non-Hodgkin lymphoma can present anywhere in the body.⁷ The typical systemic symptoms of fever, unexplained weight loss, and night sweats are most important in advanced disease. The route of dissemination of lymphomas can be through blood, lymphatic system and body cavities.⁸ There are also certain viruses which are also associated with lymphomas like Epstein Bar Virus (EBV), Human Papilloma Virus (HPV), Human Immune Deficiency Virus (HIV) and Hepatitis B virus.

The essential way of lymphoma diagnosis is morphology, immunohistochemistry, and flow cytometry.⁹ Furthermore fine-needle aspiration and core needle biopsy are often part of the initial evaluation. The Ann Arbor staging system was used for staging Hodgkin lymphoma, and was later adapted for non-Hodgkin lymphoma. It was further modified by incorporating positron emission tomography/computed tomography. Positron Emission Tomography-Computed Tomography (PET-CT) is used for fluorodeoxyglucose-avid lymphoma subtypes.¹⁰

This study aimed to evaluate patients having lymphoma with fine needle aspiration, trephine biopsy

and immunohistochemistry applied on the cut sections, in early stages as by the initial sign and symptoms with minimum intervention.

METHODOLOGY

This cross-sectional study was conducted in the Department of Pathology (Haematology) Pak Emirates Military Hospital, Rawalpindi during Apr to Jun 2023. We collected retrospective data of last 3 years of the patients, suspected to have lymphoma based on their history and clinical examination. Their bone marrow aspiration and trephine sampling had been done. Sampling was done using non-probability consecutive sampling technique. Informed consent had been taken from the patients. The study protocol was approved by the Ethical Review Committee of the Institution (ERC/ID No. 266).

All the patients presenting with complaints of (fever, weight loss and night sweats) along with physical findings of hepatosplenomegaly and lymphadenopathy were included. Their complete blood counts, peripheral film, bone marrow aspiration and trephine biopsy were done, documented and analysed. Patients with dry tap and inadequate sample obtained were excluded from the study.

Blood sample of 5 mL was taken in EDTA tube for complete blood counts and peripheral blood film examination. CBC was performed by automated haematology analyser. Bone marrow examination was done. With trephine needle bone marrow biopsy was taken. The specimen was embedded in wax and cut sections was prepared and slides were stained. If necessary, immunohistochemistry was applied to make a diagnosis. The retrospective data of 3 years was used to evaluate bone marrow trephine biopsy for lymphomas.

Statistical analysis was done using SPSS-22. Quantitative data was presented as Mean $\pm$ SD and qualitative data was presented as frequency and percentage. Chi-square test (for comparison of qualitative variables) was applied and $p \leq 0.05$ was taken as statistically significant.

RESULTS

A total of forty-three patients were included in the study, 32 (74.4%) patients were male and 11 (25.6%) were female. The mean age of the patients was 58.67 $\pm$ 16.16 years range from 14 to 85 years. Mainly the patients had history of weight loss and fever. (Table-1).

The mean of TLC was 28.18 $\pm$ 55.16 $\times 10^9$ /L, RBC 3.48 $\pm$ 0.86 $\times 10^{12}$ /L, Hb 9.63 $\pm$ 2.27 g/dL, PCV 0.9 $\pm$ 0.07 fL, MCV 85.26 $\pm$ 12.32 fL, MCH 27.93 $\pm$ 3.59 pg, MCHC 32.25 $\pm$ 2.49 g/dL, Platelet Count 144.62 $\pm$ 128.21 $\times 10^9$ /L and ESR was 52.39 $\pm$ 25.69 mm at the end of 1st hour. (Table-2).

The CD20 marker was positive in 33 (76.7%)

patients, followed by Ki67 in 24 (55.8%), CD3 in 18 (41.8%), LCA in 17 (39.5%), Pan Ck in 17 (39.5%), BCL-2 in 11 (25.6%), CD30 in 7 (16.3%), CD10 in 4 (9.3%), BCL-6 in 3 (7.0%), CD5 in 1 (2.3%), and C myc in 1 (2.3%) case. Oct-2 was not positive in any patient. (Table-3).

On bone marrow aspiration 16 (37.2%) patients had Non-Hodgkin's Lymphoma, 11 (25.6%) had Chronic Lymphocytic Leukemia, 2 (4.7%) had Diffuse Large B-Cell Lymphoma, 2 (4.7%) had ALL, 1 (2.3%) had T-Cell, and 2 patients (4.7%) showed others diagnosis like MDS. On trephine biopsy 29 (67.4%) patients had Non-Hodgkin's Lymphoma, 8 (18.6%) had Chronic Lymphocytic Lymphoma, 3 (7.0%) had Diffuse Large B-Cell Lymphoma, 1 (2.3%) had T-Cell, and 2 (4.7%) showed Inconclusive results. (Table-4).

Out of the total, 13 (30.2%) patients showed Non-Hodgkin's Lymphoma B-Cell results in both aspirate and trephine followed by CLL (3, 7.0%), and T-Cell (1, 2.3%). There were statistically significant differences between aspirate and trephine reports of the patients ($p < 0.001$). (Table-5).

Table-1: Patients' symptoms, red blood cell (RBC) morphology and grading of fibrosis (n=43)

Symptoms	Frequency	Percentage
Fever	10	23.6
Weight Loss	11	25.6
Night Sweats	2	4.7
Fever Plus Weight Loss	9	20.6
Vomiting	2	4.7
Fever, headache, constipation	2	4.7
Swelling in back neck	2	4.7
Abdominal Pain	2	4.7
Urinary Incontinence	2	4.7
COVID	1	2.3
RBC Morphology		
Anisocytosis	38	88.4
Poikilocytosis	32	74.4
Hypochromia	20	46.5
Microcytosis	34	79.1
Elliptocytes	15	34.9
Rouleaux Formation	10	23.3
Target cell	20	46.5
Ovalocytes	3	7.0
Reticulin		
Grade 1	22	51.3
Grade 2	13	30.2
Grade 3	6	13.9
Absent	2	4.7

Table-2: Complete Blood Counts of the patients

CBC	Minimum	Maximum	Mean
TLC (10^9 /L)	1.10	298.60	28.18 $\pm$ 55.16
RBC (10^{12} /L)	1.78	5.44	3.48 $\pm$ 0.86
HB (g/dL)	6.30	14.90	9.63 $\pm$ 2.27
PCV (fL)	0.20	0.45	0.29 $\pm$ 0.07
MCV (fL)	28.50	117.40	85.26 $\pm$ 12.32
MCH (pg)	19.40	39.90	27.93 $\pm$ 3.59
MCHC (g/dL)	27.20	41.60	32.25 $\pm$ 2.49
Platelet Count (10^9 /L)	10.00	639.00	144.62 $\pm$ 128.21
ESR	3.00	130.00	52.39 $\pm$ 25.69

Table-3: Interpretation of immunohistochemistry markers (n=43) [n (%)]

Markers	Positive	Negative	Inconclusive
LCA	17 (39.5)	2 (4.7)	24 (55.9)
CD3	18 (41.8)	10 (23.2)	15 (35.0)
CD20	33 (76.7)	9 (20.9)	1 (2.3)
CD30	7 (16.3)	2 (4.7)	34 (79.1)
CD5	1 (2.3)	3 (7.0)	39 (90.6)
CD10	4 (9.3)	13 (30.2)	26 (60.5)
Pan Ck	17 (39.5)	4 (9.3)	22 (51.2)
Ki67 [Low=2, Borderline=7, High grade=15]	24 (55.8)	-	19 (44.2)
Pax 5	2 (4.7)	1 (2.3)	40 (93.0)
Oct-2	0 (0)	1 (2.3)	42 (97.6)
C myc	1 (2.3)	6 (13.9)	36 (83.7)
BCL-2	11 (25.6)	5 (11.6)	27 (62.7)
BCL-6	3 (7.0)	9 (20.9)	31 (72.1)

Table-4: Diagnosis on bone marrow aspiration and bone marrow trephine biopsy (n=43)

Parameter	Frequency (%)
Bone Marrow Aspiration	
Non-Hodgkin's Lymphoma	16 (37.2)
Chronic Lymphocytic Leukaemia	11 (25.6)
Diffuse Large B-Cell Lymphoma	2 (4.7)
ALL	2 (4.7)
T-Cell	1 (2.3)
Inconclusive	6 (14.0)
Reactive	3 (7.0)
Others	2 (4.7)
Bone Marrow Trephine Biopsy	
Non-Hodgkin's Lymphoma	29 (67.4)
Chronic Lymphocytic Leukaemia	8 (18.6)
Diffuse Large B-Cell Lymphoma	3 (7.0)
T-Cell	1 (2.3)
Inconclusive	2 (4.7)

Table-5: Comparison of diagnosis on bone marrow aspiration and trephine (n=43) [n (%)]

Trephine	Aspirate									p
	Non-Hodgkin's Lymphoma B-Cell	CLL	DLBCL	ALL	T-Cell	Diluted	Reactive	Others	Total	
Non-Hodgkin's	13 (30.2)	7 (16.3)	1 (2.3)	1 (2.3)	0 (0)	4 (9.3)	2 (4.7)	1 (2.3)	29 (67.4)	<0.001
DLBCL	1 (2.3)	0 (0)	1 (2.3)	1 (2.3)	0 (0)	0 (0)	0 (0)	0 (0)	3 (7.0)	
CLL	2 (4.7)	3 (7.0)	0 (0)	0 (0)	0 (0)	2 (4.7)	1 (2.3)	0 (0)	8 (18.6)	
T-Cell	0 (0)	0 (0)	0 (0)	0 (0)	1 (2.3)	0 (0)	0 (0)	0 (0)	1 (2.3)	
Inconclusive	0 (0)	1 (2.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (2.3)	2 (4.7)	

DISCUSSION

Lymphoma is a group of histologically and biologically heterogeneous clonal malignant disease arising from the lymphoid system.¹¹ The clinical presentation ranges from indolent to an aggressive nature. Lymphoma is the most common haematological cancer.¹² Its incidence increases with age, and mostly lymphoma patients are elderly.¹³ Environmental toxins like pesticides, tobacco, fertilizers, obesity, genetic factors and autoimmune diseases also play a role in lymphoma aetiology.¹⁴

As red blood cell morphology shows anisocytosis, poikilocytosis with microcytosis and target cells, all these lead to iron deficiency anaemia which in most of the cases is associated with lymphomas. The variables of complete blood counts show vast variation in the minimum and maximum ranges.

For comparison of aspirate and trephine biopsy in diagnosis of lymphoma, 29 cases were diagnosed as non-Hodgkin lymphoma on trephine biopsy and only 13 patients had the same diagnosis on both aspirate and trephine as Non-Hodgkin's Lymphoma B-Cell. Rest of the 16 patients showed different diagnosis.

Worldwide the lymphoma diagnosis is made on morphology, immunohistochemistry and flowcytometry.¹⁵ The prognosis depends upon the International Prognostic Index, is used broadly for all subtypes of non-Hodgkin lymphoma, and the International Prognostic Score is used for Hodgkin lymphoma.¹⁶ Regarding the treatment of lymphoma, it is

the chemotherapy alone or in combination with radiotherapy.¹⁷ In old age patients who are more than 60 years of age at diagnosis have worse outcomes, nevertheless of the staging.¹⁸

There are certain chemotherapeutic agents which the National Comprehensive Cancer Network (NCCN) recommends avoiding them. As concerned to relapse for non-Hodgkin lymphoma is variable which depends upon the type which is diagnosed.¹⁹ As the most common subtype, diffuse large B-cell lymphoma, has a 40% lifetime relapse rate. For Hodgkin lymphoma lifetime relapse occurs in 10% to 15% of patients with early-stage disease but with advanced stage disease the risk increases to 40%.²⁰

In comparison to the international studies Chronic lymphocytic leukaemia, Diffuse large B cell lymphoma and T cell Lymphoma, the Non-Hodgkin B cell lymphoma are the most common then Follicular, Marginal zone, Precursor B cell and least is the Burkitt lymphoma.²¹ In our study the pattern is the same; most common is the non-Hodgkin lymphoma, Chronic lymphocytic leukaemias.

Early diagnosis of lymphoma is crucial to prevent metastasis. When disease is spread in the body it is challenging for the oncologist to treat the patient. Treatment decisions are preferably made in a multidisciplinary setting.²² The prognosis in these patients due to metastasis is really poor. Early diagnosis and confirmation with immunohistochemistry is recommended for the long survival of the patients.

CONCLUSION

Bone marrow trephine biopsy plays a pivotal role in the diagnostics evaluation of various types of lymphoma. Its complementary use along side other diagnostic modalities enhances the precision of lymphoma diagnosis, ensuring optimal patient management.

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ORIGINAL ARTICLE

FREQUENCY OF METABOLIC SYNDROME IN PATIENTS WITH FEMALE PATTERN HAIR LOSS

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Background: Female androgenetic alopecia is a common cause of non-scarring alopecia in women. There is strong pathogenetic link between metabolic syndrome (MetS) and androgenetic alopecia in men. The available evidence on the frequency of metabolic syndrome in women with androgenetic alopecia is limited while there is no local published material which necessitated the present study.

Methods: It was a hospital based cross-sectional study conducted at Dermatology Department, Jinnah Hospital, Lahore, from 1st Jan to 30th Jun 2023. Ninety-six women aged 19–45 years fulfilling the inclusion criteria were evaluated for metabolic syndrome. The frequency of metabolic syndrome was noted and compared across various subgroups based on patient's age, duration of disease and pattern of hair loss. **Results:** The mean age of the patients was 29.2±7.2 years, 66.7% of the women were aged 30 years while 33.3% were above 30 years. The mean duration of disease was 2.6±1.2 years; it was 3 years in 70 (72.9%) women, more than 3 years in 26 (27.1%). Forty-two (43.8%) women had Christmas tree pattern, 32 (33.3%) had diffuse central thinning and 22 (22.9%) had fronto-temporal recession. Metabolic syndrome was diagnosed in 57 (59.4%) women with female pattern hair loss (FPHL). When stratified, there was no statistically significant difference in the frequency of metabolic syndrome across various subgroups. **Conclusion:** A substantial proportion of patients with female pattern hair loss suffered from metabolic syndrome.

Keywords: Androgenetic Alopecia, Female Pattern Hair Loss, Metabolic Syndrome

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INTRODUCTION

Female pattern hair loss (FPHL) is the pattern of hair loss occurring in women having androgenetic alopecia, i.e., androgen dependent patterned hair loss on scalp. Its different presentations include diffuse central thinning, Christmas tree pattern, fronto-temporal recession and bitemporal thinning. Multiple factors contribute to the development of FPHL including hormonal influences, hair follicle miniaturization and genetic predisposition.¹ Studies have reported prevalence of FPHL in 3–6% of women under 30 years, increasing to 29–42% in age 70 years and above in European population and 5.6% in Asian women. There are reported incidences of 12% in females around 30 years of age and of 30–40% in age 60 and above.^{1–3}

Metabolic syndrome (MetS) is a set of diseases including high blood pressure, high blood sugar, increase waist circumference, high cholesterol and triglyceride levels.⁴ There is an increased risk of stroke, cardiovascular disease and diabetes in patients of MetS.^{5–8} Almudimeegh AM *et al*³ found 26.4% of the women with FPHL to have metabolic syndrome which was assessed according to the Adult Treatment Panel III Criteria. An Indian study by Singh S *et al*⁴ showed that 34.4% cases of FPHL were found to have MetS and correlated with onset, duration and severity of FPHL. In a study by El Sayed *et al*⁶, 45 cases of FPHL and 45 healthy controls were taken. Metabolic syndrome was

assessed according to the Adult Treatment Panel III Criteria. The study showed 68.9% cases of FPHL to have MetS.⁵

Several studies are available that demonstrate an association between male pattern hair loss and MetS but internationally only a few studies have been conducted to examine the association of FPHL and MetS. Also in Pakistan and South Asia very limited data is available. The results of our study may help in establishing FPHL as a cutaneous sign of a systemic condition like MetS and may help in early detection and treatment of MetS.

PATIENTS AND METHODS

It was a cross-sectional study conducted at Department of Dermatology, Jinnah Hospital, Lahore, from 1st Jan to 30th June 2023. Sample size of 96 cases was calculated based on the prevalence rate of MetS in a similar study⁴ with 95% confidence level, acceptable difference=0.105, and assumed proportion=0.486.⁴

Patients were selected by non-probability, purposive sampling. Female patients with aged 19–45 years, having any pattern of female pattern hair loss were included in the study. Pregnant ladies and lactating mothers, patient receiving any hormone replacement therapy including testosterone, contraceptives or chronic or acute corticosteroids therapy, smokers, patients receiving or have received treatment for polycystic

ovarian syndrome, patients having any underlying malignancy as well as cases on chemotherapy, or who had received chemotherapy over past 5 years were excluded.

Subjects fulfilling the inclusion criteria were recruited for the study after approval of Hospital Ethical Committee and informed consent. Relevant demographic details were taken and entered in the proforma. Physical examination was done for MetS, i.e., blood pressure monitoring, height, waist circumference, and weight measurement. Pattern of FPHL was noted.

Relevant investigations such as fasting lipid profile, and fasting blood glucose were done. Patient was diagnosed to have metabolic syndrome when 3 or more of the following (Adult Treatment Panel III Criteria) were present:

- BP >130/85 mmHg
- Abdominal circumference >88 Cm
- Hypertriglyceridemia >150 mg/dL
- HDL-C <50 mg/dL
- Fasting Blood Sugar >110 mg/dL

All patients were managed as per standard protocols.

Data was entered and analysed on SPSS-22. Numerical variables, i.e., age and duration of disease are presented as Mean±SD. Categorical variables, i.e., pattern of hair loss and metabolic syndrome have been presented as frequency and percentage. Data has been stratified for age, duration of disease and pattern of hair loss to address effect modifiers and post-stratification, Chi-square test was applied taking $p \leq 0.05$ as statistically significant.

RESULTS

The age of the patients ranged from 19 years to 45 years with a mean of 29.2 ± 7.2 years. Mean duration of the disease was 2.6 ± 1.2 years. The stratification of patients on the basis of age, duration of disease, and pattern of hair loss is shown in Table-1.

Table-1: Baseline characteristics [n (%)]

Characteristics	No. of Participants
Age (Years)	
≤30 Years	64 (66.7)
>30 Years	32 (33.3)
Duration of Disease (Years)	
≤3 Years	70 (72.9)
>3 Years	26 (27.1)
Pattern of Hair Loss	
Diffuse Central Thinning	32 (33.3)
Christmas Tree Pattern	42 (43.8)
Fronto-temporal Recession	22 (22.9)

Metabolic syndrome was diagnosed in 57 (59.4%) women with FPHL. When stratified, there was no statistically significant difference in the frequency of MetS across various subgroups based on patient's age ($p=0.659$), duration of disease ($p=0.793$) and pattern of hair loss ($p=0.999$). (Table-2).

Table-2 Frequency of metabolic syndrome across various subgroups of women with FPHL (n=96)

Subgroups	n	MetS [n (%)]	p
Age			
≤30 Years	64	37 (57.8)	0.659
>30 Years	32	20 (62.5)	
Duration of Disease			
≤3 Years	70	41 (58.6)	0.793
>3 Years	26	16 (61.5)	
Pattern of Hair Loss			
Diffuse Central Thinning	32	19 (59.4)	0.999
Christmas Tree Pattern	42	25 (59.5)	
Fronto-temporal Recession	22	13 (59.1)	

Chi-square test, observed differences were statistically insignificant

DISCUSSION

Female pattern hair loss is the most common form of alopecia in women. Affected women may experience psychological distress and impaired social functioning.¹⁻³ Early diagnosis and initiation of treatment are desirable because treatments are more effective to avoid progression of hair loss than stimulating regrowth.² In addition to psychological distress, there are more subtle systemic disturbances associated with androgenetic alopecia both in men and women.³

Metabolic syndrome is associated with a lot of inflammatory diseases including psoriasis, acne, hidradenitis suppurativa, lichen planus, atopic dermatitis, seborrheic dermatitis etc.^{4,5} There is link between MetS and androgenetic alopecia in men⁶ but the evidence on link between MetS and FPHL in women is scarce^{3,7,8}. The mechanism linking FPHL and MetS is not clear but studies suggest that excess of androgens causes hypertension and decrease in level of HDL cholesterol.^{3,4} As MetS increases the risk of stroke and cardiovascular diseases, this study can help in establishing FPHL as a cutaneous marker of this systemic condition and help in early detection and treatment of MetS.

In the present study, the mean age of the patients was 29.2 ± 7.2 years. HendM El-Zeftawy *et al*⁷ and Özkoca D *et al*⁹ reported similar mean age of their patients with FPHL. A study conducted by Singh S *et al*⁴ reported 31.3 ± 1.3 years as mean age of onset, while Ohn J *et al*¹⁰ reported it to be 27.3 ± 5.1 years in Korea. We observed that majority (66.7%) of the women with FPHL were aged ≤30 years while 32 (33.3%) women were aged above 30 years. Our observation is in line with that of Tandon S *et al*¹¹ who reported similar frequency of ≤30 years (79.9) and >30 years (20.1) age groups in Indian patients. Mansuri *et al*¹² reported similar distribution of ≤30 years (70.0%) and >30 years (30.0%) age groups in such patients in India.¹² Verma D *et al*¹³ who reported contrary distribution of ≤30 years (44%) and >30 years (44%) age groups in patients presenting with androgenic alopecia.

In this study, the mean duration of disease among women with FPHL was 2.6 ± 1.2 years. It was less than 3 years in 70 (72.9%) women while in 26 (27.1%)

women the disease duration was more than 3 years. This is in agreement with that of Verma D *et al*¹³ who reported that 52.0% of women having FPHL had a disease duration of 6 months to 2 years in Indian population.

In the present study, metabolic syndrome was diagnosed in 59.4% women with FPHL and they fulfilled the Adult Treatment Panel III Criteria. When stratified there was no statistically significant difference in the frequency of metabolic syndrome across various subgroups based on patient's age, duration of disease and pattern of hair loss which is in line with the findings of Singh S *et al*⁴. El Sayed *et al*⁶ observed similar frequency of metabolic syndrome in Egyptian women with FPHL and reported it to be 68.9%. Some other studies gave contrary results. Almodimeegh AM *et al*³ showed that MetS was found in 26.4% of females having FPHL whereas Singh S *et al*⁴, Verma D *et al*¹³ and Zhu H *et al*¹⁴ found it to be 34.4%, 24% and 27.87% respectively. The present study adds to already published international research evidence on the topic. The present study observed that a substantial proportion of patients with FPHL also suffer from MetS.

The limitations to the present study were that neither did we consider the effect of treatment of FPHL on associated MetS nor we considered the effect of management of concomitant MetS on the outcome of FPHL in these women. Also we did not have a control group for comparison of our results. This information could have helped in the management planning of such cases. Such a study in future is highly recommended. Timely identification and management of this co-morbid state of metabolic disarray may improve the outcome of such cases. It was a single centre study, further studies from different centers are recommended to determine the frequency of MetS in Pakistani population.

CONCLUSION

We observed that 59.4% of patients with female pattern hair loss suffered from metabolic syndrome which warrants routine screening of these women so that

timely diagnosis and management may improve the outcome of such cases.

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ORIGINAL ARTICLE

PROTECTIVE EFFECTS OF MELATONIN ON LUNG INJURIES INDUCED BY CIGARETTE SMOKE IN WISTAR ALBINO RATS

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Background: The most important risk factor for chronic obstructive pulmonary disease (COPD) and carcinoma of lung is cigarette smoking, mainly in habitual smokers. Cigarette smoke causes lung damage because it is directly inhaled into the lungs and reaches the alveoli. It is because of the deposition of its inhaled toxic particulate components and gases in the airways and alveoli that are responsible of inflammation and chronic obstruction. **Methods:** In this comparative experimental research, 60 Albino Wistar rats were used and evaluated for the toxic effects of cigarette smoke and the protective effects of Melatonin on rat's lungs simultaneously. Also, we estimated the protective effects of Melatonin on markers of inflammation and Fibrinogen serologically among negative and positive control groups and melatonin- treatment and post-treatment groups. **Results:** According to the results of the present study, it is confirmed that melatonin counterbalances the cigarette smoke (CS)-induced lung injuries in both treatment and post-treatment groups, though its effects on the treatment group exposed to cigarette smoke for only one month were slightly stronger than post-treatment group exposed to cigarette smoke for two months. The serological markers of inflammation CRP (C-reactive protein) and fibrinogen were successfully reduced with melatonin administration in both treatment and post-treatment group. **Conclusion:** In conclusion, the present study proves that melatonin has potent anti-oxidant, anti-inflammatory, immune-regulatory, and vaso-protective properties against cigarette smoke-induced lung damage in Wistar albino rats, confirmed both histopathologically and serologically.

Keyword: Lungs, Melatonin, Cigarette smoking, COPD

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INTRODUCTION

The incidence of death from lung diseases especially chronic obstructive pulmonary disease (COPD) caused by the toxic effects of cigarette smoke has increased in the past decade. Cigarette smoking is one of the major risk factors for multi-organ damage, especially lungs as cigarette smoke is directly inhaled into the lungs, responsible for mortality due to cardiorespiratory failure.¹ Cigarette smoking is also one of the leading causes of lung cancer and responsible for one-third of all cancer-related mortality. About more than 9,500 components have been explored in cigarette smoke including nicotine.²

Nicotine is a highly toxic compound and the major pharmacologically active substance present in the particulate phase of tobacco smoke and responsible for most of the damaging effects of smoking.³ Nicotine causes oxidative damage to the lungs by forming reactive oxygen free radicals, often resulting in a decrease in the concentration of Glutathione peroxidase, Catalase, and Superoxide dismutase.⁴

Melatonin (N-acetyl-5-methoxytryptamine) is an endogenous hormone mainly synthesized from the amino acid Tryptophan in the pineal gland. It is released in the dark in humans and mammals and structurally related to serotonin. It can be obtained from exogenous dietary sources such as sour cherries, walnuts, and orange juice.⁵ It has strong antioxidant property, anti-

inflammatory, anti-proliferative, anti-cancerous, anti-apoptotic (in damaged cells), and pro-apoptotic properties (in cancerous cells). Even, it has synergistic effects, if given before or along with radiotherapy and chemotherapy.^{5–7}

The anti-inflammatory effects of melatonin are produced by effective suppression of inflammatory mediators especially IL-6, TNF- α , MMP-9 in addition to inflammatory cells.⁸ Moreover, melatonin also has beneficial effects on lung fibrosis induced by cigarette smoke. This effect exerts by reducing TGF- β 1, and collagen 1 production mainly⁹ and concomitant increase in the production of anti-oxidants glutathione peroxidase and superoxide dismutase¹⁰.

As melatonin is a natural hormone, its use prophylactically and therapeutically is much safer than other drugs. It also has very few side-effects and is easily available in the market.¹⁰ Both human and animal research proved that its use as a short-term treatment as well as long-term treatment is safe. Only a few side effects, such as headache, drowsiness, and somnolence have been experienced.¹¹

The present study was carried out to estimate the beneficial effects of melatonin during and after lung injury induced by cigarette smoke in the Wistar albino rat model by histopathologic examination, and also to estimate its protective effects on C-reactive protein and fibrinogen, serologically.

MATERIAL AND METHODS

This experimental study was conducted in Department of Pathology, Isra University, Hyderabad from Jan to Jul 2023 after getting approval from the Research and Ethics Committee of Isra University. A total of 60 healthy albino Wistar rats weighing 150–250 grams were procured from the animal house of Sindh Agriculture University, Tando Jam. Samples were collected by non-probability purposive sampling technique. The animals were kept on the standard chow diet having a scientifically approved composition as per instructions of veterinary experts and water *ad libitum*.

The animals were tagged, weighed, and kept in separate stainless-steel cages as control and experimental groups. The animals were divided into four groups, each comprising 15 Wistar albino rats. Group A (Negative Control group) was given normal saline for 4 weeks. Group B (Positive Control group) was exposed to cigarette smoke in a glass chamber for 30 minutes daily for 4 weeks. Group C (Treatment group) was exposed to cigarette smoke in a glass chamber for 30 minutes daily for 4 weeks and was given Melatonin 10 mg/Kg/day, mixed with food. Group D (Post-Treatment group) was exposed to cigarette smoke in a glass chamber for 30 minutes daily for the first 4 weeks, followed by Melatonin (10 mg/Kg/day) along with cigarette smoke for the next 4 weeks.

Two millilitre of blood were drawn from heart of all animals and transferred into gel tubes. Later on All rats were sacrificed by decapitation. Inflammatory serum biomarkers¹², i.e., C-reactive proteins, and fibrinogen were estimated in the blood samples at the Pathology Laboratory of Isra University Hospital.

The lung specimens of rats were collected soon after dissection and preserved in 10% formalin for preservation for histopathological examination.

The same process was repeated with the rats of Group D at the end of the 8th week. The lung samples were passed through ascending grades of ethyl alcohol (70%, 80%, 95%), cleared in Xylene and embedded in paraffin wax. Sections measuring 5 µm in thickness were cut on microtome. These slides were stained with Haematoxylin and Eosin, and studied under light microscope at 100× magnification. Characteristic histological changes and findings in the lung samples were recorded in a predesigned proforma. Data were analysed using SPSS-21, and

$p \leq 0.05$ was considered as statistically significant. The results are presented as table and representative histological slides.

RESULTS

The variables/parameters studied in 4 groups of 15 rats each were the following:

- A. Serum Biomarkers
 - i. Serum CRP (C-reactive protein)
 - ii. Serum Fibrinogen
- B. Histopathological variables
 - i. Intra-parenchymal vascular congestion
 - ii. Number of alveolar macrophages
 - iii. Respiratory epithelial proliferation
 - iv. Alveolar destruction
 - v. Fibrosis

The serological biomarker of inflammation CRP (C-reactive protein) was successfully reduced with melatonin administration from 0.88 to 0.57 mg/dL in treatment group (Group C) and from 0.88 to 0.64 mg/dL in post-treatment group (Group D). This confirms melatonin's anti-inflammatory and antioxidant properties. Melatonin administration in the same dose successfully reduced the serum fibrinogen level from 447.1 mg/dL (positive control group, B) to 297.1 mg/dL (treatment group, C) and 312.8 mg/dL (post-treatment group, D) indicating vaso-protective effects of melatonin. (Table-1).

Administration of melatonin markedly reduced the intra-parenchymal congestion in treatment group (Group C) but its effect was less in post-treatment group (Group D). (Figure-1).

Number of alveolar macrophages was also reduced in similar fashion. (Figure-2).

Respiratory epithelial proliferation exhibited similar picture in treatment group (Group C) and post-treatment group (Group D) respectively. (Figure-3)

Alveolar destruction was much marked in the positive control group (Group A). It was lesser in treatment group (Group C) than in the post-treatment group (Group D). (Figure-4).

Figures-1, 2, 3, and 4 show that all histopathologic variables of damaging effects of exposure to cigarette smoke responded well to melatonin in the treatment group (Group C). But in post-treatment group (Group D), variables like alveolar destruction, and fibrosis responded to a limited degree, suggestive of suppressive rather than regressive/reparative effects of melatonin.

Table-1: Serum C-reactive protein and Fibrinogen levels in animal groups (n=15)

Parameters	Group A	Group B	Group C	Group D	F	p
Serum CRP (mg/dL)	0.10±0.11	0.88±0.25	0.57±0.15	0.64±0.44	9.46	0.001*
Serum Fibrinogen (mg/dL)	224.7±74	447.1±0.41	297.1±0.15	312.8±0.48	11.93	0.001*

*Statistically significant

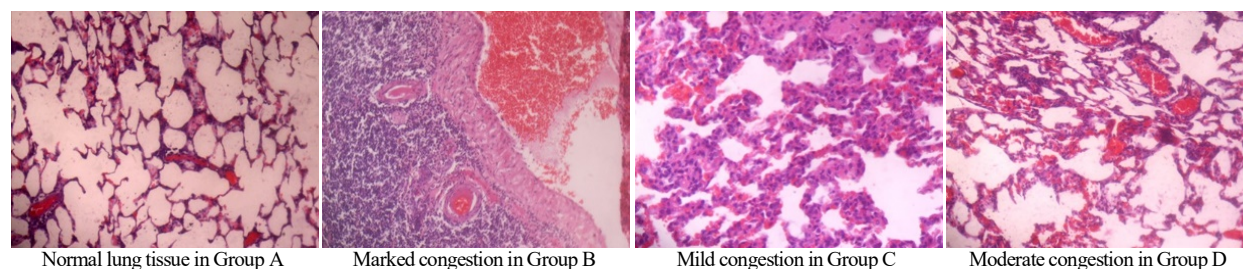


Figure-1: Intra-parenchymal congestion among various animal groups

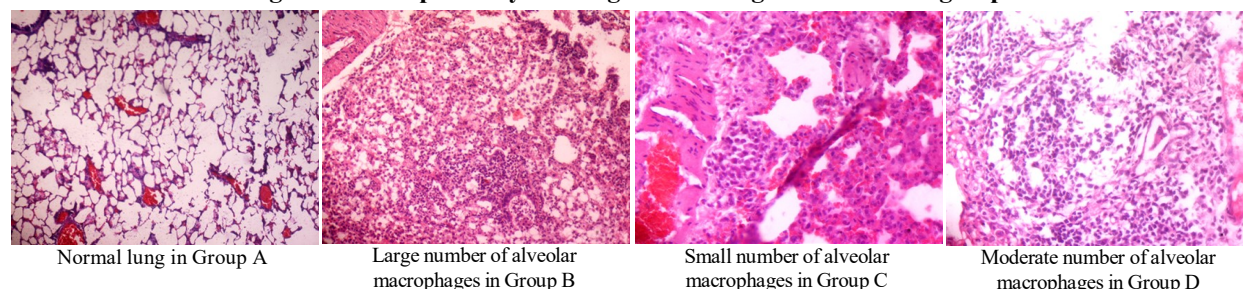


Figure-2: Number of alveolar macrophages among various animal groups

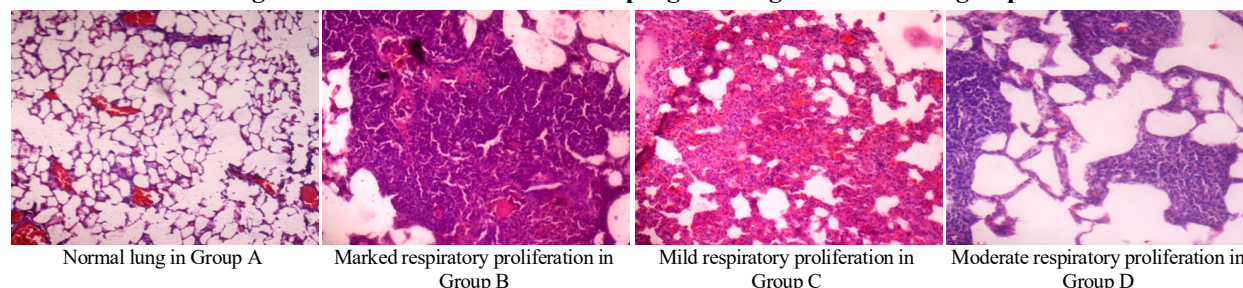


Figure-3: Respiratory epithelial proliferation among various animal groups

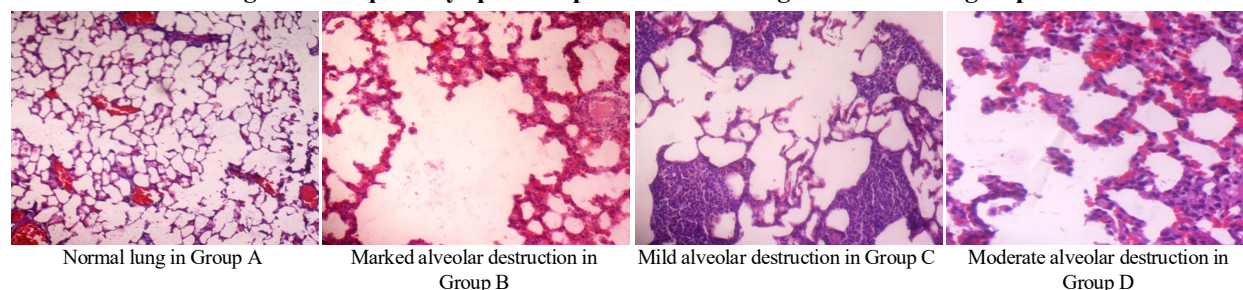


Figure-4: Alveolar destruction among various animal groups

DISCUSSION

Cigarette smoking is common all over the world. Economically, the global cost of tobacco use is two trillion dollars per year.¹³ Conventional cigarettes are the most commonly used tobacco products throughout the world especially in male adults and responsible for 7 million deaths every year.^{14,15} The conventional cigarettes deliver more harmful components and oxygen-derived free radicals than other types of tobacco products.^{16,17} In Pakistan, about half population is active adult tobacco users.¹⁸ Melatonin counterbalanced the cigarette smoke induced lung injuries in both treatment and post-treatment groups which was proved serologically and histopathologically, though the effects

of melatonin were stronger in treatment group than post-treatment because of only one month exposure to cigarette smoke.

All histopathological variables such as intra-parenchymal vascular congestion, the number of macrophages, respiratory epithelial proliferation, and alveolar destruction responded well in the treatment group suggesting that melatonin prevents these changes in the lungs due to exposure to cigarette smoke. In contrast, the post-treatment group that was exposed to CS for two months, CS had induced damaging effects and it was observed that inflammatory changes and epithelial proliferation were reversed in most of the cases while the two variables, i.e., alveolar destruction and fibrosis responded to a limited degree to melatonin

treatment. These results suggested more suppressive rather than regressive/reparative effects of melatonin. These findings are consistent with the results of many previous studies.^{1,5-9,19-26} Shin IS *et al*¹ and Shin NR *et al*⁸ proved the dose-dependent anti-inflammatory properties of melatonin in the lung tissue and broncho-alveolar lavage fluid (BALF) in CS and LPS-induced COPD mice, and in cigarette smokers.

Ma Z *et al*⁷ reported in human study that melatonin acts as an anti-mitogen by down regulating the proliferating-cell nuclear antigen (PCNA) responsible for cell division, suggesting melatonin as an anti-cancer drug in the treatment lung cancer. They also proved effects of melatonin such as induction of apoptosis in cancer cells, and inhibition of invasion and metastasis, as well as synergistic effects of melatonin with radio- and chemo-therapy. Wang Z *et al*²⁵ proved that after taking oral supplementation of 3 mg melatonin, one hour before bed time for two weeks, the blood concentrations of fibrinogen and free fatty acids were significantly reduced in smokers.

CONCLUSION

Melatonin has potent anti-inflammatory, anti-oxidant, and anti-proliferative properties against cigarette smoke-induced lung damage, confirmed histopathologically and serologically reducing serum levels of anti-inflammatory markers, C-reactive protein (CRP) and fibrinogen. It can reverse inflammatory and proliferative changes in the lungs induced by CS.

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FN: Drafting, Concept and design

SMD: Data collection and assembly, Critical revision

UH: Data collection and assembly, Drafting, Statistics

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ORIGINAL ARTICLE

ANGIOGENESIS ACCELERATION IN WOUND HEALING:
ZINC OXIDE VERSUS BETEL LEAF EXTRACTMuhammad Ali Zubair, Suhail Marfani*, Javed Mahmood**, Farheen Hameed,
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Background: Wound healing is a complex physiological process involving haemostasis, inflammation, proliferation, and remodelling. Chronic wounds pose challenges due to delayed healing. This study compares the effects of topical zinc oxide (ZnO) and betel leaf extract on angiogenesis in wound repair.

Methods: This study was carried out over 6 months from Oct 2023 to Mar 2024, at Shahida Islam Medical Complex, Lodhran. Thirty-six albino rats were divided into three groups of 12 each. A dorsal wound was created, and treatments were applied to the wound. Group A was control, and saline was applied to the wound, Group B was administered 20% ZnO on the wound, and Group C received betel leaf extract as wound dressing. Wound margin tissue was extracted, fixed, and histopathological slides were prepared. Masson trichrome stain was used to count vessels at 400× magnification under microscope. Vessel counts were analyzed via microscopy on days 3, 7, and 14. Data was analysed using one-way ANOVA. **Results:** On day 3, vessel counts were 4.3 for group A, 6.5 for group B, and 4.2 for group C. By day 7, counts increased to 5.8 for A, 11.54 for B, and 9.52 for C, and on day 14, they were 5.5 for A, 7.54 for B, and 6.57 for C. ZnO showed a significant increase in angiogenesis compared to control ($p < 0.01$) and betel leaf extract ($p < 0.05$). **Conclusion:** Zinc oxide accelerated wound healing more effectively than betel leaf extract.

Keywords: Angiogenesis, Wound repair, Zinc oxide, Betel leaves

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INTRODUCTION

Wounds especially the chronic wounds have a negative impact on the quality of life of nearly 2.5% of American population, a significant economic impact on the healthcare.¹ Chronic wounds as well as burn injuries have been increasing, and have led to an increasing cost for the national health systems of many countries. Development of new, improved, and cost-effective therapy is a huge area of academic research. It is also critical for long-term sustainability of public healthcare system.² Many materials have been used for improving wound healing and all of them have their pros and cons. Recently, nano-zinc oxide has been used in wound healing applications due to its promising microbicidal and anti-inflammatory properties as it provides a rich source of zinc ions.³ Topical zinc oxide is being used and heavily studied upon since years, and is stated that it is highly underappreciated even though clinical evidence suggests its facilitation in epithelialization, anti-infective action, and auto debridement.⁴ Established perfusion at any stage of wound repair is required for faster process, either in acute or chronic cases.⁵ Vascular system promptly begins the healing process by triggering the coagulation cascade and initiating vasoconstriction.⁶ As the cutaneous wound occurs in the body, the different inter and intra cellular pathways play role to restore the cellular and tissue integrity.^{7,8}

Zinc oxide (ZnO) is a trace element that plays a physiological function in healing. It is an essential cofactor for multiple metalloenzymes, needed for cellular repair, tissue regeneration and immune booster.⁹ Molecular and cellular function of zinc incorporates in modulating the repair process. Zinc dependent proteins take part in transcription regulation, metabolic process, and antioxidant process. Free zinc ions working as second messengers like calcium, are capable to target and stimulate many signal transduction pathways.¹⁰

Betel leaf is one of the effective leaves that can accelerate many cellular and biochemical properties that can accelerate the healing process. It is a harmless leaf composed of eugenol, anethole, chavicol and caryophyllene.¹¹ The leaves possess potent antimicrobial properties and are effective in both acute and chronic wound conditions.¹² Betel leaves have anti-inflammatory action and can trigger angiogenesis to stimulate the physiological and pathological function as well. By having impressive modulator effect on immune system, the healing process can be accelerated in any type of wound.¹³ Betel leaves contain saponins and flavonoids that are composed of antioxidant properties, and facilitate angiogenesis which results in acceleration of epithelialization process.¹⁴

The aim of this study was to compare the two different healing agents, topical application of zinc oxide and betel leaf extract.

METHODOLOGY

A quasi-experimental study was done at Shahida Islam Medical Complex, Lodhran, Pakistan for 6 month from Oct 2023 to Mar 2024 after the ethical approval from the Institutional Ethical Review Committee vide No. SIMC/HR/7727/23 dated 1st Sep 2023. Randomly selected 36 male albino rats weighing 150–200 gm were taken from animal house of the Institute. Animals were divided into 3 groups of 12 animals each and further sub-divided into 4 units according to day of sampling. The animals were kept in separate cages according to the grouping with maintenance of 12/12 hour light and dark cycle. Group A (Control group) was treated with topical application of normal saline once a day over the site of the wound. Group B was given 20% of ZnO topically once daily. Group C received the green betel leaf extract.

The extract was made with about 25–30 fresh leaves bought from the local market, dried in incubator at 40 °C for 24 hours, ground to powder form, and stored in glass container till use. The ground leaves were placed in distilled water for 24 hours and then filtered using Whatmann No. 4 filter paper. The filtered extract was placed into glass bottles.¹⁵ The dose of the betel leave extract was soaked in wound using gauze twice a day, as much as 2 drops.

The dorsal surface of the animals was shaved and cleaned with normal saline, and wounds of 2.5×2.5 Cm were prepared with a blade. The process of healing was observed by the process of angiogenesis. The samples were taken from each group on different days. The tissue was extracted from the margin of the wound, and kept in formalin. The histopathological slides were prepared to analyse the numbers of vessels and stained with Masson trichrome stain was used to calculate the numbers of vessels in specific field area under the light microscope at 400× magnification. The mean number of vessels was compared between the groups applying one-way ANOVA and $p \leq 0.05$ was considered as statistically significant.

RESULTS

The number of vessels (Mean±SD) on day 3, day 7 and day 14 is shown in Figure-1. Statistical significance (p -values) of differences in mean blood vessel counts among different groups is shown in Table-1.

Figure-2 shows histomorphological appearance of process of angiogenesis number of vessels among different groups at 400× magnification. Group A (control group) showed decreased number of vessels. Group B showed significant increase in number of vessels as compared to Group C. The Group C treated with betel leaf extract also showed increase number of vessels as compared to control group but to a lesser number as compared to Group B.

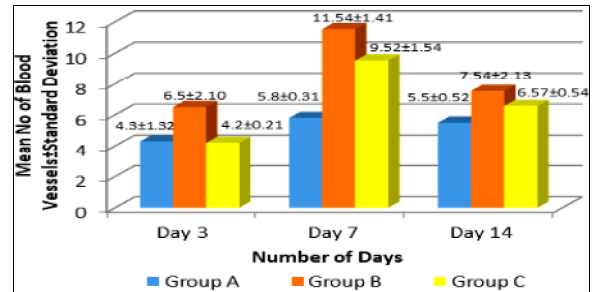


Figure-1: Number of blood vessels in different therapeutic groups on different days of sampling

Table-1: Comparison of blood vessel counts among different groups (p -values, One-way ANOVA)

Groups	Day 3	Day 7	Day 14
B versus A	0.001	0.001	0.002
B versus C	0.001	0.001	0.004
A versus C	0.08	0.001	0.04

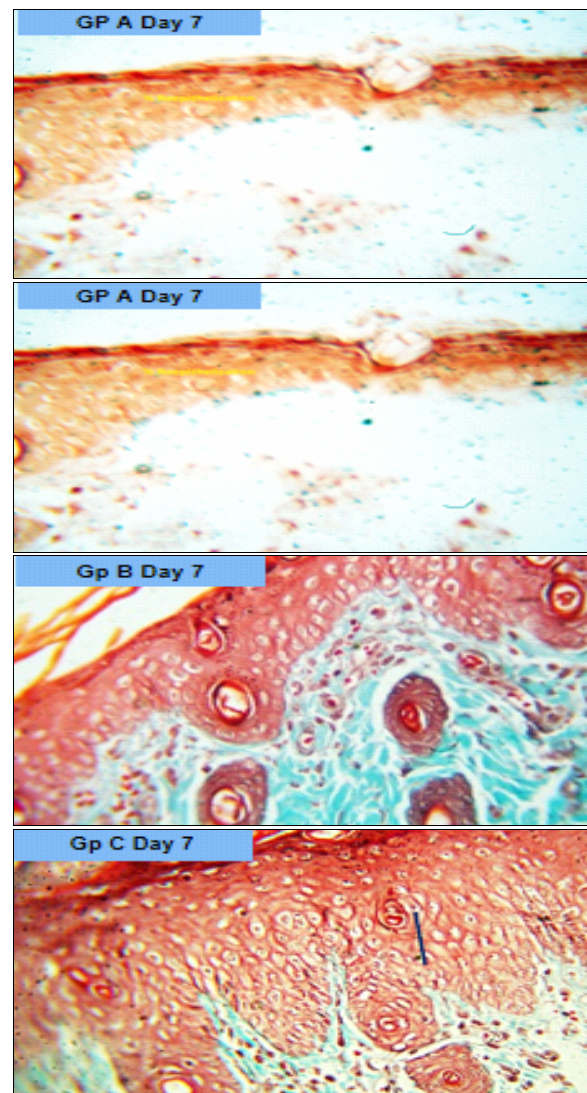


Figure-2: Photomicrograph showing process of angiogenesis among different therapeutic groups

DISCUSSION

We used betel leaf extract and compared it with topical zinc oxide to see their effects on the number of blood vessels formed. The mean number of vessels formed through angiogenesis was greater for zinc oxide topical application as compared to betel leaf extract on all days the sample was taken. A similar study reported comparable results, with an alpha score of 0.002, indicating a significant difference in injury healing when using red betel leaf extract in two doses per day as compared to a single dose. The wound healed in 6 days as compared to 8 days when used single dose per day, and 10 days when used povidone iodine alone.¹⁴ Agren *et al*¹⁶ compared the topical application of zinc oxide in two human wound models. They monitored wound healing by considering scab formation as criteria for complete healing. They found ZnO more effective in healing of wounds on different days. This supports the current study findings.

Lien *et al*¹³ observed that the betel leaf extract showed better percentage in healing of burns on 2nd, 4th, and 6th postoperative days of burns; and on excisional wounds, 5% betel leaf extract showed better healing on 7th and 15th day of wounds. This finding supports current study in which betel leaf extract showed increased angiogenesis and better wound healing compared to control group however at lesser extent than topical zinc oxide. Another study¹⁴ found that there was an increase in angiogenesis, along with new fibroblasts and macrophage formation, thus it further supports our study. The same study found that using betel leaves will lead to a significant rise in hydroxyproline content, superoxide dismutase (SOD) levels, and a decrease in the amount of malondialdehyde levels.¹⁴ A systemic review¹² was able to confirm that betel leaf has an effect on increase in blood vessel growth, collagen layer thickening, amount of epithelialization, fibrous tissue formation, weight of granulation tissue, hydroxyproline content, and ultimately inhibits bacterial cell growth.¹² These findings along with ours can confirm that betel leaf causes increase in the number of blood vessel formation, but also improves wound healing in many other ways. Betel leaf has the ability to suppress the inflammation and promote an increase in re-epithelialization.¹⁷ Suharto *et al*¹⁸ showed that betel leaf extract when applied topically enhanced wound healing and wound closed early when applied in higher concentration. They expressed that inflammatory process starts within 24 hours of wound occurrence. Fibroblasts and vascular endothelial cells begin to proliferate and form new granulation tissue which is a significant sign of healing. This is in accordance with our study where new blood vessels formed rapidly after applying betel leaf extract.

Betel leaf can be further studied by comparing different types of betel leaves such as red betel leaf or piper betel leaf.¹⁹ Future studies can also be used to explore other agents that are being currently tested in trials regarding their efficacy on wound healing and be compared to betel leaf extract.

CONCLUSION

Topical application of zinc oxide was found superior in wound healing by increasing angiogenesis on all days of sampling compared to betel leaf extract. Topical application of zinc oxide is effective for facilitating wound healing. Betel leaf extract can be considered as a suitable alternative to zinc oxide.

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ORIGINAL ARTICLE

HISTOPATHOLOGICAL SPECTRUM OF OVARIAN EPITHELIAL TUMOURS: A CROSS-SECTIONAL STUDY

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Background: Ovarian cancer (OC) poses a significant health burden, ranking 8th in women's cancers globally. Ovarian epithelial cancers (OEC) can present in a number of histopathologically distinct benign and malignant patterns. This study aims to assess the histopathological characteristics of ovarian epithelial tumours in our local setting. **Methods:** This retrospective cross-sectional study, conducted at Ayub Medical College, Abbottabad, from Jan 2022 to Dec 2023, included 111 patients with histopathologically confirmed ovarian epithelial lesions. All biopsy records of OC during the study periods were retrieved and assessed for histopathological diagnosis. Confirmed cases of OEC were re-examined and included in the study. Records of patients' associated demographic data were obtained from HMIS of Ayub Teaching Hospital. Data were analysed on SPSS-27. **Results:** The mean age at diagnosis was 53.44±10.52 years, with the majority (73, 65.8%) being multipara and having unilateral lesions. Serous cystadenoma emerged as the most prevalent (42, 37.8%) benign tumour, while serous carcinoma dominated (22, 19.8%) malignant cases. Family history emerged as a significant factor, with 60.5% of malignant cases having a positive family history ($p<0.001$). Stratification by age at diagnosis, menarche, and menopause revealed no statistically significant difference between benign and malignant lesions in all strata. **Conclusions:** Serous cystadenoma is the most common benign tumour while serous carcinoma is the most common ovarian epithelial malignancy in Hazara division. The predominance of serous cystadenoma and serous carcinoma underscores the need for further exploration of familial risk factors.

Keywords: Ovarian Cancer, Ovarian Epithelial Cancers, Histopathology, Benign, Malignant

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INTRODUCTION

Ovarian cancer (OC) ranks as the 8th most common cancer among women globally, with over 313,000 new cases reported in 2020. It accounts for significant morbidity and mortality, with a 5-year survival rate of only 48.6%.^{1,2} This dire prognosis is compounded by the fact that nearly 58% of ovarian cancer cases are diagnosed at advanced stages, where the 5-year survival rate plummets to 30.2%, compared to 92.6% for early-stage localized disease.³ Despite its lower incidence compared to breast cancer, OC exhibits a mortality rate that is three times higher, earning it the notorious label of a 'silent killer' due to its insidious growth, lack of effective screening tools, and nonspecific symptoms.^{4,5}

Histologically, ovarian cancers are broadly classified into epithelial, germ cell, and stromal tumours, with ovarian epithelial cancers constituting approximately 80% of all cases.⁶ This group is further stratified into high-grade serous, low-grade serous (borderline), mucinous, endometrioid, and clear cell tumours, with high-grade serous carcinoma accounting for nearly 75% of ovarian epithelial cancers.⁷ These tumours typically present at advanced stages, underscoring their clinical significance.

The clinical presentation of ovarian cancer is often subtle, characterized by nonspecific symptoms

such as persistent pelvic discomfort, bloating, early satiety, and abdominal distension. These vague symptoms frequently result in delayed diagnosis and poor outcome.⁸ Risk factors for ovarian cancer include non-modifiable and modifiable factors. Non-modifiable factors such as advanced age, a positive family history of ovarian or breast cancer, and genetic mutations like BRCA1 and BRCA2 significantly increase the risk.⁹ Reproductive factors like early menarche, nulliparity, and late menopause also contribute to the risk of OC.¹⁰ Modifiable factors, including obesity, smoking, sedentary lifestyles, and hormone therapy, provide opportunities for targeted preventive measures.¹¹

Understanding the histopathological spectrum of ovarian epithelial tumours is crucial for improving diagnostic precision, identifying high-risk populations, and developing effective prevention strategies. This study aims to bridge the gap in existing literature by exploring the demographic, clinical, and histopathological characteristics of ovarian epithelial tumours in a local population.

METHODOLOGY

This cross-sectional study was conducted from Jan 2022 to Dec 2023 at the Department of Pathology Ayub Medical College, Abbottabad. The sample size comprised of 111 patients, calculated using the Open-

Epi online sample size calculator taking a confidence level of 95%, a desired precision of 8% and an estimated prevalence of malignant ovarian epithelial cancers of 23.6%.¹² Consecutive sampling was used. Inclusion criteria involved patients with histopathological diagnosis of either benign or malignant ovarian epithelial lesion. Exclusion criteria included patients who could not be traced for obtaining relevant history. The study was conducted after approval of the Ethics Committee of AMTI Abbottabad. Data collection involved obtaining informed consent from the patients/attendants and collection of demographic information from patients using a proforma. Biopsy samples, fixed in 10% formalin, were prepared at the Pathology Department of Ayub Medical College Abbottabad. Sections of 4 µm thickness were cut, and stained with H&E stains for assessment under light microscope.

Malignant cells originating from the ovarian mesothelium showing nuclear stratification, hyperchromatic pleomorphic nuclei along with atypical mitotic figures, and infiltrating into the ovarian stroma with stromal reaction were considered malignant ovarian epithelial tumours. Benign lesions demonstrated well-differentiated epithelial cells with orderly growth patterns, lack of significant cellular atypia (uniform nuclei with minimal nuclear enlargement, hyperchromasia, or irregularity), and well-defined capsule.⁷ The microscopic examination was carried by two consultant clinical histopathologists having at least five years of post-fellowship experience. After discussion the final diagnoses were reported.

Data was analysed on SPSS-27. Descriptive statistics, including Mean±SD for continuous variables and frequency and percentages for categorical variables were calculated. Chi-square and student's *t*-test were applied to determine the correlation between the presence of a benign or malignant lesion and the studied parameters like age, ages at menarche and menopause, laterality and parity, and $p \leq 0.05$ was considered statistically significant.

RESULTS

Table-1 illustrates the demographic and clinical characteristics of study participants presented in the form of either Mean±SD or frequencies and percentages. The mean age of the patients at the time of diagnosis was 53.44±10.52 years. Majority (73, 65.8%) of patients were multipara, had unilateral lesion 102 (91.9%), while 23 (20.7%) patients had a positive family history of female genital system malignancy and 73 (65.8%) cases had benign lesions.

Table-2 shows the frequency of the various benign and malignant lesions with which our patients were diagnosed. Patients were diagnosed with various lesions but serous cyst adenoma (benign tumour) was encountered in 42 (37.8%) cases, followed by serous

carcinoma (malignant tumour) diagnosed in 22 (19.80%) cases and mucinous cyst adenoma (benign tumour) diagnosed in 18 (16.2%) cases.

Table-3 displays the categorical characteristics stratified by lesion histopathologic type, aiding in understanding lesion malignancy factors. None of the benign lesions had a positive family history, while 23 (60.5%) of malignant lesions did ($p < 0.001$). Laterality of lesion and parity distribution showed no significant differences between benign and malignant cases ($p = 0.953$) and ($p = 0.767$) respectively.

Table-4 displays the cross-tabulation of continuous characteristics—age at diagnosis, age at menarche, and age at menopause—stratified by lesion type (benign vs malignant). No statistically significant differences were observed for these characteristics between patients with benign and malignant lesions, ($p > 0.05$).

Table-1: Demographics and clinical characteristics of the study population

Characteristics	Mean±SD	
Age (Years)	53.44±10.52	
Age at Menarche (Years)	11.88±0.75	
Age at Menopause (Years)	48.76±3.54	
Laterality of lesion	N	%
Unilateral	102	91.9
Bilateral	9	8.1
Parity Status		
Nulliparous	14	12.6
Parity (1–3)	24	21.6
Multipara	73	65.8
Family history of female genital system malignancy		
Positive	23	20.7
Negative	88	78.3
Histopathologic type of lesion		
Benign	73	65.8
Malignant	38	34.2

Table-2: Frequency of benign and malignant lesions in our patients

Lesion	Frequency	Percentage
Serous Cyst-adenoma	42	37.80
Mucinous Cyst-adenoma	18	16.20
Serous Cyst-adenofibroma	7	6.30
Endometriotic Cysts	4	3.60
Benign Brenner's tumour	2	1.80
Serous Carcinoma	22	19.80
Mucinous Carcinoma	9	8.10
Endometrioid Carcinoma	5	4.50
Clear Cell Ca	2	1.80

Table-3: Cross-tabulation of categorical characteristics

Variable		Benign (n=73)	Malignant (n=38)	p
Laterality	Unilateral	67 (91.8%)	35 (92.1%)	0.953
	Bilateral	6 (8.2%)	3 (7.9%)	
Family history of female genital system malignancy	Positive	0 (00%)	23 (60.5%)	<0.001
	Negative	73 (100%)	15 (39.5%)	
Parity	Nulliparous	8 (10.9%)	6 (15.8%)	0.767
	Parity (1–3)	16 (21.9%)	8 (21.1%)	
	Multipara	49 (67.1%)	24 (63.1%)	

Table-4: Cross-tabulation of continuous characteristics

	Lesion type	N	Mean±SD	p
Age	Benign	73	53.18±10.218	0.717
	Malignant	38	53.95±11.213	
Menarche	Benign	73	11.8493±0.75776	0.515
	Malignant	38	11.9474±0.73328	
Menopause	Benign	55	48.5818±3.25287	0.523
	Malignant	30	49.1000±4.06287	

DISCUSSION

We investigated the histopathological spectrum of ovarian epithelial tumours among women who presented with suspected ovarian lesions. Our findings are largely consistent with previously published literature, although there may be slight variations depending on regional factors. The findings provide valuable insights into the patient characteristics, distribution of tumour types, and potential associations with clinic-pathological factors.

The mean age of our study participants at diagnosis was 53.44 years, aligning with the range reported in previous studies (49–57 years).^{13–16} The majority of patients were multiparous (65.8%), similar to observations in other studies. For instance, a study conducted in India reported a mean age of 53 years for patients with ovarian neoplasms, while another study from Saudi Arabia found that the highest frequency of ovarian neoplasms occurred in women aged 40–65 years.^{13,14} Additionally, a study from India reported that benign ovarian tumours were diagnosed mostly in patients between 20–40 years of age.¹⁵ Regarding the prevalence of unilateral lesions (91.9%), this finding corroborate well with published literature. For example, a study from Saudi Arabia found that 63.2% of ovarian neoplasms were unilateral, while another study from India reported that only about 17% of cases involved bilateral lesions.^{13,14}

Serous cystadenoma was the most frequently diagnosed benign tumour (37.8%), followed by mucinous cystadenoma (16.2%). This aligns with the established prevalence of these benign tumour types in ovarian epithelial tumours. Serous carcinoma was the most common malignant tumour (19.8%), consistent with its well-documented dominance among ovarian malignancies.

Serous cystadenoma is the most frequently diagnosed benign tumour, typically making up around 37–47% of benign ovarian tumours.^{13,17} Serous carcinoma, specifically high-grade serous carcinoma, is the most dominant form of ovarian malignancy, representing nearly 70% of cases.^{15,17} Furthermore, a study on the histopathological patterns of ovarian neoplasms in different age groups found that high-grade serous carcinoma 42 (19.8%) was the most common histological type among malignant cases while among the benign lesions, 73 (34.4%) were serous cystadenomas cases.¹⁴ A study on the global incidence and

temporal trends of OC by histologic subtype observed a consistent highest incidence of serous carcinoma among all, with 45% global incidence rate.¹⁸ This global incidence rate of the malignant tumours is followed by mucinous carcinomas (13%), endometrioid carcinomas (13%), and clear cell carcinomas (6%).¹⁸ They also reported that deviation from the general global trends of the incidence rates of the OC was most prominent in the Asian region where they have a relatively higher proportions of endometrioid, clear cell and mucinous carcinomas.

A notable finding in our study was the positive family history of female genital system malignancy in 20.7% of patients, which is higher than the prevalence reported in some studies (5–10%).^{14,16} This finding warrants further investigation in larger cohorts to determine if it represents a regional trend or is attributable to other factors. However, it is well-established understanding that family history of ovarian and breast cancer is linked to an increased risk of ovarian cancer.^{4,15} This is said to be due to the BRCA-1 and BRCA-2 mutations associated with female genital malignancies especially breast, ovarian and endometrial cancers.⁵ Our study identified a statistically significant association between family history and tumour malignancy. Patients with malignant lesions were significantly more likely to have a positive family history compared to those with benign lesions ($p<0.001$). Studies have shown that patients with malignant lesions are significantly more likely to have a positive family history compared to those with benign lesions.^{19,20} For example, a large study from Nature Scientific Reports explored familial associations of histology-specific ovarian cancer with other cancers and found that the relative risk of ovarian cancer was 2.42 in families with one ovarian cancer patient and it reached 11.36 with two affected individuals.¹⁶ Another study from Nature Communications demonstrated that family history of breast cancer showed a dose-response on ovarian cancer risk.¹⁹ This finding underscores the potential role of family history as a risk factor for ovarian cancer, highlighting the importance of family history assessment in clinical practice. However, we did not observe any significant associations between laterality, parity, age at diagnosis, age at menarche, or age at menopause and tumour malignancy. Further research with larger sample sizes and diverse populations is needed to elucidate these potential relationships more definitively.

LIMITATIONS

Our study has limitations inherent to its cross-sectional design, precluding the establishment of causal relationships between observed factors and tumour malignancy. The sample size was moderate, potentially limiting the generalization of findings.

CONCLUSION

Our study contributes to the existing knowledge on the histopathological spectrum of ovarian epithelial tumours by characterizing patient demographics, tumour distribution, and clinicopathological associations in a Pakistani population. There is predominance of serous cystadenoma among benign tumours and serous carcinoma among malignant tumours, and potential significance of family history as a risk factor. Further research is warranted to explore the observed associations and gain deeper understanding of the factors influencing ovarian tumour development and progression in diverse populations.

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ORIGINAL ARTICLE

PROTECTIVE EFFECT OF FLAXSEED OIL AGAINST EXEMESTANE
INDUCED OSTEOPOROSIS IN ANIMAL MODEL

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Background: Exemestane, an aromatase inhibitor is used as an adjuvant to chemotherapy for early breast cancer. The objective of this study was to see the effect of dietary flaxseed oil on bone mass density in mice model of osteoporosis induced by exemestane. **Methods:** This study examined three animal groups over 25 days: a Control Group on normal diet, Group A receiving exemestane (2 mg/Kg), and Group B receiving both exemestane and 10% flaxseed oil. Pre- and post-experiment weights were compared using paired *t*-tests. Serum oestradiol levels and femur trabecular diameter between the control and exemestane groups were compared unpaired *t*-tests. Chi-square test was used to analyse osteoclast cell counts between the two groups. **Results:** Exemestane caused statistically significant effect on weight loss ($p < 0.05$) in Group A and B. The flaxseed group (Group B) showed a smaller increase in trabecular diameter ($p < 0.05$) and a lesser pronounced decrease in serum oestradiol level ($p < 0.05$) as compare to Group A. There was statistically significant difference in the number of osteoclasts between groups and Group A had more osteoclasts as compared to Group B ($p < 0.05$). **Conclusion:** Combined administration of flaxseed with exemestane in mice resulted in preservation or minimal reduction of bone mass density in animal model.

Keywords: Breast cancer, Exemestane, Oestradiol, Flaxseed oil, Osteoporosis

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INTRODUCTION

Breast cancer is the third most common cancer in women around the world. The risk factors for development of breast cancer includes increasing age, obesity, family history, early menarche, low socioeconomic status, physical inactivity and use of combined oestrogen-progesterone hormone pills in menopause. Research has shown that women in their postmenopausal period have almost 10–50 fold higher levels of oestradiol in endometrium as well as in the breast cancer tissues as compared to blood.¹ The worldwide reported cases of breast cancer are around 1.3 million as stated by American Cancer Society.² Its occurrence is higher in Asian population than the developed countries of Europe and America. In South Asia, Pakistan has 2.5 times higher incidence than India and Iran, and usually patients present with advanced stage of disease resulting in high mortality.³ According to Karachi Cancer Registry, the contribution of breast cancer is 34.6% to all cancers.⁴ Projected incidence in Pakistan has been estimated to be 60.7% in 2025 in women age 60–64 years.⁵

The treatment options of breast cancer are radiotherapy, chemotherapy, hormonal therapy and surgery.⁶ Exemestane is an aromatase inhibitor, approved by FDA for breast cancer.⁷ It is a third-generation steroidal aromatase inhibitor (SAI), given to postmenopausal women having metastatic breast cancer. The drug is well tolerated by most of the patients and

has superior efficacy as compared with Tamoxifen.⁸ Exemestane irreversibly inhibits aromatase enzyme. The oral administration of Exemestane at the dosage of 10 mg/day is associated with 85–95% reduction in plasma oestrogen level.⁹ However, it results in deep suppression of circulating oestrogen concentrations in postmenopausal women, frequently below the detection limits of standard assays. This leads to decrease in bone mass in postmenopausal women, leading to increased risk of bone fractures and osteoporosis. Hormonal therapy is the treatment of choice for osteoporosis but it has some side-effects and can be shifted to non-hormonal therapy.¹⁰

Flaxseed is a commonly used natural product which has good nutritional and health effects and benefits. Flaxseed oil is rich in α -Linolenic acid (ALA) which is a plant derived Omega-3 poly unsaturated fatty acid and recommended as nutrient. Flaxseed oil also contains sodium, potassium, carbohydrate, fibre, sugars, proteins and vitamin-D. Flaxseed oil has protective role in normal functions of the body systems and also has immune boosting effects.¹¹

The present study aimed to determine the preventive role of flaxseed oil against the adverse effects of exemestane therapy in animal model.

METHODOLOGY

This was a laboratory-experimental study conducted from Feb to Jul 2022 at Khyber Girls Medical College and Khyber Medical University, Peshawar. The Animal

Ethics Committee, Khyber Medical University Guidelines were followed for the experimental study. Ethical approval was obtained from the Advanced Studies and Research Board (Reference No. ASRB001374/HS/KGMC). Sample size was calculated using G-power with an effect size of 0.25, $\alpha=0.05$, and 95% power of the study. The total sample size was 30. Female albino mice aged 6–7 weeks, weighing 20–40 grams were obtained from the National Institute of Health (NIH), Islamabad and subdivided in to 3 groups according to the study protocol (Table-1).

A simple random sampling technique was used. Healthy adult female albino mice were included while sick and/or pregnant mice were excluded from the study. Mice were provided standard laboratory pellet feed and water freely available at all times, and maintained in their respective cages for 25 days with normal light/dark cycle.

After completion of the experiment, animals were sacrificed and femur bones were collected, sectioned and stained for morphological study (measurement of osteoclast cells) and scores were given as per standard histopathologic criteria for bone study (Table-2). Blood was collected through cardiac puncture for estimation of serum oestradiol levels with ELISA method. The trabecular diameter of the femur was measured. Data was analysed on SPSS-25. Quantitative variables were presented as mean values and categorical variables as frequencies and percentages. For continuous variables like weight and oestradiol levels, paired-sample *t*-test was applied; for categorical variables like number of osteoclast cells, Chi-square test was used, and $p \leq 0.05$ was considered statistically significant.

Table-1: Distribution of mice in different groups, drugs, and food categories

Group	Animals	Food and treatment given
Control	6	Standard food and water only
A	12	Standard food and water+exemestane 1 mg/Kg/day
B	12	Standard food and water+exemestane 1 mg/Kg/day+1 mL of 10% flaxseed oil/day

Table-2: Histopathologic criteria for bone assessment

Score 0	Normal osteoblast cells (no osteoclasts)
Score 1	Few osteoclast cells (<5% of most damaged bone surfaces are lined)
Score 2	Some osteoclast cells (5–25% of the most damaged bone surfaces are lined)
Score 3	Many osteoclast cells (25–50% of most damaged bone surfaces are lined)
Score 4	Numerous osteoclast cells (> 50% of most damaged bone surfaces are lined)

RESULTS

Paired *t*-test was applied for comparison of mean weight of three groups of mice before and after the experiment. In Group A and Group B there was statistically significant effect on weight loss as

compared with Control Group ($p < 0.05$). (Table-3).

When trabecular diameter was compared between Group-A and Group-B, a significant difference was found between two groups in diameter of trabeculae ($p < 0.05$) (Table-4).

The experimental Groups A and B were categorized according to the number of osteoclasts as having 2, 3, or 4 osteoclasts in subgroup. There were statistically significant differences in the number of osteoclasts between Group A and B ($p < 0.05$). (Table- 5).

The mean serum levels of oestradiol were compared between Groups A and B with paired *t*-test. There were significant differences in mean serum level of oestradiol between two groups ($p < 0.05$). (Table-6).

Histological sections were cut for the femur bones of mice in the three different groups. The mice in Group A showed signs of adverse effects of the drugs and the bones were spongier with larger trabecular diameter on eosin and haematoxylin staining (Figure-1 A).

The mice in Group B in comparison to Group A showed little or no signs of adverse effects of the drug and the bones were less spongy and no erosion with smaller trabecular diameter on eosin and haematoxylin staining (Figure-1 B).

The large multinucleated cells in the histological section were osteoclasts representing re-modelling activity of bone in Group A, after exemestane administration to albino mice. The multinucleated cells in the histological section in Group B (Figure-1 C) were less frequent (Figure-1 D).

Table-3: Comparison of mean weight of groups pre- and post-experiment (Mean±SD)

Group	Weight before experiment	Weight after experiment	<i>t</i> -statistics	<i>p</i>
Control	27.80±2.168	27.60±2.510	1.000	<0.05
Group A	26.67±3.822	12.17±28.23	8.521	
Group B	27.50±1.732	26.08±1.730	9.530	

Table-4: Comparison of trabecular diameter between Group A and Group B (mm, Mean±SD)

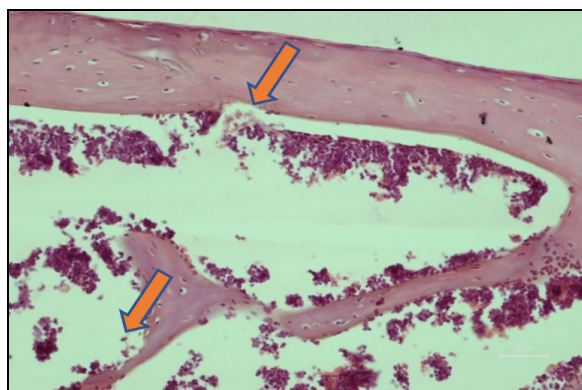
Groups	Trabecular diameter	95% CI	<i>t</i> -statistics	<i>p</i>
Group A	240.34±80.56	106.95–210.95	6.34	<0.05
Group B	81.39±32.46	105.34–212.56		

Table-5: Chi-Square test for relation between experimental groups and number of osteoclasts

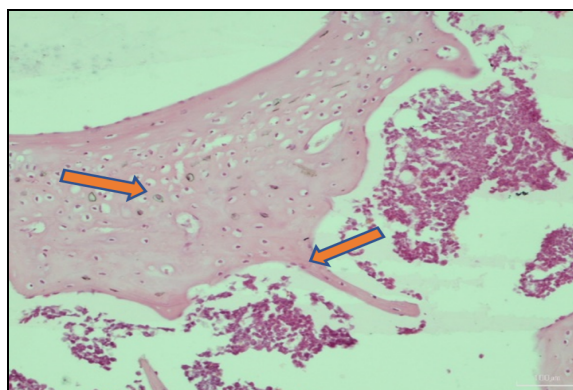
Experimental group	No. of osteoclasts in groups			Chi-square	<i>p</i>
	2	3	4		
Group A	5	7	0	11.07	<0.05
Group B	0	6	6		

Table-6: Comparison of mean oestradiol levels between group A and B

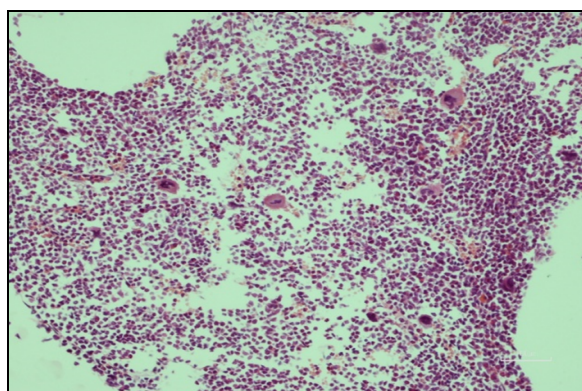
Groups	Oestradiol level (pg/ml)	95% CI	<i>t</i> -statistics	<i>p</i>
Group A	1.05±0.41	-2.14–1.03	5.94	<0.05
Group B	2.64±0.82	-2.15–1.02		



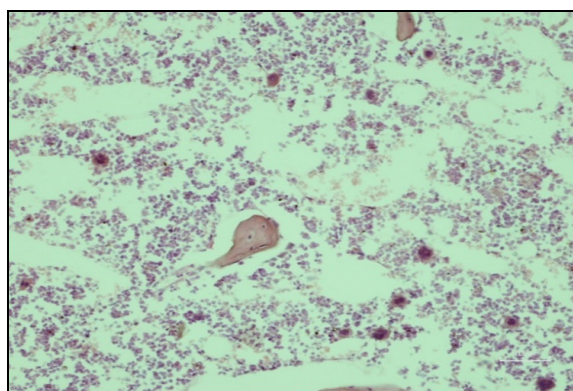
A: Histological section of femur showing large trabeculae in the exemestane only group. The arrow showing dilated trabeculae in the cortical bone with erosion of bone



B: Histological section of femur showing cortical bone in the exemestane and flaxseed treated group. The arrow showing normal trabecular bone with no signs of cortical bone erosion



C: Large multinucleated giant cells in Group-A (H & E stain)



D: Osteoclasts in Group B (H & E stain)

Figure-1: Histological assessment of bone and osteoclast cells

DISCUSSION

In the current study, there was significant weight loss in Group A and Group B when they received the drugs, compared to their weight before treatment. The weight loss was more in Group A from pre-treatment weight as compared to Group B. Weight loss in postmenopausal women with breast cancer on exemestane only was reported to be more than 2 Kg in 28% women as compared to 72% who either maintained weight or gained more than 2 Kg. These changes were reported after patients were followed for two years.¹² A study by G Francini reported significant loss of fat mass (not body weight) in obese patients after 12 months of exemestane therapy as compared to tamoxifen. Switching adjuvant therapy from tamoxifen to exemestane after 2 years may carry advantage in terms of weight loss for obese patients but this benefit may prove detrimental to patients with lower body mass index. The circulating oestrogen levels are reduced by exemestane and other aromatase inhibitors. Oestrogens have direct effect on cellular constituents of adipose tissue and adipocytes. This effect may explain the association of exemestane use with circulating oestrogen levels and that in turn explains its effects on body weight and fat mass. Another explanation for reduction

in fat mass may be explained by reduced appetite with exemestane therapy, though this change was not statistically significant.¹³ The Group B also demonstrated effect on weight loss. A trial conducted on flaxseeds using 15 gm/day suggested that flaxseed has a good impact on oestrogens which was increased in obese post menopausal women than normal weight women.¹⁴ The effect of flaxseed on BMI and fat content of breast tissue is documented by several observational and experimental studies in women with early breast cancer and without breast cancer.¹⁵

The use of flaxseed along with exemestane demonstrated effects on trabecular size. There were statistically significant differences in the trabecular size at the lower end of femur with flaxseed oil use as compared to exemestane group alone. The trabecular bone has the ability to withstand greater strain as compared to cortical bone. The BMD decreases as trabecular size increases and its mineralization decreases.¹⁶ The effect of flaxseed on BMD in Wistar rats in an experimental study demonstrated significant effects on BMD in maxilla and mandible. Flaxseed extract was more effective in increasing bone density than the group receiving Ca/Vitamin D.¹⁷ The secoisolariciresinol diglycoside (SDG), the active

metabolite in flaxseed lignans has potential oestrogenic effect on BMD in early life in female rats when oestrogens are low. This study reported that the effect of flaxseed extract on BMD in female rats was more than male rats. This is partly explained by the positive effect of lignans in addition to other flaxseed compounds on immature rats. This indicates that female rats are more sensitive than male rats to lignans and other flaxseed extract compounds.¹⁸

Exposure to purified SDG during early life has a positive effect on developing bones. This effect was demonstrated by increased bone strength in femur of female rats when measured by dual energy X-ray absorptiometry and biomechanical strength by a 3-point bending test.¹⁹ Flaxseed enhances the protective effect of low-dose oestrogen therapy on vertebral BMD, three-dimensional microarchitecture and bone strength in ovariectomized rats. Flaxseed exerts a stronger effect on bone density outcome when combined with low dose than when combined with ultra-low-dose oestrogen therapy.²⁰

There were statistically significant differences in the mean levels of serum oestradiol between two groups, as estimated with ELISA. The mean serum oestradiol level was lower in exemestane only group as compared to exemestane plus flaxseed oil group. Exemestane lowers serum oestrogen levels by inhibiting peripheral conversion of oestrogen from androgen precursors.²¹ Exemestane has demonstrated effectiveness in oestrogen receptor positive breast cancers. The lignan, a type of phytoestrogens, in flaxseed mimics the action of oestrogen in females. The level of oestrogen and progesterone in the ovariectomized rats treated with oestradiol and flaxseed oil was significantly increased as compared to rats ovariectomized only.²² Phytoestrogens in flaxseed are steroid hormones that have an oestrogen-like structure, these hormones bind to oestrogen α and β receptors and produces a response without negative effects of synthetic oestrogens thus increasing oestrogen levels. Research on phytoestrogens effect on bone health in postmenopausal women have shown promising results.²³

The number groups having 3 and 4 osteoclasts were more in Group B compared to Group A. None of the subgroups in Group A had 4 osteoclasts. The difference in the frequency of osteoclasts in groups was statistically significant. Some of the peptides isolated from flaxseed called cyclolinipeptides A-I and their related derivatives have been described as osteoclast differentiation inhibitors. These peptides are very potent inhibitors of osteoclasts differentiation. In the initiation phase of bone metabolism the osteoblast recruits osteocytes that differentiate into osteoclasts and then the bone resorption begins.²⁴ The inhibition of osteoclasts by cyclolinipeptides in the flaxseed is a possible

explanation for the increased number of osteoclasts on histology in Group B.

The increase in BMD with flaxseed is in turn supported by the increased differentiation of precursor cells into osteoblasts. This increased differentiation is mediated by Alpha Linolenic Acid (ALA) in flaxseed that in turn increases bone mass.²⁵ Another mechanism by which ALA increases BMD is to enhance the absorption of calcium from the intestine that accelerates the deposition of calcium into the bone.²⁶ Apart from increased osteoblasts, and calcium deposition in the bones, the flaxseed increases organic matrix deposition in the bone. This organic matrix involves increased deposition of collagen type 1 that increases bone strength and resistance.²⁷

The preventing the increase in the trabecular diameter, maintaining and enhancing the oestrogenic effects of its derivatives, decreasing the mitotic activity, inhibiting osteoclasts differentiation and increasing osteoblasts differentiation, increasing minerals and collagen deposition in the bones, flaxseed increases bone mass density and prevents osteoporosis.²⁸

The protective effect of flaxseed oil against the side effects of exemestane in mice is supported by the literature. This may act as a promising remedy in reducing the exemestane induced side-effects in postmenopausal women with breast cancer.

The main limitation of our study was that we did not measure bone mass density. The radiological objective evidence about the bone mass density was not compared which may have shown better results.

CONCLUSION

Administration of flaxseed oil with exemestane resulted in minimum loss or maintenance of bone mass density in animal model. A large-scale study is required to further validate our results.

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KJ: Overall supervision of research
US: Statistical analysis
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ORIGINAL ARTICLE

IMPACT OF COLONOSCOPY AND MORTALITY RATE OF COLORECTAL CANCER PATIENTS IN PAKISTAN DURING 2008–2020

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Background: Colorectal cancer (CRC) is the second neoplasm more frequent in women and the third largest in men. Its silent evolution makes early diagnosis difficult. Objective of this work was to identify data, and the impact of introduction of prognosis colonoscopy of patient with colorectal neoplasms in Pakistan. **Method:** This was an observational, quantitative, analytical, retrospective and transversal study. Patients aged ≥ 40 years with colorectal neoplasms entered into the hospital admission system and listed in the ICD chapter as neoplasms were included. Patients with morbidities outside the predetermined period, having neoplasms that are not of colorectal origin, or aged under 40 years were excluded. Analysis of the incidence and number of related deaths due to the CRC before and after the endoscopic procedure was done. **Results:** It was possible to determine the pattern of evolution of the number of hospitalizations for CRC between 2008 and 2020, considering 2013 as a milestone due to the introduction of colonoscopy. **Conclusion:** Expanding access to endoscopic examinations appears capable of promoting an even greater reduction in morbidity and mortality related to coloproctological neoplasms. This study may benefit with information more consistent with leave from the reduction of the prerequisites the realization of colonoscopy.

KEYWORDS: Colonoscopy, Colorectal neoplasms, Diagnosis, Mortality

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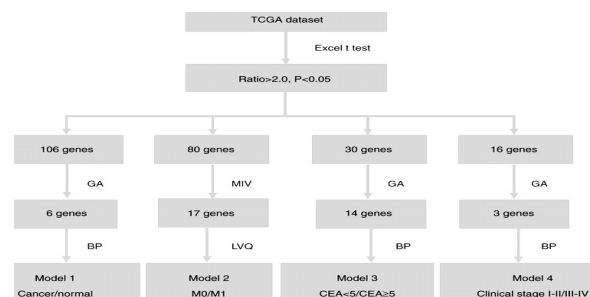
INTRODUCTION

Colorectal cancer (CRC) is a major public health problem worldwide. According to the World Health Organization (WHO), it is the second neoplasm more frequent in women and the third most frequent in men.¹ Its silent evolution makes early diagnosis difficult, giving it a worse prognosis when detected in advanced stages.²

Statistically, the CRC does not present a homogeneous incidence throughout our country, presenting cases with bigger prevalence in Baluchistan and KPK Provinces. Regarding age, the majority of population affected by colorectal neoplasia is in their 60's (average 67 years).³ Therefore, the more the age, the greater the possibility of risks of complications in relation to this neoplasia.⁴

Clinically the CRC manifests itself according to location of tumour. The cancer of colon introduces as lower bleeding, predominating occult blood in the faeces. In the advanced phases of the neoplasia in masses, they can be identified in the right hemi abdomen. There is involvement of the colonic wall vessels or tumour ulceration with haematochezia. The obstructive or semi-obstructive condition is more frequent in the left colon, evolving with changes in the evacuation habit, such as tenesmus and sensation of incomplete evacuation, with tumour progression to the rectum or in primary lesions of the rectum.⁵ These symptoms may be accompanied by weight loss, abdominal and/or pelvic pain, and anaemia which worsens with the volume of spontaneous bleeding or bowel movements. With a higher incidence between

50 and 75 years of age, the development of this pathology is associated with certain risk factors: diet rich in animal protein and/or processed foods and low in fibre, positive family history of CRC and/or adenomatous polyps, history of inflammatory bowel disease such as Crohn's colitis and chronic ulcerative colitis.⁶



Flow-chart for diagnosis of colorectal cancer

The search for blood hidden in the faeces is one of the most traditional and easy to perform among those used for the diagnosis of CRC, being used worldwide as first line of tracking of colorectal neoplasms. The technique consists of the fact that adenomatous polyps and carcinomas bleed. If bleeding is not clinically detectable, investigation of small haemorrhages can help in early diagnosis and screening of population groups at risk. However, the method has low sensitivity and low specificity, since several other intestinal alterations can cause haemorrhages, which results in a large number of false positives, making it essential to perform

radiological and/or endoscopic examinations to define the diagnosis.⁷

Discovered in the 1960s, the tumour marker CEA is one of the main markers used for forecasts of colorectal neoplasia in the clinical practice.^{8,9} This marker has low sensitivity for primary diagnostic detection. In postoperative screening of tumour resection, its sensitivity can exceed 90% in identifying loco regional recurrences or liver metastases.¹⁰

Due to the excellence of colonoscopy in investigating CRC, radiological examinations are currently relevant in the preoperative staging and postoperative monitoring of this neoplasm, investigating metastases in organs such as the liver and lung, infiltrations or extensions tumoural in the reviews postoperative.^{11,12} Among the methods used frequently are the computed tomography, the magnetic resonance and ultrasound.¹³ The colonoscopy consists of endoscopic study of the large intestine and of part of the small intestine by introducing a flexible fibre optic tube through the anus until the caecum, with terminal ileum through the ileocecal valve.¹⁴

Despite the propaedeutic relevance of colonoscopy for diagnosis of CRC, factors influence the less frequent performance of this examination in clinical practice are: i) Need of strict preparation, ii) Possibility of feeling pain during and after the exam, iii) High cost.¹⁵

The colonoscopy has been introduced in the list of diagnostic procedures. Indications for colonoscopy were lower digestive haemorrhage (different from classic anal bleeding), inflammatory bowel diseases, chronic diarrhoea, malignant or benign colon tumour, diverticular disease of the colon, colon polyps, and angio-dysplasia.^{16,17} However, there are some prerequisites for release of this propaedeutic investigation: i) Detailed clinical history with personal and family history related to the pathology, ii) Propaedeutic investigation by ultrasound, recto-sigmoidoscopy or radiological examination prior to the endoscopic examination, and iii) Request made by a general surgeon, gastroenterologist and proctologist. Patients with tumours and haematochezia have priority.

Despite the possible technical complications of colonoscopy and the limitations of its realization, this exam has revealed its fundamental importance to the detection of intestinal injuries in initial phase, especially large intestine. It allows an early therapeutic approach with removal of adenomatous polyps and the performance of tumour resection surgeries in the places where the lesions have become malignant.¹

The objective of this study was to identify the impact of introduction of prognosis colonoscopy of colorectal neoplasm cases in Pakistan, and to analyse deaths due to CRC before and after introduction of endoscopic procedure.

METHODOLOGY

This work consists of an observational, quantitative, analytical, retrospective and transversal study. These data cover the period between Jan 2008 and Dec 2020 in order to promote an analysis of parameters relative to the CRC and implementation of colonoscopy.

Inclusion criteria were: i) Both genders, ii) Age ≥ 40 years, iii) Patients presenting with colorectal neoplasms (neoplasia maligna of colon, malignant neoplasm of the recto-sigmoid junction, neoplasm of the rectum, anus and anal canal), iv) entered into the hospital admission system and v) listed in the ICD chapter as neoplasms.

The exclusion criteria were: i) Morbidities outside the predetermined period, ii) Neoplasms other than colorectal origin, iii) Patients under 40 years of age.

After selecting the database, the methodological analysis was done. (Figure-1).

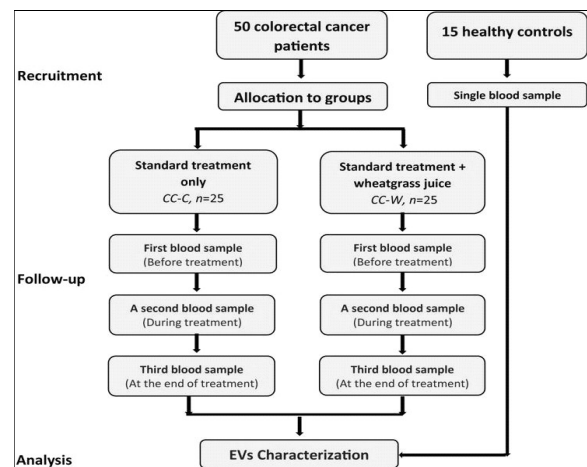


Figure-1: Flow-chart of Methodology

In the first stage, a study was carried out on the pattern of evolution of the number of hospitalizations due to CRC in the stipulated period. It was followed by the division of the sample according to sex, considering variations in the mortality rate, using the Student's *t*-test to measure and validate the observed differences; and evolution of the total number of hospitalizations age of patient the leave of 40 years, establishing itself intervals of 10 years between groups age groups. The significance of this statistical analysis was verified through the Kruskal-Wallis test.

In the second stage, sample from five Pakistan geographic regions was studied regarding:

- Variation in the mortality rate due to CRC during the period, using the ANOVA test in the Dunn method to validate the information for this analysis; and
- Evaluation of evolution of number of hospitalizations due to colorectal neoplasia, using ANOVA in Tukey's method to verify and confirm the statistical significance of the results.

RESULTS

It was possible to determine the pattern of evolution of the number of hospitalizations for CRC between 2008 and 2020, considering 2013 as a milestone due to the introduction of colonoscopy. It was observed in all the analyzed period, with exception of year of 2020, the curve referent to number total of admissions of patients aged ≥ 40 years with diagnosis of colorectal neoplasia was ascending. In 2008 there were 35,863 cases and in 2019 they were 86,009 cases representing an increase of 139.8%. (Figure-2).

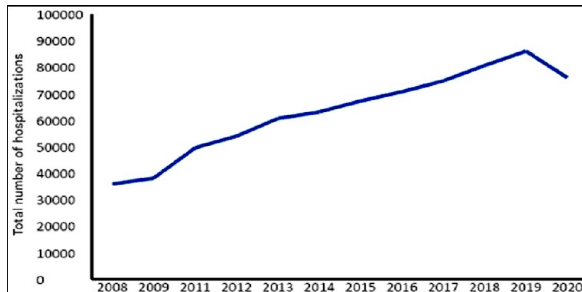


Figure-2: Number of hospitalizations with age ≥ 40 years diagnosed with colorectal neoplasia between 2008 and 2020

The analysis of data represented in Figure-2 aroused interest in the prognosis of these patients after introduction of colonoscopy in hospitals in Pakistan. For this purpose, the variation in the mortality rate due to CRC between the sexes was evaluated and the results are presented in Figures-3 and 4.

Both genders showed a decrease in the variation rate of mortality due to colorectal neoplasia in Jan 2008 (8.29% in males and 8.83% in females) to Dec 2020 (7.68% in males and 8.52% in females). However, the graphs reveal a different statistical behaviour between sexes. While there is a tendency towards balance in males, with small variations upwards or downwards since 2013, the predominance of mortality due to CRC remains in the females, and, despite the decrease with equivalence to the values with males in 2016 (8.06%), the number of deaths recovers its upward trend after 2018 (6%). (Figure-3).

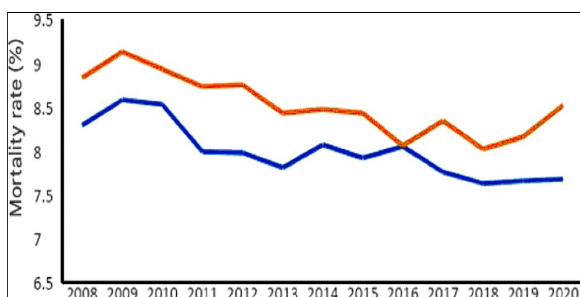


Figure-3: Mortality rate in admitted colorectal neoplasia cases aged ≥ 40 years according to gender from 2008 to 2020

In order to statistically confirm the differences observed, the results were evaluated for averages mortality rates in both genders, applying Student's *t*-test. A significant difference was confirmed between men and women regarding the average mortality rate due to colorectal neoplasms in patients over 40 years of age, with the average mortality rate in the women ($8.52 \pm 0.34\%$) being higher than the average mortality rate of men ($8.00 \pm 0.31\%$). (Figure-4).

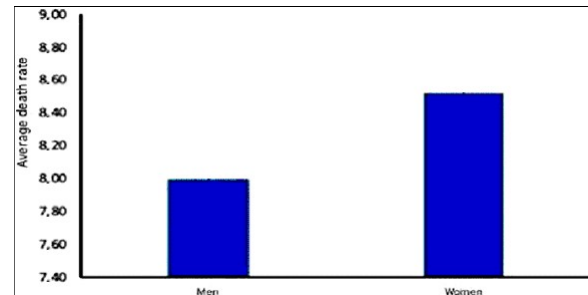


Figure-4: Average mortality rate in hospitalized cases of colorectal neoplasia aged ≥ 40 years, according to gender from 2008 to 2020. ($p < 0.001$)

After analyzing the variation in mortality rate in both sexes, considering the age as important risk factor for development of CRC, the impact of age on hospitalization due to colorectal neoplasia was evaluated in both sexes. The results are shown in Figures-5 and 6. Differences were significant ($p < 0.0001$) for the Kruskal-Wallis test between patients aged 40–49 years when compared with those aged 50–59 years, 60–69 years, and 70–79 years. Significant differences on Kruskal-Wallis test ($p < 0.0001$) between patients aged 60–69 years and those aged ≥ 80 years, and between age group 70–79 years and age group ≥ 80 years were also observed.

In order to investigate differences between hospitalizations rates for CRC among the different age groups, a statistical analysis was performed using the Kruskal-Wallis method. There were wide variations in the proportion between different groups age: 40–49 years, 50–59 years, 60–69 years, 70–79 years and ≥ 80 years. The age group with the highest number of hospitalizations was that of individuals aged 60–69 years, having peak maximum in 2019 (1,918 hospitalizations) and a drop of approximately 9% in 2020 (1,748 hospitalizations). Patients aged 70–79 years ranked in the second place when analyzing the total number of hospitalizations due to colorectal neoplasia, noticing a constant increase (78.54% hospitalizations/year) from 2008 (752 hospitalizations) to 2019. The year of 2019 presented its peak maximum (1,646 patients) and a drop of approximately 7% in 2020. The age group 50–59 years had the third highest number of hospitalizations, being peak maximum in 2019 (1,304 hospitalizations), followed a fall of 4% in 2020 (1,259 hospitalizations).

Individuals aged 40–49 years represent the group with the greatest stability in the number of statistical records in the period analyzed. Even so, a slight increase was observed from 2013 (501 hospitalizations), with a peak in 2019 (603 hospitalizations) and fall of approximately 16% in 2020 (509 hospitalizations). The age group of ≥ 80 years stands out with a tendency to grow from 2008 (417 hospitalizations) to 2019 (1,036 hospitalizations), reaching its peak that year, followed by a fall of 19.2% hospitalized cases with that colorectal cancer in 2020. Figure-5 illustrates an increase in number of total of hospitalizations due to CRC in the period of 2008–2020.

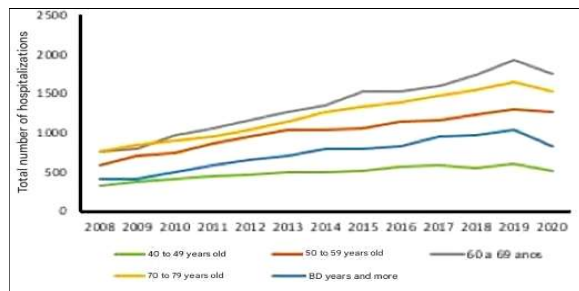


Figure-5: Number of hospitalizations due to colorectal neoplasia, according to age group, from 2008 to 2020

The second stage of analysis statistics sought to evaluate possible differences between CRC mortality rates among the five geographic regions of Pakistan. There were significant differences on ANOVA in comparison of mortality rate due to colorectal neoplasia between Sind and Baluchistan provinces ($p < 0.05$). There were also significant differences in average mortality rates due to CRC between the Federal Capital region and the Baluchistan Province ($p < 0.01$), between Punjab and Baluchistan ($p < 0.05$).

Figure-6 shows that the Federal Capital region had the highest mortality rate due to colorectal neoplasia among patients ≥ 40 years of age admitted to hospitals affiliated with public hospitals, with the maximum peak recorded between 2016 and 2017 (12.8%). After that period, there was a fall of 19%, passing by to 10.72% with tendency towards equilibrium, around 11%. The Punjab province had its maximum peak in mortality rate in 2012 (11.86%) and a drop of 17% in 2013, going at 9.84%. After the year 2013, when the colonoscopy was introduced, there was a drop in mortality from CRC, falling to 7.77% in 2017 with a light increase of 9% between 2017 and 2019. The KPK Province registered the biggest rate mortality rate due to this pathology in 2008 (13.3%) and in subsequent years showed a downward trend, reducing from 13.3% to 10.12% until 2019, where there was a slight increase, reaching 10.8% in 2020. The Baluchistan Province had the lowest rate of mortality, 9.4% in 2008 with reduction of 37% from 2008 to 2012, reaching values of 6.16% this year and,

after 2013, it continued with small variations, maintaining the tendency to progressive decline. The Sind Province was the one that presented the greatest variations. Its maximum mortality peak was in 2010 (11.52%) with a decrease accentuated in 2011 (7.97%) and a considerable increase in 2012 (10.76%). After this period, there was a fall until 2016 (41.2%) with a peak in 2019 (10.72%), returning to lower records in 2020 (9.47%).

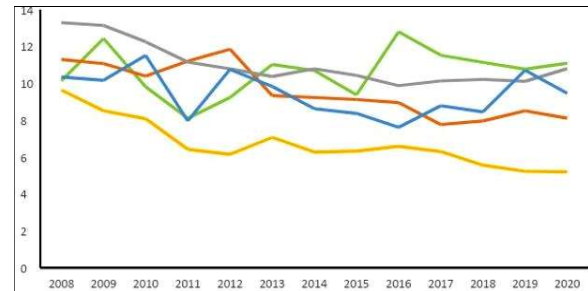


Figure-6: Rate of mortality in hospitalized colorectal neoplasia patients aged ≥ 40 years from 2008 to 2020

Illustrated in Figure-7, ANOVA test using the Dunn method confirmed the significant differences between the mean mortality rate due to CRC in Baluchistan Province (mean mortality rate = $6.09 \pm 0.65\%$) compared to other four provinces of Pakistan. There were smaller differences between themselves, with the highest average in the KPK (mean mortality rate = $9.61 \pm 0.35\%$) and Federal Capital regions in relation to those in Punjab (mean mortality rate = $7.94 \pm 0.62\%$) and Sind (mean mortality rate = $8.66 \pm 0.57\%$).

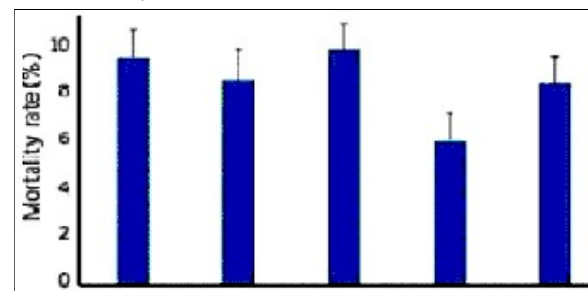


Figure-7: Averages rate of mortality due to colorectal neoplasia in hospitalized patients aged ≥ 40 years from 2008 to 2020

The total number of hospitalizations due to colorectal neoplasia in different Pakistan regions aims to situate mortality rates in relation to the number of highly complex procedures for treating CRC. These data can be seen in Figures-8 and 9.

There were significant differences ($p < 0.0001$), when comparing the average hospitalization between the Federal Capital Area and Punjab, KPK and Baluchistan respectively, ($p < 0.05$). Differences were significant ($p < 0.0001$), when comparing the average of

hospitalization between Punjab and Sind. Significant differences were also observed when comparing the average hospitalization between Sind and KPK and Baluchistan, respectively ($p < 0.05$).

The results shown in Figure-9 show significant differences between the average number of hospitalizations due to colorectal neoplasia between the KPK ($14,362.31 \pm 6,213.85$ hospitalizations) and Baluchistan ($9,756.15 \pm 4,670.70$ hospitalizations) in relation to Punjab ($3,425.85 \pm 1,759.22$ hospitalizations), Sind ($1,815.38 \pm 705.47$ hospitalizations), and Federal Capital Area (546.15 ± 222.85 hospitalizations).

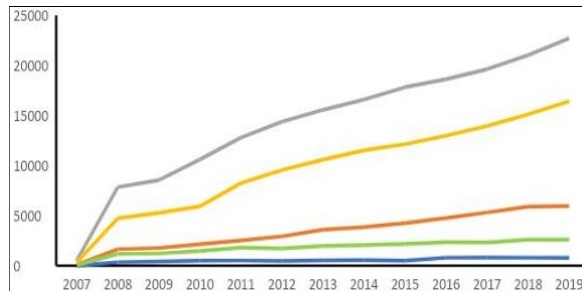


Figure-8: Number of hospitalized cases of colorectal neoplasia aged ≥ 40 years from 2008 to 2020

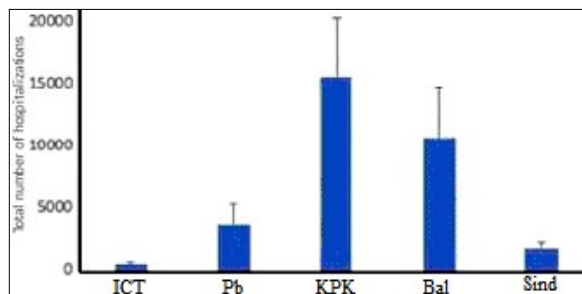


Figure-9: Number of hospitalizations due to colorectal neoplasia from 2008 to 2020

From the statistical analysis, the facts revealed are: i) Bigger number of hospitalizations due to CRC after the integration of colonoscopy in hospitals, ii) Reduction in mortality from this pathology in the same period, regardless of sex of these patients, iii) maintenance of colorectal neoplasms patients despite of endoscopic method, and iv) increase in the number of patients diagnosed.

DISCUSSION

Most cases of colorectal malignant neoplasia originate from adenomatous polyps. These polyps commonly originate from the intestinal mucosa, depending on their size and appearance. They can evolve into malignancy, which occurs through two pathways: one related to the adenoma-carcinoma sequence and the other linked to the serrated-sessile adenoma. In both, somatic mutations occur in several genes, such as APC, TP53 and KRAS that is common in the elderly population.¹⁶

Adenoma-carcinoma is responsible for the majority of the CRC, being 85% of neoplasms as origin through of polyps adenomatous conventional present over the course of 10 years, the cells of these polyps undergo approximately 60 mutations, becoming chromosomally unstable and evolve to the carcinoma. The sessile serrated adenoma accounts for about 15% of colorectal tumours. In this pathway, for a period yet not determined, occur approximately 700 mutations, affect cellular proteins, and cause microsatellite instability, culminating in the malignancy of polypoid lesions.¹⁷

Increasing incidence rates of CRC in 50–54-year-olds parallel the well-documented increases in early-onset CRC, and the same, as-yet-unknown mechanisms contributing to increasing incidence rates at ages younger than 50 years may also contribute to increasing rates in this age group. These mechanisms remain puzzling because most young adults diagnosed with CRC have no known risk factors or predisposing conditions. Patients with age equal too or greater than 50 years are considered to be at medium risk for colorectal neoplasia, since over 50 years of age the chances of patients developing colorectal neoplasia increase exponentially.¹⁸

However, patients with a family history are framed as of high risk to that pathology independently from the age. CRC screening methods are usually performed using three tests: search for occult blood in the faeces, radiological and endoscopic exams. To one diagnosis more precisely, these tests are associated in clinical practice⁶, in addition to complementary tests being used, such as serum carcinoma-embryonic antigen (CEA) dosage, which also has propaedeutic relevance in colorectal patients in Pakistan.¹⁹

Regarding endoscopic examinations, colonoscopy is the gold standard in the diagnosis and detection of adenomatous polyps and intestinal carcinomas. For this examination to be successful, the patient must have undergone appropriate colon preparation, providing good technical execution by the examining physician. Colonoscopy has high sensitivity and reasonable specificity when it comes to the detection of adenomatous polyps. It enables the visualization of injuries neoplastic in very initials phase. In addition, the colonoscopy allows early removal of the adenomas, as one form of prevention of development of colorectal neoplasms, colonic mucosa or to the neoplasm itself or polyp during the colonoscopy.²⁰

After the year 2013, the year colonoscopy was introduced in our hospitals, there has been a constant but less pronounced increase in the number of hospitalizations until 2019. Comparing the years preceding 2013, the rate of hospitalization had been accentuated and less stable. It is also observed that in 2020 there has been a fall in number of hospitalizations arising from of colorectal neoplasms, (10,037 cases)

which is relevant to previous studies being carried out. The present study showed a predominance in the number of hospitalizations in the group of patients with age between 60 and 69 years, registering the bigger number average of hospitalizations due to colorectal neoplasia (1337.77 hospitalizations), followed by the age groups 70–79 years and 50–59 years. Another significant difference is the reduction in the average number of hospitalizations among the patients aged 40–49 years (486.15 hospitalizations) and ≥ 80 years (729.23 hospitalizations), if compared to the remaining age groups in the same period. Our observations are in agreement with the previous work carried out globally. A large number of menopausal women suffer from this disease globally.²¹

When analyzing the total number of hospitalizations for CRC in the period from 2008 to 2020 in different regions of Pakistan, no change was recorded in the pattern of evolution of the graphs from the date of integration of colonoscopy in 2013. It was found that the KPK Province region was the one with the most patients hospitalized for colon neoplasia, where its maximum peak was in 2019 (22,703 hospitalizations) with a considerable drop in 2020 (19,280 hospitalizations), despite a constant growth trend in the analyzed period. The Baluchistan Province showed growth throughout the analyzed period, presenting its maximum peak (16,423 hospitalizations) in 2019 and a downward trend the fall year of 2020 (15,131 hospitalizations). The Federal Capital Area with a discreet trend in the growth during the years analyzed, had a peak (5,961 hospitalizations) in the year of 2019 and this trend remained constant. Our findings are similar to data which has been found in other studies with reference to global data about colorectal neoplasia.^{22,23}

CONCLUSION

After the implementation of colonoscopy in the list of procedures, there was a significant increase in the number of hospitalizations associated with colorectal cancer (CRC) with a significant reduction in the mortality rate related to this neoplasia. The role of colonoscopy is: i) in the early detection of colorectal neoplasia or in less advanced stages as compare to laboratory and radiological propaedeutic methods, and ii) greater assertiveness in diagnosis, combined with biopsy of lesions accompanied by anatomic-pathological study. Despite being significant, the impact of introduction of this endoscopic method still reflects the heterogeneous development of technological resources for the health area in different geographic regions of the country. The majority of hospitalizations for CRC are in the KPK and Baluchistan Provinces of Pakistan, with predominance from the first, also registers high indexes of mortality in relation to the others. Probably because it

attracts more complex cases, despite the high hospitalization rates, the KPK does not show a reduction in mortality rates like the Baluchistan. The behaviour pattern of subpopulation in Baluchistan, combined with broad access to diagnostic resources, reflects more accurately the conditions for diagnosis and prognosis of CRC after 2013: an increase in the number of hospitalizations with a reduction in the mortality rate. Access to endoscopic examination appears capable of promoting an even greater reduction in morbidity and mortality related to coloproctological neoplasms.

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HM: Data collection, Initial write-up

SB: Statistical analysis, Tabulation

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ORIGINAL ARTICLE

COMPARISON OF IRON ALONE VERSUS IRON COMBINED WITH VITAMIN A FOR MANAGING IRON DEFICIENCY ANAEMIA

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Background: Iron deficiency is the most prevalent nutritional deficiency and a significant global public health concern. This study aims to rapidly reduce the prevalence of iron deficiency anaemia (IDA) and prevent related cognitive impairments in children. Objective was to compare the effectiveness of iron alone versus iron combined with Vitamin-A in reducing iron deficiency anaemia in children (aged 6–59 months of age). **Methods:** This randomized controlled trial over 6 months conducted in Department of Paediatrics, PAC Hospital, Kamra. Study included 104 children (52 in each group), aged 6–59 months, of either gender, with haemoglobin levels below 11 g/dL and serum ferritin levels below 12 ng/mL. Participants were randomly assigned to two groups: Group A received iron plus Vitamin A, while Group B received only iron therapy. After three months, patients were assessed for changes in haemoglobin concentration and ferritin levels as ferritin stores replenish in specified period. Data were recorded using a specially designed form. **Results:** In Group A, the mean age was 37 ± 7.10 months, and in Group B, it was 40 ± 6.25 months. In Group A, 27 (52%) children experienced a reduction in IDA, and in Group B, 15 (29%) children showed a reduction in IDA. Older children in Group A and those having literate mothers showed better improvement in IDA in Group A. **Conclusion:** Combining iron with Vitamin A is more effective than iron alone in reducing iron deficiency anaemia in children.

Keywords: Iron plus Vitamin A, Iron therapy, Iron Deficiency Anaemia, IDA

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INTRODUCTION

Iron deficiency Anaemia (IDA) is a widespread nutritional problem and a significant global health concern.^{1–3} It has an incidence of 39% between 0–4 years of age and 48.1% between 5 and 14 years.⁴ Nationwide, the rate of iron deficiency anaemia (IDA) in children varies from 15.3% to 62.5%.⁵ High risk groups for IDA include infants, pre-schoolers, and women of reproductive age.²

IDA progresses in stages, starting with the depletion of iron reserves. It is marked by reduced serum ferritin, low mean corpuscular volume (MCV), and low mean corpuscular haemoglobin (MCH).⁶ As iron stores become exhausted, decreased haemoglobin levels lead to anaemia. Common causes of IDA in children include inadequate dietary intake, excessive milk consumption during periods of rapid growth, low birth weight, worm infestations, and gastrointestinal losses. Symptoms may include loss of appetite, angular stomatitis, irritability, dysphagia, pica, and increased susceptibility to infections. Importantly, IDA can lead to cognitive impairments, particularly during first two years of life.^{7,8}

Oral ferrous salts are a common treatment for IDA, but treatment often extends for 6 months to a year due to the slow increase in haemoglobin concentration and ferritin stores. Challenges such as poor compliance, lengthy treatment duration, altered bowel habits, disrupted iron absorption, and ineffective counselling can

hinder outcomes. Given the magnitude of the problem and the limited effectiveness of prolonged oral iron therapy, new interventions are needed to rapidly increase haemoglobin levels and reduce the duration of IDA treatment.^{9,10}

Studies suggest that combining iron with Vitamin A supplements is more effective than iron alone in treating IDA.¹¹ This effectiveness is attributed to Vitamin A's role in enhancing iron absorption, metabolism, and erythropoiesis.¹² Besides Vitamin A is also related to concentrations of IGF type 1 (Insulin like growth factor), that helps to mobilize body iron stores.¹ A study in Malaysia showed that children receiving Vitamin A supplements experienced a more significant increase in haemoglobin levels after three months compared to those who did not receive Vitamin A. The treatment group also saw a notable reduction in IDA prevalence (22.4%) compared to the control group (5.6%).¹³

This study aims to rapidly decrease IDA prevalence to mitigate long-term cognitive impairments in children. Another study at Brazil revealed that Vitamin A supplementation is a safeguarding tool against anaemia and stunting especially in underdeveloped countries.^{1,2}

METHODOLOGY

This controlled trial was conducted from 1 Oct 2023 to 1 Apr 2024, at the Outpatient Department of Paediatrics,

Pakistan Aeronautical Complex, Kamra. A sample size of 104 was calculated by G*Power calculator, with 52 children in each group, achieving 80% power with a 5% significance level. The expected reduction in IDA after three months of treatment was 22.4% for the iron plus Vitamin A group and 5.6% for the iron-only group.¹⁴

Children aged 6 to 59 months, of either gender, presenting with haemoglobin levels below 11 g/dL and serum ferritin levels below 12 ng/mL, were included. Children with co morbidities (congenital heart disease, celiac disease, Thalassemia Major, inflammatory bowel disease, malabsorption syndromes, chronic diarrhoea, haemorrhoids, and bleeding disorders) and infectious diseases (pneumonia, otitis media, liver diseases) were excluded from study. All children were ensured to be on healthy diet according to their age, they were also de-wormed before starting the treatment.

The study was performed only on patients whose parents agreed to their participation and approved to the informed consent. Demographic variables (name, age, and gender) were recorded. Children were randomly assigned to two groups: Group A received iron plus Vitamin A, while Group B received only iron. Vitamin A was administered at a dose of 500 IU three times a week for three months. Both groups were treated with iron supplements in the form of ferrous salts for three months. Follow-up was conducted in the outpatient department every 2 weeks to ensure compliance and address issues regarding availability of medicines and any side effect. After three months, patients were evaluated for changes in haemoglobin and ferritin levels by blood samples to assess the reduction in IDA. At the time of sampling it was ensured that patients are not suffering acutely from any illness as it may alter the ferritin results.

Data were entered and analysed using SPSS-20. Quantitative variables like age, haemoglobin concentration, and ferritin levels were presented as means with standard deviations. Qualitative variables such as gender and reduction in IDA were presented as frequencies and percentages. Chi-square tests were used for comparing IDA reduction between groups, with $p \leq 0.05$ considered significant. Stratification controlled for age, gender, and maternal education.

Children who failed to respond to oral iron therapy were investigated for other disorders like celiac disease, Thalassemia syndromes, Megaloblastic anaemia, worm infestations, H pylori gastritis and inflammatory bowel disease. After ruling out these disorders, the candidates were considered for intravenous iron therapy.⁹ Although it was an infrequent option in the past, but now with availability of new iron products, it allows for better replacement of total iron deficit. The new products are safe and approved by FDA like iron sucrose therapy.¹⁰

RESULTS

Mean age of children in Group A was 37 ± 7.10 months, 16 (31%) children were aged 6–30 months, and 36 (69%) were aged 31–59 months. Mean age of children in Group B was 40.0 ± 6.95 months, 17 (33%) children were aged 6–30 months, and 35 (67%) were aged 31–59 months. Group A had 21 (40%) males and 31 (60%) females, while Group B had 23 (44%) males and 29 (56%) females. In Group A, 19 (37%) children weighed ≤ 10 Kg and 33 (63%) weighed > 10 Kg, with a mean weight 12 ± 3.56 Kg. In Group B, 20 (38%) children weighed ≤ 10 Kg and 32 (62%) weighed > 10 Kg, with a mean weight 13 ± 4.19 Kg. Eighteen mothers of Group A children were ‘literate’ while 34 were ‘illiterate’. Twenty mothers of Group B children were ‘literate’ and 32 were ‘illiterate’. (Table-1).

Group A showed more significant improvements compared to Group B in serum haemoglobin and ferritin levels. Pre-treatment, both groups had similar values. Post-treatment, Group A had a significantly higher increase both in haemoglobin (2.72 g/dL vs 0.63 g/dL) and ferritin (3.46 ng/mL vs 1.71 ng/mL) ($p=0.0001$) (Table-2, 3).

In Group A, 27 (52%) children showed a reduction in IDA compared to Group B with 15 (29%) only ($p=0.016$) (Table-4). Overall, the addition of Vitamin A to iron treatment proved more effective in improving iron status and reducing IDA.

Maternal education played a significant role in our patients’ treatment. It was easy for us to make them follow medical advice and to ensure timely medical care in case of complications. Reduction in IDA among the two groups was also stratified by age, gender, and maternal education. Older children in Group A and those having literate mothers showed better improvement in IDA in Group A. (Table-5).

Table-1: Basic demographics

Variables	Group A	Group B
Age (Months)		
6–30	16 (31.0%)	17 (33.0%)
31–59	36 (69.0%)	35 (67.0%)
Mean $\pm$ SD	37.0 $\pm$ 7.10	40.0 $\pm$ 6.95
Gender		
Male	21 (40.0%)	23 (44.0%)
Female	31 (60.0%)	29 (56.0%)
Weight (Kg)		
≤ 10 Kg	19 (37.0%)	20 (38.0%)
> 10 Kg	33 (63.0%)	32 (62.0%)
Mean $\pm$ SD	12 $\pm$ 3.56	13 $\pm$ 4.19
Mother’s education		
Educated	18 (35.0%)	20 (38.0%)
Uneducated	34 (65.0%)	32 (62.0%)

Table-2: Serum haemoglobin concentration (g/dL)

Haemoglobin Level	Group A	Group B	p
Pre treatment	6.09 $\pm$ 2.24	6.28 $\pm$ 2.10	0.6564
Post treatment	8.8 $\pm$ 1.76	6.91 $\pm$ 1.88	0.0001
Rise in Hb level	2.72 $\pm$ 0.48	0.63 $\pm$ 0.22	0.0001

Table-3: Serum ferritin level (ng/mL)

Serum Ferritin Level	Group A	Group B	p
Pre treatment	8.31±3.47	8.11±2.94	0.7518
Post treatment	11.77±2.51	9.82±2.64	0.0002
Rise in Hb level	3.46 ±0.96	1.71±0.30	0.0001

Table-4: Reduction in IDA [n (%)]

Reduction in IDA	Group A	Group B
Yes	27 (52%)	15 (29%)
No	25 (48%)	37 (71%)

Table-5: Stratification of reduction in IDA with variables

Variables	Reduction	Group A	Group B	<i>p</i>
Age				
6–30 months	Yes	8	5	0.2263
	No	8	12	
31–59 months	Yes	19	10	0.0380
	No	17	25	
Gender				
Male	Yes	11	7	0.1391
	No	10	16	
Female	Yes	16	8	0.0576
	No	15	21	
Mothers' education				
Educated	Yes	9	6	0.2078
	No	9	14	
Uneducated	Yes	18	9	0.0404
	No	16	23	

DISCUSSION

Our study was focused to imply the effects of Iron therapy with and without Vitamin A supplementation in managing iron deficiency anaemia. The results from this study revealed that adding Vitamin A to iron considerably improved the haemoglobin and ferritin levels along with overall improvement in Iron deficiency anaemia in children. Furthermore maternal education was also found to be an important contributing factor in establishing better compliance to therapy and improved consequences.

One of the major results in our research was the noteworthy improvement in both serum haemoglobin and ferritin in patients who received iron plus Vitamin A (Group A) in comparison to those who received Iron therapy alone (Group B). The mean rise in haemoglobin level in Group A was 2.72 g/dL notably higher than 0.63 g/dL increase noted in Group B ($p<0.0001$). This data lines up many studies in recent years that have also shown synergistic effects of Vitamin A on Iron absorption & metabolism, improving anaemia.^{14,15} Vitamin A is also believed to improve gut absorption of iron. Vitamin A has been shown to improve the intestinal absorption of iron, that also leads to improved response in Group A patients.¹⁶

On the other-hand in Group B that only received iron therapy, a smaller increase in haemoglobin and ferritin levels were observed. This emphasizes the tentative limitation Of iron alone to improve anaemia especially in children who have concurrent deficiencies

of other micro-nutrients as well like vitamin A. previous studies have also shown similar results that isolated iron treatment may be less efficient in improving anaemia in areas where multiple micro-deficiencies prevalent.¹⁷

One more essential finding of our study was the higher rate of iron deficiency anaemia reduction in Group A, i.e., 52% as compared to Group B, i.e., 29% ($p=0.016$). This study supports the design that Vitamin A can magnify the efficacy of iron therapy in managing Nutritional anaemia. A similar study was performed by Kumar *et al*, Implying that patients receiving combined iron and vitamin A had a higher resolution rate of iron deficiency anaemia than those receiving isolated iron.¹⁸ This result is in harmony with the theory that vitamin A plays a vital role in enhancing iron usage, thereby improving anaemia.¹⁹

Recent data also reveals that micro-nutrient deficiencies like vitamin A and iron are very common in young population particularly in under developed countries and combined therapy may be more effective than dealing with individual deficiencies.⁴ Our finding also supports the evidence of beneficial response of combined therapy thereby decreasing the national burden of Iron deficiency anaemia.

Our study highlights crucial role of maternal education in managing iron deficiency anaemia in children. Educated mothers are more likely to comply with treatment plans and seek early medical care. Educated mothers are better informed about the benefits of iron supplementation and fortification, and can recognize early symptoms of anaemia, leading to improved health outcomes for their children. Maternal education is a key component of public health programs aimed at reducing iron deficiency anaemia, particularly in young children.

The age range in our study was balanced enough between the two groups falling in range of 31 to 59 months. This is the most vulnerable group due to rapid growth, development and higher nutritional requirements. There was no considerable difference noted on the base of age or gender. However some studies did reveal a greater prevalence of iron deficiency anaemia in females due to menstrual blood loss. Gender was not contributing factor in our study but still a large sample may have helped us to give a better insight.

Al-Mekhlafi HM *et al* found that anaemia was more common in children aged 10 years or younger compared to those older than 10 years. There was no significant difference in the prevalence of anaemia between male and female children.¹²

Iron deficiency anaemia is a notable problem of our country. It is easily treatable by simple measures like iron supplementation, food fortification, and maternal awareness programs. A study conducted in Pakistan⁸ revealed that the prevalence of IDA in Pakistani children represents a moderate burden on country economy. The

affected children more often belonged to mother having IDA and living in areas where food security is undermined. It requires both vertical and horizontal programs like iron supplementation and food fortification to alleviate the burden.

Although our study proved to be successful in establishing a role of iron and vitamin A but still there are some limitations to look upon. Our study duration was short and a long follow-up data would have been more helpful to establish that haemoglobin and ferritin levels remained consistent with time. Long follow-up would also be helpful to ensure better healthy lifestyle. A detailed study would also be useful to ponder on confounding factors like dietary pattern, iron stores, and presence of underlying conditions that influence therapy response.

CONCLUSION

Combining iron with Vitamin A is more effective than iron alone in reducing IDA. Children who fail to respond to iron treatment altogether should be evaluated for other underlying disorders. Emphasis on vertical and horizontal programs like iron supplementation and food fortification is required to alleviate the burden on national basis.

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ORIGINAL ARTICLE

FREQUENCY OF AORTIC ROOT DILATATION IN PATIENTS WITH LONG STANDING ESSENTIAL HYPERTENSION

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Background: Aortic root dilatation poses a potential risk factor for development of aortic dissection. In people with hypertension, aortic root dilatation is connected to left ventricular hypertrophy, indicating the susceptibility to cardiac damage. Dilatation of ascending aorta is a major risk factor for development of aortic dissection and/or rupture of aorta if not treated surgically. It signifies 50% of all thoracic aneurysms. Objective of this study was to determine the frequency of aortic root dilatation in patients with long standing essential hypertension. **Methods:** One hundred and three patients with essential hypertension were selected for the study. The size of aortic root was measured by two-dimensional echocardiography calculating the maximum distance between the anterior and posterior aortic root walls' two leading edges at the end diastole. A parasternal long axis perspective was used to take images of the proximal aortic root. **Results:** The patients' age was 51 ± 6.7 years, duration of hypertension was 10.7 ± 2.9 years and weight of 87.5 ± 6.5 Kg. Out of these patients, 79.6% were male and 20.4% were female, and out of the total, 19.4% had aortic root dilatation. Frequency of aortic dilatation was significantly higher in people older than 50 years, or having hypertension for more than 10 years ($p < 0.005$). **Conclusion:** There was a high prevalence of aortic root dilatation in patients with essential hypertension.

Keywords: Hypertension, Longstanding, Aortic root, Dilatation

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INTRODUCTION

Elevated blood pressure remains a continual global issue that poses a significant threat to global health. The possibility of cardiovascular illnesses like ischemic heart disease and stroke is increased in hypertension (HTN), either alone or in conjunction with other metabolic disorders like obesity and diabetes.¹ Hypertension is coupled with atherosclerosis and end-organ damage.² Hypertension poses serious health concerns globally. The true incidence of hypertension is under reported due to its silent nature.³

Both males and females with cardiovascular risk factors have an increased risk of developing hypertension in later age.⁴ Age, Body Mass Index (BMI), and arterial blood pressure, especially systolic blood pressure (SBP) are important prognostic factors for hypertension. Higher BMI, age, higher triglyceride levels, higher fasting blood glucose level, higher systolic and diastolic blood pressure blood pressure insulin resistance and high total cholesterol level are all connected with a greater occurrence of hypertension in both genders. However, the only significant markers in females are the waist-to-hip ratio and diastolic blood pressure.⁴ Obesity, sedentary lifestyle, and a family history are prevalent risk factors for hypertension.⁵ People who drink soft water over extended periods of time are more likely to develop hypertension.⁶

One important risk factor for the development of aortic dissection is aortic root dilatation (ARD).⁷ In

the hypertensive population, ARD is linked to left ventricular hypertrophy, indicating a greater burden of cardiac injury.⁸ According to a study, 22% of patients with chronic essential hypertension had aortic root dilatation.⁹ The American College of Cardiology advises taking measurements in a person at least twice on different dates to diagnose hypertension. Additional readings should only be taken if the first two readings differ by ≥ 10 mmHg. The average of the previous two readings of blood pressure is then noted.¹⁰

Ambulatory blood pressure monitoring is the most accurate method to diagnose hypertension which also aids in identifying persons with silent hypertension as well as white coat effect. Elevated sympathetic activity and obesity has a major role in pathogenesis of hypertension due to a combination of behavioural (high salt intake), psychological (mental stress), and lifestyle (physical inactivity) factors, as well as genetic influences (family history). Forty to fifty percent of middle-aged obese persons have hypertension. The sympathetic nervous system, especially the renal sympathetic nervous system, is more active in obese individuals due to a number of factors, such as hyperleptinemia and high sodium consumption, which causes hypertension. However, epidemiological research suggests that a significant risk factor for the development of essential hypertension is mental stress.¹¹

There is a considerable risk of dissection or aortic rupture when the ascending aorta dilates as a

result of hypertension. It is responsible for half of all thoracic aneurysms. Based on aetiology and surgical management, it can be divided into two distinct types: 1) the aortic root aneurysm, which signifies the first part, the aortic root, which includes the sinuses of Valsalva, and 2) the supra-ventricular aortic aneurysms, located above the sinuses of Valsalva up to the brachiocephalic trunk. The supra-ventricular aneurysms can be corrected with a supra-coronary tube graft while aortic root aneurysms need replacement of aortic valve. The aorta in humans enlarges with aging too, but the prevalence of aortic root dilatation among individuals with long standing essential hypertension has not been proven in studies performed in general population. This study is targeted at determining the prevalence of aortic root dilatation in patients with chronic essential hypertension.

MATERIAL AND METHODS

This cross-sectional study was carried out in Department of Cardiology, Ayub Teaching Hospital, Abbottabad from 15 Nov 2020 to 15 May 2021. A 95% confidence interval, an 8% margin of error, and an anticipated frequency of 22% aortic root dilatation in individuals with chronic essential hypertension yielded a sample size of 103 using WHO sample size software⁹. Non-probability sequential sampling was done. People of both genders between ages 30–70 years with essential hypertension for more than 5 years were selected for study. Those with non-hypertensive cardiovascular disease, valvulopathy, and diabetes were excluded.

Informed consent was obtained from all patients. Basic demographics were recorded, including age, gender, length of hypertension, and weight. Two-dimensional echocardiography was conducted while the patient was at rest and in the left lateral position. Devereux's method was used to measure left ventricular mass (LVM) which made use of the interventricular septum (IVS), inferolateral wall (ILW) thickness, and end-diastolic left ventricular internal diameter (LVIDd). To compute relative wall thickness (RWT), the formula $(2 \times \text{ILW})/\text{LVIDd}$ was utilized. When a female's left ventricular mass index for height was greater than 46.7 g/m or that of a male was greater than 49.2 g/m, it was considered left ventricular hypertrophy. Aortic root size was calculated using two-dimensional echocardiography by measuring the maximum distance between the anterior and posterior aortic root walls at the end of diastole. From a parasternal long axis perspective, images of the proximal aortic root were recorded.

Aortic root diameter was calculated and documented on specifically created proforma and SPSS-22 was used for data analysis. Qualitative variables, e.g., gender and aortic root dilatation were presented as frequencies and percentages. For quantitative parameters such as age, weight, and duration of hypertension, Mean $\pm$ SD was measured. Aortic root

dilatation was categorized according to weight, age, gender, and duration of hypertension. Chi-square test was used for mean comparison, and a $p \leq 0.05$ was considered as significant.

RESULTS

The mean age of the study population was 51 ± 6.7 years, with a range of 30–70 years. Mean duration of hypertension was 10.7 ± 2.9 years and mean weight was 87.5 ± 6.5 Kg. Male patients were 79.6% and females were 20.4%. Aortic root dilatation was observed in 19.4% patients (Table-1).

Frequency of aortic dilatation was significantly higher in people older than 50 years, or having hypertension for more than 10 years ($p < 0.005$). There were no significant differences between males and females. The frequency of aortic dilatation was more in people weighing > 80 Kg, but the differences were not significant. (Table-2).

Table-1: Frequency of hypertensive patients with aortic root dilatation (n=103)

Aortic Root Dilatation	Frequency	Percentage
Yes	20	19.4
No	83	80.6

Table-2: Aortic root dilatation stratified in different parameters

Variables	Aortic Root Dilation		<i>p</i>
	Yes	No	
Age groups (Years)			
30–50	2 (5.1%)	37 (94.9%)	0.004
51–70	18 (28.1%)	46 (71.9%)	
Gender			
Male	16 (19.5%)	66 (80.5%)	0.962
Female	4 (19%)	17 (81%)	
Duration of hypertension (Years)			
5–10	1 (1.9%)	52 (98.1%)	0.000
>10	19 (38%)	31 (62%)	
Weight (Kg)			
≤80	1 (7.1%)	13 (92.9%)	0.212
>80	19 (21.3%)	70 (78.7%)	

DISCUSSION

Prevalence of ARD in arterial hypertension has been the subject of numerous published investigations. Some studies^{12,13} suggest that prevalence of this syndrome ranges from 4 to 11.8%, although our observations of up to 17% is significantly greater. Many factors including age, gender, weight, and systemic blood pressure affect size of thoracic aorta. In autopsies and imaging investigations, age-related dilatation of the ascending aorta and aortic root has been documented.

IN a study in India¹⁴ the aortic diameter was greater in hypertensives compared to normotensive individuals at annulus and sinotubular junction. Aging has a complex role in the metabolic, structural, and functional changes in major arteries and micro vessels in conjunction with aggravating factors such as heredity, disease, and environmental factors. In our study, age and HTN were

significant parameters affecting the aortic size. On applying multivariate analysis of the dependent variables, weight was most strongly related to the aortic size. Kim *et al*¹⁵ observed that body surface area is inversely related to the aortic size. This is different from Pearce WH *et al*¹⁶, who noted that BSA is a better predictor of size than height or weight. They reported that obese women were more likely to have ARD if they had a high stroke volume index, high diastolic blood pressure, a lower baseline systolic blood pressure, and a high average diastolic blood pressure at follow-up ($p<0.05$). However, normalized ARD was linked to non-obese females with lower baseline systolic blood pressure, average follow-up diastolic blood pressure, reduced stroke volume index, and longer follow-up duration ($p<0.05$).¹⁶ We observed that in both genders, age and aortic root diameters correlated positively at all levels which is in accordance with Akintunde *et al*.¹⁸ Vandroux *et al*¹⁹ reported that age positively correlated with ARD in a large cohort among the normotensive Beninese population. Vríz *et al*²⁰ noted a comparable result in 1,043 healthy adults of age range between 16–92 years. In both these studies, the absolute aortic root diameters of males were greater than those of females. We observed the same pattern in our study.

CONCLUSION

The prevalence of aortic root dilatation in patients with essential hypertension is larger than previously reported, reaching a maximum of 19.4%. Our findings therefore point to the value of selecting the diagnostic criterion correctly which must be used on a single patient in order to define ARD.

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ORIGINAL ARTICLE

NERVE CONDUCTION STUDY PATTERNS IN EARLY
GUILLAIN BARRÉ SYNDROME

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Background: Nerve conduction studies (NCS) in early Guillain-Barré Syndrome (GBS) help differentiate demyelinating from axonal subtypes, though findings may be subtle or normal initially. Objective of this study was to explore the NCS findings in patients presenting with early GBS. **Methods:** This cross-sectional study was conducted at Department of Physiology, Shahid Zulfiqar Ali Bhutto Medical University, and Department of Neurology, Pakistan Institute of Medical Sciences, Islamabad from May 2023 to February 2024. Data was collected from 94 patients after written informed consent and analysed using SPSS-23. Chi-square test was applied taking $p \leq 0.05$ as significant. **Results:** Twenty-one 21 cases had normal Nerve Conduction Study, 73 NCS cases showed abnormal findings out of which 13.7% cases had normal motor conduction velocities and 86.3% had abnormal conduction velocities. Moreover, 20.5% cases had normal while 79.5% cases had abnormal distal motor latencies; 15.1% cases out of the abnormal NCS cases had normal and 84.9% cases had abnormal F-waves. Out of 74 abnormal NCS, 28.8% had normal compound motor action potential and 71.2% had abnormal compound motor action potential ($p=0.0001$). There was significant association ($p \leq 0.05$) between NCS and electrophysiological parameters. **Conclusion:** GBS is common in 13–65 years age group with male predominance. NCS findings were abnormal in 77.7% of cases presenting early. Early electrophysiological parameters in NCS, elucidate their diagnostic significance and offer insight into pathophysiology of nerve disorders.

Keywords: Guillain-Barré syndrome, Nerve conduction study, Electrophysiological studies

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INTRODUCTION

Guillain-Barré Syndrome (GBS) is an uncommon yet severe post-infectious immune-mediated neuropathy characterized by the autoimmune attack on nerves within the peripheral nervous system. This leads to symptoms like numbness, tingling, and weakness, which may escalate to paralysis.¹

Nerve conduction studies (NCS) serve as a critical electrophysiological tool for evaluating the functionality of both cranial and peripheral nervous systems. They play a pivotal role in diagnosing and characterizing disorders affecting nerve roots, peripheral nerves, muscles, and neuromuscular junctions.² NCS enables the assessment of motor, sensory, or mixed nerve functions. These studies involve nerve stimulation with recording electrodes placed over distal muscles, cutaneous sensory nerves, or entire mixed nerves, providing comprehensive diagnostic information.² There are several parameters of NCS including motor conduction velocities (MCV), F-waves, Distal Motor Latency (MDL) and Compound Motor Action potential (CMAP) and Sensory nerve action potential (SNAP).^{3,4}

F-wave studies are commonly employed to assess nerve conduction velocity, especially for detecting conduction issues in proximal nerve regions. The F-wave refers to a compound action potential elicited by supramaximal antidromic stimulation of a motor nerve. The absence or slowing of F-waves can indicate an isolated conduction abnormality, particularly

in the early stages of GBS.⁴ This observation underscores the importance of F-wave studies as a sensitive tool for detecting early nerve conduction abnormalities.^{4,5}

The motor conduction velocity (MCV) test determines the speed of electrical impulses along a nerve, aiding in the detection of nerve damage.⁶ The latency refers to the time delay between the initial stimulation from the first electrode and the detection by the downstream electrode.⁷ A compound muscle action potential (CMAP) is the combined action potential recorded from a muscle during a motor nerve conduction study. It occurs when stimulation at any point along the motor pathway is adequate to activate some or all of the muscle fibers in that muscle. The compound muscle action potential (CMAP) involves stimulating a nerve fibre and observing the corresponding muscle response. In cases of peripheral neuropathies, alterations in the CMAP signal are commonly observed.⁸

Various electrophysiological characteristics exist to categorize different variants of GBS.⁹ Accurate diagnosis of each variety requires electrophysiological testing; however this can only be done if it is performed soon after the onset of symptoms.¹⁰ Cranial nerves involvement is also common in GBS.¹¹ There may be single or multiple cranial nerves involvement and this also indicate severity of disease.¹¹ There are two approved methods to treat GBS, i.e., plasma exchange

(PLEX)/intravenous immunoglobulins (IVIGs).^{12,13} Few data is available on NCS findings in early GBS. Our study aims to focus on changes in electrophysiological studies during early presentation of GBS.

MATERIAL AND METHODS

We conducted this cross sectional study at the Neurology unit of Pakistan Institute of Medical Sciences (PIMS), Islamabad from May 2023 to February 2024 after obtaining approval from the ethical review board of Shaheed Zulfiqar Ali Bhutto Medical University (SZABMU). Study was approved by AS&RB of SZABMU. Sample size of 94 was calculated using WHO sample size calculator. Both male and female patients provisionally diagnosed with GBS with the age range of 13 to 65 years were included for this study by using non-probability consecutive sampling technique after taking written consent. The patients with diabetes mellitus, renal impairment, peripheral nerve disease, and myopathy were excluded from the study.

After recording patients' demographic details and physical examination suspected GBS cases NCS evaluation was done by Neuro-physician by using Nicolet Natus EDX 1000 NCS/EMG/EP Machine. Following parameters were measured, i.e., Motor conduction velocities (MCV), MDL, F-wave, Compound Motor Action potential (CMAP) of peripheral nerves including median, ulnar and radial nerves in upper limbs whereas tibial, peroneal and sural nerves of lower limbs. Other parameters including types of GBS, Ventilator support, cranial nerves involved and treatment options [plasma exchange (PLEX)/intravenous immunoglobulins (IVIGs)] also studied.

The data was analysed in SPSS-23. Mean±SD were determined for age of the patients. Frequency and percentages were determined as categorical data such as gender, NCS, i.e., motor conduction velocities (MCV), distal motor latency (MDL), F-wave, compound motor action potential (CMAP), types of GBS, Ventilator support, cranial nerves involved and treatment options. Patients were broadly divided into two groups, i.e., Group A comprised of patients with normal NCS and Group B of patients with abnormal NCS. Chi square test of association was applied taking $p \leq 0.05$ as significant and with a confidence interval of 95%.

RESULTS

There were 50 males and 44 females with mean age of 39.77 ± 16.43 . Table-1 is showing GBS types according to demyelination or axonal pattern on NCS. In our study there 30 cases (32.0%) of Acute inflammatory demyelinating polyneuropathy (AIDP), 25 cases (26.6%) of Acute motor axonal neuropathy (AMAN), 18 cases (19.1%) of acute motor sensory axonal neuropathy (AMSAN) variant and there were 21 cases (22.3%) having normal NCS.

We also did association of NCS with electrophysiological findings showing that 21 (100.0%) cases were having normal motor conduction velocities, distal motor latencies, F-waves and compound motor action potential with respect to normal NCS in all these 21 cases. Whereas with respect to abnormal NCS 73 cases, 10 (13.7%) cases were having normal motor conduction velocities and 63 (86.3%) had abnormal conduction velocities. Out of abnormal 73 NCS cases 15 (20.5%) cases had normal and 58 (79.5%) cases had abnormal distal motor latencies respectively. Out of abnormal 73 NCS cases 11 (15.1%) cases had normal and 62 (84.9%) cases had abnormal F-waves respectively. We found that out of 74 abnormal NCS, 21 (28.8%) had normal compound motor action potential and 52 (71.2%) had abnormal compound motor action potential. ($p=0.0001$). (Table-2).

Table-3 shows Association of NCS with respect to ventilator support showed that out of 94 cases 21 patient which were having normal NCS showed 4 (19.1%) required ventilator and 17 (80.9%) had not required ventilator. Whereas 73 patients which were having abnormal NCS showed 17 (23.3%) required ventilator and 56 (76.7%) had not required ventilator. Association of NCS with cranial nerves involvement showed that out of 94 cases 21 patients which were having normal NCS showed 8 (38.1%) with single cranial nerve involvement, 2 (9.5%) cases with multiple and 11 (52.4%) without cranial nerve involvement. Whereas 73 patients which were having abnormal NCS showed 23 (31.5%) with single cranial nerve involvement, 10 (13.7%) cases with multiple and 40 (54.8%) without cranial nerve involvement. Out of 94 cases 88 (93.6%) opted for plasmapheresis, i.e., PLEX whereas only 6 (6.4%) opted IVIGs as treatment option.

Table-1: Types of GBS

GBS Type	Frequency	Percentage
AIDP	30	32.0
AMAN	25	26.6
AMSAN	18	19.1
None	21	22.3

Table-2: Association of NCS with electrophysiological parameters

Electrophysiological findings	Normal		Abnormal		p
	n	%	n	%	
Motor Conduction Velocities (MCV)					
Normal	21	100.0	10	13.7	0.0001
Abnormal	0	0.0	63	86.3	
Distal Motor Latency (MDL)					
Normal	21	100.0	15	20.5	0.0001
Abnormal	0	0.0	58	79.5	
F-wave					
Normal	21	100.0	11	15.1	0.0001
Abnormal	0	0.0	62	84.9	
Compound Motor Action potential (CMAP)					
Normal	21	100.0	21	28.8	0.0001
Abnormal	0	0.0%	52	71.2	

Table-3: Association of NCS with ventilator support and cranial nerves involved

Characteristics	Normal (n=21)	Abnormal (n=73)	<i>p</i>
Ventilator support			
Required	4 (19.0%)	17 (23.3%)	0.17
Not required	17 (81.0%)	56 (76.7%)	
Cranial Nerves Involved			
Single	8 (38.1%)	23 (31.5%)	0.46
Multiple	2 (9.5%)	10 (13.7%)	
None	11 (52.4%)	40 (54.8%)	

DISCUSSION

Average age of patients diagnosed with Guillain-Barré Syndrome (GBS) in our study was found to be 39.77±16.42 years which was closely near to a study conducted in Pakistan showing mean age of 41.27 years.¹⁴ In our study it was observed that 50 individuals (53.2%) were males, while the remaining 44 (46.8%) were females. This was comparable to other studies.^{14,15} We found that NCS test was abnormal in 73 (77%) patients, which is in agreement to a study which reported abnormal NCS in 80.64% patients.¹⁶

We observed that MCV was abnormal in 86.3% patients, distal motor latency (MDL) was abnormal in 79.5% patients, about 84.9% patients showed abnormal F-wave, and 71.2% patients showed abnormal Compound Motor Action Potential (CMAP). Our findings are almost similar to Brohi H *et al*¹⁶. Another study¹⁷ reported that CMAP was abnormal in 41.75% of their subjects which is lower than our findings. Most prevalent variant in our study was AIDP which is in contrast to a study conducted in Pakistan¹⁵ in which most common was AMAN variant.

Ventilator support in our patients was needed by 22.3% patients. These values were comparable to a study conducted in Pakistan¹⁸ in which 13% cases required ventilator support which is lower than our study. Shang P *et al*¹⁹ showed that 30% patients needed mechanical ventilation in their study which is quite high as compared to our study. Another study²⁰ showed that 22% patients required mechanical ventilation which is in agreement to our study.

Out of 94 cases 54.2% showed no cranial nerve involvement, 33.0% showed single cranial nerve involvement, and 12.8% showed multiple affected cranial nerves. A study²¹ showed that 34% patients showed cranial nerves involvement which is lower as compared to our study. Another study¹¹ showed 62.3% showing cranial nerves involvement (21% with single whereas 41% showed multiple cranial nerve involvement) whereas 38% with no cranial nerve involvement which is lower than our study.

There are 2 approved treatment options available for treatment of GBS, i.e., IVIGs and plasmapheresis. In our study, 93.6% cases opted for plasmapheresis, i.e., PLEX, whereas only 6.4% opted

IVIGs as treatment option. Our results were in contrast to studies conducted in India where 50% cases opted for PLEX.^{21,22} We found significant association of NCS with MCV, Distal Motor Latency (MDL), F-wave, Compound Motor Action Potential (CMAP), and NCS with GBS Type. However we could not find any significant association of ventilator support and cranial nerves involvement. These results are different from another study conducted in Pakistan¹⁶ in which there was no significant association of NCS with electrophysiological parameters.

CONCLUSION

GBS is common in 13–65 years age group with male predominance. NCS findings were abnormal in 77.7% of cases presenting early. Early electrophysiological parameters in NCS, elucidate their diagnostic significance and offer insight into pathophysiology of nerve disorders.

LIMITATIONS & RECOMMENDATIONS

Since we conducted this study in a single centre, it is recommended that such studies must be conducted at multiple centres with Neurology Departments to further explore the diagnostic options along with electrophysiological parameters for diagnosis of GBS.

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ORIGINAL ARTICLE

A HETEROZYGOUS *BCL11B* GENE VARIANT INDUCES SCID AND HYPO-IMMUNOGLOBULINEMIA IN A PAKISTANI FAMILYMaryam Shafaq, Muhammad Imran, Rahma Zahid Butt*, Hira Pervez Kiyani**, Sidra Ashfaq***, Syed Irfan Raza[†]

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Background: The gene B-cell lymphoma/leukaemia 11B (*BCL11B*) which encodes the zinc finger transcription factor plays a crucial role in the development of the central nervous system and T cell differentiation by regulating the expression of numerous genes in early development. *BCL11B* gene defects lead to neurological and immunologic disease manifestations. **Methods:** In this study, we enrolled a 2-month-old male patient from a highly consanguine Pakistani family segregating autosomal dominant type of Primary Atopic Disorders (PADs) along with low immunoglobulin levels. Detailed clinical evaluations were performed by expert paediatrician. Whole blood samples were collected in EDTA tubes for genetic analysis. Detailed laboratory tests including complete blood count, and serum immunoglobulin levels, were performed. **Results:** Genetic analysis revealed four mutations in different genes. A heterozygous missense mutation [(c.2035C>T; p.Pro679Ser), ENST00000357195.8] in the gene *BCL11B* gene was found segregating (parents and healthy siblings were homozygous normal while patient was heterozygous) in the family. Flow cytometry and serum immunoglobulin estimation revealed Elevated Total Leukocyte Counts (TLC) and low serum immunoglobulins. **Conclusion:** The current study describes a novel heterozygous missense mutation in gene *BCL11B*. Clinical investigations revealed novel findings with novel clinical manifestations including short-term neurological seizures and recurrent pneumonia.

Keywords: DNA Sanger sequencing, *BCL11B* gene, Hypo-immunoglobulinemia, Complete Blood Count, CBC

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INTRODUCTION

Severe Combined Immune Deficiency (SCID) and Neuro-Developmental Disorders (NDD) are a group of monogenic disorders characterized by dysregulated allergic effector responses, leading to heightened susceptibility to atopic diseases such as asthma, eczema, food allergies, allergic rhinitis, intellectual disability and language disorders.¹ Major clinical features include severe atopic dermatitis, food allergies, allergic asthma, urticaria, eosinophilia, and elevated IgE. PAD manifestations include comorbid immunodeficiency and immune dysregulation such as systemic lupus erythematosus (SLE) and autoimmune vasculitis as is the case in some patients with dominant-negative STAT3 variants.¹ Due to the heterogeneous presentation of PADs, it can often be difficult to differentiate monogenic from polygenic aetiologies. In recent years, the rapid development of genome sequencing technology has provided an effective solution in the diagnosis of such heterogeneous disorders with varied clinical presentations.

The gene B-cell lymphoma/leukaemia 11B (*BCL11B*, OMIM 617237) is localized on chromosome 14q32 which encodes a zinc finger

transcription factor protein essential for development of T-cell, skin, and teeth. Alternate *BCL11B* splicing yields four isoforms with variable length of protein. The isoform 1 (NM_138576.4; NP_612808.1), consist of four exons and encodes 894 six zinc finger C2H2-type domains.^{2,3}

The protein is involved in proliferation, apoptosis, and T-cell receptor modulation by controlling DNA-binding transcription and directing haematopoietic progenitor migration.⁴ Heterozygous mutations of *BCL11B* have been identified in some patients with developmental disorders and severe immunodeficiency (SCID, IMD49) with dysmorphic features, skin abnormalities, and global developmental delay.^{5,6} *BCL11B* knockout mice (without functional *BCL11B*) showed lacking the functional T-cell lineage by affecting the differentiation and function of thymic lymphocytes.⁷

In the current study, we report a 2-months-old male patient belonging to a family of Pakistani origin with severe combined immunodeficiency and mild neurological seizures. Genetic analysis of the patient revealed a missense mutation in the gene *BCL11B*.

METHODOLOGY

To conduct this research study ethical approval was obtained from the Institutional Review Board (IRB) of HBS Medical College (EC20/4), Islamabad, Pakistan. Informed written consent was obtained from the parents and healthy siblings.

The current research study was a ‘family-based genetic association study’. To find the inheritance pattern of the disease considering the shared genetic background among family members, the DNA sequencing data was analysed using BioEdit—a biological sequence alignment editor.

In the current genetic study, a three-generation highly consanguineous Pakistani family was selected with a positive family history of SCID. A 2-year-old male child was first hospitalized at age 2 months with birth weight 5.1 Kg with symptoms of cough, chest congestion, and recurrent pneumonia along with his healthy parents and siblings were enrolled in this study (Figure-1A). The patient was admitted to Department of Paediatrics, Combined Military Hospital, Rawalpindi. An expert team of paediatricians performed detailed clinical investigations. Laboratory tests were ordered including blood complete picture (CP), serum immunoglobulin levels, and flow cytometry. While taking family history mother of the patient disclosed that a male child with similar disease symptoms died at the age of two months. Based on this crucial information physician suggested a positive family history and there might be involvement of genetics. To rule out immune deficiency or related genetic anomalies, genetic testing was ordered.

Patient’s peripheral blood sample (5 mL) was acquired in EDTA tubes (BD Vacutainer® EDTA Tubes, Becton Dickinson, UK). Lymphocyte subset analysis was performed in the Department of Immunology, Armed Forces Institute of Pathology, using flow cytometry with a standard protocol BD FACSCanto™ II Clinical Flow Cytometry System (Becton Dickinson, San Jose, USA). To estimate serum immunoglobulin levels 2 mL blood samples were acquired in a serum separator tube (SST) BD FACSCanto™ II Clinical Flow Cytometry System (Becton Dickinson, San Jose, USA).

To perform genetic analysis 3 mL blood samples were obtained from patients in EDTA tubes. DNA extraction and genetic analysis was performed at Alpha Genomic Islamabad. The identified pathogenic variant was tested in the healthy family members using exon-specific primers DNA Sanger sequencing.

RESULTS

We enrolled one 2-month-old male patient. The family history was positive with one patient who died at two

months of age with similar disease manifestations. According to pedigree, the patient belonged to a four-generation family where the parents are first cousins with a recessive mode of inheritance of disease (Figure-1A). A patient with a severe productive cough at the age of two months and 10 days was admitted to CMH Rawalpindi, Pakistan. He was suffering from chest congestion for 14 days and fever (101.5 °F). For a few minutes, he suffered focal seizures.

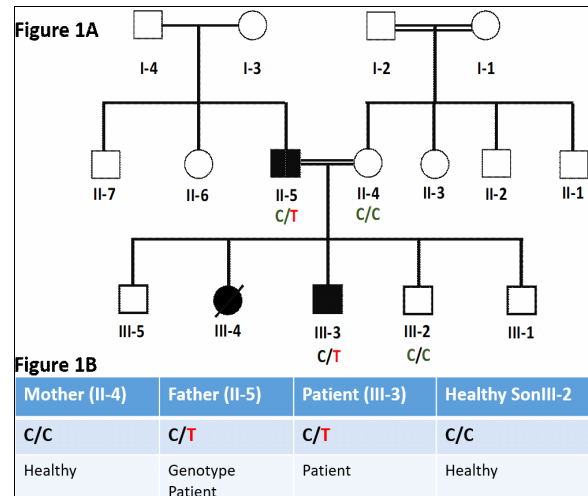


Figure-1: A=Family Pedigree and B=Genotype

Figure-1A: Family Pedigree. Circles represent females while squares represent males. Filled squares and circles characterize patients. Parents with double lines display cousin marriages. Figure-1B presents *BCL11B* SNP analysis. C/C genotypes are homozygous and denote healthy while the C/T genotype is heterozygous and represents patient (III-3). Mother II-4 is homozygous healthy with (C/C) genotype while Father (II-5) is genotypically heterozygous (C/T) which is a disease genotype with low disease manifestations due to low penetrance. III-3 is a heterozygous (C/T) patient. Male sibling III-2 with genotype C/C is healthy.

Blood complete picture test revealed persistent raised total leukocyte count (TLC, 88.7 counts/ μ L). Platelet counts were also elevated (1,099 cells/ μ L). Monocytes (4.9 cells/ μ L), Basophils (0.2 cells/ μ L), and Haemoglobin levels (11.8 g/dL) were within the reference range. (Table-1). Serum Immunoglobulin levels were very low (Hypogammaglobulinemia) including (IgA<0.15; IgM 0.3, IgG 2.26, and IgE 4.0). (Table-2). Due to persistent high levels of TLC lymphocyte subset analysis could not be performed.

Family history, disease symptoms, and low serum immunoglobulin levels showed possible involvement of genetics in this family. Next Generation Sequencing (NGS) revealed multiple mutations as shown in Table 3. Genetic analysis showed interesting variants in multiple genes. A missense variant (c.593C>T; p.Thr198Met) in the gene *C2orf81* on chromosome 2p23.2 was identified which

was initially considered a pathogenic. A second missense variant (c.593C>T, p.Thr198Met) is identified in the gene *V/SIR* located on chromosome 10q22.1. Both of these variants were non segregating with disease manifestations within the family. Another interesting heterozygous missense variant (c.2035C>T; p.Pro679Ser) is observed in gene *BCL11B* located on chromosome 14q32.2. SNP-based segregation studies confirmed the involvement of the *BCL11B* variant (c.2035C>T; p.Pro679Ser) in this family. Father was with the patient's heterozygous (C/T) genotype while the mother and one of the siblings were homozygous (T/T). Due to low disease penetrance father showed only a few disease symptoms while the patient (III-3) showed full disease symptoms. The healthy sibling had homozygous health (C/C) (Figure-1B).

Table-1: Blood count of the patients

Test	Patient	Reference Range
Total Leukocyte Count (TLC)	88.7	5.0–19.0 Cells/ μ L
Absolute Neutrophil Count (ANC)		
Monocyte	4.9	5–9 Cells/ μ L
Basophil	0.2	0–1 Cells/ μ L
Eosinophils	0.5	1–6 Cells/ μ L
Absolute Lymphocyte Count (ALC)		
Haemoglobin	11.8	11.5–16.5 g/dL
Platelets	1,099	210–500 cells/ μ L

Table-2: Serum immunoglobulin levels

Test	Patient	Reference Range
Serum IgM	0.3	0.5–3.0 mg/dL
Serum IgG	2.26	6.5–16 mg/dL
Serum IgA	<0.15	0.4–3.5 mg/dL
Serum IgE	4.0	$\leq$ 13 IU/mL

Table-2: Patient gene variants

Gene Name	Chromosome	c.DNA Change	Amino Acid Change	READS	CAAD
<i>C2orf81</i>	2p23.2.	c.451G>T	p.Glu151Ter	242	33
<i>V/SIR</i>	10q22.1	c.593C>T	p.Thr198Met	169	26.2
<i>IFIH1</i>	2q24	c.1943del	p.Gly648ValfsTer16	111 22	111 22
<i>BCL11B</i>	14q32.2	c.2035C>T	p.Pro679Ser	170	18.75

c.DNA=Complementary DNA, CAAD=Combined Annotation Dependent Depletion

DISCUSSION

In the current research study, we enrolled a highly consanguineous Pakistani family with low immunoglobulin levels and probably low levels of circulatory T-cells. The gene *BCL11B* encodes a transcription factor called C2H2-type zinc finger protein, which plays an important role in T-cell development, especially during the early stages and development of the central nervous system. The protein functions both as a gene activator and repressor which affects cell differentiation and apoptosis.⁸

The B-cell leukaemia/lymphoma 11B (*BCL11B*) is located on chromosome 14q32.2. The gene *BCL11B* (isoform-1) encodes 894 amino acids through four functional exons which compose six Zinc finger C2H2-type domains (OMIM, 617237).² To date, there are 17 variants of the *BCL11B* gene identified in 19 patients through different studies. The major types of *BCL11B* genetic variation include frameshift, non-sense, missense, and complex chromosome rearrangement. However, there is still no report on splicing variation.^{8,9} Mutations in *BCL11B* are associated with (T^h1⁺NK⁺) type of Severe Combined Immune Deficiency and neurological seizures regulating the expression of numerous genes.^{8,10}

In the current study, we identified a novel mutation in the gene *BCL11B* (c.2035C>T; p.Pro679Ser). This mutation lies in exon four of the gene which encodes all six zinc finger domains, which are crucial for the protein's DNA binding function. The missense mutation possibly affects the zinc finger domains which lead to affect the protein function.

The current study revealed raised TLC levels

along with low levels of immunoglobulin levels. These findings are in contrast to those already reported in the previous reports where immunoglobulin levels and TLC levels were within the reference range.^{8,10,11} Due to elevated total leukocyte count, Flow-cytometry was not performed. Based on previously published findings we can predict the possible T-cell deficiency. As for the disease symptoms are a severe cough and recurrent episodes of pneumonia and seizure attacks match with the findings reported earlier.^{8,10–12}

CONCLUSION

SCID, with or without low immunoglobulin levels, is a rare genetic disorder characterized by a severe deficiency of both B cells and T lymphocytes, while natural killer (NK) cells may remain present, and immunoglobulin levels can vary. Genetic analysis in patients exhibiting T-cell or B-cell deficiency in association with or without low immunoglobulin levels can confirm the disease diagnosis.

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ORIGINAL ARTICLE

EVALUATION OF BONE MARROW ASPIRATE, TREPHINE BIOPSY AND IMMUNOHISTOCHEMISTRY IN NON- HAEMOPOIETIC MALIGNANCIES

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Background: Bone marrow involvement in non-haematopoietic malignancies is rare but clinically significant. Accurate diagnosis is crucial for assessing disease progression and prognosis. This study evaluates the diagnostic effectiveness of bone marrow aspiration and trephine biopsy in detecting non-haematopoietic malignancies. Objective of this study was to assess bone marrow aspiration and trephine biopsy findings in metastatic solid tumours and confirm diagnoses using immunohistochemistry. **Methods:** This cross-sectional study was conducted at Pathology (Haematology) Department, Pak Emirates Military Hospital, Rawalpindi, from Apr 2021 to Jun 2023. Data were collected from patients undergoing bone marrow biopsy who were provisionally diagnosed with metastatic solid tumours. Statistical analysis was performed using SPSS-22, with quantitative data expressed as Mean $\pm$ SD and qualitative data as frequencies and percentages. Chi-square test was applied and $p \leq 0.05$ was considered statistically significant. **Results:** There were 21 patients in the study, 18 (85.7%) male and 3 (14.3%) female. The mean age was 63.81 $\pm$ 11.82 years (range: 40–86). Common symptoms included pallor (52.4%), weight loss (28.6%), and fever (19.0%). Bone marrow trephine biopsy revealed metastatic tumours in 8 patients (38.1%), anaemia of chronic disease in 3 (14.3%), peripheral platelet destruction in 1 (4.8%), and reactive changes in 6 (28.26%). **Conclusion:** Metastatic solid tumours were found in 38.1% of cases, highlighting the importance of bone marrow evaluation in disease monitoring and prognosis. Understanding marrow metastasis patterns supports research and targeted therapies.

Keywords: Bone Marrow, Immunohistochemistry, Trephine Biopsy

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INTRODUCTION

Bone marrow examination is a convenient and beneficial analytical technique in Pathology practice for the diagnosis of both haematological disorders as well as non-haematological solid malignancies. The bone marrow examination helps in either confirmation of the already formed diagnosis or may provide the earlier unanticipated diagnosis.¹

Bone marrow metastatic malignancies are usually all non-haematological solid tumours that originate in other parts of the body and metastasize to the bone marrow through blood or lymphatic circulation.²

Many frequently arising solid tumour reveals great incidence of bone marrow involvement in their later stages.³ In adults, the most common metastatic solid tumours seen in bone marrow are from breast, prostate, lung and gastrointestinal tract and in children from neuroblastoma. Diagnosis of metastatic solid tumours in bone marrow can be challenging, as in certain cases they may not always produce visible symptoms and may not show up on imaging studies.⁴ In some cases of advanced disease, bone marrow metastasis is diagnosed before the primary tumour site due to cytopenias.⁵

Bone marrow aspiration is adequate for diagnosing megaloblastic anaemia and the majority of haematological malignancies. On the other hand, bone marrow trephine biopsy is better suited for identifying conditions involving focal marrow abnormalities, such as lymphoproliferative disorders, lymphoma staging, metastatic tumours, granulomatous diseases, and hypoplastic marrow. To ensure the highest level of diagnostic precision, performing both procedures together is strongly advised.⁶ Bone marrow trephine biopsy provides a way to directly visualize and analyse the bone marrow tissue for the presence of cancer cells. The biopsy allows for the discovery of small deposits of tumour cells that may not be detectable by other diagnostic methods. The biopsy can help determine the extent of tumour involvement in the bone marrow, which is important for determining the stage of the cancer and planning appropriate treatment. Bone marrow involvement in solid tumours is a poor prognostic factor and can influence treatment decisions. Majority of cancer deaths occur as a result of expansion of irredeemable metastasis.⁷

In this article, we will discuss role of bone marrow trephine biopsy in diagnosing metastatic solid tumours in bone marrow and reliability of bone marrow trephine biopsy in diagnosis of metastatic solid tumours.

METHODOLOGY

It was a cross-sectional study, carried out at the Department of Pathology (Haematology), Pak Emirates Military Hospital, Rawalpindi for a duration of 2 years, from Apr 2021 to Jun 2023, after taking approval from the Ethical Review Committee (ERC/ID/265). A total of 745 bone marrow biopsies performed during the study period were analysed. A total of 21 cases of suspected metastatic bone marrow infiltration were retrieved. Non probability consecutive sampling technique was used.

Inclusion criteria was patients of age 45 to 75 years, of both genders, with suspicion of metastatic solid tumour in bone marrow trephine which was later confirmed by immunohistochemistry. Exclusion criteria was no confirmed evidence of solid metastatic bone marrow tumour on aspiration.

The patients' history, physical findings, haematological and bone marrow trephine findings were reviewed retrospectively and were noted down on proforma. Clinical profile included information about patients' age, gender, physical findings and provisional diagnosis on aspiration. Peripheral blood counts and blood films were reviewed. Bone marrow aspirations and subsequent trephine biopsies were performed in all the patients. The bone marrow aspiration findings were correlated with trephine biopsy results. Results were later confirmed by applying relevant immunohisto-chemistry markers.

Data was analysed on SPSS-22. Quantitative data was represented using Mean $\pm$ SD and qualitative data was represented by using percentage and frequency. Chi-square test (for comparison of qualitative variables) was applied and $p \leq 0.05$ was taken as statistically significant.

RESULTS

A total of 21 patients were included in the study, 18 (85.7%) patients were male and 3 (14.3%) were female. The mean age of the patients was 63.81 $\pm$ 11.82 years ranging from 40 to 86 years. Majority of the patients had pallor history 11 (52.4%) followed by weight loss 6 (28.6%) and fever 4 (19.0%). Of the total lymph nodes biopsy was done in 8 (38.1%) patients and not done in 13 (61.9%) patients. The detail of patients' history and lymph nodes biopsy shown in Table-1. However, out of 21 patients, CRP raised in 11 patients (52.4%), Serum Alk Phosphatase in 7 patients (33.3%), and LHD raised in 13 patients (61.9%).

The CK7 marker was positive in 10 patients (47.6%) followed by Pan Ck (n=9; 42.9%), PAS (n=8; 38.1%), CK20 (n=8; 38.1%) and can 5.2 (n=3; 14.3%). The following table shows Immunohistochemistry markers applied (Table-2).

Eight (38.1%) patients had metastatic tumour, 3 (14.3%) had anaemia of chronic disorder, 1 (4.8%) had peripheral destruction of platelets, 1 (4.8%) had iron deficiency anaemia, 1 (4.8%) had mixed deficiency anaemia, and 1 patient (4.8%) had few clumps of atypical non-haemopoetic cells seen. The details of aspiration results are shown in Table-3.

Out of 21 Patients, 8 (38.09%) patients had metastatic tumour/metastatic carcinoma, 3 (14.3%) patients had adenocarcinoma prostate infiltrating bone marrow, 1 (4.8%) patient had peripheral destruction of platelets, and 3 (14.3%) patients had anaemia of chronic disorder. The details of trephine reports/results are shown in Table-4.

Out of the total, 6 (28.57%) patients showed metastatic tumour/metastatic carcinoma in both aspirate and trephine, followed by anaemia of chronic disorder (2, 9.6%), and reactive change (4, 19.0%). There were statistical significant differences between aspirate and trephine reports of the patients ($p=0.003$) as shown in Table-5.

Table-1: The detail of patient's history and lymph nodes biopsy (n=21)

Variables	Frequency	Percentage
Patient's history		
Pallor	11	52.4
Weight loss	6	28.6
Fever	4	19.0
Lymph nodes biopsy		
Yes	8	38.1
No	13	61.9

Table-2: Immunohistochemistry markers in patients (n=21) [n (%)]

Markers	Positive	Negative	Inconclusive
Pan Ck	9 (42.9)	7 (33.3)	5 (23.8)
PAS	8 (38.1)	4 (19.0)	9 (42.9)
Can 5.2	3 (14.3)	9 (42.9)	9 (42.9)
CK 7	10 (47.6)	3 (14.3)	8 (38.1)
CK 20	8 (38.1)	4 (19.0)	9 (42.9)

Table-3: Diagnosis on bone marrow aspiration (n=21)

Entities	Frequency	Percentage
Diluted Marrow	2	9.5
Metastatic tumour	8	38.1
Anaemia of chronic disorder	3	14.3
Peripheral destruction of platelets	1	4.8
Reactive changes	4	19.0
Iron deficiency anaemia	1	4.8
Mixed deficiency anaemia	1	4.8
Few clumps of atypical non-hemopoetic cells seen	1	4.8

Table-4: Diagnosis on bone marrow trephine biopsy (n=21)

Entities	Frequency (%)
Adenocarcinoma prostate infiltrating marrow	3 (14.3)
Metastatic tumour/metastatic carcinoma	8 (38.09)
Reactive change	6 (28.6)
Peripheral destruction of platelets	1 (4.8)
Anaemia of chronic disorder	3 (14.3)

Table-5: Comparison of diagnosis on bone marrow aspiration and trephine (n=21) [n (%)]

Aspiration	Trephine						p
	Adenocarcinoma prostate infiltrating marrow	Metastatic tumour/metastatic carcinoma	Reactive change	Peripheral destruction of platelets	Anaemia of chronic disorder	Total	
Diluted Marrow	1 (4.8)	1 (4.8)	0 (0)	0 (0)	0 (0)	2 (9.6)	0.003
Metastatic Tumour	2 (9.5)	6 (28.57)	0 (0)	0 (0)	0 (0)	8 (38.2)	
Anaemia of chronic disorder	0 (0)	0 (0)	1 (4.8)	0 (0)	2 (9.6)	3 (14.3)	
Peripheral destruction of platelets	0 (0)	0 (0)	0 (0)	1 (4.8)	0 (0)	1 (4.8)	
Reactive changes	0 (0)	0 (0)	4 (19.0)	0 (0)	0 (0)	4 (19.0)	
Iron deficiency anaemia	0 (0)	0 (0)	0 (0)	0 (0)	1 (4.8)	1 (4.8)	
Mixed deficiency anaemia	0 (0)	0 (0)	1 (4.8)	0 (0)	0 (0)	1 (4.8)	
Few clumps of Atypical non-hemopoetic cells seen	0 (0)	1 (4.8)	0 (0)	0 (0)	0 (0)	1 (4.8)	
Total	3 (14.3)	8 (38.09)	6 (28.6)	1 (4.8)	3 (4.3)	21 (100)	

DISCUSSION

Bone marrow is a rare yet important site for metastasis of non-haematopoietic solid tumours, affecting less than 10% of patients with metastatic disease and being associated with a poor prognosis.⁸ Bone marrow assessment for metastasis detection is conducted in two scenarios: as part of the staging process in patients identified with non-haematological malignancies or to endorse metastases in suspected cases, indicated by abnormal peripheral blood results or radiologically suspected cases of bone marrow involvement.¹ Most common reported non-haematological malignancies which results in bone marrow metastasis include those arising in the prostate, breast, lung and neuroblastoma.⁹

Present study focused on the clinical features of the patients with solid cancers and bone marrow metastasis detected on bone marrow trephine biopsy. The mean age of the patients was 63.81±11.82 years and ranged from 40 to 86 years. Khan *et al*¹⁰ showed mean age of their subjects as 40±11.5 years. Patients in our study were of the same median age as was detected in a similar study conducted in Taiwan¹¹.

In our study, prostatic adenocarcinoma (14.3%) was the most common solid cancer with bone marrow metastasis, whereas in a similar study by Hung YS *et al*¹¹ the most common primary tumour location was found to be stomach (39%) followed by prostate (19%) and lung (15%). In Kucukzeybek BB *et al*¹² study most cases were from primary breast carcinoma. The smaller percentage of cases of primary tumour in our study is related to the invasive nature of the procedure due to which mostly terminally ill patients and their physicians find this procedure of little to no help in treatment of the patient.

Clinically, in present study the most common symptoms experienced by the patient was body aches and bone pain (42%), followed by fever (28%) and weight loss (23%). The results in this study were in coherence with the results of parallel studies conducted by Yu-Shin Hung *et al*¹¹ and Chauhan K *et al*.¹³

The single biochemical marker raised in majority of the cases in present study was LDH whereas

in a study conducted by Arya L *et al*¹⁴ ALP was found to be elevated in almost all cases of patients of bone marrow metastasis of solid tumours. In contrast, serum LDH was raised as established by few case reports and case series in the background of BM involvement by a solid organ cancer.¹⁵⁻¹⁷

It is always endorsed to execute both aspiration and biopsy and these are considered harmonizing to each other.¹⁸ In the present study, out of total, 6 (28.6%) patients showed metastatic tumour both in aspirate and trephine biopsy. In the study by Rani HS *et al*¹ one case of metastatic malignancy was missed on trephine biopsy but detected on bone marrow aspiration owing to sparse spreading of the tumour. In the present study, primary origin of all diagnosed metastatic tumours was confirmed by immunohistochemistry.

CONCLUSION

Trephine biopsy has higher sensitivity than bone marrow aspirates in diagnosis of marrow metastasis and should be performed in all cases for detection of bone marrow metastasis along with augmentation by relevant immunohistochemistry markers.

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ORIGINAL ARTICLE

SHIELDING EFFECTS OF CORN SILK EXTRACT AGAINST
DOXORUBICIN INDUCED HEPATOTOXICITY
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Background: Doxorubicin is a known anticancer drug. This study evaluated the protective effects of corn silk extract on hepatotoxicity induced by Doxorubicin in albino rats. **Methods:** This randomized control trial was conducted in animal house of Anatomy Department, PGMI, Lahore, for one year in 2022. Thirty healthy adult albino rats of either sex, aging 6–8 weeks with 180–220 g of weight were randomly allotted to three groups 1 (control), 2 (experimental group-1), and 3 (experimental group-2) (n=10) that were given standard rat feed and distilled water *ad libitum*. Intraperitoneally 1.2 mg/Kg body weight distilled water and doxorubicin were given to group 1 and group 2+3 for 21 days respectively. Group-3 was also given corn silk extract 400 mg/Kg/body weight by oral gavage for 21 days. On 22nd day, blood was drawn for biochemical analysis of alkaline phosphatase and gamma glutamyl transpeptidase, and liver were preserved for tissue processing and histological analysis. **Results:** Serum alkaline phosphatase of group 1 was 134.70±15.18, group 2 was 328.30±18.05, and group 3 was 229.9±17.9. The serum gamma glutamyl transpeptidase of group 1 was 2.6±1.4, group 2 was 17.63±1.4, and group 3 was 9.6±1.2 ($p<0.01$). Histological analysis showed that group 1 rats had normal liver histology. Sinusoidal congestion and granuloma formation was observed in all rats of group 2. While in group 3, minimal sinusoidal congestion and granuloma formation was seen. **Conclusion:** Corn silk extract has protective effects against doxorubicin induced liver damage.

Keywords: Congestion, Corn silk extract, Doxorubicin, Hepatotoxicity, Sinusoid

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INTRODUCTION

Global burden of cancer is increasing day by day at an alarming rate. According to the report of International Agency for Research on Cancer, more than 19 million new cancers have been reported in 2020.¹ Traditional treatment strategies for cancer include surgical resection of the mass, radiotherapy and chemotherapy. There are 100 different types of chemotherapeutic drugs that kill the cancer cells.² One of the most commonly used drug is Doxorubicin (Dox) an anthracycline based antibiotic with anticancer properties. It is extracted from the bacterium *Streptomyces peucetius*.³ It is available in Pakistan in the form of injectables and in the dose of 10 mg, 20 mg and 50 mg. It is used in treatment of various types of cancers, e.g., breast, urinary bladder, lungs, thyroid gland, ovaries, stomach, soft tissue, bone sarcomas, neuroblastoma, Wilm's tumour, Hodgkin's and Non-Hodgkin's lymphoma, acute lymphoblastic leukaemia and acute myeloid leukaemia.^{4,5} DOX is also known as the red devil, based on its bright red colour with characteristics and side-effects, including alopecia, myelosuppression, vomiting, soreness of mouth, hepatotoxicity, nephrotoxicity and cardiotoxicity.^{6,7} The biochemical mechanism of action of Doxorubicin lies in its ability to intercalate into the DNA leading to inhibition of topoisomerase-II-mediated DNA replication, transcription and repair, release of free

radicals and production of lipid peroxidation which damages cellular membrane and oxidative trauma which initiates the pathway of cell death or apoptosis.^{3,8,9} It also induces genetic mutations which produces histological as well as biochemical changes in various organs like liver, pancreas, kidneys, heart, testes and stomach.^{10,11}

Liver is the second largest and most significant organ of the body, which performs vital role in protein synthesis, storage, and metabolism of fats, carbohydrates and hormones, detoxification of drugs and toxins, excretion and metabolism of bilirubin.¹²

The administration of Dox has severe hepatotoxic effects. According to the literature the histological and biochemical changes including the presence of focal granulomatous lesion, cellular infiltrates, perivenular fibrosis and fat accumulation in liver cells and abnormal levels of liver enzymes including alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP) and gamma glutamyl transpeptidase (GGT).^{13–15}

Corn silk or maize silk is a yellow strand like structure that arises from the stigma of the female maize flower. It is silky soft thread, about 10 to 20 Cm in length and sweet in taste. It contains carbohydrates, strong anti-oxidants (Vit E, C and flavonoids), proteins, minerals (I, Ca, K, Mg and Na), volatile oils, alkaloids, tannins, saponins and steroids.¹⁶ It is used as an anti-diabetic ,

anti-fungal, diuretic and uricosuric agent and hence used all over the world to treat urinary tract infections, cystitis, gout, nephritis, prostatitis, gonorrhoea, benign prostatic hyperplasia, hypertension and obesity.¹⁷

The natural antioxidant properties of corn silk extract eliminate free radicals and lipid peroxidation in the body. These characteristics are more obliging and less harmful than synthetic antioxidants like butylated hydroxy-anisole (BHA) and butylated hydroxytoluene (BHT).¹⁶ Cornsilk extract is safe to use and acts on liver, pancreas, kidney, heart and fat tissues. Protective effects of corn silk extract on liver tissue are proposed to be due to its anti-oxidant properties.¹⁸

This study was designed to evaluate the ameliorating effects of corn silk extract against doxorubicin induced hepatotoxicity.

MATERIAL AND METHODS

It was a laboratory based randomized controlled trial conducted at the animal house and histology laboratory of Postgraduate Medical Institute Lahore in 2022. The study protocol was accepted by Advanced Studies and Research Board of University of Health Sciences, Lahore, and Ethical Committee of PGMI. Thirty, healthy adult albino rats of either sex, aging 6–8 weeks, weighing 180–220 g were taken. The animals were randomly divided into three groups of 10 each; 1 (control), 2 (experimental 1) and 3 (experimental 2). The animals were kept at 23–25 °C, 50–55% humidity, and normal photoperiod of 12–12 hours light/dark cycle and given standard rat feed and distilled water *ad libitum*, and were allowed to acclimatize for 1 week. Animals were weighed at start and end of experiment.

The rats of Group 1 (Control group) were given standard rat feed and distilled water *ad libitum* with additional distilled water I.P. in the dose of 1.2 mg/Kg/body weight every 3rd day for 21 days. The rats of Group 2 (Experimental group 1) were given Doxorubicin I.P. in the dose of 1.2 mg/Kg /body weight every 3rd day for 21 days.¹⁹ The rats of Group 3 (Experimental group 2) were given Doxorubicin I.P. at the dose of 1.2 mg/Kg/body weight every 3rd day for 21 days and corn silk extract 400 mg/Kg/body weight orally for 21 days consecutively.²⁰

After 24 hours of administration of the last dose at 22nd day, ~5 mL of blood was drawn from each rat via single intra-cardiac puncture and stored for biochemical analysis. Then animals were sacrificed and livers were removed, fixed in 10% formalin²¹, and stored for tissue processing and morphological and histological study.

For serum extraction, vacutainers were centrifuged at 1300 g for 10 minutes.²² Serum was aspirated by help of micropipette and dispensed into

pre-labelled eppendorf tubes. Biochemical analysis of liver enzymes was done by colorimetric method (micro lab 300). For quantitative determination of serum ALP levels, lab kit of TRUE chemie based on IFCC method (LOT# A1322041 and linearity up to 650 U/L) was used. Whereas, for estimation of GGT, lab kit of Elabscience (Cat# E-BC-K126-M, detection range 0.88–399.4 U/L) was used. All the results were noted down for data analysis on SPSS.

For tissue processing and histological analysis, tissue samples were dehydrated and cleaned with xylene and embedded in paraffin blocks. Longitudinal sections of 5–7 micrometer thickness were obtained. Slides were stained with haematoxylin and eosin (H&E).

RESULTS

Results of biochemical parameters (serum ALP and GGT) were analysed by one-way ANOVA by using SPSS-22 and expressed as Mean±SD, ($p < 0.05$) (Table-1). Inter group comparison was done by post Hock Tukey's HSD test and expressed in Table-2.

Table-1: Serum ALT and GGT level (Mean±SD)

Groups	ALP	GGT	p
Group 1	134.70±15.18	2.6±1.4	<0.05
Group 2	328.30±18.05	17.63±1.4	<0.05
Group 3	229.90±17.984	9.6±1.2	<0.05

Table-2: Intergroup comparison by Post Hoc Tukey's HSD test

Variables	Group vs Group		p
Alkaline Phosphatase (U/L)	Group-1	Group 2	<0.001
		Group 3	
	Group 2	Group 3	
Gamma glutamyl transpeptidase (U/L)	Group 1	Group 2	<0.001
		Group 3	
	Group 2	Group 3	

Group 1 (Control group) showed normal histology of liver, i.e., typical cellular structures with preserved cytoplasm and distinct nucleus, well defined central vein, sinusoidal spaces and normal portal triad (Figure-1).

Group 2, animals administered with Doxorubicin showed multiple alterations including deranged structure of hepatocytes, dilatation and congestion of central vein and sinusoids with increased infiltration of inflammatory cells within the portal triad and oedema (Figure-2).

Group 3, animals treated with corn silk extract alongside Doxorubicin showed improvement in the hepatocyte architecture disrupted by Doxorubicin exposure. These animals exhibited significantly fewer alterations compared to the extensive liver damage observed in group 2. Corn silk extract administration effectively prevented swelling, inflammatory cells infiltration, necrosis, central vein and sinusoidal congestion (Figure-3).

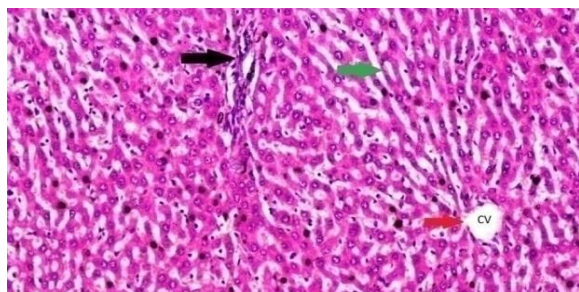


Figure-1: Photomicrograph of liver from Control group showing normal structure of hepatocytes, portal triad (black arrow), sinusoidal spaces (green arrow) and central vein (red arrow). (H&E stain, ×20)

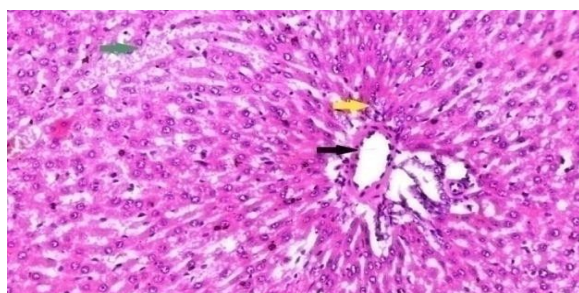


Figure-2: Photomicrograph of liver from Experimental group 2 showing deranged structure of hepatocytes including dilated and congested central vein (black arrow), infiltrate/Granuloma formation (yellow arrow) and sinusoidal congestion (green arrow). (H&E stain, ×20)

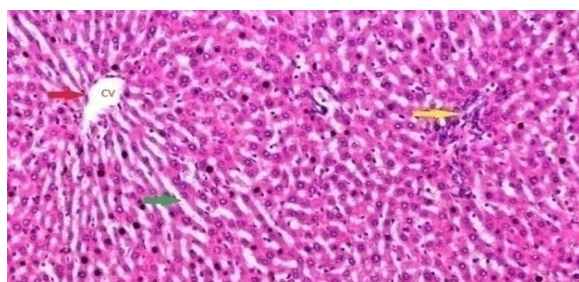


Figure-3: Experimental group 3 shows improvement compared to group 2. The central vein (red arrow) and sinusoidal congestion is reduced (green arrow) and inflammatory cell infiltration (yellow arrow) is also decreased. (H&E stain, ×20)

DISCUSSION

Rising occurrence of cancer in the developed and developing countries of the world has become a matter of concern. According to the WHO cancer data of 2019 cancer is the leading cause of mortality in more than 100 countries of the world.¹ Advancements in medical knowledge has evolved the management of cancer, e.g., same day surgery, short hospital stays, radio ablation, targeted drug therapy, personalized medication along with ambulatory home care, life style modifications and follow ups.²³

One of the commonly used anti-cancer drug is doxorubicin which has potential side-effects on various organs of the body particularly on liver because liver is the main organ that metabolizes and detoxifies the drugs. Hence it remains exposed to the toxic metabolites of these drugs and any damage to the liver structure and function is clinically significant.²⁴ Hepatotoxic effects of drugs can be prevented by using natural products, i.e., crude extracts and bioactive substances.²⁵ One of these compounds is natural corn silk extract which is enriched with vitamins, minerals and strong anti-oxidant agents. Researches have proven its strong ameliorating effects against oxidative stress induced by various chemotherapeutic drugs like paclitaxel and cisplatin.^{26,27}

Results of our study showed significant elevation of serum ALP and GGT levels, degeneration of hepatic cords, vacuolated cells with round to oval and elliptical shaped nuclei and dilated sinusoidal space, dilated and congested central vein and granuloma formation in animals of group 2. These results are in accordance with the study of Bilgic *et al*²⁸ in which 20 mg/Kg of DOX administered via I.P. injection for 6 days caused degenerative changes in hepatocytes, elevation of AST, ALT, and malendialdehyde levels and reduced catalase, glutathione peroxidase and super oxide dismutase in male Sprague Dawley rats. The study of Belhan S *et al*²⁹ showed that single I.P. dose of 20 mg/Kg DXR reduced anti-oxidant enzymes levels and elevated malondialdehyde levels. From the results of these studies we can propose that the biochemical basis of doxorubicin induced liver damage is due to induction of free radicals and oxidative stress.

In our study the group C showed significant improvement in liver histology (significant reduction in sinusoidal congestion, granuloma formation and cellular infiltration) and reduction of serum ALP and GGT levels towards normal. These results are similar to the study of Barakat AI *et al*²⁶ in which corn silk extract in a dose of 400 mg/Kg given orally for 7 days restored the oxidative damage which occurred in rat lungs induced by chemotherapeutic drug paclitaxel. These properties of corn silk are due to its bioactive component flavonoids, e.g., maysin, ax-4-OH maysin, 3-methoxymaysin and vitamin ascorbic acid that have strong anticancer, anti-inflammatory, and antioxidant properties.³⁰

CONCLUSION

Corn silk extract ameliorates the toxic effects of doxorubicin on the histology and biochemical functions of liver. Corn silk extract can be recommended as a protective agent against doxorubicin-induced hepatic damage due to its easy availability, low cost, rapid and safe dietary administration in cancer patients.

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ORIGINAL ARTICLE

ROLE OF ZINC SUPPLEMENTATION IN RECOVERY OF CHILDREN WITH MEASLES AND THE DURATION OF HOSPITAL STAY: AN INTERVENTIONAL STUDY IN A TERTIARY CARE HOSPITAL

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Background: Measles contribute to considerable childhood morbidity and mortality. Emerging research suggests zinc supplementation may enhance immune function and play a role in reducing hospital stay in children with measles by shortening recovery time. Objective of this study was to determine the role of zinc supplementation in recovery of children with measles in terms of duration of hospital stay. **Methods:** This study was conducted in Department of Paediatrics, Ayub Teaching Hospital, Abbottabad from Oct 2021 to Apr 2022. A total of 117 children of either gender with signs and symptoms of measles were enrolled in this study using convenient sampling technique, after taking an informed consent from the parents or patients. All patients received zinc supplement on admission to hospital. A specially designed proforma was used for data collection. Demographic data, duration of disease, hospital stay, and vaccination status were recorded. Data was analysed on SPSS-19. **Results:** Among the 117 children, 34% were females. Age varied from 5 months to 12 years (Mean age=21.95 months), and weight varied from 4 to 30 Kg (Mean weight=9.31 Kg). Majority of the patients (56.4%) were unvaccinated while 21.4% were partially vaccinated, and 11% were fully vaccinated. Duration of hospital stay varied between 1 to 13 days (Mean stay=3.15 days). **Conclusion:** The role of zinc in reducing the duration of hospital stay in children with measles is inconclusive. Children who had history of vaccination against measles had better recovery.

Keywords: Measles, Maculopapular rash, Hospital stay, Childhood Respiratory Tract Infection, Conjunctivitis

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INTRODUCTION

Measles is caused by single-stranded RNA virus in the genus Morbilli-virus. It is highly contagious and is a notifiable childhood infectious disease.¹⁻³ Measles spreads through droplets infection in prodromal phase. Measles is an important cause of childhood morbidity and mortality, despite increasing vaccine coverage. The World Health Organization (WHO) estimates that before the introduction of measles vaccine in 1963 and widespread vaccination, major epidemics occurred approximately every two to three years and caused around 2.6 million deaths annually. According to WHO, 535,000 children died of measles in 2000.²

Global measles vaccination has significantly reduced deaths due to measles. Between 2000 and 2022, estimated measles related deaths reduced from 800,000 to approximately 107,500 (87% reduction).² In 2020, measles cases in Pakistan were 2,747. Cases reported from Pakistan fluctuated substantially in recent years, increasing through 2001–2020 to an estimated 2,747 in 2020.⁴ Vaccination rates remain low. Zinc plays a significant role in the maintenance of normal immunological functions. Zinc is said to be crucial for effective innate and acquired immunity.⁵ In children, zinc supplementation is reported to reduce morbidity, mortality, and recovery time from acute infectious

diseases.^{6,7} About 107,500 people died of measles in 2023 most being children younger than 5 years of age.⁸ Children with measles and associated complications pose a significant health threat, as vaccination rates remain low in our province. It is important to examine the potential role of zinc supplementation in controlling the symptoms and mortality from measles in children.

MATERIAL AND METHODS

This study was carried out at the Department of Paediatrics, Ayub Teaching Hospital, Abbottabad from Oct 2021 to Apr 2022. Ethical approval was obtained from the Institutional Review Board of Ayub Medical College and Teaching Hospital, Abbottabad, Pakistan. Sampling technique applied was convenient sampling. Data regarding demographic as well as other variables was collected on a proforma. Diagnosis was made on history and physical examination. Age, gender, duration of symptoms and weight were recorded. All measles patients included in our study received zinc supplementation in dose of 1 mg/Kg/day. Duration of symptoms and hospital stay was recorded. Parents were interviewed for history of contact and vaccination. The data were input and analysed using SPSS-19. Frequency and percentage were computed for qualitative variables. Mean±SD was presented for quantitative variables like age, weight, duration of symptoms and hospital stay.

Post stratification Chi-square test was applied and $p \leq 0.05$ was considered statistically significant.

RESULTS

Of the 117 patients, 66% were males. Mean age was 21.95 months (5 months–12 years). Mean weight was 9.31 Kg. Patients presented between day 4 and 26 of appearance of clinical symptoms with mean time of presentation at day 9. Mean hospital stay was 3.15 days and varied between 1 to 13 days (Table-1).

Among them, 77 (66%) patients had a positive contact history and 28 (24%) patients had no history of exposure (exposure unknown in 12). Majority (56%) of the patients were unvaccinated, 21% were partially vaccinated and 11% were fully vaccinated. (Table-2).

A Pearson product-moment correlation coefficient was computed to assess the relationship between vaccination status & duration of hospital stay. There was a weak negative correlation between the two variables, $r = -0.20$, $n = 117$, however the relationship was significant. ($p < 0.05$) Children who had received measles vaccination had shorter recovery time (Table 3).

Fever was the most common presentation (100% patients) followed by with cough and body rash (80.34% and 86.32% respectively) (Table-4).

Table-1: Descriptive statistics (n=117)

Parameters	Mean±SD	Minimum	Maximum
Age (Months)	21.95±22.31	5	144
Weight (Kg)	9.31±4.27	4	30
Duration of signs/symptoms	9.15±3.49	4	26
Duration of hospital stay	3.15±1.93	1	13

Table-2: Frequency distribution of exposure, vaccination status and mean hospital stay (n=117)

Patients status	Frequency (%)	Mean hospital stay (days)
Positive history of exposure	77 (66)	3.11
No history of exposure	28 (24)	3.29
Unknown exposure	12 (10)	3.15
Unvaccinated	65 (56)	3.55
Partially vaccinated	25 (21)	2.62
Fully vaccinated	13 (11)	2.60
Unknown vaccination status	14 (12)	2.79

Table-3: Correlation of vaccination status and duration of hospital stay (n=117)

Correlations	Pearson Correlation Coefficient r	p
Vaccination status	-0.201	0.030*
Duration of hospital stay		

*Significant

Table-4: Clinical presentation of the patients

Clinical presentation	Number	Percentage
Fever	117	100
Cough	94	80.34
Body rash	101	86.32
Diarrhoea	59	50.43
Oral ulcer	55	47.00
Conjunctivitis	71	60.68

DISCUSSION

Because measles is so contagious, it can easily cross borders. Globally, a 20% surge in measles cases is reported in 2023 (10.3 million people infected) attributed to a decline in measles vaccination rates and low vaccinations during the COVID-19 pandemic. Pakistan is on top of list in countries with measles out breaks.^{2,9}

The contagious nature of measles was evident from our study group as 77 (66%) patients had a positive contact history. Vaccination rates were low. Mean hospital stay was 3.15 days. A retrospective cohort study of 267 children with measles from Uganda reported a median length of hospital stay as 4.0 days and less than 7 days for majority.¹⁰ A study from Turkey reported 38 children hospitalized with measles with a mean duration of hospital stay 5.13±2.67 days.¹¹

A Cochrane review found only one randomized controlled trial from India, involving 85 children with measles and pneumonia. Their study showed no significant differences in mortality or time to fever resolution between the zinc and placebo groups. Evidence to support or reject the impact of zinc supplementation in children with measles wasn't enough. Due to the very low quality of the available evidence, no definitive conclusions could be made regarding the effects of zinc supplementation on clinical outcomes in children with measles.¹²

Mahalanabis D *et al*¹³ found that children with severe measles and pneumonia treated with antibiotics and vitamin A did not show any additional benefit from receiving a zinc supplement. Their study concluded that zinc supplementation did not provide a clinically significant benefit when added to the standard treatment of antibiotics and vitamin A.

Naseer A *et al*¹⁴ aimed at role of zinc supplementation in treating measles pneumonia in children, the average duration of illness was reported as 132.81±8.36 hours. Their conclusion was that duration of illness with zinc supplementation was similar to that observed in previous studies and highlighted the need for additional trials to confirm their findings.

In our study a coincidental finding was that clinical outcome was related to the vaccination status of patients. Data showed that vaccinated patients had a shorter duration of hospital stay. A higher mortality among unvaccinated children with measles was also reported in one study from Uganda.¹⁰ Burström B *et al*¹⁵ from rural Kenya Africa noted same.¹⁵ They emphasized the importance of raising the measles vaccination coverage considerably to reduce mortality rates, as well as alternative ways to protect infants. Children who have not been previously vaccinated against measles infection and are infected with it experience a significantly higher mortality rate as

compared to those who were vaccinated. Rather than being a natural selection process that eliminates the weakest children, measles seems to worsen the health of many, leading to increased mortality later on. In regions with high case fatality rates, measles vaccination should be an essential component of primary healthcare.¹⁶

The role of zinc in measles remains uncertain. The available overall quality of evidence on this topic is considered low. The Cochrane review¹² highlighted the need for more robust research to conclusively determine the benefits of zinc supplementation in treating measles in children.

CONCLUSION

The role of zinc in reducing the duration of hospital stay in children with measles remains inconclusive. Children vaccinated for measles were found to have faster recovery as evident from shorter duration of stay.

SUGGESTIONS

Further large scale studies are required to ascertain the role of zinc in reducing the hospital stay, improving symptoms, and reducing mortality in children with measles. Studies may be done to ascertain the outcome of measles in vaccinated versus unvaccinated children.

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ORIGINAL ARTICLE

TRENDS OF SEDATIVE-HYPNOTICS CONSUMPTION AMONGST THE MEDICAL STUDENT POPULATION OF MULTAN

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Background: Medical curriculum has a notoriety of causing work life imbalance and frequent burn outs. To overcome this mental health dilemma the medical students resort to consumption of sedatives. This study aimed to evaluate the tendency of medical students in Multan towards usage of benzodiazepine with emphasis on understanding prevalence rates, drug consumption pattern, and associated socioeconomic factors. **Methods:** This study was conducted under collaboration of Pharmacology Departments of Nishtar Medical University and Bakhtawar Amin Medical and Dental College, Multan. Data was collected through a modified questionnaire from 400 undergraduate medical students. The sample population was categorized into two groups based on benzodiazepine users and non-users. Data was analysed and interpreted using SPSS-25. **Results:** On gender comparison female user exhibited a lower odds ratio (OR=1.67, $p=0.047$). In academic perspective the senior students exhibited varied odds of consumption. Parental with higher literacy level were associated with reduced odds of sedative use (OR=0.48, $p=0.035$). Individual lifestyle including smoking habits, exercise, and intake of stimulants also displayed significant associations. Students with previous history of drug consumption expressed less intent towards continuation (OR=0.53, $p=0.01$). **Conclusion:** The number of female students consuming sedatives was significantly higher than their male counterparts. The progression through academic years significantly correlates with increased sedative consumption particularly peaking in 4th year. Students reporting poor sleep quality were less likely to consume sedatives, a finding that challenges assumptions about the direct relationship between sleep quality and sedative use.

Keywords: Anxiety, Benzodiazepine, Insomnia, Medical Students

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INTRODUCTION

Benzodiazepines form the principal drug group of sedative-hypnotics. These drugs bind to molecular component of gamma amino butyric acid (GABA) receptors in neuronal membranes in central nervous system. Benzodiazepines activate receptors by increasing the frequency of chloride ion passage helping in sleep onset and to alleviate anxiety.¹ Most commonly referred as sleeping pills, these drugs are globally popular for overcoming insomnia and anxiety disorders.²

With an increasing prevalence of complains associated with altered sleep pattern and sleep hygiene a dangerous raise in non-prescription trade and misuse has also raised concerns. One of the major cohorts which are exposed to unwarranted risk of sedative hypnotic consumption is undergraduate medical students.^{3,4}

Curriculum burden of medical students are hard-hitting. Students need to attend lengthy overwhelming lectures/practical/discussion/wards and other academic activities throughout the year. Their exam pattern has a higher difficulty index where they have to appear not only in written examination but also

in practical and viva voce.⁵ To prepare these professional exams they have to spend long hours of self-study which demands sleepless nights often. It can alter their sleeping habits and may bring in complaints of insomnia and anxiety.⁶ Some students avail cognitive approaches like stress management methods, improved assertiveness skills, time management plans and counselling sessions to manage their respective anxiety and insomnia.⁷

Nonetheless, some students opt for medical formulas to alleviate their indications. Sedative hypnotics are the most commonly used drug by medical students for this purpose. The use of these drugs by them may allure them to become habitual which may lead to numerous undesirable consequences such as drowsiness, poor physical synchronization, lack to focus and lethargy.⁸

Studies conducted in different constituency of world have evaluated prevalence of sedative drugs usage in the medical student population of their region and identified various confounding variables.^{9,10} Aim of this study was to evaluate popularity of benzodiazepine in medical students of Multan, Pakistan and to inculcate our outcome with related studies.

METHODOLOGY

This study was conducted in the Department of Pharmacology, Nishtar Medical University, Multan, in collaboration with Department of Pharmacology, Bakhtawar Amin Medical and Dental College, Multan. The study was ethically reviewed by Institutional Review Board of Nishtar Medical University (IRB letter No. 6985).

A questionnaire that was used in previous similar study¹¹ was integrated in this study with minor modifications. Four-hundred students of MBBS (1st year–5th year) were selected in this study through non-probability convenient sampling. Participants were divided into two groups: sedative users and non-users.⁸ Informed written consent was obtained before enrolling them in the study.

Statistical analyses were done using SPSS-25. Demographic and routine life factors were expressed as percentages and differences between two groups were compared through Chi-square test with $p \leq 0.05$ considered as statistically significant.

RESULTS

The analysis of sedative use among medical students reveals several key insights related to gender, academic year, GPA, marital status, parental education, income level, and residence. Females showed a significantly lower odds ratio (OR=1.67, $p=0.047$) of sedative use as compared to males.

Academic performance and year in school also significantly influenced sedative use; students in their 4th year exhibited the lowest odds (OR=0.29, $p=0.003$) compared to 1st year students, suggesting that academic pressure may affect sedative use differently across years.

Parental education level, particularly fathers with a Master's degree, was associated with a reduced odds of sedative use (OR=0.48, $p=0.035$). Income level and residence did not significantly impact sedative use, although trends suggested varying effects.

Overall, these findings highlight the complex interplay between academic stress, gender, marital status, and family background in the prevalence of sedative use among medical students, suggesting targeted interventions could be beneficial. (Table-1).

Analysis of sedative use among medical students in relation to lifestyle habits and sleep patterns reveals multifaceted insights. Smoking and exercise routine significantly influence sedative consumption; non-smokers and those who do not exercise regularly exhibiting higher odds of sedative use, although the relationship with smoking was not statistically significant. Consumption of stimulants, including coffee, tea, cola drinks, and others, showed varied odds of sedative use, with none showing a significant impact.

Interestingly, during non-exam periods, students who occasionally experienced stress had significantly higher odds of sedative use, suggesting acute stressors might prompt sedative consumption. Conversely, during exam periods, students rarely stressed showed significantly lower odds, indicating perhaps a habituation effect or different coping mechanisms.

Study habits and sleep patterns, including hours spent studying, sleeping, time needed to fall asleep, and sleep quality, demonstrated complex associations with sedative use. Notably, those with a night and day sleeping pattern showed significantly higher odds of using sedatives, highlighting the impact of irregular sleep patterns on sedative use.

These findings underscore the intricate relationships between lifestyle, sleep habits, and sedative consumption among medical students, suggesting that interventions aimed at improving sleep hygiene and stress management could potentially reduce the reliance on sedatives. (Table-2).

Table-3 provides a nuanced exploration of the relationship between sleep quality, presence of sleeping disorders, and the use of sedative drugs among medical students. Data suggest that students who describe their sleep quality as poor have significantly lower odds (OR=0.54, $p=0.013$) of sedative use compared to those who report good sleep quality, indicating a possible under use of sedatives among students struggling with sleep quality or an indication of sedatives not being the primary solution for their sleep issues.

Regarding sleeping disorders, the odds ratios reveal no significant association between having specific disorders like insomnia, sleep apnoea, restless legs syndrome, narcolepsy, or none and the use of sedatives, with insomnia being used as the reference category. This suggests that the presence of these specific sleeping disorders does not significantly influence sedative use among this population, possibly reflecting a cautious approach to sedative use regardless of diagnosed sleep conditions.

Interestingly, students who have used sedative drugs or other sleep aids since entering medical college are less likely to do so again compared to their counterparts who haven't (OR=0.53, $p=0.01$), suggesting a potential reconsideration or discontinuation after initial use. This could reflect a variety of factors including efficacy, side effects, or changing perceptions of sedative use over time.

Overall, these findings underscore the complex interplay between sleep quality, the presence of sleeping disorders, and the propensity towards sedative use among medical students, highlighting the need for targeted interventions to address sleep health without over-reliance on sedatives.

Table-1: Factors influencing sedative use among medical students: A comparative analysis

Characteristics	Sedative use, n (%)		OR	p
	Yes	No		
Gender				
Male	60 (27.3)	160 (72.7)	1	
Female	33 (18.3)	147 (81.7)	1.67	0.047
Academic year				
1 st	7 (10.9)	57 (89.1)	1	
2 nd	21 (22.8)	71 (77.2)	0.42	0.088
3 rd	19 (25.0)	57 (75.0)	0.37	0.048
4 th	42 (30.0)	98 (70.0)	0.29	0.003
5 th	4 (14.3)	24 (85.7)	0.74	0.73
cGP in previous year				
2.5 or less	10 (13.2)	66 (86.8)	1	
2.5-3	26 (23.2)	86 (76.8)	0.50	0.093
3-3.5	30 (31.3)	66 (68.8)	0.33	0.006
3.5-4	15 (26.8)	41 (73.2)	0.41	0.071
4- 4.5	12 (20.0)	48 (80.0)	0.61	0.35
Fathers' level of education				
High school	16 (15.4)	88 (84.6)	1	
Bachelors	41 (25.6)	119 (74.4)	0.53	0.066
Masters	33 (27.5)	87 (72.5)	0.48	0.035
PhD	3 (18.8)	13 (81.3)	0.79	0.720
Mothers' level of education				
High school	27 (25.0)	81 (75.0)	1	
Bachelors	19 (20.7)	73 (79.3)	1.28	0.503
Masters	47 (25.0)	141 (75.0)	1.00	1.000
PhD	0 (0.0)	12 (100.0)	-	-
Average family income per month in PKR				
1000 to 5000	18(20.5)	70 (79.5)	1	
5000 to 10,000	20(16.1)	104 (83.9)	1.34	0.469
10,000 to 15,000	22(32.4)	46 (67.6)	0.54	0.100
15,000 to 20,000	18(23.7)	58 (76.3)	0.83	0.706
More than 20,000	15(34.1)	29 (65.9)	0.50	0.094
Residence				
Home	51(20.9)	193 (79.1)	1	
Hostel	42(26.9)	114 (73.1)	0.72	0.204

Table-2: Associations between lifestyle habits, sleep patterns, and sedative use among medical students

Characteristics	Sedative use, n (%)		OR	p
	Yes	No		
Are you a smoker?				
Yes	48 (21.1)	180 (78.9)	1	
No	45 (26.2)	127 (73.8)	0.75	0.23
Do you exercise regularly?				
Yes	52 (19.4)	216 (80.6)	1	
No	41 (31.1)	91 (68.9)	0.53	0.01
Do you use any type of stimulants including?				
Coffee	19 (25.0)	57 (75.0)	1	
Tea	33 (17.6)	155 (82.4)	1.57	0.18
Cola drinks	18 (30.0)	42 (70.0)	0.78	0.56
Others	23 (30.3)	53 (69.7)	0.77	0.59
Do you use sedatives during non-exam periods?				
Never	9 (32.1)	19 (67.9)	1	
Rarely	67 (27.5)	177 (72.5)	1.25	0.66
Occasionally	5 (6.6)	71 (93.4)	6.73	0.002
Always	12 (23.1)	40 (76.9)	1.58	0.43
Do you use sedatives during exam periods?				
Never	16 (15.4)	88 (84.6)	1	
Rarely	24 (33.3)	48 (66.7)	0.36	0.006
Occasionally	47 (26.1)	133 (73.9)	0.51	0.039
Always	6 (13.6)	38 (86.4)	1.15	1.00
On average how much time do you spend studying per week?				
4-6	16 (26.7)	44 (73.3)	1	
6-8	40 (18.2)	180 (81.8)	1.64	0.15

8-10	37 (30.8)	83 (69.2)	0.82	0.61
On average how many hours do you sleep per day?				
4-6	34 (23.0)	114 (77.0)	1	
6-8	15 (14.4)	89 (85.6)	1.77	0.11
8-10	44 (29.7)	104 (70.3)	0.70	0.23
On average how much time do you need to fall asleep?				
0-30 min	16 (15.4)	88 (84.6)	1	
30-60 min	41 (25.0)	123 (75.0)	0.55	0.067
> 60 min	36 (27.3)	96 (72.7)	0.48	0.039
Can you specify your sleeping pattern?				
Night only	72 (30.0)	168 (70.0)	1	
Night and day	21 (13.1)	139 (86.9)	2.84	<0.001
Excellent	52 (31.0)	116 (69.0)	0.96	0.91

Table-3: Influence of sleep quality and disorders on sedative use among medical students

Characteristics	Sedative use, n (%)		OR	p
	Yes	No		
How can you describe your sleep quality?				
Excellent	52 (31)	116 (61)		
Good	20 (18.5)	88 (81.5)	1	
Poor	21 (16.9)	103 (83.1)	0.54	0.013
Do you experience any of these sleeping disorders?				
Insomnia	12 (23.1)	40 (76.9)	1	
Sleep apnoea	11 (22.9)	37 (77.1)	1.01	1.00
Restless legs syndrome	19 (15.8)	101 (84.2)	1.59	0.28
Narcolepsy	43 (32.6)	89 (67.4)	0.62	0.22
None	8 (16.7)	40 (83.3)	1.50	0.46
Since entering the college of medicine have you ever used sedative drugs or any other drugs that help you fall asleep?				
Yes	46 (20.5)	178 (79.5)	1	
No	47 (26.7)	129 (73.3)	0.53	0.01

DISCUSSION

Benzodiazepines augment the neuro-inhibitory action of GABA which grants it the therapeutic potential against anxiety, panic and sleep disorders. However they acts as a double edged sword as with increasing dose it tones down the skeletal muscles and triggers dopamine outbursts turning on the reward and pleasure centers in ventral tegmental area of brain.⁷ Hence, while keeping in consideration their side-effects including memory and cognitive impairment, respiratory depression, behavioural disorders and blood pressure changes, it is also imperative to highlight the most controversial yet harrowing issue of its addiction and misuse.¹²

Global statistics mark an age cohort of 18-24 years as the biggest consumer of sleep industry market amongst which medical students are the most susceptible individuals owing to their work routine with reports of overburdening, frequent burnout episodes and attacks of severe panic and anxiety.⁴ This cross-sectional study conducted in Pakistan is a continuum to the surveys taking place globally to assess the frequency of the medical students consuming these sedative hypnotics.

In our study the total ratio of students taking benzodiazepines came out to be an alarming total of 23%. This percentage was in line with a recent study in Iran where 20% of the study participants admitted to

use the pills.¹³ However, both ours and Iranian study were in a complete contrast with a study conducted in Serbian students where though 19% males and 12.8% females reported with complete burn out but the benzodiazepines consumption was reported by 3.4% only, close to the one in Ethiopian medical students with 6.2% of total medicos.¹⁴ While compiling the respective gender ratio of all other aforementioned studies no remarkable difference was seen. However, in our study male students showed higher relative compliance. The varying numbers amongst these studies can be owing to their significant association with factors including use of other stimulant drugs, smoking and alcohol whereas our study could not develop any such association.

The social factors raising the consumption bar were students living in hostels which were in line with a Spanish online survey with 6,798 volunteers; these were all diverse students with a considerable fraction hosteller medical students who reported a massive inclination towards acquiring diazepam and lorazepam without a prescription.¹⁵

Economically, children belonging to household with middle class income and literate background, pertaining to both parents education, were at higher stake. On exactly similar lines, a research⁷ was published by a North Eastern Ethiopian University validating our findings, this research had widens the scope of research by underlining the concurrent abuse of antihistamines as well.

Last and more important aspect to be covered is academic position of these students, as a series of direct questions helps us categorize the students grades, and it turned out that the students who came in the window of average scorers with 8–10 hours of study schedule had highest rate of frequent pill popping with a higher trend in exam period for which the curricular burden is the main malefactor. The students who exercise regularly demonstrated an ability to overcome this urge with a lower ratio in comparison. A descriptive study conducted in India had exact aforementioned results with higher occurrence in average scorers; the study also depicted multi-tiered factors similar to ours like gender, place to stay and seniority.¹⁶

Allied sleeping habits affect the number too, here we observed a mixed interplay of sleep aspects as majority sedative hypnotic users marked variable responses with the greater credit to 8–10 hours of night sleep with excellent quality. It is worth mentioning that the users also shared the problem of narcolepsy and insomnia as well, which is polls apart in sleep architecture. Benzodiazepines do improve the sleep quality with no awakening episode; time for stage 3, however, may be reduced.¹⁷ We were unable to find more studies commenting in these points so this was a

rather novel part of the study which may lead further to better understanding with more data compiled.

This study adds onto the pool of global data alarming the authorities regarding the speedily trending use of benzodiazepines as not merely mental health aids but also as an illicit substance abuse. It also warrants a change in medical education system which buds so much anxiety in young brain that they resort to such ‘short-cuts’. Such studies should be conducted on massive level to identify precise magnitude of problem and address it as a matter of utmost importance and urgency.

CONCLUSION

The number of female students consuming sedatives was significantly higher than their male counterparts. The progression through academic years significantly correlates with increased sedative consumption particularly peaking in 4th year. Students reporting poor sleep quality were less likely to consume sedatives, a finding that challenges assumptions about the direct relationship between sleep quality and sedative use.

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ORIGINAL ARTICLE

SUICIDAL IDEATION AND SELF HARM DURING PREGNANCY
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Background: Pregnancy can sometimes give way to darker emotions, as the complex interplay of hormonal, psychological, and social factors increases the risk of suicidal thoughts and self-harm behaviours. This study aims to determine the frequency of suicidal ideation, self-harm and factors contributing it among pregnant females presenting for antenatal care. **Methods:** It was a cross-sectional study conducted at Nishtar Hospital, Multan, and DG Khan Medical College, Dera Ghazi Khan, from May 2023 to Nov 2023 after taking approval from Ethical Review Board. Data was collected from 160 responders at 12–36 weeks of gestation after informed consent. Pregnant women with severe medical complications that may require immediate care and women who present an immediate risk of self-harm or suicide were excluded. Data was analysed on SPSS-22. **Results:** Total 16 out of 160 had thought of self-harming. Frequency of suicidal ideation was 10%. The 10th question of Edinburgh Post-natal Depression Score (EPDS) ‘The thoughts of harming myself’ was answered as ‘never’ by 75.63%, ‘hardly ever’ by 6.25% ‘sometimes’ by 8.13% and ‘yes, quite sometimes’ by 10%. **Conclusion:** Women who are depressed and had some form of domestic violence are at greater risk of having suicidal ideation. Attention should be given to establish mental health programs for pregnant women.

Keywords: Perinatal, suicidal ideation, self-harm, domestic violence

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INTRODUCTION

Suicidal ideation, often a precursor to suicide, commonly called suicidal ideas or thoughts, is a broad term used to describe non-active suicidality including preoccupation, contemplations or wishes of death.¹ Suicidal ideation is an important and often overlooked possible antecedent for mortality among high risk population such as pregnant women.² Pregnancy is a major and complex occasion in a woman's life that is related to significant physiological, social, and mental changes.² Suicide is a leading cause of maternal death during pregnancy and up to a year after birth.³ In high income countries suicide accounts for 5–20% of maternal deaths. In comparison, among low and middle-income countries the combined occurrence of pregnancy-related deaths attributed to suicide is 1.7%.¹

Studies indicate that pregnant women who endorsed suicidal fantasies are more likely to have current and lifetime co-morbid depressive and anxiety disorders than did women without suicidal ideation.⁴ Women who undertake self-annihilation during pregnancy have a high rate of abortion, miscarriage, preterm delivery, and hospitalization.⁵ Suicidal preoccupation in women is not only associated with poorer mother-infant bonding but also associated with impaired emotional and cognitive development and growth of children.^{6,7} Lack of support, co-morbid mental illness, have low educational attainment, unemployment, unplanned pregnancy, previous suicide attempts, family history of suicide, and co-morbid chronic physical illness

are main factors of self-destructive thinking during pregnancy in developing countries.^{8–10}

Main aspiration of this study is to identify pregnant women who are at high risk of suicide and help in developing different and suitable intervention programs to decrease maternal mortality and morbidity. The aim of this study was to determine socio-demographic factors that affect mental health in pregnant women. This study is intended to assess the proportion of suicidal ideation and self-harm among pregnant women attending perinatal services at two main hospitals of South Punjab.

METHODOLOGY

It was a descriptive, cross-sectional study done at outpatient department of Nishtar Hospital Multan and DG Khan Teaching Hospital from May to Nov 2023 after taking approval from Ethical Review Board. Participants were selected through non-probability convenient sampling technique. The sample size was calculated from the formula: $n = (Za/2)^2 / p(1-p)/d^2$ where n is minimum sample size required for study, Z=standard normal variant, which for 5% type 1 error is 1.96. The p is prevalence of suicidal ideation attempt taken from previous study ($p=11.8$)² and d is the absolute precision or tolerable margin of error ($d=5$), $n=160$.

A total of 160 pregnant ladies, 18–45 years old, at 12–36 weeks of gestation were included in the study after informed consent. Pregnant women with severe medical complications that may require

immediate and intensive remedial care and pregnant women who present an immediate risk of self-harm or suicide were excluded from the study. Data was collected from Gynaecology out-patients through self-administered questionnaire in local language of respondents. Edinburgh Postnatal Depression Scale (EDPS) score was used to identify women who may have depression. It comprises of 10 questions. Each answer was given a score of 0–3. The maximum score was 30. The 10 question was related to self harm thoughts. It was compiled and analysed on SPSS-22.

RESULTS

The sample consisted of 160 pregnant women. Majority (54.4%) of them fell in age group 25–35 years. Mean age of the subjects was 31.5 ± 7.66 (Range: 13–40.5) years. Mean gestational age was 25.9 ± 8.30 weeks. Most (123, 76.9%) of the women were housewives and dependent on husband for living. Thirty-seven (23.1%) were working women, 65 (40.9%) women had family income of less than Rs. 30,000, 75 (47.2%) had monthly income Rs. 30,000–70,000, and 20 (12.4%) women had family income >Rs. 70,000. Women living in urban areas were 81 (50.6%) and 79 (49.4%) were from rural area. Twelve (7.5%) women presented during first trimester (<13 weeks), 75 (46.9%) presented during 2nd trimester (13–26 weeks), and 73 (45.6%) presented in 3rd trimester (27–36 weeks). (Table-1).

Women having 1st time pregnancy were 29 (18.1%), and multigravida were 131 (81.9%). Most of them lived in joint family system 108 (67.5%). The 10th question of EPDS ‘The thoughts of harming myself’ was answered as ‘never’ by 75.63%, ‘hardly ever’ by 6.25%, ‘sometimes’ by 8.13% and ‘yes, quite sometimes’ by 10%. Out of 160 pregnant women, 35 (21.8%) had an EPDS score ≥ 10 showing moderate risk of depression and anxiety during pregnancy. (Table-2).

Bivariate analysis was done and Chi-Square test was applied for data analysis. Odds ratio with 95% confidence interval were calculated. There was significant association between suicidal ideation in pregnancy and previous history of postpartum depression ($p=0.03$). Total 57 (35.6%) women experienced post-partum depression, out of which, 13 (22.8%) pregnant women experienced suicidal ideation. A total of 61 (38.1%) women had previous history of miscarriage. Only 9 (14.5%) women experienced self-harm thoughts. Total 103 (64.4%) females had previous history of still birth and 13 (12.6%) women had self-harm thoughts. Ninety-six women had history of domestic violence, out of which 7 (7.3%) women answered our question of suicidal ideation as ‘yes’. (Table-3).

Table-1: Demographic and psychosocial factors among pregnant women of the study (n=160)

Variables	Categories	n	%
Age (Years)	16–24	27	16.87
	25–34	87	54.35
	35–44	42	26.3
	45	4	2.5
History of domestic violence	Yes	96	60.0
	No	64	40.0
History of postpartum depression	Yes	57	35.6
	No	103	64.4
Family history of psychiatric illness	Yes	28	17.5
	No	132	82.5
Previous miscarriages	Yes	61	38.1
	No	99	61.9
Monthly family income (Rs. $\times 1,000$)	<30	65	40.9
	30–70	75	47.2
	>70	20	12.4
Residence	Rural	81	50.6
	Urban	79	49.4
Gestational Age at presentation (Weeks)	<13	12	7.5
	13–26	75	46.9
	27–36	73	45.6
Occupation	House-wife	123	76.9
	Working	37	23.1

Table-2: EPDS Score among the participants

EPDS score	N	%
1–3	7	4.4
4–6	90	56.3
7–9	28	17.5
≥ 10	35	21.8

Table-3: Association of socio-demographic variables with suicidal ideation during pregnancy

Categories	Suicidal ideation		OR (95% CI)	p
	Yes [n (%)]	No [n (%)]		
Previous Miscarriages				
Yes	9 (14.75)	52 (85.2)	2.71 (0.41, 18.0)	0.285
No	7 (7.1)	92 (92.9)	1	
Previous History of Still Birth				
Yes	13 (12.6)	90 (87.4)	2.67 (0.27, 25.8)	0.382
No	3 (5.3)	54 (94.7)	1	
History of Domestic Violence				
Yes	7 (7.3)	89 (92.7)	0.40 (0.06, 2.6)	0.336
No	9 (14.1)	55 (85.9)	1	
History of Postpartum Depression				
Yes	13 (22.8)	44 (77.2)	8.85 (0.90, 86.6)	0.03
No	3 (2.9)	100 (97.1)	1	

DISCUSSION

The present study indicates a high burden of suicidal risk and self-harm among pregnant women with far-reaching consequences for maternal and infant health, among the two teaching hospitals of South Punjab, Pakistan. This issue has not been given importance in statistical data of maternal morbidity and mortality. Majority of women experienced self-harm thoughts were having history of domestic violence and previous history of postnatal depression. Notably, the withdrawal of progesterone on the postpartum period has been implicated in post natal depression, which may also contribute to suicidal ideation. Another important finding was that suicidal behaviour was higher among women who had formal education. Lack of tolerance for existing social structure may lead to be in more conflicted relation with a restrictive society. Women living in urban areas are more likely to have suicidal thoughts.

Nevertheless, percentage of women with suicidal ideation found in our study is almost consistent with available data from other low resource countries. For example, suicidal ideation is common among 4% of Brazilian pregnant women.¹ In Spain, 2.5% of Maternal mortality was secondary to suicide.⁴ Another study on 23 countries distributing on six continents, including Asia, North America and South America, found that 8% prevalence of suicidal ideation.⁶ Prevalence among pregnant women in Jimma Medical Centre, Ethiopia was 13.3%.⁸ Prevalence of suicidal ideation at public hospitals in Southern Ethiopia was 11.8%.² These results are quite similar to our study where feeling of self-harm is reported in 10% pregnant ladies. Mean age of our study was 31.5 years while another study done in Ethiopia⁸, mean age was 25.5 years among the participants. In our study, domestic violence is reported in 60% of participants which is quite high while in another study, it was only 22.5%.⁸

According to study in Sri Lanka, feeling of self-harm was 2%.¹¹ According to study conducted in Canada, suicidal ideation was 10.4% quite close to our results.⁸ According to study, in South Africa, thoughts of self-harm was 5.9% among pregnant females.¹² Another study including 18 sub-Saharan African countries showed Self harm and suicidal ideation prevalence of 10.3% like our study.¹³ The prevalence rate of self-harm was 23.5% in a study conducted in times of pandemic.¹⁴ This is almost double than our results. We only found postpartum depression significantly correlated with suicidal thoughts in pregnancy. Although various factors like family history of psychiatric illness, personal history of psychiatric illness, parity, monthly income, were also associated with suicidal ideation in another study in Ethiopia.^{2,15}

This study has several limitations. The sample size was relatively small, which may limit the generalization of the findings. Since our study was based on patient questionnaire data, we had no data on successful suicidal attempts.

The findings of our study have significant implications for the development of targeted interventions and policy initiatives. Routine screening and comprehensive risk assessment for mental health condition should be considered during antenatal and postnatal care.

CONCLUSION

Hidden burden of maternal mental health is major concern for many under-developed and developing countries with same social background. Maternal mental health issue should be utmost priority among low income countries. Future research should investigate the effectiveness of interventions aimed to reduce suicidal ideation and self-harm thoughts during pregnancy.

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ORIGINAL ARTICLE

QUALITY OF LIFE IN MIGRAINE PATIENTS AND ASSOCIATION WITH AGE, GENDER, EDUCATION LEVEL AND MARITAL STATUS OF PATIENTS IN A TERTIARY CARE HOSPITAL

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Background: Migraines are globally prevalent and contribute to be the foremost neurological problems for which patients frequently seek medical care. Disabilities caused by migraine are one of the main causes contributing to the poor quality of life (QOL) among patients with migraine. This study aimed to determine the QOL in migraine patients. **Methods:** It was an observational cross-sectional study conducted in Ayub Teaching Hospital, Abbottabad, from Jan to Jul 2024. Both age and sex matched male and female aged 18–50 years, with diagnosis of migraine were included. WHO-BREF scoring through software was administered. Socio-demographic data, age, gender, family and socioeconomic history was recorded for each patient. **Results:** Mean age of the subjects was 37.91 ± 8.48 years. There were 41 (41%) male and 59 (59%) were female cases. Sixty-three (63%) cases had mild to moderate, and 37 (37%) cases had severe migraine. Mean physical health score was 22.86 ± 2.67 , mean psychological health score was 20.96 ± 2.08 , mean social relationship score was 10.52 ± 2.02 , mean environment score was 28.08 ± 1.02 , and mean total WHOQOL-BREF score was 82.42 ± 4.31 with minimum and maximum total score as 72 and 94. **Conclusion:** The patients with migraine have lower health related quality of life (HRQOL).

Keywords: Migraine, Quality of life, QOL, WHO QOL-BREF scoring

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INTRODUCTION

Migraine is classified as one of the primary headaches in which patients experience recurrent attacks of throbbing headaches, usually associated with pre attack of nausea, vomiting, flashes of light and photophobia lasting hours to days.¹ A specific diagnostic criteria for diagnosis of migraine is specified by International Classification of Headache Disorders 3rd edition.² Migraine is one of the most common presenting neurological complaint in medical OPD. Reduced quality of life (QOL) was reported in patients with migraine when compared with age- and sex-matched people without migraine.

Migraine is often a permanent devastating neurological condition. It not only has serious effects on physical health of patients but also affects patients' daily life domains in terms of their interpersonal relationships, psychological well-being, perceived QOL, emotional status and economic load. Migraine is the 21st principal cause of disability adjusted life-years (DALYs) worldwide, 10th in the Western Europe and 6th leading cause worldwide, in the age group 25–39 years.³

According to report of Global Burden of Disease (2015), migraine was hierarchical the 7th principal cause of the years lived with disability (YLD) in migraine population and 1st foremost cause of years lived with disability in patients less than fifty years of age.³ The WHO definition of QOL is 'an individual's perception of their position in life in context of culture and value systems in which they live and in relation to

their goals, expectations, standards and concerns.' This definition takes into account different facets of quality of life as well as overall quality of life and health.

Migraine patients have reported limitations with respect to their learning in occupational skills, educational prospects and other daily life activities performance and poor quality of sleep all as the top leading cause of disability among all neurological disorders.⁴ Migraine has a significant and debilitating impact on academic, social, physical, and occupational functioning of the sufferer. Migraine frequent episodes not only significantly impair an individual's capability to function during that period but can also reduce the QOL in between the episodes.⁵ This finding appeared to be accurate because chronic migraine (CM) affects the physical, psychological, emotional, and social lives of CM patients. Negative impact of migraine attack (even moderate attacks) has been reported between 50–73% of CM patients on the family and social relationships, even during the in between periods of attacks when patient is having subtle fear about the occurrence of next migraine attack. This is accompanied by marked functional impairment and affects their academic, physical, social, and occupational performance. More than 90% of the sufferers either are not able to go to their work or cannot concentrate or perform properly during the period of migraine attack.⁶

In Pakistan⁷ in 2017, the prevalence of migraine during 1 year (adjusted age and gender wise) was 22.5% (male 18.0%, female 26.9%), tension-type

headache (TTH) was 44.6% (male 51.2%, female 37.9%), and prevalence of chronic migraine was more in women by a ratio of 3:2. However TTH was found to be more prevalent in male patients by ratio of 4:3 ($p=0.026$). Over the years, frequency and severity of migraine headaches has increased.⁷

Migraine and all other headaches were found age-related, peaking between 40 and 49 years of age; though, tension-type headache was more associated with stresses, peaked nearly one decade earlier. Higher levels of education and socioeconomic status had negative association with migraine prevalence.⁷

People suffering from migraine frequently report poor quality of life (QOL) even during a period free of attack, than healthy people.⁸ The global burden of diseases, injuries, and risk factors study continues to categorize migraine as a leading cause of incapacity worldwide, predominantly in individuals who are younger than 50 years.¹ Patients of migraines frequently report poor life quality during attack-free periods than healthy individuals.⁹

Migraine is also associated with considerable disability, especially among females and those living in poor socioeconomic rural populaces. Migraine alone is 7th of almost disabling neurological disorders in men, and 3rd in women aged 15–49 years.¹⁰ A survey conducted on 4,943 Saudi people revealed that migraine was present in 1,333 (26.9%) individuals, with female predominance (1:2.9), like earlier surveys.¹¹ Another cross-sectional community-based survey with a sample size of 15,523 individuals, conducted in Kuwait, discovered that 23% people were diagnosed as intermittent migraine patients, and 5.4% as cases of chronic type of headache, with a mean age of 34.56 ± 10.17 years. The prevalence of episodic migraine was 31.71% in female, surpassing over in males 14.88% ($p < 0.01$) corresponding to previous data.¹² In a recent study from Pakistan, out of 238 patients with different types of headaches, 137 (79.19%) patients had migraines among whom migraine without aura was present in 113 (65.3%) patients.¹³ Another study¹⁴ reported that among 6.6% of patients, migraine with aura was present and migraine without aura was seen among 26.1% of patients. Millions of days are lost at work and school each year in the UK and USA by migraine, and a huge loss is estimated to be caused by migraine in the field of healthcare and overall productivity. The purpose of our study was to explore the quality of life in patients suffering from migraine in a tertiary care hospital.

MATERIAL AND METHODS

This observational, cross-sectional study was conducted in the Ayub Teaching Hospital Abbottabad for 6 months after approval from Institutional Ethical Review Committee vide No. RC/EA-2023/210 Dated 31 Dec 2023. Both male and female, age and sex matched

patients, aged 18–50 years, with diagnosis of migraine according to ICH criteria were included. Subjects with other types of headaches secondary to tension/cluster headaches, underlying ENT pathologies, sinusitis, ocular pathology, structural lesions of brain etc. were excluded. Subjects who had other disabling co-morbid systemic conditions like cardiac failure, respiratory failure, malignancy etc. was excluded. Those with history of cerebrovascular accident (CVA), head injury, or sensory/ motor neurological deficit on examination were excluded.

Using WHO sample size Calculator, keeping Confidence level of 95% population mean 83.4, with Standard deviation of 11.4, sample size was 100 in total.¹⁶ Sampling was done by consecutive non-probability sampling. Informed written consent was taken from the patients willing to participate in this study and fulfilling the criteria. WHO brief version of original scoring system consisting of 100 responses (0–100 score) was used. Responses were recorded on questionnaire for 26 responses about four domains of patient's life that is physical, social, psychological and environmental health domain. Final scoring was done as per operational definition, transformed on 0–100 scale.⁴

Demographic data, age, gender was recorded for each patient. Data was entered and analysed using SPSS-23. Descriptive statistics of the participants were calculated. For qualitative variables, percentage was calculated. For quantitative variables Mean $\pm$ SD were calculated. Effect modifiers were controlled through stratification. Independent samples Student's *t*-test was used to compare effect modifiers, and $p \leq 0.05$ was taken as significant.

RESULTS

Mean age of the patients was 37.91 ± 8.48 (Range: 18–50) years. There were 38 (38%) cases 18–35 years old, and 62 (62%) cases 36–50 years old, 41 (41%) were males. Sixty-three (63%) cases had mild to moderate and 37 (37%) cases had severe migraine. There were 25 (25%) cases educated < 12 and 75 (75%) cases received ≥ 12 years education, 34 (34%) were married. (Table-1).

Table-1: Descriptive statistics of patients

Variables	Frequency (N)	Percentage (%)
Age (Years)		
18–35 years	38	38 %
36–50 years	38	62%
Gender		
Male	41	41%
Female	59	59%
Severity of disease		
Mild-moderate	63	63%
Severe	37	37%
Level of education		
< 12 years	25	25%
> 12 year	75	75%
Marital status		
Married	35	35%
Unmarried	65	65%

The mean physical health score was 22.86 ± 2.67 , mean psychological health score was 20.96 ± 2.08 , and mean social relationship score was 10.52 ± 2.02 . Mean environment score was 28.08 ± 1.02 and mean total WHOQOL-BREF score was 82.42 ± 4.31 with minimum and maximum total score as 72 and 94. The mean physical health score, psychological health score, social relationship score, environment score and total WHOQOL-BREF score was statistically similar in age groups and gender ($p > 0.05$).

The psychological health score, social relationship score, environment score and total WHOQOL-BREF score was statistically similar with respect to severity of migraine, and mean physical health

score was significantly higher in cases who had severe migraine ($p < 0.05$).

The mean physical health score, social relationship score, environment score and total WHOQOL-BREF score was statistically same with respect to education level, $p > 0.05$. The mean psychological health score was higher in cases who had < 12 years of education, $p < 0.05$.

The mean physical health score, psychological Health score, social relationship scores and total WHOQOL-BREF score was found statistically same in both married/single groups, $p > 0.05$. The mean environment score was statistically higher in married cases ($p < 0.05$) (Table -2).

Table-2: Comparison of mean WHO QOL-BREF scoring and different study groups (Mean $\pm$ SD)

Groups	Physical Health Score	Psychological Health Score	Social Relationship Score	Environmental Score	Total WHOQOL-BREF Score
Age					
18-35 Years	23.32 $\pm$ 2.53	20.87 $\pm$ 2.07	10.61 $\pm$ 0.97	28.47 $\pm$ 1.90	83.26 $\pm$ 3.95
36-50 Years	22.58 $\pm$ 2.73	21.02 $\pm$ 2.11	10.47 $\pm$ 1.08	27.84 $\pm$ 2.07	81.90 $\pm$ 4.46
t-test	1.344	-0.343	0.640	1.537	1.543
p	0.182	0.733	0.523	0.127	0.126
Gender					
Male	22.93 $\pm$ 2.67	21.17 $\pm$ 2.11	10.44 $\pm$ 1.03	27.85 $\pm$ 1.98	82.39 $\pm$ 4.18
Female	22.81 $\pm$ 2.69	20.81 $\pm$ 2.07	10.44 $\pm$ 1.05	28.24 $\pm$ 2.05	82.44 $\pm$ 4.43
t-test	22.81	0.842	-0.648	28.24	82.44
p	0.836	0.402	0.519	0.353	0.954
Severity of migraine					
Mild-Moderate	22.35 $\pm$ 2.46	21.16 $\pm$ 2.07	10.59 $\pm$ 1.03	27.89 $\pm$ 2.08	81.98 $\pm$ 4.21
Severe	23.73 $\pm$ 2.82	20.62 $\pm$ 2.10	10.41 $\pm$ 1.07	28.41 $\pm$ 1.89	83.16 $\pm$ 4.43
t-test	-2.569	1.248	0.844	-1.239	-1.326
p	0.012*	0.215	0.401	0.218	0.188
Marital Status					
Married	22.82 $\pm$ 2.54	21.21 $\pm$ 2.04	10.29 $\pm$ 1.06	28.74 $\pm$ 1.93	83.06 $\pm$ 4.05
Unmarried	22.88 $\pm$ 2.75	20.83 $\pm$ 2.11	10.64 $\pm$ 1.02	27.74 $\pm$ 1.99	82.09 $\pm$ 4.43
t-test	-0.098	0.846	1.571	2.385	1.065
p	0.922	0.400	0.119	0.019*	0.289
Educational status					
<12 Years	22.36 $\pm$ 2.31	21.84 $\pm$ 2.06	10.60 $\pm$ 1.04	28.32 $\pm$ 2.04	83.12 $\pm$ 3.83
>12 Years	23.03 $\pm$ 2.77	20.67 $\pm$ 2.02	10.49 $\pm$ 1.04	28.00 $\pm$ 2.02	82.19 $\pm$ 4.45
t-test	1.083	2.502	0.443	0.685	0.938
p	0.281	0.014*	0.659	0.495	0.351

*Significant

DISCUSSION

Migraine is reported as a devastating neurological disorder that is presented as moderate-severe headaches, being unilateral and throbbing most often, accompanied by sensitivity to light and sound nausea, and vomiting. With chronic migraine (CM) and frequent attacks, the patients suffered significant effect on psychological, social, academic, and occupational domains of life. Migraine is undoubtedly reported as the principal cause of infirmity among neurological disorders, responsible for over one half of all the Years Lived with Disability (YLDs). The recurrent attacks are associated with noteworthy functional impairments, involving both physical and psychological health of patient during or after an immediate migraine attack.⁷⁻¹⁰ The severity of CM and incidence may increase over time, and attacks incline to occur more frequently for at least 15 days a month.¹¹ It has been tiered as the 3rd most common disease in the world in both males and females.

Commonly, they have poor well-being and very low health related quality of life (HRQOL) during the attacks and period following immediately after an attack a when compared with age and gender-matched healthy controls.¹¹ In comparative studies, migraine sufferers have worse personal well-being and QOL.¹²

Migraine is highly associated with numerous psychological disorders, including depression, severe anxiety, and bipolar affective disorders.¹⁰ Considerable evidence is available in literature that migraine can have serious implications on QOL, affecting patients to the extent that they can't perform activities of daily life easily. A study from Saudi Arabia showed Health Related Quality of Life lower than those of the general population and worsened consistently with increased migraine severity.¹¹ Migraine is often a lifelong neurological condition. A poorly documented part of chronic migraine's burden is that it not only affects physical health but also has effects on patients' daily

lives in terms of their interpersonal relationships, perceived QOL, emotional status and economic burden.^{13,14}

In our study the physical health score, the psychological health score, social relationship score, environmental score and total WHO-QOL BREF score had no significant differences in relation to both age groups which is not consistent with some other studies^{5,15}. Younger age group appeared to be significantly associated with lower QOL scores because of the younger people being more physically, and socially active and are involved in multiple work-related activities.¹⁶ The mean physical health score, psychological health score, social relationship score, environment score and total WHOQOL-BREF score in our study were statistically similar in both gender. These findings are not consistent with other studies where female predominance of CM was found 3:1.¹⁶ Estimates of prevalence of CM range from 1 to 3%.¹⁷ In another study, the prevalence of migraine was found to be higher in females.¹⁸ In another study in KSA, migraine was found to be more common in women, and in town areas, though it was related to high redundancy rates.

The third parameter of our study was severity of disease where significant differences were seen in physical health score, while psychological, social, environmental and total WHO-QOL BREF score were not statistically different. A comparative analysis of migraine with other chronic diseases like diabetes, hypertension, back pain, arthritis, anxiety and depression predicted that the effect of CM on HRQOL is comparable to the impact of depression, and even more severe than those of other above mentioned chronic disorders. The headache disorders are also commonly associated with impaired quality of life (QOL) across European countries.²⁰ Impact in a variety of domains including social relationship and environmental, both on migraineurs and on other people (their partners, colleagues and children) is quite high.²¹

The mean physical health score, social relationship score, environment score, and total WHOQOL-BREF score did not vary with education level. The mean psychological health score was higher in cases who had <12 years of education. These findings are not consistent with findings of a study⁶ on medical students and interns where incidence was higher who reported a drop in their educational performance and ability to concentrate and attend the classes during episode of migraine. However, our study has similarities with findings of another study²⁰ conducted to investigate the QOL and the level of migraine-associated disability. Significant number of patients reported negative impacts on educational attainment, the limitations to physical activity or temporary debility leading to decreased earning potential, workplace performance and careers.²¹ Lower scores had positive association with younger age

groups, prolonged disease duration, increased frequency of chronic migraine attacks, and co-morbid chronic illnesses.²²

The mean physical health score, psychological health score, social relationship scores and total WHOQOL-BREF score was statistically similar in married/single groups. The mean environment score was statistically higher in married cases. Some studies have reported that people with migraine often feel that CM lessens their supposed credibility and charge in the home, workplace as well as their interpersonal relationships and parenting capabilities.^{22,23} Many aspects of dealing with their family, colleagues and friends, social gatherings, leisure time events, performance and concentration on daily life activities at home or at work contribute to low QOL.²⁴ The migraineurs mostly conveyed being affected negatively for socialization, feeling energetic or frustrated, feelings of shyness, being a burden to others.²⁵

Assessment of QOL in migraine patients is essential in their headache management. The clinicians do not play ample role to discover the patients' QOL and its negative impact. Migraine is a chronic illness, where the attacks are unpredictable. Patients feeling restricted, try to limit their normal daily activities in order to prevent attacks, which eventually results in their low QOL.²⁶ There is substantial evidence available that medical care provided to CM sufferers is usually suboptimal. The majority of patients in this context are undertreated and not managed properly in other health related domains.^{27,28}

CONCLUSION

Chronic migraine patients have quite lower health related quality of life. The healthcare professionals should routinely assess this and related infirmity to determine whether these patients are receiving an effective and adequate treatment.

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ORIGINAL ARTICLE

PREVALENCE AND FACTORS ASSOCIATED WITH STRESS
AMONGST NURSES IN TERTIARY CARE HOSPITALS IN PESHAWARShakir Ullah Khan, Hamza Munir, Aman Ullah*, Nawabzada Khan**, Nisar Khan***,
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Background: Nursing is a challenging profession worldwide, frequently linked to significant stress levels stemming from various aspects of the healthcare setting. This study aimed to assess the prevalence of stress and identify its contributing factors among nurses employed in tertiary care hospitals in Peshawar, Pakistan. **Methods:** This cross-sectional study was conducted in Hayatabad Medical Complex (HMC) and Khyber Teaching Hospital (KTH), Peshawar. Participants meeting the inclusion criteria were invited to participate. Data were collected using a self-administered questionnaire adapted from the Spanish version of the Nursing Stress Scale. The data were analysed on SPSS-24. **Results:** The study involved 366 nurses with mean age 31.06 ± 6.54 years, 23.5% were male and 76.5% were female. A total of 56.0% of nurses indicated trouble with the Matron/Supervisor/Head Nurse, and 75.4% of nurses acknowledge that political influence by others can cause stress during duty. Furthermore, 60.7% of nurses indicated that they could not get holidays on their wish. Nearly 82.0% agreed that they experienced job stress, and 76.0% experienced stress personally. **Conclusion:** Stress was prevalent in nurses, with primary stressors identified as low salaries, political influence, supervision challenges, and inadequate holiday allowances.

Keywords: Occupational stress, Nurse, Job satisfaction, Workload, General health, Pakistan

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INTRODUCTION

Stress is how our body reacts to challenging situations, whether mental, physical, or emotional. Two key systems manage this reaction: the sympathetic nervous system, and the hypothalamus–pituitary–adrenocortical axis (HPA axis). Stress can come from internal sources, like personality traits, or external ones, like difficult situations. Research has identified three main types: acute (short-term), episodic (recurring), and chronic (long-term).¹ In contemporary organizational contexts, stress has emerged as one of the key determinants affecting individual efficacy and satisfaction; stress has had an impact on both our physical and mental health.²

Changes in occupational activities, job-related variables, or location can cause job stress³, can lead to behavioural, emotional, and physical problems, various malignancies, immune system problems, musculoskeletal and cardiovascular issues, as well as increased risk of accidents and injuries among the physical repercussions. Work absenteeism, smoking, sleep issues, alcohol and drug abuse, and addiction are all behavioural effects of occupational stress.⁴ The following traits of occupational stress apply to it: it is persistent, challenging to adjust to, frequently resulting in emotional swings, and challenging to modify with the demands of the working environment.⁵

Nursing professionals experience a variety of work-related stressors; some have to do with personality, while others have to do with the job, organizational structure, or customer service.⁶ This

environment is also plagued by complex interpersonal relationships with the multi-professional team, daily contact with suffering, high-performance standards for the team, and worries about patient safety. There is a lack of understanding and autonomy, and this environment is devalued.⁷ The nursing field is renowned for its challenging working conditions, demanding workloads, and generally low socioeconomic level.⁸ Studies show that stress in nurses can lead to depression, patient isolation, absence, and a decline in their qualifications.⁹ The literature has extensively researched nursing job performance since it indicates the calibre of care provided and, as a result, patient outcomes and satisfaction. Examining occupational stress and its related causes is one of the crucial steps that hospitals should do to enhance working conditions and reduce the work stress of nurses.¹⁰

Stress in nursing profession also contributes to absenteeism and high turnover rates.¹¹ Nurses frequently encounter occupational stress, are exposed to psychological abuse at work, and develop burnout and secondary traumatic stress.¹² Data collected from different healthcare systems and different cultures such as USA, Europe, and Asia, indicates that job stress tends to affect nursing profession worldwide.¹³ A concern of global health is the incidence of occupational stress among nursing professions.¹⁴ Since nursing work performance reflects the standard of care delivered and, consequently, patient outcomes and satisfaction, the field of nursing has undergone substantial research. One

of the key actions that hospitals should do is to improve working conditions and decrease workload of nurses and associated factors. Pakistan is one of the 57 nations with the worst labour shortages, prevalence of stressed among nurses are highly contextual which varies from organization, hence needed to be addressed at local level for necessary solution.¹⁵ According to the Joint Learning Report from 2004 and the World Health Report from 2006, Pakistan is one of the 57 countries with the most serious workforce shortages. Nurses who are under stress tend to disregard the law and, as a result, warning indicators.¹⁶ It harms people's bodies, minds, spirits, relationships, and cultures, which has myriad negative effects on people and their families. The aim of current study was to find out the prevalence and the associated factors with the stress among nurses working in teaching hospitals in Peshawar.

METHODOLOGY

This descriptive cross-sectional study was carried out at two tertiary care hospitals in Peshawar, i.e., Hayatabad Medical Complex (HMC) and Khyber Teaching Hospital (KTH) from 1st January 2022 to 30th June 2022. A total of 366 nurses were selected through convenience sampling technique. Nurses working and available for data collection in the hospital were included in the study, while nurses who were not willing to participate were excluded. Participants who met the inclusion criteria were invited to participate in the study after receiving approval from the BOSAR Committee. All willing study participants were required to sign a written informed consent. Data were collected through a self-administered, pretested, validated, structured questionnaire. The questionnaire was translated into Urdu. It included three parts. The first part of the questionnaire measured the demographic information of the nurses. The second part of the questionnaire was a brief Nursing Stress Scale used to assess stressors in nursing. The third part consisted of questions about the factors which can cause stress in the study subjects.

Analysis was conducted using SPSS-24. For each survey question, frequency analysis was performed. Frequencies and percentages were calculated for all categorical variables, while means and standard deviations were calculated for all continuous variables.

RESULTS

The mean age of all enrolled 366 nurses was 31.06±6.54 years. Out of the total, 86 (23.5%) were male and 280 (76.5%) were female, 177 (48.4%) were married, 125 (34.2%) were unmarried, 47 (12.8%) were ever married, and 17 (4.6%) were if ever married. (Table-1).

We also examined the core stress indicators in nurses during the job. Nine percent of nurses indicated something unexpected happened, 33.9% revealed their inability to control important tasks, and approximately 28% stated nervousness during their jobs (Table-2).

Many nurses report experiencing challenges and stressors in their work environment. Specifically, a majority (56.0%) indicated they were facing trouble with the Matron/Supervisor/Head Nurse, while slightly over half (54.4%) felt insecure. A significant number of nurses (75.4%) acknowledge that political influence by others can cause stress in their duties, and 71.6% attribute stress to management influence. A notable portion (60.7%) indicated they could not take holidays as they wished. The overwhelming majority (82.0%) agree that most nurses feel stress during their job, and a substantial portion (76.0%) personally experience stress as nurses (Table-3).

Table-1: Demographic characteristics (n=366)

Characteristic	Frequency (n)	Percentage (%)
Gender		
Male	86	23.5
Female	280	76.5
Marital Status		
Married	177	48.4
Unmarried	125	34.2
Ever Married	47	12.8
If Ever Married	17	4.6
Education Level		
MS	108	29.5
BS	119	32.5
BSc	91	24.9
Diploma	48	13.1
Experience		
<1 Year	14	3.8
1-5 Years	277	75.7
6-10 Years	66	18.0
11-15 Years	9	2.5

Table-2. Core indicators of perceived level of stress among nurses working in selected hospitals [n (%)]

Question	Never	Almost	Sometimes	Fairly often	Very often
In the recent past, how often have you been upset because of something that happened unexpectedly?	119 (32.5)	72 (19.7)	95 (26.0)	47 (12.8)	33 (9.0)
In the recent past, how often have you felt that you could not control the important tasks in your life?	55 (15.0)	69 (18.9)	124 (33.9)	71 (19.4)	47 (12.8)
In the recent past, how often have you felt nervous and stressed?	39 (10.7)	50 (13.7)	88 (24.0)	101 (27.6)	88 (24.0)
In the recent past, how often have you felt confident about your ability to handle your problems?	49 (13.4)	41 (11.2)	118 (32.2)	86 (23.5)	72 (19.7)
In the recent past, how often have you felt that things were going your way?	65 (17.8)	66 (18.0)	121 (33.1)	70 (19.1)	44 (12.0)
In the recent past, how often have you found that you could not cope with	85 (23.2)	75 (20.5)	94 (25.7)	50 (13.7)	62 (16.9)
All the things that you had to do?	88 (24.0)	57 (15.6)	90 (24.6)	82 (22.4)	49 (13.4)

Table-3: Factors that cause stress in nurses [n (%)]

Question	Yes	No
Are you having trouble with the Matron/Supervisor/Head nurse?	205 (56.0)	161 (44.0)
Do you feel secure at your job?	199 (54.4)	167 (45.6)
Can political influence by others cause stress in your duty?	276 (75.4)	90 (24.6)
Does management influence stress in your duty?	262 (71.6)	104 (28.4)
Do you take your holidays as you wish?	144 (39.3)	222 (60.7)
Do you agree that most nurses feel stressed during their jobs?	300 (82.0)	66 (18.0)
As a Nurse, do you feel stressed?	278 (76.0)	88 (24.0)

DISCUSSION

Within the ever-evolving healthcare landscape, nurses serve as the cornerstone of patient care, playing a pivotal role in the healthcare system. Nonetheless, the demanding nature of their profession frequently subjects them to elevated levels of stress, posing significant implications not only for their personal well-being. It is also for the calibre of patient care they deliver.¹⁷ By pinpointing particular stressors and comprehending their effects on nurses' welfare and professional efficacy, healthcare administrators and policymakers can formulate tailored interventions to alleviate stress and foster a more conducive work environment for nurses.

In our study findings, it emerged that 'Perceived stress' exhibited the highest prevalence of respondents reporting elevated levels of stress. This indicates that a significant proportion of participants in our study reported experiencing stress 'Fairly often' or 'Very often' within the context of 'Perceived stress 3'. As a result, 'Perceived stress 3' emerged as the figure indicating the highest stress level among those examined in our study. In a study¹⁸ it was found that the majority (81%) of participants, agreed that nurses experience stress during their job. The remaining indicated that they did not feel stressed while performing their job duties. In another study carried out in India, 49% nurses indicated experiencing frequent stress due to uncertainties regarding treatment protocols, and 48% of nurses reported frequent stress attributed to patient interactions, while the majority, accounting for 59%, identified workload as the primary cause of stress.¹⁹

In the present study the marital status of nurses was examined, revealing that 177 (48.4%) were currently married, 125 (34.2%) were unmarried, 47 (12.8%) had previously been married, and 17 (4.6%) had a history of past marriages. This distribution highlights the diversity of marital statuses within the nursing demographic under investigation. For many married nurses, balancing the demands of their profession with family responsibilities can be stressful. Nurses often work long hours and irregular shifts and face emotionally taxing situations, all of which can contribute to stress.

Adding family obligations on top of this can create additional pressure. Being married can also provide sources of support and resilience in dealing with stress.²⁰ A supportive spouse can offer understanding,

encouragement, and practical assistance, which can help alleviate some of the stress associated with the nursing profession. Marriage can introduce stressors unrelated to work, such as financial concerns, relationship conflicts, or parenting responsibilities. These additional stressors can compound the challenges faced by married nurses.²¹

Many nurses report experiencing challenges and stressors in their work environment. Specifically, a majority indicated they were facing trouble with the Matron/Supervisor/Head Nurse while slightly over half felt insecure. This suggests potential issues in leadership and management within healthcare settings. This aligns with previous research indicating that poor leadership and communication can contribute to nurse dissatisfaction and stress.²² The high percentage of nurses feeling insecure in their jobs underscores the importance of providing a supportive and stable work environment to foster nurse well-being and job satisfaction.²³

A significant number of nurses acknowledged that political influence by others can cause stress in their duties, and a similar percentage attribute stress to management influences. This emphasizes the need for effective organizational policies and practices to mitigate external pressures and promote a positive work culture.²⁴ Labrague *et al*²⁵ showed that nurses perceive a high level of political influence, including organizational politics in pay scales and promotion.²⁵ A notable portion indicated they could not take holidays as they wished. The inability raises concerns about work-life balance and the potential impact on nurse burnout and overall job satisfaction.²⁶

An overwhelming majority agree that most nurses feel stress during their jobs, and a substantial portion personally experience stress as nurses, underscoring the pervasive nature of stress within the nursing profession. This highlights the urgent need for targeted interventions and support mechanisms to address stressors and promote nurses' mental health and well-being.¹⁸

Our results contradict the results of Hoedl *et al*²⁷ who stated that the nurses experience mild stress due to increased working hours during COVID-19 pandemic. Our findings highlight the prevalence of challenges and stressors the nurses face in their work environment, and shed light on several key areas of concern.

CONCLUSION

Stress is widespread among nurses in Peshawar's tertiary care hospitals, with inadequate salaries, political interference, supervisory issues, and insufficient leave allowances being major contributing factors. Tackling these stressors is essential to enhancing nurses' well-being and improving the quality of patient care.

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ORIGINAL ARTICLE

EXHUMATION: A GATEWAY TO THE HIDDEN JUSTICE, FORENSIC AND PHYSIOLOGICAL TALE TO THE TRUTH

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Background: Exhumation is important in medico-legal investigations and is under debate since long for its usefulness. Critics also try to relate these with the religion and label it as destroying the human dead body as this is a belief that we don't achieve desired results by exhumation. This study was carried out to assess the usefulness of exhumation and its outcome in our set-up and circumstances. **Methods:** This retrospective study was conducted in Ayub Teaching Hospital and supervised cases of Benazir Bhutto Shaheed Teaching Hospital, Abbottabad. Total 27 cases were studied in a 2 year time. Medico-legal aspects were studied keeping in view the physiological process in the body and anatomical destruction after death. **Results:** We were able to detect cause of death in 53% cases (23% burning, 19% firearm, 4% drowning, and 7% cut-throat cases); 33% in 0–20 years, 45% in age group 20–40 years, 8% in 40–60 years, and 14% in >60 years. Major reason for doing exhumation was to find out the cause of death (in 47% cases), followed by identity and not doing initial post mortem in 19% cases, and re-autopsy in 15% of cases. **Conclusion:** Exhumation should be ordered and conducted as it helps the investigators in determining the reasons behind the death and solve the mystery in determining the cause of death after doing this procedure and aid in administration of justice.

Keywords: Exhumation, Section 176 CrPc, Re-post mortem, Forensic, Physiology, Anatomy

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INTRODUCTION

Exhumation is the process of disinterment of dead body once it is buried.¹ Exhumation is performed to find out the cause of death, to establish personal identity, shifting the body from one place to other, in cases of suspicious circumstances, or cases when autopsy is challenged and it is conducted as re-autopsy/2nd autopsy and is carried out under the rules of the land throughout the world.²

Changes that affect the body after death are a result of complex physiochemical and environmental processes. The post mortem changes can be used to determine the time since death (TSD) or post mortem interval (PMI). PMI is essential aspect of medico-legal investigations as it helps in determining the facts when a crime had been committed. These changes are affected by the factors within the cadaver and outside it. These factors affect the onset and either increases the rate of post mortem changes or retard it. Factors that accelerate the rate of post mortem changes include hot and humid climate, presence of body fat, open injuries on the body, sepsis or infection, and location of the cadaver in open.³ Different countries have different rules for the time period of exhumation with limits of performing autopsies. In Pakistan and India it is executed under section 176 CrPc^{4,5} reproduced below:

"1) Inquiry by Magistrate into cause of death.

2) Power to disinter corpses: 'Whenever such Magistrate considers it expedient to make an examination of the dead body of any person who has been already interred, in order to

discover the cause of his death, the Magistrate may cause the body to be disinterred and examined.'

In Pakistan there is no time limit for the exhumation. There has been constant debate about the timing of the exhumation in Pakistan reasoning that there is no benefit of doing exhumation after some time limit. It is also argued by giving the example of limitation act that if there is no time limit the time period should be fixed for three years. In other concerned cases it has been argued that there is no benefit of doing this exercise after so much time elapsed. But the courts and the legislature are not accepting this as in majority of cases and as per Pakistani law there is no time limit for exhumation.

For exhumation logistics and necessary equipment and material especially for taking relevant samples for toxicological purposes and other investigations are required.⁶ It is a team work starting from the judicial involvement regarding the decision taking for conducting exhumation where concerned magistrate has the sole responsibility for identifying and permitting the exhumation of the grave. Then comes the police with responsibility of security and cordoning-off the grave. The municipal officials are involved for opening the grave and transferring the dead body. Most importantly are doctors, morticians, paramedics, technicians and the other concerned staff. Moreover all necessary equipment and the sampling items should be accompanied keeping in mind radiological findings, help from dentistry, anatomy input wherever needed.⁷⁻⁹

Permission of exhumation is a debatable topic whether we should adopt this procedure or not. As per existing rules mere suspicion of the foul play is enough to open the grave and initiating the order of exhumation.

Exhumation is the comprehensive process to know cause of death, time since death, individuality of person and other reasons varying from country to country.¹⁰ This study was carried out to assess the usefulness of exhumation and its outcome in our set-up and circumstances.

MATERIAL AND METHODS

This study was conducted from Aug 2021 to Aug 2023 and a total of 27 cases were studied in Ayub Teaching Hospital and Benazir Bhutto Shaheed Hospital, Abbottabad under supervision by Standing Medical Board of Ayub Medical College. Permission was taken from the hospital and the concerned authorities regarding ethical approval of the study. Data was collected on a performa specially designed considering initial inquest/investigations by police, autopsy/exhumation document, demographic data, motive/purpose behind the exhumation, conflict with police report, conclusion of exhumation, court proceedings and other significant findings.

Data was analysed on Microsoft Excel and tabulated as frequency and percentage.

RESULTS

All bodies that were exhumed were in advanced state of putrefaction and physiological process of death were in process with 5 bodies in skeletonized state and 6 bodies at varying degree of putrefaction. In majority of the cases (22 cases out of 27 cases), time elapsed in exhumation was between 1 to 5 months, while the remaining 5 cases were disinterred after 6 months.

It was noted that wherever the bodies were exposed to water the process of putrefaction was in advanced stage. Other significant findings are tabulated below. Majority (14 out of 27) of cases showed the manner of death as homicidal. The male to female ratio was 1:0.8.

Nine victims (33%) bodies were aged 0–20 years, followed by 12 (45%) in age group 20–40 years, 2 (8%) were in age group 40–60 years, 4 (14%) cases were ≥60 years. (Table-1).

In 19% of the cases reason for exhumation was that initial post-mortem was not done! Fifteen percent cases were of re-autopsy, 19% exhumations were done for determination of identity, and 13% were done to explore the cause of death. (Table-2).

In cases where positive findings could be collected, major cause of death was burns followed by the firearm injuries, cut throat and drowning. In 13 (47%) cases the cause of death could not be ascertained due to various reasons. (Table-3).

Table-1: Age stratification of the victims

Age in years	Cases	Percentage
0–20	9	33
20–40	12	45
40–60	2	8
>60	4	14

Table-2: Purpose/reasons for exhumation

Reason	Cases	Percentage
Initial Post-mortem not done	5	19
Re autopsy	4	15
Identity of the victim	5	19
To know cause of death	13	47

Table-3: Ascertained cause of death

Determined cause of death	Cases	Percentage
Burns	6	23
Fire Arm Injury	5	19
Cut Throat	2	7
Drowning	1	4
Un-identified	13	47

DISCUSSION

The process of exhumation, which involves the legal disinterment of a body, is generally avoided unless necessary, and it is usually carried out under specific legal frameworks, such as Section 176 of the Code of Criminal Procedure. This section allows for exhumation when there is a requirement to investigate suspicious deaths, especially when foul play is suspected or when the initial post-mortem is deemed inconclusive or unsatisfactory.

In many cases, a thorough and well-conducted initial post-mortem can prevent the need for exhumation. However, if new evidence or suspicions of foul play arise after the burial, or if the first autopsy results are questioned, exhumation may become necessary to re-examine the body for additional evidence. The decision to exhume is typically guided by legal authorities and is based on the specific circumstances of the case, taking into account both the medical and legal necessity.

Our study analysed 27 cases of exhumation, most of the victims fell in the age group 20–40 years. This aligns with findings by Maqsood *et al*¹, Mughal MI *et al*², and Hamayun *et al*¹⁰ who observed average ages of 32, 15, and 21 years, respectively.

Bardale³ identified the cause of death in 16 cases (66.6%), which is comparable to Grellner *et al*⁴, where the cause of death was confirmed in 78% cases. This is also consistent with Humayun's study¹⁰ which showed 42.83% positive determination and 57.14% being inconclusive. Verhoff *et al*⁵, provided recommendations for compensation in medical insurance cases with a success rate of 99.2%. The cause of death determination is the most important step in exhumation and for the investigators as all investigations and trial is limited to this aspect where, for one reason or another, initial investigations failed to do that with the suspicion of foul play. In majority of cases initially there is no suspicion of homicide and

body is buried without autopsy and later on circumstances give suspicion and for confirmation of suspicion of foul play this procedure is performed.

In majority of our cases the time elapsed was between 1 and 5 months, similar to findings of Ammani *et al*⁶ where 70% of exhumations were carried out in 1 to 6 months. Maqsood *et al*¹ concluded that time interval between burial and exhumation was 1 to 6 months while results of Mughal² showed this time period to be 6 months. Climatic changes, soil condition, body temperature, and cause of death influence the body in the grave. We found that skeletonized bodies in the majority of the cases showed advanced stage of putrefaction. Hamayun *et al*⁷ also mentioned the importance of various factors on the condition of the body found in the grave. It is worth mentioning that the time elapsed is one of the biggest limitation in conducting the autopsy but fractures, firearm marks on the bones may even be appreciated on the body after putrefaction of soft tissues.

Deaths were homicidal in nature where cause of death was determined in 53% cases. It is similar to findings of Bardale *et al*³, and Suresh *et al*⁸. It is especially important that many cases of foul play are homicidal in nature and it is still possible to get the results by exhumation and conclude them. Albano *et al*⁹ and Willeman *et al*¹⁰ also studied the homicidal pattern of death in about 64% of the cases and argued in favour of doing the exhumation whenever needed. Ueland *et al*¹¹ reported multiple stabbing, and they differentiated homicidal wound from suicidal wound and solved the difficult cases by exhumation. Rani M *et al*¹² identified a victim of homicide on exhumation and identification of the personal belongings recovered with/on the body after two years of missing. This shows importance of exhumation in establishing the identity of the victim.

On many occasions even the body is initially gone through the process of autopsy and failed to label it homicidal mainly due to casual approach of examiner, limited resources of investigations, lack of medico-legal knowledge though there is positive cause of death due to homicide. In these cases exhumation is very important that completes investigation and solves mystery of death in unusual circumstances.

CONCLUSION

Despite the limitations of the exhumation process, it can still yield crucial results and help solve important mysteries regarding the cause of death that might otherwise remain unresolved. Performing an initial post mortem can often eliminate the need for exhumation. However, exhumation remains vital for ensuring justice and uncovering the truth.

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ORIGINAL ARTICLE

EMPATHY DEVELOPMENT IN UNDERGRADUATE MEDICAL STUDENTS: COMPARATIVE STUDY BETWEEN HEALTH EMPATHY MAPS AND NARRATIVE MEDICINE

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Background: Empathy is a crucial skill in medical education, enhancing patient care and physician well-being. The aim of the study was to compare the effectiveness of Health empathy maps and Narrative medicine exercises to foster empathy in undergraduate medical students.

Methods: This experimental study was carried out in a private medical college of Rawalpindi for a period of 6 months where 130 medical students were recruited and empathy scores were calculated by Jefferson scale of physician empathy student version. Participants were randomly allocated into two groups: Group 1 practiced the HEM after attending relevant workshops, whereas Group 2 wrote patient interaction-based narratives after attending the relevant workshops. CONSORT guidelines (2010) were maintained for research protocol. The post test scores were compared with pretest scores. Unpaired t test was used to compare the data between the two groups as the data was normally distributed (using Shapiro Wilk test). **Results:** The students demonstrated improved scores on Jefferson Scale of Physician Empathy (JSPE-S), assessing various facets of empathy. The Empathy Mapping group had high mean score 107.80 ± 12.72 as compared to Narrative Medicine group (101.51 ± 16.49). Both the groups had statistically significant difference as $p=0.016$. The resultant better technique proved to be the empathy maps.

Conclusion: Our findings suggested that empathy maps prove to be an efficient way of fostering empathy in medical students. This strategy would benefit the patients in terms of treatment compliance and the doctors by reducing their stress and burnout.

Keywords: Empathy, Health empathy maps, Narrative medicine

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INTRODUCTION

Person centred medicine is emphasized as an innovative approach in health care.¹ According to the Institute of Medicine, person-centred care is ‘care that is respectful and responsive to individual patient preferences, needs and values, and ensuring that patient values guide all clinical decisions’.

Empathy is a milestone in person centred medicine. Clinical empathy can be defined as understanding patient’s circumstances, perspective and emotions, moreover interpreting their thoughts and feelings.² Nurturing empathy in healthcare professionals contributes significantly to improved patient outcomes that is why evaluating the effectiveness of teaching practices in diverse cultural contexts is essential, as the concept of professionalism varies across cultures.³

As regards health professionals the most influential understanding of empathy can be attributed to the work of Carl Rogers, a renowned psychotherapist known for his groundbreaking contributions to the therapeutic bond between clients and therapists. His client-centred approach, which aligned with the patient-centred approach, played a pivotal role in shaping our comprehension of empathy in the context of therapy.⁴

According to Morse *et al*, empathy has four fundamental constructs.⁵ Emotive empathy is experiencing and sharing in another’s psychological state and emotions. Moral empathy is the inner altruistic guide that serves as a motivation for practicing empathy, leading individuals to consider the welfare of others. Cognitive empathy is to objectively identify and understand another individual’s emotions and perspective without necessarily sharing the same emotional experience. Behavioural empathy is delivering the insight of another’s perspective through improved communication.

Empathy is a multidimensional construct which has to be developed and assessed in different ways.⁶ That is the reason behind the constant improvement of empathy assessment indices currently in use.⁷ Development of an empathic physician requires nurturing attitude, competency and behaviour. The competencies to be achieved are empathetic skills which could include being a good listener, communication skills and skills to build a trustful relationship.⁸

According to researchers’ empathy can be considered as a multidimensional construct.⁹ The four key areas, emotive, moral, cognitive and behavioural,

can be analysed through concept analysis of health empathy maps filled by the students after patient encounters. The basic rationale to conduct the experiment is to compare teaching strategies devised to inculcate empathy in undergraduate medical students and to analyse their effectiveness in Asian cultural context. Enhancing the empathetic skills and teaching strategies can have positive role on patient safety. Exhibiting empathy can be different in eastern and western diaspora. Lack of resources and strenuous workload are considered the key inhibiting factors in displaying empathy and compassion.¹⁰

Empathy maps are addressing the constructs of empathy triad thus it can prove to be a cost effective and a structured way of interviewing patients in a busy hospital setting.

METHODOLOGY

The study was conducted after the approval from the Ethical review committee, Foundation University Medical College, letter No. FF/FUMC/215-280-1 Phy/23. A randomized control trial was conducted for 6 months from Feb to Jul 2023 after approval of synopsis, using a parallel study design.

Sample size was calculated as 128 using the G Power Sample size calculator. 3rd year MBBS students were enrolled in the current academic session. The students were selected to attend the workshops designed after the literature review, and provided written informed consent.

Simple randomized sampling was done to tackle the confounders affecting the results. It eliminates the bias as every student has an equal chance of participation in either intervention.¹¹ Randomization was achieved through a lottery method to ensure equal probability of assignment to either intervention group. After confirming participants' eligibility based on the inclusion criteria and giving an informed consent, each participant was assigned a code. These codes were then placed into a container, and a neutral third party drew the codes randomly to assign participants to either Group 1 (HEM) or Group 2 (Narrative Medicine). This process ensured that neither the participants nor the researcher had prior knowledge of group allocation, maintaining blinding and minimizing selection bias.

There were two groups with 65 participants in each group. An intervention was introduced in both groups. Autonomy was maintained by allowing the students to have full right to refuse from participating or if at any time they want to withdraw from the study they are allowed to do so. The confidentiality was maintained throughout the data collection process as it was collected through Google forms and correspondence was through emails.

A pilot test involving 10 students was conducted to assess the feasibility, appropriateness, and

accuracy of the validated tool Jefferson's Scale of Physician Empathy (Student version) (JSPE-S). Feedback from the students revealed challenges in understanding two questions in the sociodemographic questionnaire, prompting the need to rephrase those questions for improved comprehension. Additionally, the students were requested to engage in the interventions and reported that the instruments were user-friendly and practical. This pre-testing helped in the overall enhancement of the clarity and reproducibility of the methodology.¹²

The workshops were designed based on a thorough literature review highlighting the importance of communication skills and understanding nonverbal cues for empathy development. Both interventions incorporated experiential learning, a proven method to foster empathy in healthcare professionals.¹³ A true experimental design was employed to compare the efficacy of two approaches—Health Empathy Map (HEM) training and narrative medicine exercises—in enhancing empathy among 3rd year MBBS students.¹⁴ There were reasons identified in various researches and this year was chosen due to previous literature highlighting a decline in empathy among medical students during the transition to the clinical cycle.¹⁵

The study included two phases. In Phase 1, all participants completed a baseline questionnaire that included socio-demographic items and the Jefferson Empathy Scale (student version). In Phase 2, participants were randomly divided into two groups, with both groups attending workshops focused on communication skills and the recognition of verbal and nonverbal cues. The only difference between the interventions was the specific training provided:

Group 1 (G1): Students were introduced to the Health Empathy Map (HEM), a tool designed to develop empathy by exploring patients' emotions, needs, and perspectives. They practiced completing the HEM during patient interactions.

Group 2 (G2): Students were trained in narrative medicine, which involved writing reflective narratives based on their patient interactions without using a structured tool like the HEM.

At the end of the three-week discipline, all participants completed the Jefferson Empathy Scale (student version) again to measure any changes in empathy levels. CONSORT guidelines were followed to ensure methodological rigor, and all collected data—questionnaires, maps, and narratives—were anonymised using participant registration numbers.

The Health Empathy Map (HEM) consists of four quadrants corresponding to the core components of empathy: perspective-taking, emotional sharing, and empathetic concern.¹⁶ Each quadrant was associated with specific prompts:

1. 'What would you feel if you were in this person's shoes?'
2. 'What is your perception of this person's needs and desires, both current and future?'
3. 'How do I feel knowing this person's history?'
4. 'How can I help this person?'

At the centre of the map, students placed an emoji reflecting the patient's emotions based on their interpretation.

Narrative medicine emphasizes storytelling as a means to enhance healthcare professionals' understanding of illness. In this study, students were guided to write narratives reflecting on their patient interactions. Unlike the HEM, narrative medicine exercises were unstructured and relied on students' individual reflections without predefined prompts.

This design allowed a direct comparison between the structured approach of the HEM and the open-ended reflective process of narrative medicine, while maintaining consistency in the foundational content of the workshops.

At the start of the study, we filled out the socio-demographic questionnaires to take into account the confounding variables. After the first intervention there was a cross-over design and the participants were subsequently subjected to the second intervention.¹⁷

Social desirability bias, defined as the tendency to provide answers that portray one self positively, was minimized by ensuring complete confidentiality for participants, including students and patients. Responses were anonymised through coded data collection and handled discreetly to reduce the likelihood of altered answers influenced by social expectations.¹⁸

There is risk of contamination after the workshops because the students might discuss their interventions. The participants were blinded to the specific teaching strategy being used by designing the workshops in a way that were similar in all aspects including the training on communication skills and interviewing skills. The strategies were implemented in separate clinical wards. The data was also analyzed for signs of contamination bias.

RESULTS

Collected data was processed through SPSS-26, using standard protocol analysis. Normality of data was checked by Shapiro-Wilk test. The data was found to be normally distributed. Baseline variables were analysed descriptively using frequencies and percentages for qualitative variables like gender, chronic illness, medico-legal encounter and mean with standard deviation for continuous variables like age and Pre and Post Jefferson Scale of Physician Empathy (JSPE-S) scores. Statistically significant differences in JSPE-S scores before and after intervention were

assessed in both the groups using Paired sample *t*-test for HEM group and narrative medicine group. The independent *t*-test was used for the comparison of empathy mapping and narrative medicine groups, and $p \leq 0.05$ was considered significant.

A total of one hundred and fifty-eight (158) medical students were included, twenty-eight (28) did not complete the follow up then excluded from the analysis, so data of 130 medical students were included in the final analysis. Out of 130 students, 35 (26.9%) students were male and 95 (73.1%) were females. Mean age of the students was 21.16 ± 0.97 years range from 19 to 25 years. Of the total, 15 (11.5%) had chronic illness and 115 (88.5%) did not suffer from any chronic ailment. However, 44 (33.8%) students had family history of chronic illness and 86 (66.2%) students did not have family history of chronic illness. Eleven (8.5%) students had medico-legal encounter and 119 (91.5%) did not have medico-legal encounter.

The Jefferson scale of empathy was used to compare the effectiveness on the changes in the empathy of the undergraduate medical students. The JSPE-S consists of 20 items on a 7-point Likert scale (1=Strongly disagree, 2=Slightly disagree, 3=Disagree, 4=Neutral, 5=Agree, 6=Slightly agree, 7=Strongly agree). The Cronbach Alpha of our study was 0.852 which shows that it is a validated tool for our study population.

After the pre-test (before the intervention) the students were divided into two groups. The first group was subjected to empathy mapping workshop and the second group to narrative medicine workshop. Both groups had improvement after intervention. (Table-1).

The mean overall empathy level across all the data points was 60.53 ± 10.69 range from 33.00 to 86.00 for pre-test (before intervention) and 104.65 ± 15.01 range from 63.00 to 132.00 for post-test. There were statistically significant differences between pre and post intervention (overall) ($p < 0.001$).

Group 1 (empathy mapping) and Group 2 (narrative medicine) showed significant differences among pre and post intervention ($p < 0.001$). Table-2 shows a comparison of the mean scores of narrative medicines as compared to empathy mapping.

Empathy is a multidimensional construct and Jefferson scale of empathy questionnaire corresponds to three parts, Perspective Taking, Compassionate care and Standing in the Patient's shoes. The first part Perspective taking corresponds with these 10 questions (2, 4, 9, 20, 13, 16, 10, 5, 17, 15), the second part Compassionate care encloses 8 questions (11, 7, 12, 19, 1, 8, 14, 18) and the third part Standing in the Patient's shoes holds only two question (3, 6). All the parts were improved after the intervention with high significant $p < 0.001$. (Figure-1).

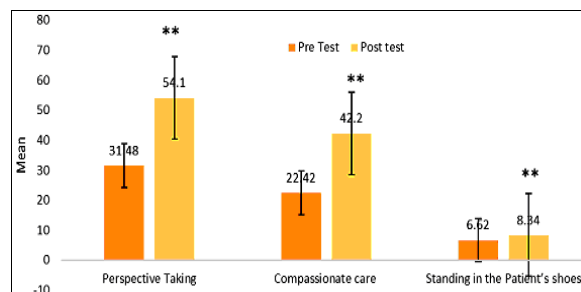
Table-1: Individual item responses on the JSPE-S for both pre and post intervention

No.	Question	Empathy Mapping		Narrative Medicine	
		Pre-Test	Post-Test	Pre-Test	Post-Test
1.	Healthcare providers' understanding of their patients' feelings and the feelings of their patients' families does not influence treatment outcomes	2.94±1.82	5.51±1.42	2.80±1.76	5.14±1.90
2.	Patients feel better when their healthcare providers understand their feelings	3.02±2.04	3.71±1.99	3.38±2.21	5.32±1.82
3.	It is difficult for a healthcare provider to view things from patients' perspectives	2.77±1.54	3.45±1.46	3.42±1.37	4.28±1.70
4.	Understanding body language is as important as verbal communication in healthcare provider-patient relationships	3.85±2.79	5.85±1.41	3.11±2.21	6.08±1.16
5.	A healthcare provider's sense of humour contributes to a better clinical outcome	2.60±1.51	4.80±13.8	3.12±1.99	5.28±1.12
6.	Because people are different, it is difficult to see things from patients' perspectives	3.45±1.83	4.34±1.34	3.62±1.63	4.63±1.25
7.	Attention to patients' emotions is not important in-patient interview	2.48±1.65	5.58±1.28	2.34±1.20	5.54±1.51
8.	Attentiveness of patients' personal experiences does not influence treatment outcomes	3.12±1.58	5.03±1.34	3.14±1.28	5.11±1.33
9.	Health care providers should try to stand in their patients' shoes when providing care to them	2.60±1.28	5.34±1.52	2.71±1.28	5.49±1.34
10.	Patients value a healthcare provider's understanding of their feelings which is therapeutic in its own right	2.68±1.87	5.37±1.51	2.37±1.02	5.54±1.34
11.	Patients' illnesses can be cured only by targeted treatment; therefore, healthcare providers' emotional ties with their patients do not have a significant influence in treatment outcomes	3.29±1.62	5.06±1.51	3.12±1.25	5.08±1.44
12.	Asking patients about what is happening in their personal lives is not helpful in understanding their physical complaints	2.86±1.44	5.02±1.35	2.78±1.15	5.18±1.58
13.	Healthcare providers should try to understand what is going on in their patients' minds by paying attention to their non-verbal cues and body language	2.62±1.62	5.48±1.38	4.75±1.93	5.68±1.18
14.	I believe that emotion has no place in the treatment of medical illness	2.45±1.39	5.68±1.22	2.60±1.27	5.65±1.32
15.	Empathy is a therapeutic skill without which a healthcare providers' success is limited	2.26±1.30	5.32±1.82	2.37±1.26	5.82±1.19
16.	Healthcare providers' understanding of the emotional status of their patients, as well as that of their families is one important component of the healthcare provider – patient relationship	5.46±1.23	5.37±1.38	5.62±0.94	5.86±1.15
17.	Healthcare providers should try to think like their patients in order to render better care	2.95±1.28	5.03±1.59	3.35±1.35	5.35±1.41
18.	Healthcare providers should not allow themselves to be influenced by strong personal bonds between patients and their family members	2.97±1.47	4.74±1.48	3.05±1.42	5.28±1.38
19.	I do not enjoy reading non-medical literature or the arts	2.86±1.71	5.23±1.56	2.23±1.37	5.60±1.48
20.	I believe that empathy is an important factor in patients' treatment	2.26±1.31	5.62±1.51	1.89±1.06	5.91±1.23

Table-2: Comparison of pre and post-test with respect to empathy mapping and narrative medicine

Study Parameters	Pre-Test	Post-test	p
Empathy Mapping	61.76±10.82	107.80±12.72	<0.001**
Narrative Medicine	59.29±10.50	101.51±16.49	<0.001**

Paired t-test, **highly significant


Figure-1: Comparison of pre and post mean response for constructs of empathy

DISCUSSION

The main aim of the study was to compare the effectiveness of empathy maps and narrative medicine to develop empathy in undergraduate medical students. This experimental design considered the challenges faced by the physicians working in a condition of high workload, stressful conditions and underdeveloped health care settings.¹⁹ The study provides evidence that providing framework to medical humanities would help in shaping the future physicians and nurture empathy in them.

Empathy maps have been used in business models to develop the personas of clients and to better understand the needs and desires of the users.²⁰ In another study empathy maps have been used to identify the professional needs in an organization in nursing profession.²¹ There has been a slight modification to develop health empathy maps and have proven to be effective to inculcate empathy in medical residents. This study yielded significant results but there was a lack of comparative group. In a recent study empathy map have proved to enhance communication skills in medical students.²² Hence, there was a gap for studies with larger sample size and a comparative group to fully understand the potential role of health empathy maps.

This is the first study with a comparative group of narrative exercises and the sample was also sufficient with 130, 3rd year MBBS students. Sixty-five students attended the HEM group and the other 65 attended the narrative medicine group. Both the groups had a pre-test before the intervention and after four weeks of intervention they had had a post-test. The results of this study are promising with significant increase after four weeks of intervention in both the groups. The results proved that the scores of the students in the HEM group improved remarkably. Health empathy maps proved to be more effective and more cost-effective, time efficient, more structured and focused activity that can be carried out in busy hospital settings as compared to narrative medicine exercises.

Stress and burnout are two other factors that decrease physicians' empathy as they go up the ladder. There is a fine line that has to be attained where a doctor sets up boundaries for their objectivity. These areas need to be nurtured along at the clerkship levels and practiced along the undergraduate and postgraduate journey to master these soft skills. Non empathic doctors were proven to be linked to stress and excessive workload.²³ The need to practice skills for developing empathy can prove to reduce stress and manage anxiety in the undergraduate medical students.

Similar studies have been conducted where empathy development curricula have shown promising results in medical residents.²⁴ Such trials have demonstrated that right teaching strategies will prove beneficial in inculcating empathic behaviours in medical students towards their patients. Empathy mapping thus proves to be an effective way of reciprocating the humanistic approach of literary activities such as narrative writings.

CONCLUSION

Health empathy maps provide a structured way for undergraduate and postgraduate medical students to enhance their doctor-patient relationship by fostering empathy for their patient. These empathy maps could win a permanent place in portfolios and thus provide a way to reflect back on the professional growth of a student during a semester or throughout the year. Practicing health empathy maps allows the healthcare workers not only to improve their empathy levels but also enhances their communication skill, team building, decision making and leadership qualities.

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ORIGINAL ARTICLE

USE OF ARTIFICIAL INTELLIGENCE TOOLS AMONG MEDICAL AND DENTAL STUDENTS AT AYUB MEDICAL COLLEGE, ABBOTTABAD

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Background: Artificial intelligence (AI) is increasingly being used for education. The objectives of this study were to determine the usage of artificial intelligence tools for academic purposes among the students of Ayub Medical College and comparison of AI use between medical and dental students. **Methods:** This comparative cross-sectional study was conducted at Ayub Medical College from 15 Aug to 20 Nov 2023. The sample size was 304 comprising of 209 medical and 95 dental students. A self-designed questionnaire on Google Forms was electronically distributed to participants for data collection. The questionnaire included questions about demographic variables, information about usage of artificial intelligence tools for different purposes and their disadvantages. Frequencies and percentages were calculated. Mean and standard deviation was calculated for age. Comparisons between medical and dental students were done using Chi-square and Fischer Exact tests. **Results:** The most frequently used artificial intelligence tool was ChatGPT, 81.3% in medical students and 84.2% in dental students. In comparing medical and dental students, there were statistically significant differences in use of AI for developing research project ($p<0.001$), its different steps ($p=0.002$), and prescription writing ($p=0.026$). **Conclusions:** The greater use of ChatGPT among medical and dental students was highlighted. The students used the artificial intelligence tools for many purposes but were primarily used for acquiring information about medical and dental knowledge. They were also aware about the limitations of artificial intelligence.

Keywords: Artificial Intelligence, AI, Dentistry, Medicine, Education, Undergraduate, Trends

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INTRODUCTION

Artificial intelligence (AI) is the science of the creation of systems for the performance of intelligent tasks like decision-making, learning and judgment.¹ It has applications within healthcare in all specialties² especially in the developed world and has the potential to revolutionize medicine in the developing countries³. It is also increasingly being used for the betterment of medical and dental education.⁴ One such open-source freeware AI Chatbot which is being increasingly utilized by healthcare students and healthcare professionals alike is Chat Generative Pre-trained Transformer (ChatGPT).⁵ Another AI software used is Microsoft Copilot.⁶

The importance of incorporating AI into the curriculum in medical schools has been highlighted by piloting it in the curriculums of University of Toronto and Harvard Medical School as acquiring information about AI use is beneficial to medical students.⁷ ChatGPT can have advantages in medical education such as quick access to information, clinical reasoning, better personalized learning, comprehension of complex concepts, and improvement of clinical skills by teaching medical and dental procedures through simulations.⁸ It helps the teachers in scoring papers and providing teaching assistance.⁶ Therefore, ChatGPT and other AI software have presented a

paradigm shift in education⁵ and have the ability to potentially modernize the educational system¹.

The AI software also have their limitations. ChatGPT causes decreased logical and critical thinking in students.⁵ It often gives biased and inaccurate information.⁶ Its use has caused greater dependency on AI.⁵ Students might also be tempted to cheat more and it challenges academic integrity.⁹ There are confidentiality, transparency and privacy concerns about the information given to such software.² So use of AI in medical education has to be done carefully while monitoring its use, and its purpose should be to enhance human abilities not to replace them.¹⁰

The objectives of this study were to determine the frequencies of AI tools usage in medical and dental students and to compare the usage between them in Ayub Medical College. This study will help the medical teachers to understand the patterns of AI usage in the medical and dental students, and the academic purposes they are using them for. With this information they can better develop strategies for the correct and safe use of AI tools by the students in their curricular activities.

METHODOLOGY

The study was a comparative cross-sectional study conducted at Ayub Medical College, Abbottabad, Pakistan from 15th August to 20th November 2023.

Medical and dental students of Ayub Medical College were included in the study. The students who had not used AI tools were excluded. The sample size of 304 was calculated using OpenEpi sample size calculator using confidence level of 95%, population size of 1,450, and 51% percentage frequency of outcome factor in the population.¹¹ The dental students comprised 1/3rd of the total sample size. The sampling technique used was non-probability quota sampling where two groups of medical and dental students were devised and sampling performed. Ethical approval was taken from the Medical Teaching Institution Abbottabad Institutional Ethical Review Committee Approval Code/Ref. No. RC-EA-2023/121. Informed written consent was taken from the participants. A questionnaire designed on Google Forms was electronically distributed to them via WhatsApp to collect the data. It included questions about demographic variables, information about usage of AI tools for different purposes and their disadvantages.

Data analysis was performed on SPSS-21. Frequencies and percentages were calculated. Mean and standard deviation was calculated for the variable of age. Comparisons between the medical and dental students were done with Chi-square test, and $p \leq 0.05$ was considered statistically significant.

RESULTS

The sample size was 304 comprising of 209 medical students (137 males and 72 females) and 95 dental students (27 males and 68 females) belonging to all professional years. Mean age of participants was 22.1 ± 1.7 years. The most frequently used AI tool was ChatGPT (81.3% in medical students and 84.2% in dental students). Other AI software used included Microsoft Copilot, Gamma AI, Grammarly, AI Chat, Ask AI, Ideogram, Midjourney, Adobe AI, Leonardo AI, Snapchat AI, WhatsApp AI, DALL-E, Stable Diffusion, Pebble AI, Gemini, Tome AI, Pi, Poe, and Quillbot.

Comparing questions for pattern of usage of AI tools for academic tasks, there were no significant differences between medical and dental students (Table-1).

Statistically significant differences were seen in Questions 1 ($p < 0.001$) and 2 ($p = 0.002$) when comparing questions for pattern of usage of AI tools for research tasks (Table-2).

Comparing questions for pattern of usage of AI tools for clinical learning, there were statistically significant differences in Questions 4 ($p = 0.026$) regarding use of AI in prescription writing. (Table-3).

There were no statistically significant differences in medical and dental students regarding limitations/disadvantages of AI. (Table-4).

Table-1: Use of AI tools for academic purposes

Table 1: Use of AI tools for academic purposes					
Questions/Answers	Medical students		Dental students		p
	n	%	n	%	
1. Use of AI tools for updated medical/dental knowledge					
Yes	123	58.9	55	57.9	0.900
No	86	41.1	40	42.1	
2. Use of AI tools for understanding of complex medical/dental concepts					
Yes	95	45.5	44	46.3	0.902
No	114	54.5	51	53.7	
3. Use of AI tools for writing assignments					
Yes	104	49.8	57	60.0	0.108
No	105	50.2	38	40.0	
4. Use of AI tools for doing which assignments' steps					
For ideas generation about assignment	104	49.8	43	45.3	0.145
For grammar check and sentence structuring	15	7.2	9	9.5	
For providing feedback or scoring your assignment	3	1.4	1	1.1	
Commanding AI tools to do the whole assignment for you	6	2.9	9	9.5	
Not using AI tools at all for assignments	81	38.8	33	34.7	
5. Use of AI tools for drawing medical/dental diagrams					
Yes	13	6.2	12	12.6	0.072
No	196	93.8	83	87.4	
6. Use of AI tools to solve question papers					
Yes	434	16.3	23	24.2	0.11
No	175	83.7	72	75.8	
7. Use of AI tools to make PowerPoint presentations					
Yes	144	21.1	26	27.4	0.24
No	165	78.9	69	72.6	

Table-2: Use of AI tools for research purposes

Table 2: Use of AI tools for research purposes					
Questions/Answers	Medical students		Dental students		p
	n	%	n	%	
1. Use of AI tools as an aid for developing a research project					
Yes	80	38.3	14	14.7	0.000
No	129	61.7	81	85.3	
2. Use of AI tools for which step of research project					
Outline of research project	48	60.0	9	64.3	0.002
Questionnaire development	4	5.0	1	7.1	
Data analysis	21	26.25	4	28.6	
Write-up	7	8.75	0	0	

Table-3: Use of AI tools for clinical learning

Table 1: Use of AI tools for clinical learning					
Questions/Answers	Medical students		Dental students		p
	n	%	n	%	
1. Use of AI tools for enhancement of problem solving and clinical reasoning skills					
Yes	72	34.4	40	42.1	0.203
No	137	65.6	55	57.9	
2. Use of AI tools for practicing communication techniques and skills					
Yes	60	28.7	24	25.3	0.582
No	149	71.3	71	74.7	
3. Use of AI tools for practicing virtual simulated patient interactions					
Yes	14	6.7	11	11.6	0.177
No	195	93.3	84	88.4	
4. Use of AI tools for medical/dental prescription writing					
Yes	20	9.6	18	18.9	0.026
No	189	90.4	77	81.1	
5. Use of AI tools for generating history-taking questions for patients					

Yes	24	11.5	17	17.9	0.148
No	185	88.5	78	82.1	
6. Use of AI tools for generating health education information for patients					
Yes	38	18.2	21	22.1	0.437
No	171	81.8	74	77.9	
7. Use of AI tools for interpreting radiographs					
Yes	18	8.6	11	11.6	0.407
No	191	91.4	84	88.4	
8. Use of AI tools for learning clinical procedures					
Yes	31	14.8	17	17.9	0.501
No	178	85.2	78	82.1	

Table-4: Opinion about disadvantages of AI tools

Questions	Medical students		Dental students		p
	n	%	n	%	
1. Do you think AI tools provide the right kind of information and resources related to your medical/dental search?					
Yes	114	54.5	49	51.6	0.072
No	26	12.4	5	5.3	
Not Sure	69	33.0	41	43.2	
2. Do you feel you are getting too dependent on AI tools for academics?					
Yes	31	14.8	23	24.2	0.124
No	154	73.7	64	67.4	
Not Sure	24	11.5	8	8.4	
3. Do you feel the frequent use of AI tools decreases your critical thinking ability and creative skills?					
Yes	108	51.7	56	58.9	0.207
No	67	32.1	21	22.1	
Not Sure	34	16.3	18	18.9	
4. Do you feel that AI tools should be allowed in institutions as an additional source of learning without replacing human teachers?					
Yes	140	67.0	62	65.3	0.945
No	47	22.5	23	24.2	
Not Sure	22	10.5	10	10.5	
5. Do you feel privacy concerns while using AI tools?					
Yes	81	38.8	34	35.8	0.647
No	79	37.8	34	35.8	
Not Sure	49	23.4	27	28.4	

DISCUSSION

This study demonstrated the pattern of AI use among the medical and dental students of Ayub Medical College. The most used AI tool by students was ChatGPT (81.3% in medical students and 84.2% in dental students). This is in contrast to two studies where frequencies were 11.3%¹² and 15.9%¹³. A systematic review¹⁴ mentioned AI tools usage in medical students from 11.3–45%. Previous studies assessed familiarity and knowledge about an overall concept of AI in medical education, and not just about ChatGPT like in this study. Knowledge about AI ranged from 47%¹⁵, 70%¹⁶, 56.7%¹⁷ and 68.8%¹⁸ in four studies, last two of which were local studies. In a systematic review¹⁴ AI knowledge ranged from 5.9–92%.

In the present study, 67% medical students and 65.3% dental students agreed that AI knowledge should be taught as part of curriculum. Previous studies showed a similar high percentage trend with 82.1%¹¹, 59.7%¹³, 68.4%¹⁵, 85.3%¹⁶, 74.1%¹⁸, 78.1%¹⁹, 77.7%²⁰, and 64.1%²¹ in eight studies, last three of which were local studies. A study by Bisdas *et al*³ showed a frequency of

85.6% and compared the medical and the dental students, and like this study ($p=0.945$) the difference was statistically insignificant ($p=0.747$).³

Some medical schools piloted AI inclusion in curriculum like Harvard Medical School, University of Toronto, Ulsan University, and Yonsei University. Teaching competencies of AI in medical education in their respective curriculums were all different with a lack of a standard curriculum, and so a need was felt for such a curriculum.²²

In the current study, 58.9% medical students and 57.9% dental students used AI tools to keep themselves updated about their medical knowledge. This was similar to a study by Kalaimani *et al*¹³ where 53.4% dental students did this. In our study, 11.6% dental students used AI to interpret radiographs and this was unlike the study by Kalaimani *et al*¹³ where 44.1% used it for this purpose.

In this study, 38.8% medical students and 35.8% dental students felt privacy concerns with AI use. A general awareness among medical students about the limitations of AI was depicted with frequencies of 67.6%¹¹, 63.4%¹⁵, and 48.3%¹⁹ in three studies. In a study by Al Saad *et al*¹⁵, 61.6% medical students had privacy concerns in contrast to this study.

There were significant differences between the medical and dental students regarding use of AI as an aid to research project development, the usage in the various steps of research and in the usage for prescription writing. The medical students (38.3%) used AI more than the dental students (14.7%) in developing a research project. Both categories of students (59.5% medical, and 64.3% dental) used AI for developing an outline of the research project. This could be because undergraduate students are not well trained in AI use for research so can mostly use it for the easiest task of making an outline. The dental students (18.39%) used AI more than the medical students (9.6%) in writing prescriptions. This could be so because in Ayub Medical College, dental students are dealing directly and independently with patients earlier than medical students and so write more prescriptions for which they can use AI to help them with.

A scoping review by Lee *et al*²² gave some recommendations for the incorporation of AI in the curriculum. These include teaching students the basics of AI and use of its tools, its uses and its effect on clinical reasoning, advantages and dangers and communicating AI findings to patients.

It is recommended that awareness about AI use in medical education should be spread through educational activities in medical and dental students. The curriculum should be enriched by incorporating AI topics within it. Teachers can help students to use AI as a means of gathering medical information. The performance of the students should be assessed in

collecting medical information.²³

The limitations of the study included using a non-random sampling technique and not including participants from other medical and dental colleges.

CONCLUSION

The mostly frequently used AI tool among medical and dental students was ChatGPT. A greater proportion of the students used them for finding information about medical and dental topics. AI tools were mostly used for idea generation. In developing a research project they were used mostly for crafting an outline. The majority of the students were also concerned about the consequences of limitations of AI. Future studies should focus on interventional studies when piloting an AI educational program in the curriculum.

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ORIGINAL ARTICLE

NORMAL VALUES OF SKULL BASE ANGLES USING STANDARD AND MODIFIED MRI TECHNIQUE IN PAKISTANI POPULATION

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Background: Different techniques are used to measure skull base angle on sagittal images of MRI to diagnose platybasia and basilar kyphosis. Objective of this study was to determine the normal values of skull base angle using standard and modified methods of magnetic resonance imaging in Pakistani population. **Methods:** This cross-sectional, observational, descriptive study was conducted in Radiology Department, Wah Medical College, Wah Cantt. It comprised 700 subjects, including 336 females and 364 males (children and adults). Midline sagittal T1-weighted MR images were evaluated to measure the standard and modified skull base angles on Picture Archive and Communication System (PACS Links). All subjects underwent MR imaging on a 1.5 Tesla scanner, equipped with an 8-channel head coil. Data was collected on prescribed proforma and analysed using SPSS-23. **Results:** The standard MRI method gave a mean angle of $129.9 \pm 5.12^\circ$ (range $108.1-148.3^\circ$) compared to mean angle of $121.3 \pm 5.06^\circ$ (range $107.1-136.2^\circ$) obtained by the modified method; the difference of 8.6° between the mean angles given by these two methods is highly significant ($p=0.002$). **Conclusion:** Pakistani population has wider basal angle range as compared to the western population.

Keywords: MRI, Standard method, Modified method, Angular craniometry

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INTRODUCTION

Measurement of skull base angle is used for the diagnosis of platybasia and basilar kyphosis. Traditionally, basal angle measurements were based on plain skull images.^{1,2} With the advent and generalization of MR imaging, traditional radiography method has been replaced and is rarely used. Modified MRI techniques produce lower basal angles than those reported by using traditional radiography.¹

The size and shape of skull differs among different races. Differences occur in head shape, height of nasal root, jaw thickness, slope of forehead and size of brow ridge.³ Skull base angle is not affected by change in the position of the patient. It is determined by extending a line across the anterior cranial fossa from the nasion to the tip of the dorsum sella and a second connecting line drawn along the posterior margin of the clivus.⁴

Increase in the angle beyond the normal range, leads to flattening of the skull base and is termed Platybasia⁵. Decrease in skull-base angle is known as basilar kyphosis. Basilar invagination and platybasia often appear together, in a variety of congenital craniofacial anomalies, such as cleidocranial dysostosis, osteogenesis imperfecta, Arnold-Chiari malformation and in acquired disorders, such as rickets, osteomalacia, senile atrophy, hyperparathyroidism, Paget disease, trauma and local bone destruction.

As an isolated finding most patients are asymptomatic, and platybasia is not important

clinically. No typical treatment is indicated unless there is compression or impingement of nerve roots or brain parenchyma. In some patients, posterior decompression, usually in conjunction with fusion of the skull to the upper cervical spine may be required.⁵

Most of the studies giving range of normal values for skull base angle are done on Western population.^{6,7} The purpose of our study was to determine the normal range of skull base angle in our local population and compare it with other studies, using both standard and modified MRI techniques.

METHODOLOGY

This study was carried out in the Radiology Department of Wah Medical College, POF Hospital, Wah, from Apr to Nov 2021. T1-weighted sagittal images of the skull base of 700 consecutive cases were evaluated to measure the basal angle irrespective of the patient's age and gender. Cases with skull base tumours, infections, skull base fracture, congenital or acquired musculoskeletal dysplasia and craniovertebral assimilation abnormalities were excluded to determine a normalized standard.

Standard skull base angle was measured by taking the angle between the line ensuing from nasion and terminating at the centre of pituitary fossa and second line starting from the anterior margin of foramen magnum and ending at the centre of pituitary fossa as done by McGregor.⁸ For measurement of modified skull base angles the landmarks provided by Keats and Cohen were used.^{5,9}

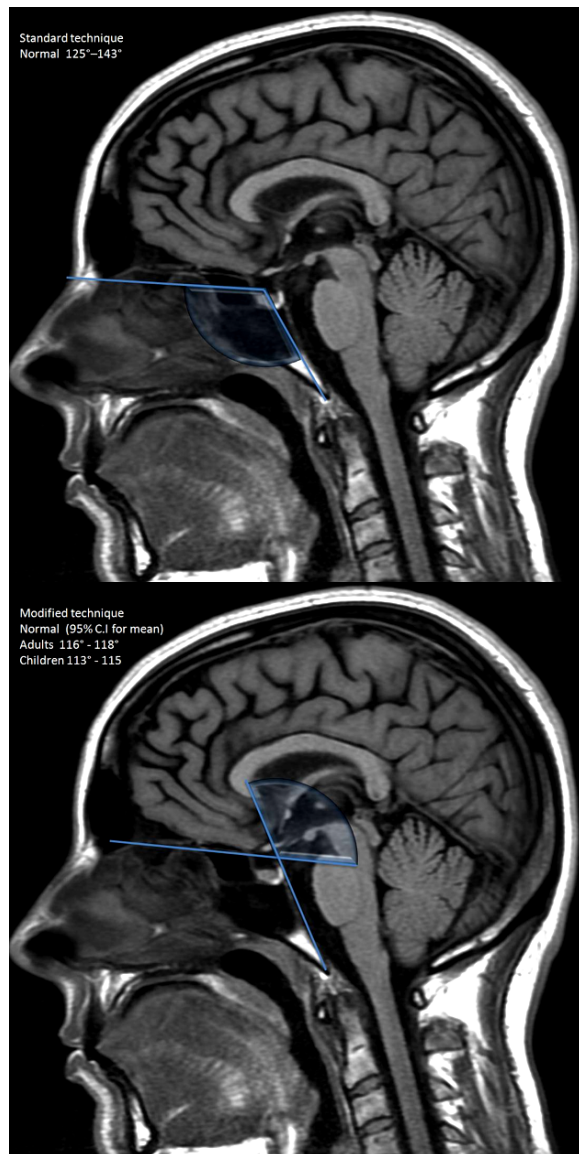


Figure-1: Measurement of skull base angle (Standard and Modified techniques)

Basic statistics were calculated for age and MRI angles of skull by two methods. Normal ranges with Mean $\pm$ SE were reported. Correlations were calculated between the angles from the two methods. Paired *t*-test was used to test significance of difference between the mean angles of the two methods for all subjects as well as for female and male subjects. Two-way tables were made showing means for gender-by MRI methods, age groups (children vs adults) by MRI methods, and age groups by combination of method and gender. Unequal samples *t*-test was used to test the difference in angles of female and male subjects, children and adults of all subjects, and children and adults of the two gender classes.

Confidence interval and standard error of differences between two means for independent

samples *t*-test were calculated. To test the effects of four combinations of gender and age groups considered as treatments on MRI angles measurement from the standard and modified methods, one way analysis of variance (ANOVA) was used.

RESULTS

The standard MRI method gave a mean angle of 129.9 $\pm$ 5.12° (range: 108.1–148.3°) as compared to mean angle of 121.3 $\pm$ 5.06° (range 107.1–136.2°) obtained by the modified method. The difference of 8.6° between the angles given by the two methods is highly significant using paired *t*-test. The 95% confidence interval for the mean difference was 8.5–8.9. Paired *t*-test also showed significant differences (8.7°) between the angles of the two MRI methods in females and in male subjects ($p=0.002$). (Table-1).

The angles of the two methods of MRI were very highly correlated ($r=0.8430$, 0.7649 , and 0.8996 for all, female and male patients respectively) showing that the results of the modified method are as reliable as the standard method. The scatter plot and regression line of the angles of modified method on the angles of the standard method of the patients with a coefficient of determination (R^2 of 0.7715) also show close relationship between the reading of angles of the two MRI methods, because the regression model for modified method is explaining 77% of the variation in data from the standard method. (Figure-2).

Male subjects had lower angles than female subjects with difference highly significant in standard method and modified method. Differences in mean angles of children and adults were also highly significant with children having wider angles than adults. (Table-2).

Student's *t*-test for differences between children and adults showed that differences were significant in males and not significant in females in case of standard method. Showing that boys on the average had wider angles than men while the angle of girls were slightly wider than women and the difference between girls and women did not reach significant level in standard method based on unequal sample non-paired *t*-tests. (Table-3).

As children had wider angles than adults, we explored if there is a clear trend of angle with age or not, for this purpose a frequency table for different age groups was prepared and averages of the skull angles for the different age groups were calculated. The results for females and males are slightly different, especially in children and old age groups. It has implications for normal range of skull angles in our population. There should be separate recommendations for normal angle ranges of skull for boys, girls, men and women. (Table-4).

Table-1: Results of paired *t*-test for the angles of standard and modified MRI

Subjects	Angle given by		Mean difference (95% CI)	<i>t</i>	<i>p</i>
	Standard method	Modified method			
Over all (n=700)	129.9±5.1°	121.3±5.06°	8.6° (8.5, 8.9)	91.97	0.002
Female (n=336)	130.7±5.0°	122.0±4.7°	8.7° (8.4, 8.9)	59.60	0.001
Male (n=364)	129.2±5.1°	120.5±5.3°	8.7° (8.4, 8.9)	70.04	0.001

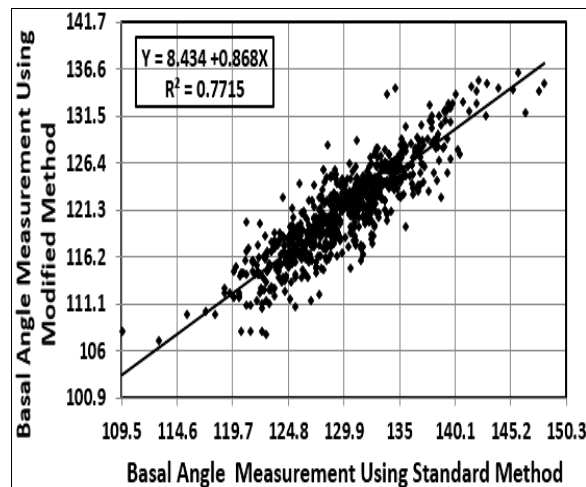


Figure-2: Relationship of basal angle measurement by standard and modified methods of MRI

Table-2: Mean angle of MRI for two-way tables, method by gender and method by age-category

Gender	Gender		Difference	Age categories		Difference
	Female (n=336)	Male (n=364)		Children (n=96)	Adults (n=604)	
Standard	130.7	129.2	1.5*	132.3	129.6	2.7*
Modified	122.0	120.5	1.5*	124.1	120.8	3.3*

**p*<0.01

Table-3: MRI angle of gender and age categories using standard and modified method

	Number		Standard			Modified		
	Female	Male	Female	Male	Diff.	Female	Male	Diff.
Children	41	55	131.64	132.78	-1.15	123.40	124.55	-1.15
Adults	295	309	130.56	128.58	1.98	121.85	119.81	2.04
Diff.			1.08	4.20		1.55	5.54	

Table-4: Averages of the skull angles for the different age groups

Age (Years)	Female			Male		
	n	Standard (degrees)	Modified (degrees)	n	Standard (degrees)	Modified (degrees)
1-14	41	131.64	123.40	55	132.78	124.55
15-24	41	130.49	122.42	35	128.83	120.72
25-34	44	129.78	121.41	25	127.80	119.11
35-44	42	131.41	123.41	32	128.94	120.20
45-54	70	130.81	121.45	55	129.27	120.35
55-64	41	129.60	120.37	77	128.02	119.15
65-74	35	130.13	121.51	51	128.26	119.77
75-84	19	132.17	123.16	29	129.37	120.03
85-91	2	134.40	124.90	5	128.42	117.88

DISCUSSION

This study documents and compares the normal range and mean of skull base angle by both the Standard and Modified MRI methods for both genders and different age groups. A mean basal angle of 129.9±5.1° obtained by standard method and a mean basal angle of 121.3±5.06° obtained by the modified method may be considered normal. Angles greater than the given values may be associated with clinical diagnosis of platybasia. However further research is mandatory.

Koenigsberg¹¹ retrospectively studied midline sagittal images of 200 adults and 50 children using both the standard and modified methods. They reported mean angles of 129±6° for adults and mean angles of 127±5° for children with the standard MRI technique, and the modified technique gave values of 117±6° for adults and 114±5° for children. The modified method gave values significantly lower than those of standard MR imaging and traditional radiography. A study similar to our study was conducted in Thai population¹² involving 200 adults and 50 children and found Modified MRI basal angle range of 100.5–130° for adults and 102–130.5° for children. Their mean basal angle was 121.65±5.1° and they found no statistically significant difference between male and female subjects or between children and adults. Botelho¹³ and Ferreira¹⁴ in their studies of angular craniometry on craniocervical junction malformation found a mean basal angle of 118.71±7.11° and a range of 107–132°. They suggested a diagnosis of platybasia when the basal angle exceeds 133° and found that an enlarged BA was associated with a more acute Clivus canal angle and larger Boogards angle. In recent studies¹⁵⁻¹⁸ it was further discussed that Clivopalate angle gives complementary information to clivoaxial and clivodens angle.

CONCLUSION

Pakistani population has wider skull base angle as compared to the western population. Modified MR imaging technique provides a better tool to evaluate platybasia and basilar kyphosis. Our observed values may be used as a guide for the potential range of normal skull base angles in Pakistani population.

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RA: Analysis and interpretation

KI: Drafting and reviewing

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ORIGINAL ARTICLE

COMPARISON OF SALIVARY ANTIOXIDANT LEVEL
IN SMOKERS AND NON-SMOKERS

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Background: Saliva is the first biological medium encountered during inhalation of cigarette smoke. Uric acid in saliva possesses free-radical-scavenging properties which are produced by cigarette smoke and contributes approximately 70–85% of the total antioxidative capacity of saliva. Objective of this study was to determine and compare the salivary uric acid (SUA) level in cigarette smokers and non-smokers amongst the patients visiting Sardar Begum Dental Teaching Hospital, Peshawar. **Methods:** It was a comparative study done on 118 individuals, (59 in each group of cigarette smokers and non-smokers). Non-probability convenience sampling was used. The data was collected using structured proforma and analysed using SPSS-25. For the association of quantitative variables with smoking independent sample *t*-test was used. Chi-square test was used for qualitative variables, and $p \leq 0.05$ was considered statistically significant. **Results:** Mean salivary uric acid level in cigarettes smokers was 2.7 ± 1.02 mg/dL as compared to 4.7 ± 0.91 mg/dL in non-smokers. **Conclusion:** Salivary uric acid level was found significantly lower in cigarette smokers compared with non-smokers.

Keywords: Antioxidant, Oxidative stress, Saliva, Uric acid

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INTRODUCTION

Saliva is the first line of biological medium that meets outside components like food, drink, or inhaled volatile substances consumed in daily routine. Whole saliva is a bio-mixture of major and minor salivary gland secretions.¹ Water content of saliva is around 99% with electrolytes and proteins. These components collectively are accountable for functions such as digestion, buffering, taste, balance of re-mineralization, and anti-microorganisms. Salivary monitoring is an important factor as its composition reflects the disease severity and response to treatment.^{1,2}

Early detection of COVID-19 is possible from saliva by gathering oral swabs and testing RNA among susceptible individuals.³ Saliva plays a key role in maintenance of oral mucosa and oral health. It protects the soft and hard tissues from damage. Saliva not only lubricates the oral mucosa but also protects the oral cavity and oral mucosa from different types of harmful chemicals and microorganisms.^{4–7} Salivary immunological defence system is predominant because of protein defence and secretory immunoglobulin A. This defence system is mainly composed of uric acid, albumin, caeruloplasmin, ascorbic acid, lactoferrin, agglutinin, glutathione, lysozyme, and transferrin.^{2,8}

Uric acid, ascorbic acid and albumins are the essential antioxidant elements of saliva. However, uric acid proved to be the foremost antioxidant present in saliva and exhibits a concentration which is similar to

that of serum as well as has accepted clinical importance in examining oxidative stress.^{1,9} Uric acid contributes around 70–85% of the total salivary antioxidant capacity.⁹ Salivary uric acid (SUA) is able to chelate transition metals and to counter with biological oxidants such as reactive oxygen species, hydroxyl radicals, hypochlorous acid, and reactive nitrogen species. The SUA is achieved as an end-product in purine catabolism. Increased concentration of SUA is believed as a hallmark in oxidative stress monitoring.¹⁰ Saliva represents as a first line defence against oxidative stress, and carries protective effects against oxidants, microorganisms, and toxins. Antioxidants eradicate the free radicals generated from the oxidative stress which form as a product or by-product during functional activities and cellular metabolism. Highly active free radicals due to their unpaired electrons carry a tendency to react with healthy tissues and their ingredients causing oxidative stress.^{11,12} Oxidative stress can harm DNA, RNA, lipids, and proteins increasing risk of oral diseases and cancer, cardiovascular diseases, and autism. Antioxidants reduce the oxidative damage directly by countering with free radicals and indirectly by inhibiting free radical-generating enzymes activity.

Among defensive systems, salivary antioxidant activity may be affected by cigarette smoking.^{7,13} Imbalance between oxidants and antioxidants makes smokers more susceptible to oxidative stress.¹⁴ Oxidative stress can originate from human body itself as well as from external sources. Cigarette smoking leads to

formation of numerous hazardous compounds.¹⁵ These compounds and their metabolites may initiate oxidative stress by creation of reactive species or beginning of radical chain reactions.^{16,17}

This study aimed to evaluate the comparison of salivary uric acid level in cigarette smokers and non-smokers visiting Sardar Begum Dental Teaching Hospital, Peshawar, Pakistan.

MATERIAL AND METHODS

It was a comparative, cross-sectional study between cigarette smokers and non-smokers for assessing association of smoking with salivary uric acid level. Study was done from 23rd Aug to 22nd Dec 2020 after approval from Ethical Committee and Board of Advance Studies and Research. Non-probability convenience sampling was used to recruit all eligible subjects. Subjects' saliva was collected from Outpatient Department, Sardar Begum Dental College and Hospital, Peshawar, and procedure was carried out in Department of Pathology, Khyber Teaching Hospital, Peshawar. Study population consisted of all male patients aged 20–60 years. Sample size was 118 using WHO Sample Size calculator. Participants were male cigarette smokers and non-smokers aged 20–60 years. Participants having any systemic diseases, drug users, or having a malignancy were excluded.

Total of 118 subjects fulfilling the inclusion criteria, were enrolled in this study after informed consent. Data was recorded on a structured Performa. The enrolled subjects were divided into cigarette smokers and non-smoker groups. Each group included 59 subjects. The record included demographics, smoking status, and salivary uric acid level.

About 2.5 mL clear saliva was collected from each subject in a saliva collection tube by spitting method¹⁸. Supernatants of saliva was achieved by centrifuging the collected saliva at 4,000 rpm for 10 minutes.¹⁹ Salivary uric acid was determined with colorimetric method²⁰ on Cobas Mira Autoanalyzer (HITACHI (Roche Pakistan Limited) C 501).

Quantitative variables were calculated as Mean±SD, and qualitative variable as frequencies and percentage. For the association of quantitative variables with smoking, independent sample *t*-test was used and for qualitative variables Chi-Square test was used. The data was analysed on SPSS-25, and $p \leq 0.05$ was considered statistically significant.

RESULTS

There were 59 smokers and 59 non-smokers in a total of 118 patients. All subjects were male with mean age 38.19±11.30 years (Range: 20–60). Mean age of cigarette smokers was 40.37±11.0 years, and mean age of non-smokers was 36.02±11.23 years. Independent *t*-test showed that there was a statistically significant

difference in age between the two groups ($p < 0.05$). (Table-1).

Mean salivary uric acid level in cigarettes smokers was 2.73±1.02 mg/dL compared to 4.73±0.91 mg/dL in non-smokers. There was a statistically significant difference in salivary uric acid levels between the two groups ($p < 0.001$). (Table-2).

Table-1: Ages of the study subjects (Mean±SD)

Group	N	Age (Years)	<i>p</i>
Smokers	59	40.37±11.0	<0.05
Non-smokers	59	36.02±11.23	
Total	118	38.19±11.30	

Table-2: Salivary uric acid level in study subjects

Group	N	Salivary Uric Acid (mg/dL)	<i>p</i>
Smokers	59	2.73±1.02	<0.001
Non-smoker	59	4.73±0.91	

DISCUSSION

Smoking is a risk factor for oral diseases such as periodontal disease, oral cancer, alveolar bone loss and black hairy tongue. Pregnant women's smoking is also a risk factor for cleft lip and palate in the baby. Cigarette smoking has a direct correlation with DNA damage.²¹

The current study corroborates findings of Hanna *et al*²¹ which assessed serum uric acid levels in smokers and non-smokers. They concluded that although serum uric acid level in smokers did not reach the lower reference range, it is significantly lower than the non-smoker group ($p < 0.001$).²¹

Our findings are in agreement with Greabu *et al*²² that indicated significant decrease in important salivary antioxidant levels and the loss of activity of salivary enzymes with antioxidant actions can be considered as one of the mechanisms by which the toxic effects of cigarette smoke initiate oral inflammatory diseases, promote precancerous transformations and destroy the oral cavity homeostasis.²²

The findings of present study contrasted with a study by Zappacosta *et al*²³ which showed that there was no statistical difference in SUA level in cigarette smokers and non-smokers. The results of present study were also in contrast with Fawaz Pullishery *et al*⁷ who reported that the SUA levels with number of cigarettes smoked in tobacco users and non-tobacco users had no significant differences.

The results of present study were in agreement with Miricescu *et al*²⁴ who observed that SUA level was significantly less in smokers compared to controls. They observed that decrease SUA levels were found in periodontitis and oral lichen planus, and smoking may initiate oxidative stress which causes toxic effects of free radicals, and can in turn initiate precancerous transformations, oral diseases and destroy the oral cavity homeostasis.²⁴

The results of present study are in agreement with Fatima *et al*¹ who evaluated that the SUA level in smokers with periodontitis and periodontally healthy individuals and concluded with significantly decrease SUA level in smokers. According to them the reduced level of salivary antioxidant could be a direct underlying factor in periodontal and other oral disease.¹

This study also corroborates with A. Abdolahinia *et al*²⁵ who showed an association of smoking with the age. Their analysis presented a significant correlation between age and number of daily cigarette smoking. The current study also agrees with Abdolsamadi *et al*²⁶ that there is a parallel relationship between smoking and age as the smoking increase with age.²⁶ Same result was observed by Dube *et al*²⁷ that there was a significant association between age and smoking as cigarette smoking increases with age and puts adverse effects on physical health.

LIMITATIONS

Number of cigarettes, chronicity of smoking and effect of smoking cessation was not considered in this study.

CONCLUSION

Cigarette smokers had significantly low uric acid level in their saliva as compared to non-smokers.

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