

AJSMU

Volume 9

Issue 2

July - December 2023



ANNALS of JINNAH SINDH MEDICAL UNIVERSITY



HEC Recognized 'Y' Category Journal

Indexed and abstracted in PMDC, Pak Medinet, CAB Abstracts, Global Health UK, Pakistan Science Abstracts and WHO Index Medicus for the Eastern Mediterranean Region (IMEMR)





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Published by: The Registrar, Jinnah Sindh Medical University, Rafiqui H.J. Shaheed Road, Karachi.



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Artificial intelligence in Medical Education: Advantages and Challenges

Masood Jawaid

How to cite: Jawaid M. Artificial intelligence in medical education: advantages and challenges. Ann Jinnah Sindh Med Uni. 2023; 9(2):32-33
DOI 10.46663/ajsmu.v9i2.32-33

Artificial intelligence is rapidly transforming medical education. With the increasing need for clinicians to use such tools, medical education must prepare future healthcare professionals for the evolving landscape of artificial intelligence¹.

The evolving digitalization of the medical curriculum and the collaboration between data scientists and physicians offer opportunities for the increased use of AI systems in medical education. As technology continues to advance, the potential uses of AI in medical education will continue to expand. This includes the integration of AI with immersive technologies such as virtual reality and augmented reality, offering new avenues for learning in medical education². The University of Texas at San Antonio, in conjunction with UT Health San Antonio was the first university to offer a dual degree program in AI and medicine, demonstrating a proactive approach to the integration of these two fields³.

The incorporation of AI into teaching, learning and assessment in the field of medical education is crucial to prepare future healthcare professionals. By leveraging AI, faculty can enhance the development and delivery of educational content⁴. AI-based systems can help instructors to develop personalized learning materials to support individual students' needs, ultimately fostering a more engaging and effective learning environment. By incorporating AI into the development of learning content, our organization has significantly reduced its workload. The robust system allows us to create individualized learning experiences for more than 2000 healthcare professionals with diverse learning needs. AI can contribute to addressing the shortage of faculty by providing innovative methods for teaching future doctors. Through virtual simulations, AI-driven tools can immerse students in realistic clinical scenarios, allowing them to hone their clinical reasoning skills in a safe and controlled environment⁵.

AI can play a pivotal role in easing the workload of medical educators by automating routine tasks like replying to emails, administrative work, marking assignments, developing assignments, enabling them to focus on more strategic and impactful aspects of teaching¹. Now we are witnessing the AI Assistant powered by ChatGPT for different learning platforms. All this can lead to more efficient and effective use of educators' time and resources, ultimately enhancing the overall learning experience for the students. Artificial intelligence holds numerous benefits for medical students by offering transformative opportunities for learning, skill development, and professional growth. One of the key advantages of AI in medical education is its capacity to provide personalized, adaptive learning experiences. With AI-driven educational tools, students can receive tailored learning materials and support, catering to their unique learning styles and knowledge gaps⁶. AI can facilitate comprehensive and realistic clinical simulations, allowing students to practice and enhance their clinical reasoning abilities in a simulated yet authentic environment. These virtual scenarios not only provide valuable hands-on experience but also contribute to patient safety by ensuring that students are well-prepared and competent before entering real clinical settings⁷.

AI can aid medical students in staying abreast of the latest advancements in the healthcare sector. By leveraging AI-powered educational platforms, students can access current and relevant information, keeping pace with the rapidly evolving healthcare landscape and expanding their knowledge base effectively. One such advance and free tool is *scispace* which is now reshaping how we review recent literature and navigate evidence-based clinical decisions⁸.

The integration of artificial intelligence in medical education undoubtedly brings numerous advantages, but it also poses significant challenges and potential threats that need to be carefully addressed. One of the primary challenges of implementing AI in medical education is the need for faculty and institutions to adapt to rapidly evolving technology. This includes providing adequate training and resources for educators

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to effectively integrate AI tools into their teaching methods. Additionally, ensuring the ethical and responsible use of AI in medical education is a paramount concern. It is essential to establish guidelines and regulations to govern the use of AI-driven technologies in medical learning to mitigate the risk of potential biases or inaccurate outcomes.

There is a risk that reliance on AI may lead to a reduction in critical thinking and clinical reasoning skills among medical students as the students may become overly reliant on AI tools for diagnosis and decision-making, potentially compromising their ability to think independently and critically analyze⁹. Another looming threat is the potential for data privacy and security breaches as AI-driven systems generate and analyze vast amounts of sensitive medical data. Institutions will need to invest in robust data protection measures to safeguard patient information and maintain the integrity and confidentiality of medical records. Thus integration of AI in medical education requires substantial investment and resources, which may pose financial challenges for many educational institutions, especially those with limited funding.

As the landscape of medical education continues to embrace AI, it is crucial for educators, institutions, and policymakers to proactively address these challenges and develop comprehensive strategies to harness the benefits of AI while mitigating potential risks. By promoting responsible AI use, ensuring ongoing faculty training, and safeguarding data security, the field of medical education can effectively navigate the challenges and maximize the potential of AI to train future healthcare professionals effectively. To substantiate the effectiveness of AI, comparative studies with traditional teaching methods are essential, requiring a substantial sample size for probabilistic results. This necessitates a robust and comprehensive approach to evaluating the role of AI in medical education, highlighting the need for extensive research and validation.

In Pakistan, where many of us are struggling to incorporate effective utilization of technology in education and we are far from their optimal usage, AI is another big entity to tackle. One area of focus should be on adapting AI applications to the specific needs and challenges of the Pakistani healthcare system. This involves identifying areas where AI can address existing gaps in medical education. Furthermore, the development of AI-driven educational tools tailored to the cultural, linguistic, and societal context of Pakistan can optimize their effectiveness and relevance in medical training. This involves collaboration between educators, AI developers, and healthcare professionals to ensure that the content and applications align with the unique requirements of the local healthcare landscape. This includes providing sufficient resources

and training for educators to effectively utilize AI tools and platforms, as well as establishing robust data security measures to protect sensitive patient information. To navigate the financial challenges associated with AI implementation, partnerships with public and private stakeholders, international organizations, and governmental support may be beneficial in securing the necessary investment for AI integration in medical education. Moreover, fostering a culture of responsible AI use, research, and innovation within medical education institutions in Pakistan is essential. This involves promoting interdisciplinary collaboration, ethical guidelines, and continuous evaluation to ensure that AI applications align with the highest standards of patient care and educational excellence.

By carefully addressing these considerations and strategically planning for the integration of AI in medical education, Pakistan can harness the potential of AI to overcome existing challenges, enhance educational outcomes, and ultimately contribute to the advancement of healthcare provision in the country.

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The Functional Outcome of Philos Plate Fixation in Patients with Proximal Humerus Fracture

Pervez Ali¹, Muzafer Husain², Fahad Jatoi², Dost Mohammad², Iftikhar Memon²,
Ejaz Matlo², Mian Sajjad³, and Riaz Elahi Khoso²

ABSTRACT

Objective: To determine the functional outcome of PHILOS plate fixation in patients with proximal Humerus fracture.

Methodology: A prospective observational study was conducted at the Department of Orthopedic Surgery, Jinnah Postgraduate Medical Centre, Karachi as well as Neurospinal and Medical Institute (NMI), Karachi between June 2016, and January 2021. Post-operative patients with proximal humerus fracture treated with Philos plate fixation were enrolled. Detailed history and physical examination were recorded. Patients were followed up to 12 weeks to determine functional outcome. Constant-Murley Score was used to assess the functional outcome as either satisfactory or unsatisfactory.

Results: A total of 304 patients were recruited in the study with a mean age of 51.42±7.939 years (range; 30-60). 216 (71.05%) patients who were managed with Philos plate for the management of fracture of the proximal humerus had satisfactory outcomes while only about 88 (28.95%) patients had unsatisfactory outcomes. 54 (17.76%) patients with an unsatisfactory outcome were older than 45 years. Body Mass Index was not significantly associated with patient outcome. However, delay in presentation of more than three days was significantly associated with unsatisfactory outcome in patients as 82 out of 90 patients with delay > 3 days had unsatisfactory outcome at the end of the study (p=0.0002).

Conclusion: We reported that philos plate fixation provided adequate stability and overall good functional outcome. Further large-scale studies should be conducted to ascertain our findings and assess the long-term complications.

Key Words: Constant-murley score, philos plate fixation, proximal humerus fracture

How to cite: Ali P, Husain M, Jatoi F, Mohammad D, Memon I, Matlo E, Sajjad M, Khoso RE. The functional outcome of philos plate fixation in patients with proximal humerus fracture. Ann Jinnah Sindh Med Uni. 2023;9(2):34-37

DOI 10.46663/ajsmu.v9i2.34-37

INTRODUCTION

Fractures of the proximal humerus are very frequent, resulting in approximately five percent of all fractures¹. According to published statistics, it is estimated that the occurrence of proximal humerus fractures has increased to three times since 1970².

Despite the advancements in the field of orthopedic surgery, surgical intervention for unstable fractures are still considered as a challenge³. There is no consensus

for the most optimum treatment option for the management of humerus fractures. There are several techniques and procedures for the management of these fractures, however, none is established as the most superior⁴.

Proximal humeral locking plates, such as the Proximal Humeral Interlocking Osteosynthesis (PHILOS) plate have shown a positive outcome in the management of humeral fractures. The Philos fixation procedure is specific to the site of injury. The plate is adjusted to the proximal humerus and the use of locking screws eliminates the need for a plate-to-bone compression and conserves blood supply to the bone. The insertion of polyaxial screws into the head of the humerus allows support in multiple planes^{5,6}.

The present study was designed with the view that the data on this topic is meager and no recent study is available in Pakistan, therefore this study generated local data and added the current knowledge to the pool of already developed literature. Hence steps towards

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better management of patients with proximal humerus fracture can be taken.

METHODOLOGY

A prospective observational study was undertaken at the Department of Orthopedic Surgery, Jinnah Postgraduate Medical Centre, Karachi for three years from June 2017 to May 2020. Permission from the ethical review committee was sorted prior to conducting the study with ethical approval (IRB No.F.2-81/2021-GENL/64326/JPMC). Informed verbal and written consent were taken from the patients before their induction in the study.

All patients with fracture of proximal humerus from 30 to 60 years of age, duration of less than seven days and either gender were included in the study. Patients with infected wound at fracture site, open wound, pathological fracture, diabetes mellitus, coronary heart disease, malignancy, recurrent fractures, polytrauma, pseudoarthrosis or bilateral fracture were excluded. A non-probability consecutive sampling was used to recruit participants in the study. A thorough clinical history regarding the cause, mode, and duration of fracture along with sociodemographic of the patients was documented on a predefined pro forma. Surgery was performed by a consultant having more than 5 years of experience in orthopedics. The primary outcome variable was the postoperative functional outcome of patients which was assessed by the 12th week of surgery. It was based on the Constant–Murley Score (CMS)^{7,8}. The Constant scores of 0 to 55 is termed as “poor”, 56 to 70 as “moderate”, 71 to 85 as “good”, and >86 is “excellent”. For the purpose of this study, the presence of a good or/and excellent functional outcome was considered as a satisfactory outcome. Whereas any score below 71 was considered as an unsatisfactory outcome.

Data was analyzed via SPSS version 26. Frequency and percentage were calculated for gender, functional outcome. Mean $\pm$ standard deviation was calculated for age, weight, height, BMI, Constant–Murley score and duration of fracture. Stratification was done to control effect modifiers like age, BMI, duration of fracture and gender. Post stratification chi square test was applied, p less than or equal to 0.095 will be taken as significant.

RESULTS

A total of 304 patients were recruited in the study with a mean age of 51.42 ± 7.939 years (range; 30–60).

Sociodemographic and clinical parameters of the study population are presented in Table 1.

Table 1: Characteristics of Study Participants (n=304)

Characteristics	n (%)	Mean $\pm$ SD
Age (in years)		51.42 $\pm$ 7.939
Age Groups		
< 45 years	106 (34.87%)	
> 45 years	198 (65.13%)	
Gender		
Male	118 (38.82%)	
Female	186 (61.18%)	
Constant score		60.45 $\pm$ 15.68
Weight (in Kg)		69.75 $\pm$ 17.98
Height (in meters)		1.5 $\pm$ 0.258
BMI (Kg/m ²)		26.12 $\pm$ 4.06
Distribution of BMI		
< 23	126 (41.45%)	
> 23	178 (58.55%)	
Duration of Disease/ Trauma		
< 3 days	216 (71.05%)	
> 3 days	88 (28.95%)	

In the present study, we reported that 216 (71.05%) patients who were managed with Philos plate for the management of fracture of the proximal humerus had satisfactory outcomes while only about 88 (28.95%) patients had unsatisfactory outcomes (Figure 1).

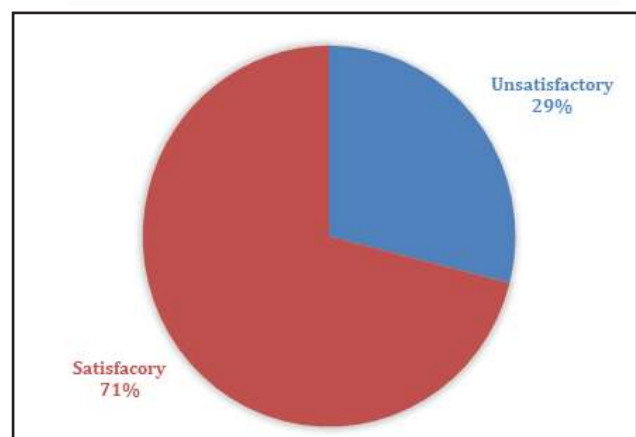


Figure 1: Functional Outcome in Patients with Proximal Humerus Fracture

We revealed that 28 (9.21%) patients had excellent, 188 (61.84%) had good, 10 (3.29%) had moderate, while 78 (25.66%) patients had poor outcomes (Figure 2).

Present study evaluated factors associated with poor/unsatisfactory outcomes in patients managed with



Figure 2: Functional Outcome According to Constant-Murley Score (CMS)

the Philos plate (Table 2). It was found that the majority of the patients with unsatisfactory outcome i.e., 54 (17.76%) were older than 45 years. However, the difference was insignificant ($p=0.32$). More females as compared to males had unsatisfactory outcomes, 62 (20.39%) and 26 (8.55%), respectively. Body Mass Index was not significantly associated with patient outcome. However, delay in presentation of more than three days was significantly associated with unsatisfactory outcome in patients as 82 out of 90 patients with delay > 3 days had unsatisfactory outcome at the end of the study ($p=0.0002$) (Table 2).

Surgical intervention for unstable fractures, however, is still challenging for the orthopedic surgeons, despite the advancement in the field¹⁰. Management of severe fractures without opting for surgical treatment is linked with poor outcomes¹¹. Several surgical techniques such as wiring and plating for complicated fractures is a testament to the lack of superiority of any one single method¹². Most of the surgical techniques result in complications, including hardware failure, mal-unions, osteonecrosis, or rotator cuff impairment. The present study evaluated the functional outcome of Philos plate fixation in patients with proximal Humerus fracture in our population. We reported an overall satisfactory functional outcome postoperatively in our study population with a mean Constant-Murley Score of 60.45 ± 15.68 . Our study findings are in accordance with recent literature^{13,14}. Ganesh et al., revealed that the mean Constant-Murley score (CMS) in their cohort of patients at the end of 3mo was 22.5 at 6mo 56 and at the end of 1yr was 73.3¹³.

Proximal humeral locking plates have shown a positive outcome in the management of the majority of the humeral fractures¹⁵. However, despite the benefits of the Philos fixation, certain studies have associated it with construct failure and need of recurrent surgery in patients over the age of 65¹⁶.

Table 2: Factors Associated with Patient Outcome in the Study

Variable	Patient Outcome			P-value
	Total	Satisfactory Outcome	Unsatisfactory Outcome	
Age Groups				0.32
Below 45 years	106 (34.87%)	70 (23.03%)	36 (11.84%)	
45 years or above	198 (65.13%)	144 (47.37%)	54 (17.76%)	
Gender				0.17
Male	118 (38.82%)	92 (30.26%)	26 (8.55%)	
Female	186 (61.18%)	124 (40.79%)	62 (20.39%)	
Distribution of BMI				0.192
< 23	126 (41.45%)	84 (27.63%)	44 (14.47%)	
> 23	178 (58.55%)	131 (43.42%)	44 (14.47%)	
Delay in Presentation				0.002*
< 3 days	214 (71.05%)	166 (54.61%)	48 (15.79%)	
> 3 days	90 (28.95%)	48 (15.79%)	42 (13.82%)	

* p-value is significant at < 0.095

DISCUSSION

Fractures of the proximal humerus are very frequent, resulting in 5-9% of all fractures⁷. It is the most common fracture in the elderly population⁸. Such fractures are generally stable and can be treated conservatively⁹.

In another study conducted at the Krishna Hospital and Research center Karad, it was revealed that 80 percent of the patients with proximal humerus fractures which were managed via Philos plate had excellent or good functional outcome¹⁷.

Philos plate has also been favored by surgeons because it is a minimally invasive procedure. It allows for an indirect reduction of the fracture, therefore reducing the probability of avascular necrosis and minimizing immobilization time, limiting the possibility of a frozen shoulder. Additionally, it is a fixating device, which is highly stable in osteoporotic bones. Our study is supported by the earlier published literature and highlights the significance of Philos plate fixation in the management of fractures of proximal humerus.

However, as true with any research our study also had certain apparent limitations. Firstly, since it was a single center study, the sample population was undiversified and had similar socio demographics hence, the applicability of the findings on a larger Pakistani population is not logical. Moreover, due to a lack of resources we were unable to keep a long-term follow-up of patients for more than 12 weeks.

CONCLUSION

We reported that Philos plate fixation provided adequate stability and overall, a good functional outcome. A delay in presentation was associated with a poor functional outcome. Further large-scale studies should be conducted to ascertain our findings and assess the long-term complications.

Conflict of interest: Authors declare that there is no conflict of interest.

Authors' Contribution: PA: Principal investigator and worked on Paper Writing, Data Interpretation, literature review, MB: Worked on Data collection, and Literature review, FJ: Helped in paper writing, DM, IM, EM, MS, and RE: worked on Data Collection.

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Cytopathological Spectrum of Pleural Fluid Effusion at a Tertiary Care Hospital of Karachi

Syed Mehmood Hasan¹, Asma Shabbir¹, Nazish Jaffar¹, and Syed Muhammad Hasan²

ABSTRACT

Objective: To evaluate the spectrum of pleural fluid effusion received for the period of one year at a tertiary care centre of Karachi

Methodology: This descriptive study was conducted after ethical approval at the Burhani Hospital Laboratory, Karachi. Data of cytopathologically diagnosed pleural fluid effusion was collected from the records available between June 2018 and May 2019. Relevant data pertaining registration number, age, gender of the patients, and diagnosis were recorded. Data was entered and analysed using SPSS version 21.

Results: Of the total 59 cases received, 44 (74.4%) were males and 12 (25.5%) were females. Age range was between 35 and 70 years with mean value = 55 ± 12.8 . Out of 59 cases, 15 (25.4%) were mixed inflammatory infiltrate, 12 (20.3%) showed chronic inflammatory cells, 08 (13.5%) had predominantly neutrophils, 06 (10%) were with atypical cells, 04 (6.7%) presented with adenocarcinoma, and 14 (23.7%) were labelled as inadequate/haemorrhagic samples.

Conclusion: Majority of the pleural effusion samples were adequate. Inflammatory lesions were more common in comparison to malignancy. Adenocarcinoma was the most common neoplastic entity. Pleural fluid cytology is an effective initial diagnostic modality in Pakistani health care setup.

Key Words: Adenocarcinoma, atypical, inflammatory cells, pleural effusion

How to cite: Hassan SM, Shabbir A, Jaffar N, Hasan SM. Cytopathological spectrum of pleural fluid effusion at a tertiary care hospital of Karachi. *Ann Jinnah Sindh Med Uni.* 2023;9(2):38-41

DOI 10.46663/ajsmu.v9i2.38-41

INTRODUCTION

Pleural fluid effusion refers to the accumulation of excess fluid within the pleural cavity. About a million people suffer from pleural effusion each year. Only in the United States, there are 1.3 million new cases each year. The common factors responsible for pleural effusion (PE) include conditions leading to volume overload, pulmonary infections, pleural infections, congestive heart failure, and malignancy^{1,2}. In almost half of the patients with pleural effusion, the underlying cause is direct involvement of pleura by a metastatic tumor. The indirect pleural involvement in malignancy may be due to pulmonary embolism, post radiotherapy effect, or hypoproteinemia³.

Pleural fluid cytology is the one of the traditional and reliable methods of analysis of pleural fluid. In addition, cytological examination of pleural fluid is helpful in diagnosis of malignancy⁴. In suspicious cases of malignant pleural effusions (MPE), the yield of pleural fluid cytology is reported to be 60%. However, a definitive diagnosis on cytology may not be obtained due to overlapping of cells, indistinct histological features, inflammatory infiltrate, and decreased number of representative cells⁵. Mycobacterium tuberculosis is endemic in Pakistan and the microbiological yield of acid fast bacilli is reported to be around 50%. Pleural fluid cytology is also helpful in distinguishing tuberculous pleural effusion (TPE) from MPE⁶. Limited published literature from Karachi region is available regarding pleural fluid cytology. The aim of this study was to observe the spectrum of pleural fluid cytology cases received in the period of one year at a tertiary care centre of Karachi.

METHODOLOGY

This descriptive study was conducted after ethical approval (IRB no: JSMU/IRB/2019/-273) and Lab approval at Burhani Diagnostic and Laboratory, Karachi.

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Data was collected from the records available between June 2018 and May 2019. All cytological diagnosed cases of pleural fluid reported during one year were included in the study. Relevant data pertaining to registration number, age, gender of the patients, and diagnosis were recorded. Cases with incomplete data were excluded from the study. Data was entered and analysis was done using SPSS version 21.

RESULTS

For the period of one year, 59 cases of pleural fluid cytology were recorded. Males were 44 (74.5%) and females were 15 (25.4%). M:F ratio in our study was 2.9:1. Age range was between 35 and 70 years with mean value = 55 ± 12.8 (Table 1).

Table 1: Distribution of Histological Findings in Pleural Fluid Cytology (n=59)

S. No.	Histological Finding	N (%)
1.	Mixed Inflammatory infiltrate	15 (25.4)
2.	Chronic inflammation	12 (20.3)
3.	Predominantly neutrophils	08 (13.5)
4.	Atypical cells	06 (10)
5.	Adenocarcinoma	04 (6.7)
6.	Inadequate / haemorrhagic samples	14 (23.7)
7.	Total	59 (100)

From the total 10 cases of mixed inflammatory infiltrate, 10 were males and 5 were females. Out of 12 cases of chronic inflammatory infiltrate, 10 were males and 2 were females. Predominantly neutrophils were seen in 5 cases of males and 3 females. Out of the total 14 cases with inadequate and haemorrhagic samples, 9 were males and 5 were females. Whereas, all the cases with atypical cells and adenocarcinoma were seen only in males (Figures 1 and 2).

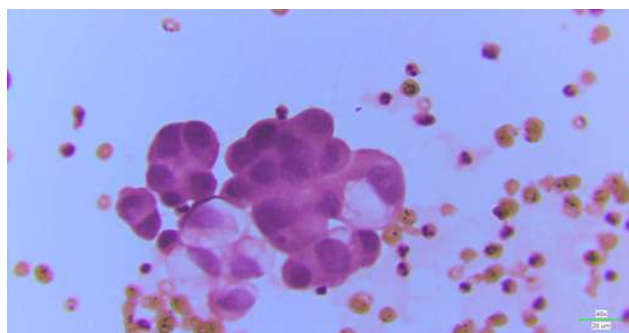


Figure 1: Photomicrograph of Pleural Fluid Showing Atypical Cells (H&E, 40X)

Four cases diagnosed as adenocarcinoma and six with atypical cell population, were further advised for clinical correlation and immunohistochemistry analysis to rule out the primary site.

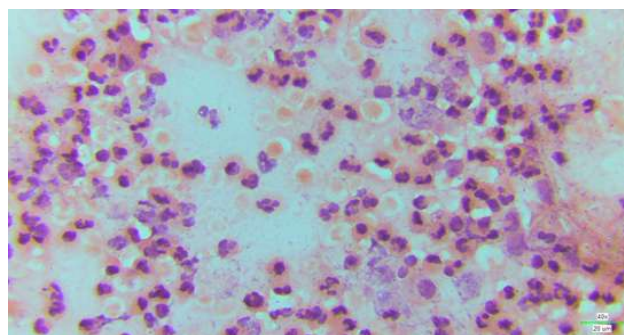


Figure 2: Photomicrograph of Pleural Fluid Showing Predominantly Acute Inflammatory Cells (H&E, 20X)

DISCUSSION

Pleural effusion can occur in multiple diseases and pose a diagnostic challenge. Pleural fluid cytology is a traditional and useful diagnostic modality for the initial investigation of pleural effusion. In resource poor countries like Pakistan, where expensive diagnostic approach is not available at majority of health care centres, pleural fluid cytology remains the first line of investigation. It provides the right pathway for further diagnostic workup and correct management of the patient⁷.

M: F ratio in our study was 2.9:1. Karachi cancer registry consolidated data of 5 year from 2017-2021 stated lung cancer to be in the top 5-10 most frequent preventable cancer list⁸. Additionally shaukat khanum cancer registry showed a declining trend for this malignancy placing it at 9th position for males. However, the recent declining trend revealed this malignancy to be at 9th position for males. Furthermore, in female population of Pakistan, lung cancer remains uncommon.

In one year's duration, we received 59 pleural cytology samples. About 6.7% cases were diagnosed as adenocarcinoma. Shaukat Khanum cancer registry, 2018, reported adenocarcinoma of lung as the most frequent subtype followed by squamous cell carcinoma in the local population⁹. A research from Peshawar showed a total of 39.1% malignant pleural effusions out of which 11.2% were malignant mesothelioma while the rest were secondary malignancies metastasized to lungs. These results may attributed to the fact that this study used both cytology and pleural biopsy for diagnosis¹⁰.

Moreover, 10% of the samples in the current series showed atypical cells. The confirmed diagnosis of the primary site of the neoplastic changes in these cells remained uncertain. Further work up including Immunohistochemistry and an inquest for primary site of malignancy was advised. The suspicious cell

population poses diagnostic challenge to pathologists. Reactive mesothelial cells, clumping of cells, increased number of inflammatory infiltrate, tend to overlap with suspicious malignant cells to create difficulty in leading to definitive diagnosis¹¹. However, another study on fluid cytology stressed upon the significance of utilization of ancillary techniques to accurately diagnose a malignancy instead of only relying on cytomorphologic features¹².

A considerable number of smears were infiltrated by inflammatory cells. About 25.4% samples showed mixed inflammatory cells, followed by 20.3% and 13.5% predominantly lymphocytic and neutrophilic infiltrate respectively. Acute inflammatory cells are mostly observed in cases with recent infection, pneumonia or empyema. A Turkish study is in agreement with our findings with 8.3% parapneumonic inflammation¹³. Lymphocytic infiltrate may indicate presence of tuberculous lesion in the background. In agreement with our study, an Indian research also recorded 21.8% lymphocytic and 10.9% mixed inflammatory cell population¹⁴. Since tuberculosis is prevalent in Pakistani population, , another study on the local population reported 44% chronic or granulomatous inflammation, supporting our findings¹⁵.

About 23.7% samples either had inadequate number of cells or were haemorrhagic. Although haemorrhage may be an indication of a malignant lesion, yet not all haemorrhagic smears can be concluded as cancerous. Presence of definitive nuclear pleomorphism, altered nuclear cytoplasmic ratio, and other parameters are still required for a definitive diagnosis¹². Other studies also reported a smaller percentage of inconclusive sampling. A Turkish and a Pakistani study revealed about 10% of inadequate samples each^{13,15}.

One of the limitations of the current study was a smaller sample size and another was that we could not follow up the patients who were advised for further immunohistochemistry and clinical correlation. Hence, we were unable to provide definitive diagnosis of these cases. However, we have attempted to identify the pleural cytology cases commonly diagnosed in our setup. Further studies are advised to be conducted on a larger population and not just on pleural fluid but other fluids also to highlight the importance of cytology.

CONCLUSION

Majority of the pleural effusion samples were adequate. Inflammatory lesions were more common in comparison to malignancy. Adenocarcinoma was the most common neoplastic entity. Pleural fluid cytology

is an effective initial diagnostic modality in Pakistani health care setup.

Conflict of interest: Authors declare that there is no conflict of interest.

Authors' Contribution: SMH conceived the idea and literature review, AS did statistics and manuscript writing, NJ did manuscript writing & editing. SMH did critical review & final approval.

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The Significance of Screening Mammography: A Preliminary Study

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ABSTRACT

Objectives: To assess the effectiveness of clinical breast examination, breast self-examination, and mammography screening in reducing breast cancer mortality in older, average-risk women, as well as the risks associated with screening

Methodology: A one-day mammography screening camp was held in Mirpur, Azad Jammu and Kashmir, Pakistan. This cross-sectional research included fifty women who were 40 years of age or older. A questionnaire on breast cancer screening attitudes was evaluated for validity. Bilateral mammography was performed after a clinical examination, and the results were reported using the ACR-BIRADS 5th edition guidelines.

Results: Mammography was performed on 50 women ranging in age from 40 to 65 years old, with a mean age of 48.6 years. Breast cancer screening behaviours (breast awareness, clinical breast examination, and mammography) were found to be substantially related to attitudes toward general health check-ups and perceived barriers to mammographic screening.

Conclusion: Regardless of cost constraints, a national programme for the diagnosis of breast cancer must include mammography, a tried-and-true screening technology. Mammography camps can be a very effective way to spread knowledge about these services and encourage people to use them.

Key Words: Breast cancer awareness, mammography, risk assessment, screening

How to cite: Khurshid F, Zia I, Ayesha UA, Yaqoob F, Khurshid H, Zia A, Khurshid A. The significance of screening mammography: A preliminary study. *Ann Jinnah Sindh Med Uni.* 2023;9(2):42-48

DOI 10.46663/ajsmu.v9i2.42-48

INTRODUCTION

Breast cancer, the second leading cause of death for women worldwide after lung cancer, is a frequent and expensive malignancy. With estimated yearly costs of 88 billion dollars and an average cost of almost 1.5 million dollars per affected woman, it imposes a

significant financial burden¹⁻³. Cancer's high mortality rate is mostly a result of the disease's late diagnosis, as survival is inversely correlated with the cancer's stage at diagnosis⁴. Breast cancer awareness is lacking. Women frequently visit hospitals near the end of their lives, at times of high mortality⁵.

Prevalence estimates in Pakistan are merely the tip of the iceberg⁶. Breast cancer incidence has increased by 50-100% in the last 20 years, according to trend analysis⁷. Male breast cancer is uncommon, accounting for less than 1% of all breast cancers, but there has been an increase in the frequency of the disease⁸. Breast cancer is distinct in that it can be quickly identified and caught early. However, because risk factors, access to systematic screening programmes and options for effective treatment vary across the world, so do breast cancer incidence, mortality, and survival rates⁷.

Early detection via screening programmes can lessen the morbidity and death linked to breast cancer⁹. It improves survival prospects, reduces the need for invasive therapy, and raises the likelihood that the disease will be successfully treated and cured^{10,11}. The two main actions for enhancing prognosis outcomes

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have been recognized as ensuring the availability of early diagnostic and screening services and acting quickly¹². According to studies, when compared to industrialized countries, most developing countries have poor prognosis and high mortality rate as a result of diagnosis of breast cancer in advanced stages^{13, 14}.

Breast self-examination (BSE), clinical breast examination (CBE), and mammography are all crucial preventive methods for the early detection of breast cancer. In low-resource areas with limited access to diagnostic facilities, it is crucial to empower women with BSE while screening for breast cancer^{15,16}. Despite the fact that mammography is frequently employed and advised for breast cancer screening, new screening techniques have emerged that have not been well examined for their effects on breast cancer mortality. The effectiveness of these tools in reducing breast cancer mortality is uncertain, despite their implementation in community settings^{13,17,18}. The aim of the study is to evaluate the benefits and dangers of clinical breast examination, breast self-examination, and mammography in lowering the death rate from breast cancer in older, average-risk women.

METHODOLOGY

In August 2023, a one-day screening mammography camp was held at the Aliya Begum Diagnostic and Cancer Care Centre in Mirpur, Azad Jammu and Kashmir (AJK). Since there is no ethical committee in the hospital, this study received ethical approval from the owner of the hospital where the camp is located. Informed consent was obtained to ensure that the study was conducted in accordance with ethical considerations such as participant confidentiality, and ensuring disclosure. The camp's objectives were to raise breast cancer awareness and encourage screening among women over 40. By handing out flyers and spreading the news, the public was made aware of the camp. The camp was performed in accordance with the ethical standards required for a prospective observational study. The study's goal was to observe the participants' outcomes and experiences. The camp's overall goal was to raise awareness and deliver free mammography services to the target community.

The convenience sample method was utilized in this investigation, including 50 willing participants. Instead of choosing the participants at random, they were chosen based on their willingness to participate. A complete clinical examination and pertinent history-taking were done before the mammogram. In a cross-sectional design, data was collected at a given point

in time in a cross-sectional manner, allowing for the investigation of correlations between variables.

Three elements made up the data collection tool: demographic information (such as age, marital status, economic situation, and level of education); questions to determine awareness of suggested screening methods for early breast cancer detection (including breast self-examination and mammography); and the Breast Cancer Screening Belief Questionnaire (BCSBQ) for screening. The 13-item Breast Cancer Screening Belief Questionnaire (BCSBQ) was developed by Kwok et al. in 2010. It looks at how women perceive several aspects of breast cancer screening, such as attitudes towards general health assessments, knowledge, attitudes, and perceptions of the disease, as well as screening practices like mammography. The survey uses a Likert-type scale with a range of 1 to 5, and converts the total score into a scale from 0-100. The BCSBQ is regarded as a credible and useful measure for examining women's perceptions about breast cancer screening¹⁹.

Before having bilateral mammography on a GE Senographe DS digital mammography machine, study participants gave written informed consent. Mammograms were performed using the proper compression forces, and for each breast, both medio-lateral oblique and cranio-caudal images were obtained. To guarantee the best possible imaging quality, the machine was set to the Automatic Optimization of Parameters (AOP) mode²⁰. The mammograms in the console were examined by a radiologist with five years' experience in breast imaging, and they were then reported in compliance with the guidelines of the 2013 ACR-BIRADS 5th edition. Any mass, asymmetry, architectural distortion, calcifications, or ancillary findings were recorded using words from the fifth edition of ACR BIRADS²¹. Due to the use of X-rays during the procedure, all the women received information on the procedure and the safety of mammography.

The Statistical Package for the Social Sciences (commonly known as IBM SPSS version 20) was used to compile a summary of the characteristics of the participating women using descriptive statistics and exploratory factor analysis (EFA). The present study employed appropriate statistical tests, such as correlation analysis or regression analysis, to evaluate the relationship between breast cancer screening behaviours (breast awareness, clinical breast examination, and mammography), attitudes towards general health check-ups, and perceived barriers to mammographic screening.

RESULTS

Fifty women of all ages 40 to 65 (mean 48.6 years), participated in the study. All of these women were Mirpur, AJK residents who were married. Forty per cent of the women reported having a job, compared to 54% who were unemployed. This suggests a mixture of employed and unemployed study participants. In terms of education, 10% of the women had only completed their primary or elementary education, 20% had post-graduate degrees, and 30% had graduated. Table 1 illustrates the participants' various educational backgrounds.

Table 1: Demographic Characteristics of Participants

Characteristics	Number	%	Mean	Standard Deviation	Variance
Age					
40-44	15	30%	48.6	7.021	49.305
45-49	13	26%			
50-54	12	24%			
55-59	5	10%			
60-64	5	10%			
Marital Status					
Married	50	100%	1.0000	0	0
Unmarried	0				
Employment					
Employed	20	40%	1.6500	0.58714	0.345
Unemployed	27	54%			
Retired	3	6%			
Education					
Uneducated	10	20%	3.8000	1.88065	3.537
Primary	5	10%			
Middle	5	10%			
Secondary	0	0			
Higher Secondary	5	10%			
Graduate	15	30%			
Post-Graduate	10	20%			
Residence					
Mirpur	50	100%	1.0000	0	0

The frequency of mammography screenings, clinical breast examination frequency, and attitudes toward general health checkups were all significantly linked with perceived barriers to mammographic screening and breast cancer awareness. The distribution of participants by awareness about breast cancer screening and frequency of practice is shown in Table 2. The findings reveal that most participants were aware of breast cancer. The study also showed that the individuals held false beliefs and concepts that were based on myths.

Female participants reported different levels of breast awareness: 20% reported annual awareness, 30% reported once every few month awareness, and 26% reported monthly awareness. Unexpectedly, 24% of interviewees said they had never been breast conscious. However, just 4% of the patient who were already diagnosed with breast cancer had had diagnostic mammogram rather than screening mammogram, while

Table 2: The Frequency of Breast Cancer Screening Practices

Breast Cancer Screening Practices	Frequency	Percentage	Mean	Standard Deviation	Variance
Breast Awareness					
1. Once a Month	13	26%	2.45	1.145	1.31
2. Once every few months	15	30%			
3. Once a year	10	20%			
4. Never	12	24%			
Clinical Breast Examination					
1. A year or less	13	26%	2.90	1.29	1.67
2. More than a year or less than 2 years	5	10%			
3. Two to three years or more than three years	7	14%			
4. Never had one	25	50%			
Mammogram					
1. Once a year	2	4%	3.85	0.67	0.45
2. Once every two years	0	0			
3. Once every three years or more	0	0			
4. Never	48	96%			

the other 96% had never had one. For early identification and prevention, routine mammograms and breast awareness are essential. It is alarming that many of the participants did not consider these exams important. To raise awareness and guarantee prompt identification and action, it is crucial to emphasize the value of routine breast exams and mammograms.

According to the analysis of the Breast Cancer Screening Belief Questionnaire (BCSBQ), the study's participants showed a positive attitude towards and a knowledge of the usefulness of routine physicals. Divergent perspectives on breast cancer were expressed, including disagreements over whether it should be considered a death sentence, different opinions on its prognosis and degree of suffering, and disagreements over the notion that little can be done to lower mortality rates. There were differences in individuals' perceptions of how fate affected the onset of breast cancer. Participants disagreed on worries about pain, transportation issues, language hurdles, and potential embarrassment, but overall they did not view mammographic screening to be significantly hampered. Regarding the degree of embarrassment connected to getting a mammogram, some conflicting views were noticed (Table 3 and Table 4).

Table 5 shows the various breast components detected during mammography in the study participants. Eighteen women exhibited fat density parenchyma, a sign of less dense breast tissue. Fifteen women had mixed-density parenchyma, or tissue that was both fatty and thick. There was a higher percentage of thick

Table 3. The Breast Cancer Screening Beliefs Questionnaire

Attitude Towards General Health Checkup	Score 01 Strongly Agree	Score 02 Agree	Score 03 Neutral	Score 04 Disagree	Score 05 Strongly Disagree
1: If I feel well, it is not necessary to have a health check-up.	15	5	3	25	2
2: If I follow a healthy lifestyle such as a balanced diet and regular exercise, I don't feel it is necessary to have a regular health check-up.	10	2	8	30	0
3: I see a doctor or have my health check-up only when I have a health problem.	23	27	0	0	0
4: If I feel healthy, I do not need to see the doctor.	17	23	3	7	0
Knowledge and Perceptions about Breast Cancer					
5: Breast cancer is like a death sentence; if you get it, you will surely die from it.	0	7	0	30	13
6: Breast cancer cannot be cured; you can only prolong the suffering.	0	13	7	23	7
7: Even if breast cancer is detected early, there is very little a woman can do to reduce the chances of dying from it.	3	12	0	30	5
8: If a woman is fated to get breast cancer, she will get breast cancer; there is nothing she can do to change fate.	5	15	10	17	3
Barriers to Mammographic Screening					
9: I'm worried that having a mammogram will hurt my breasts.	3	12	5	18	12
10: It would be difficult to arrange transportation for getting a mammogram.	3	2	3	37	5
11: I don't want to have a mammogram because I can't speak English.	0	0	0	33	17
12: I don't want to go for a mammogram because I would need to take off my clothes and expose my breasts.	0	3	5	32	10
13: Having a mammogram is embarrassing.	2	15	8	20	5

Table 4: Evaluation of BCSBQ

BCSBQ Components	Factors	Mean	Standard Deviation
Attitude Towards General Health Checkup			
1: If I feel well, it is not necessary to have a health check-up.	0.880	2.90	1.447
2: If I follow a healthy lifestyle such as a balanced diet and regular exercise, I don't feel it is necessary to have a regular health check-up.	0.863	3.25	1.251
3: I see a doctor or have my health check-up only when I have a health problem.	0.852	1.55	0.51
4: If I feel healthy, I do not need to see the doctor.	0.636/0.567	2.00	1.026
Knowledge and Perception about Breast Cancer			
5: Breast cancer is like a death sentence; if you get it, you will surely die from it.	0.761	3.95	0.945
6: Breast cancer cannot be cured; you can only prolong the suffering.	0.617/0.442	3.50	1.051
7: Even if breast cancer is detected early, there is very little a woman can do to reduce the chances of dying from it.	0.733	3.45	1.146
8: If a woman is fated to get breast cancer, she will get breast cancer; there is nothing she can do to change fate.	0.680	2.95	1.146
Barriers to Mammographic Screening			
9: I'm worried that having a mammogram will hurt my breasts.	0.787	3.50	1.277
10: It would be difficult to arrange transportation for getting a mammogram.	0.857	3.80	0.894
11: I don't want to have a mammogram because I can't speak English.	0.744/0.404	4.35	0.489
12: I don't want to go for a mammogram because I would need to take off my clothes and expose my breasts.	0.838	4.00	0.725
13: Having a mammogram is embarrassing.	0.750	3.35	1.04

tissue in four women's parenchymas that were heterogeneously dense. Seven of the women had hyperdense breast tissue, which is defined by significantly increased tissue density. For a woman with a history of left breast cancer, there were no indications of recurrent disease found in the left mastectomy bed. However, a right-side sono-mammogram revealed tiny breast cysts, which produced a BIRADS Category II sono-mammogram result. This classification denotes benign results with a low likelihood of cancer.

Table 5: Breast Composition on Mammography

Breast Composition	Number of Participants
Fat Density Parenchyma	18
Mixed Density Parenchyma	15
Heterogeneously Dense Parenchyma	10
Hyperdense	7

The following results were obtained during the evaluation using bilateral mammography: 4% of the females had new cases of breast cancer. 10% of patients were given the go-ahead to undergo brief interval follow-up because of likely benign findings. A low suspicion of malignancy was indicated by the BIRADS II findings in 44% of the cases. Forty per cent of the women received BIRADS I results, suggesting a normal interpretation of the mammography with no obvious abnormalities found (Table 6).

Table 6: ACRBIRADS: American College of Radiology-Breast Imaging Reporting and Data Systems

ACR-BIRADS Category	Number of Females	Percentage
BIRADS I	20	40%
BIRADS II	22	44%
BIRADS III	5	10%
BIRADS IV	0	0
BIRADS V	3	6%

DISCUSSION

Due to its high incidence and prevalence, overcrowded healthcare systems, and elevated direct medical costs, breast cancer has been acknowledged as a significant public health issue in both developed and developing countries⁶. The high number of unrecognized/hidden cases in Pakistan seriously jeopardizes the survival rate of female cancer patients. The conservative nature of the society, women's hesitation to seek medical care, customs, illiteracy, ignorance, and the absence of a widespread screening programme can all be linked to this. In India, the incidence rates of breast cancer have drastically grown with each passing year^{5,22,23}.

The multifactorial etiology of breast cancer involves a wide range of endogenous (hormonal/genetic) and

exogenous (drugs, radiation) factors. Additional risk variables include age, parity, breastfeeding practices, hormone therapy, dietary considerations, alcohol consumption, family history, age at menarche, menopausal status, genetic mutations, and benign breast disease. Typical symptoms of breast cancer include a tumor in the breast and bloody nipple leaking^{7,25-27}.

Early identification of breast cancer is crucial, particularly in developing countries with low income. Breast self-examination (BSE) has been suggested as an early detection technique. BSE is an inexpensive, straightforward, and non-invasive procedure that women can carry out on their own. It raises awareness, promotes healthy lifestyle choices, and may help find tiny tumors. However, there is some disagreement regarding the efficacy of BSE, and there are obstacles like lack of knowledge, time restraints, and cultural attitudes. To clarify the role of BSE and remove these obstacles, more studies are required^{7,17,18}. More female participants were attracted to the screening camp held as part of this study. This illustrates the importance of educating the public about these medical disorders by using word-of-mouth, healthcare professionals, handouts, etc. to combat ignorance, reluctance, and taboo. Without the awareness raised by the camp, the majority of the girls would not have had mammograms and clinical breast exams.

Screening techniques that reduce mortality include mammography and clinical breast examination. There is little evidence, however, that a clinical breast examination utilized as a stand-alone screening method reduces mortality. The methods used in clinical examinations are also not standardized²⁴.

There were a total of 13 items on the BCSBQ, with three components being 'attitude toward health checks,,', 'knowledge and perception regarding breast cancer', and 'mammography screening practice.' One being highly agree and five being strongly disagree, were used to rate the questions on a 5-point Likert scale. The better one understands breast cancer and the more one is willing to view physical examinations and breast cancer screening, the higher one's score is.

The CBCSBQ, in particular, looked into the use of mammography as a breast cancer screening method. Mammography is the most sensitive for detecting lesions in breasts with distributed fibroglandular densities or breasts that are completely fatty. The sensitivity of film screen mammography decreases when the breast parenchymal composition is dense. Breast density is also associated with an increased chance of developing breast cancer. The sensitivity of detecting lesions in dense breasts has improved with

digital mammography. MRI and ultrasonography support the screening regimen but cannot be utilized in place of mammography as the primary screening modality^{5,22,23}.

According to Bleyer and Welch's study, there is a high danger of over-diagnosis and overtreatment with mammography as a screening tool, but it has little effect on lowering mortality rates²⁵.

However, in a country like Pakistan where many instances are only found in the later stages, resulting in higher mortality rates, such a technique can only boost survival. There are rules governing the use of mammography as a screening method in many US states, and these laws are periodically amended as necessary²⁴. A mammography-based breast cancer screening programme in Pakistan is alleged to not be financially feasible⁵. It is the right time to start a thorough breast cancer screening programme in the current environment with Pakistan's expanding economy and focus on gender sensitivity. We can address societal taboos and hesitations and ensure that early identification of breast cancer has the greatest impact by holding mammography camps, clinical breast examinations, and awareness programmes. In order to advance gender equality and protect women's health, it is administratively necessary to increase mammography services and resource allocation. Some of the limitations of the study were that the sample size was small. There were only 50 women of a given age and location. Another important limitation of this study was the lack of a control group. These results may not apply to the entire population of women who have mammograms. Future research should aim to include larger, more diverse samples to examine the relationship between mental health, demographic variables, and the impact of associated factors on women's mammography experience.

CONCLUSION

In conclusion, this preliminary study highlights the importance of breast cancer screening for early detection and reduction of mortality. Priority should be given to including mammography in national screening, as it is a proven and reliable method despite financial constraints. Mammography camps can be an effective tool to raise awareness and encourage the use of screening services. In addition, addressing issues such as transportation, language and culture, breast awareness and routine screening are important to increase treatment, evaluate and ultimately reduce the burden. By implementing comprehensive screening and educating women about the importance of early

detection, we can make a positive impact in preventing breast cancer and saving lives.

Conflict of interest: Authors declare that there is no conflict of interest.

Authors' Contribution: FK: Study design and concept, manuscript writing, data collection, analysis and literature review, IZ: Data collection, UAA: Discussion writing, FY: Literature review, abstract writing, AK, AZ: Literature review, HK: Data analysis

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In Vitro Assessment and Application of Kinetics Models on Dissolution Profile of Several Brands of Clopidogrel (75Mg) Available in Karachi, Pakistan

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ABSTRACT

Objective: This investigation focuses on conducting a comprehensive in vitro standard evaluation of ten different Clopidogrel (75mg) brands available in Karachi, Pakistan.

Methodology: The study involved pharmacopoeial tests on ten Clopidogrel tablet brands, including weight variation, friability, disintegration, dissolution, and assay. Non-pharmacopoeial tests measured thickness, diameter, and hardness. Model-dependent approaches used first-order kinetics, Higuchi, Hixson Crowell, and Weibull models. Model-independent approaches calculated the difference (f1) and similarity (f2) factors for dissolution data analysis.

Results: The average weight variation of all ten coded Clopidogrel brands fell within the USP specification of $\pm 7.5\%$ deviation (225.9-356.1 mg). Measurements of hardness, thickness, and diameter met specified limits outlined by USP. Disintegration times of 2-8 minutes complied with the USP standard for film-coated tablets. Friability ranged from 0.02% to 0.3%, within the standard limit, indicating tablets' sufficient mechanical strength. Assay studies revealed Clopidogrel content within the range of 98.7% to 101.30%, aligning with USP assay monograph limits. Multiple point dissolution studies in 0.1N HCl showed drug release ranging from 90.9% to 99.8%, meeting USP specifications. Similarity factor (f1) and dissimilarity factor (f2) values were within limits, reinforcing bioequivalence. Kinetic models, including first order, Hixson and Crowell were applied, and all coded brands demonstrated acceptable r² values.

Conclusion: The study successfully implemented pharmacopoeial and non-pharmacopoeial tests on ten Clopidogrel (75mg) brands available in Karachi, Pakistan, demonstrating compliance with USP specifications. While the results suggest the suitability of these formulations for therapeutic use, a larger-scale study is recommended for further validation and understanding.

Key Words: Comparison, in vitro, kinetics approaches, model dependent

How to cite: Akhtar H, Ahmed KZ, Batool H, Sheikh A, Rafique W, Mansoor N. In vitro assessment and application of kinetics models on dissolution profile of several brands of clopidogrel (75mg) available in Karachi, Pakistan. Ann Jinnah Sindh Med Uni. 2023;9(2):49-55

DOI 10.46663/ajsmu.v9i2.49-55

INTRODUCTION

In-vitro testing encompasses all activities aimed at gathering additional information and data about any product. This information is crucial for product improvement and the establishment of regulations and standards¹. The manufacturing processes of pharmaceuticals significantly affect the quality of the

product's. One crucial technique for determining product acceptability is "dissolution testing," which evaluates a drug product's lot-to-lot quality and guides the development of new formulations².

Tablets, as a dosage form, are generally more stable than other forms, such as liquids, and have a longer shelf life, making them easier to store and transport³. Clopidogrel, a thienopyridine, falls within the category of substances that have low solubility/high permeability according to bio pharmaceuticals classification. Due to its low water solubility, Clopidogrel exhibits a relatively low oral bioavailability (less than 50%) and is activated by enzymes, particularly CYP2C19 and CYP3A4 enzymes⁴⁻⁶.

In this context, the study focuses on the assessment of various formulations of Clopidogrel (75mg) available

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in the market in Karachi, Pakistan. The tablets' physical attributes, such as weight variation, hardness, thickness, diameter, friability, and disintegration time, are examined to ensure compliance with pharmacopeial standards. Additionally, multiple-point dissolution studies are conducted to evaluate the kinetics of drug release from the formulations.

The growing number of formulations in the market emphasizes the need for rigorous quality control measures, comparing each product against standards outlined in Pharmacopoeia. Tablets must possess sufficient mechanical strength to withstand handling, storage, and transport, while also ensuring consistent drug release in the gastrointestinal tract^{7,8}. The study introduces ten designed formulations evaluated for various parameters, including hardness, friability, thickness, assay, wetting time, disintegration time, and in-vitro drug release. The primary objective is to conduct pharmacopeial and non-pharmacopeial tests on several brands of Clopidogrel available in Pakistan nationally and apply various kinetic models to assess the multiple-point dissolution profile of Clopidogrel film coated tablets of (75mg).

METHODOLOGY

Ten products of Clopidogrel USP 75mg brands were randomly selected from the local market in Karachi, Pakistan, and each was assigned a unique identification code (C1, C2, and C3...C10). This study does not involve any human or animal studies therefore ethical review from IRB is exempted.

Software used: Microsoft Excel 2016 was employed for data analysis, while the dissolution profile evaluation and application of kinetic models were conducted using the DD Solver add-in programme.

Instruments and Reagents: The study utilized the following equipment: Vernier Caliper (Seiko, China), Friability Tester (Curio FB 2020, Pakistan), Digital Hardness Tester, USP Basket Rack Assembly (DA 6D, Veego, India), USP Type 2 Paddle Dissolution Apparatus (Curio, Pakistan), UV Spectrophotometer (Shimadzu, Japan), Analytical balance (Shimadzu, Japan). Additionally, hydrochloric acid and distilled water were used as reagents.

Pharmacopoeial tests

Weight variation test: To initiate the weight variation test, ten Clopidogrel tablets from each of the ten brands were individually and randomly selected for weighing. The tablets were carefully chosen to ensure a representative sample. This average weight was then compared with the specific weight of each tablet to

identify any variations. The estimation of weight variation was determined using the formula:

$$\text{Weight Variation} = (\text{Individual weight-average weight}) \times 100 / \text{average weight} \text{ (Table 1)}$$

Table 1: Limits of weight variation according to USP

Mean weight of tablet	Percentage difference
<130mg	±10%
>130mg and < 324mg	±7.5%
>324mg	±5%

The test criteria state that if no tablet deviates by more than twice the specified percentage limit, and no more than two tablets exceed the designated percentage, the tablet passes the weight variation test^{9,10}.

Friability: The friability test for tablets was conducted using a Roche friabilator. Ten tablets were selected, and their initial weights were collectively recorded. Subsequently, these tablets were placed in the friabilator and rotated at 25 revolutions per minute (rpm) for a duration of 4 minutes. Upon completion of the test, the same ten tablets were removed, and their final weights were recorded. This procedure was repeated for tablets from other coded brands. Friability was determined using the following formula:

$$\%F = (1 - W/W_0) \times 100 \%$$

Friability of tablets <1% are treated as satisfactory¹¹.

Disintegration Test: The disintegration test is a crucial evaluation, providing insights into the tablet's ability to break down into smaller particles, facilitating proper absorption of the drug. Adherence to the disintegration time limit is essential for ensuring the efficacy and quality of the tablets. Six tablets were chosen from each coded brand to assess their disintegration properties. For the test, each tablet was placed within an open-ended tube on a wire mesh fixed at one of its ends⁶. One tablet was introduced into each of the six tubes comprising the assembly for the coded brand. The test was conducted using distilled water at a temperature of $37 \pm 2^\circ\text{C}$, and the duration of the test was set at 15 minutes.

Upon complete disintegration of the tablets, the time was meticulously noted. It is worth mentioning that, according to the United States Pharmacopeia (USP) guidelines, the disintegration time should not exceed 15 minutes for tablets to meet the specified standards¹². This procedure was replicated for tablets from other coded brands, including C2, C3, C4.....C10.

Dissolution: The dissolution test was conducted using a USP Type II dissolution apparatus operating at 50 rpm. The dissolution medium for Clopidogrel comprised

0.1N HCl. Samples were withdrawn at specific time intervals, including 5 minutes, 10 minutes, 15 minutes, 20 minutes, 25 minutes, and 30 minutes. To maintain sink conditions, 10ml of freshly prepared 0.1 N HCl was added each time a sample was withdrawn.

The absorbance of each sample at the designated time points was measured using a UV spectrophotometer at a wavelength of 270nm, with 0.1N HCl serving as the blank. This rigorous testing protocol ensures accurate monitoring of the drug dissolution profile, crucial for assessing the tablet's performance and adherence to dissolution specifications.

Assay: The assay test was conducted using a 0.1N HCl solution. One tablet was placed in a 50ml volumetric flask containing 0.1N HCl. The mixture was sonicated for 5 minutes, followed by the transfer of 5ml of the solution into another 50ml volumetric flask. This was then diluted to 0.1N HCl, and sufficient volume was added to achieve the required total volume.

Subsequently, the solution was passed through a 0.45µm filter, discarding the initial 5ml. The absorbance of the filtered solution was measured using a UV spectrophotometer at 270nm. This entire process was repeated for all selected Clopidogrel brands. The obtained results were then compared to assess the uniformity of content against the established standard. This meticulous assay test ensures accurate determination of the drug content, a critical parameter for evaluating the quality and consistency of the tablets.

Non-pharmacopoeial tests

Thickness and diameter: Non-pharmacopoeial tests were conducted to assess tablet characteristics, specifically thickness and diameter. Vernier calipers were employed for precise measurements. Ten tablets from each brand were individually measured by sliding them between the jaws of the Vernier calipers. It is essential to note that the tablet thickness should fall within a ±5% variation of a standard value, ensuring compliance with quality standards¹³. These non-pharmacopoeial tests provide valuable insights into tablet properties, and the obtained measurements were meticulously recorded and analyzed using Microsoft Excel 2019 for comprehensive evaluation and comparison among different tablet brands.

Hardness: Tablet hardness is a crucial parameter guiding both product development and quality control. Ten tablets from each product were individually tested using a digital hardness tester, and their hardness values were recorded. The acceptable range for hardness is set between 4 to 10 kg.

Model Dependent Approach

First order kinetics model: This model illustrates the absorption and elimination characteristics of the compound. First-order kinetic reactions are concentration-dependent, with higher concentrations resulting in greater elimination per unit time. Q_0 and Q_t represent the initial dosage form quantity and the amount released at time t , respectively.

$$\text{Log } Q = \text{Log } Q_0 - \frac{kt}{2.303} \quad (2)$$

Here,

t = time

K = First Order Rate constant

Higuchi model: This model illustrates the release mechanism of a drug from tablet matrices and is applicable to porous systems. It establishes a direct proportionality between the total amount of drug release and the square root of the time period.

Here

K_{HZ} represents Higuchi constant

$$Q = kt^{\frac{1}{2}} \quad (3)$$

Hixson Crowell model: This model expresses the change in diameter and surface area during drug release of tablet. It depicts the dissolution rate as a function of time for a decrease in surface area of solid.

$$Q_0^{1/3} - Q_t^{1/3} = K_{HC} \times t \quad (4)$$

Here,

K_{HC} represents Hixson–Crowell Rate constant

Weibull model: This model is presented to compare drug release profile of matrix system. It is also useful in comparing release patterns of matrix system.

Weibull model described for different dissolution mechanisms.

$$m = 1 - \exp \left[-\frac{(t-T_i)^\beta}{\alpha} \right] \quad (5)$$

M = drug's dissolution rate as a function of time t

M_0 = entire quantity of drug released

T = lag time

Eq.5 can be written as:

$$\text{Log}[-\ln(1 - m)] = b \log(t - T_i) - \log \alpha \quad (6)$$

Model Independent Approaches: The following

formulas will be used to get the dissolution data's difference factor (f_1) and similarity factor (f_2).

$$f_1 = \left[\frac{\sum_{t=1}^n (R_t - T_t)}{\sum_{t=1}^n R_t} \right] \times 100 \quad (7)$$

$$f_2 = 50 \times \log \left\{ \left[1 + \left(\frac{1}{N} \right) \sum (R_i - T_i)^2 \right]^{-0.5} \right\} \times 100 \quad (8)$$

n = number of samples

R_t = per cent release of the reference drug

T_t = per cent release of test drug

The Limit of Difference factor (f_1) is between 0 and 15, while the Similarity factor (f_2) is between 50 and 100.

RESULTS

Table 2: Pharmacopoeial and non - Pharmacopoeial Test for Clopidogrel (75mg)

S.No	Formulation Codes	Weight (mg) Mean $\pm$ SD	Thickness (mm) Mean $\pm$ SD	Diameter (mm) Mean $\pm$ SD	Hardness (kg) Mean $\pm$ SD	Friability (%)	Disintegration Time (minutes) (not >15 minutes)
1	C1	287.5 $\pm$ 3.930	4.82 $\pm$ 0.04	9.8975 $\pm$ 0.06	5.465 $\pm$ 0.24	0.10	4 minutes 22 seconds
2	C2	306.3 $\pm$ 3.226	3.545 $\pm$ 0.15	9.8 $\pm$ 0.03	5.802 $\pm$ 0.65	0.16	2 minutes 22 seconds
3	C3	319.7 $\pm$ 3.067	4.38 $\pm$ 0.04	10.35 $\pm$ 0.05	5.192 $\pm$ 0.34	0.09	2 minutes 57 seconds
4	C4	309.9 $\pm$ 4.805	1.85 $\pm$ 0.04	10 $\pm$ 0.06	5.63 $\pm$ 0.41	0.03	6 minutes 8 seconds
5	C5	225.9 $\pm$ 2.3	3.64 $\pm$ 0.08	8.925 $\pm$ 0.04	6.63 $\pm$ 0.77	0.3	4 minutes 8 seconds
6	C6	350.8 $\pm$ 5.582	4.905 $\pm$ 0.01	10.075 $\pm$ 0.04	4.967 $\pm$ 0.56	0.02	3 minutes 45 seconds
7	C7	251.3 $\pm$ 5.367	3.625 $\pm$ 0.34	9.075 $\pm$ 0.04	5.215 $\pm$ 0.34	0.03	8 minutes
8	C8	258.7 $\pm$ 2.794	4.105 $\pm$ 0.01	8.825 $\pm$ 0.04	5.192 $\pm$ 0.97	0.07	6 minutes 50 seconds
9	C9	356.1 $\pm$ 6.518	3.32 $\pm$ 0.071	10.5 $\pm$ 0.08	6.337 $\pm$ 1.067	0.02	7 minutes 20 seconds
10	C10	291.2 $\pm$ 2.785	3.3 $\pm$ 0.01	9.925 $\pm$ 0.04	5.577 $\pm$ 0.041	0.03	2 minutes 1 seconds

Table 3: Assay Test of Clopidogrel (75mg)

No. of Tablets	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10
10	99.80%	100.10%	99.60%	101.20%	100.40%	99.90%	101.40%	98.70%	101.90%	100.20%
10	99.60%	101.20%	100.20%	100.40%	100.10%	98.70%	101.10%	99.60%	101.10%	100.10%
10	100.10%	99.90%	101.40%	99.90%	99.50%	99.90%	101.40%	99.60%	99.60%	99.60%
MEAN	99.83%	100.40%	100.40%	100.50%	100.00%	99.50%	101.30%	99.30%	100.87%	99.97%
SD	$\pm$ 0.002	$\pm$ 0.005	$\pm$ 0.007	$\pm$ 0.005	$\pm$ 0.003	$\pm$ 0.005	$\pm$ 0.001	$\pm$ 0.004	$\pm$ 0.009	$\pm$ 0.002

Table 4: f_1 and f_2 Tests with Reference Formulation C7

Similarity (f_2) and Dissimilarity (f_1) factor at 0.1N HCl	C1	C2	C3	C4	C5	C6	C8	C9	C10
f_1 difference factor	9	4	6	6	6	6	5	7	8
f_2 similarity factor	61	74	66	67	67	69	70	66	63

DISCUSSION

This study aimed to assess different brands of 75 mg clopidogrel available in Karachi, Pakistan, focusing on parameters crucial for good manufacturing practice, such as tablet size and dosage uniformity¹⁴. According to the USP, for tablets weighing more than 130 mg, the mean weight variation should be within $\pm 7.5\%$, with not more than two tablets deviating from the average weight, as indicated in Table 1.

The average weight of clopidogrel tablets in this study ranged from 225.9 to 356.1 mg, as presented in Table 2¹⁵. Hardness is a key parameter indicating the force required to break a tablet, influencing handling, dissolution, and disintegration. USP recommends tablet hardness between 4 to 10 kg¹⁶. The tablets in our study exhibited hardness in the range of 4.967 to 6.63 kg, ensuring sufficient mechanical strength. Using Vernier

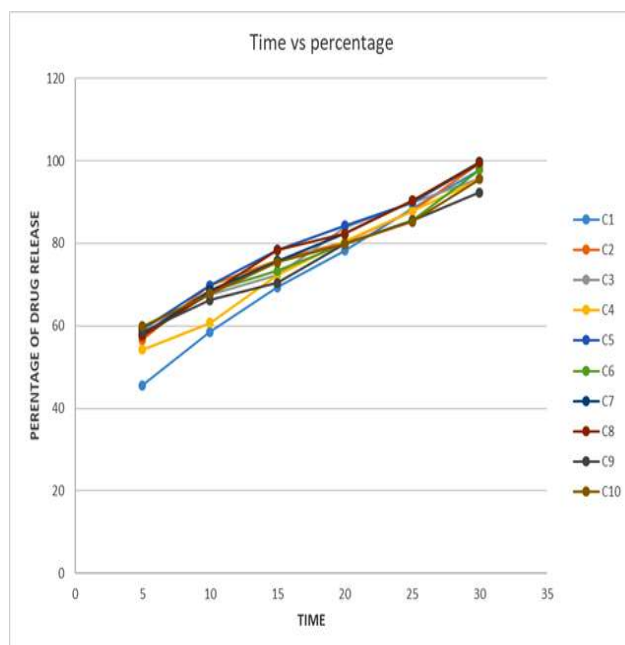


Figure 1: Graphical Presentation of Multiple Point Dissolution Studies of Clopidogrel 75mg at 0.1N HCL.

USP standard (90.0% to 110.0%), ranging from 98.7% to 101.30% (Table 3). The In-vitro Dissolution method is becoming an increasingly valuable tool for predicting bioavailability, often replacing expensive in vivo clinical research to demonstrate bioequivalence. It stands as one of the most crucial quality control tests for pharmaceutical dosage forms²⁰. In this study, we examined ten different brands of Clopidogrel for dissolution in 0.1N HCl. For each brand, ten milliliters of sample were drawn at multiple time points (5, 10, 15, 20, 25, and 30 minutes) during the dissolution of 75 mg Clopidogrel tablets. The percentage of drug release was analyzed using a UV spectrophotometer at a wavelength of 270nm, with 0.1 N HCl as the blank. Figure 1 illustrates the graphical presentation of drug release at these multiple time intervals in 0.1 N HCL. At the 30-minute mark, the drug release from tablets of all selected brands was within the specified limit of the USP (not less than 80% in 30 minutes), ranging from 90.9% to 99.8%. In the past Clopidogrel quality test were conducted depending

Table 5: Release Kinetics of Coded Tablets of Clopidogrel (75mg)

Coded Tablets	First Order		Higuchi		Hixon Crowell		Weibull Model		
	r^2	$k_1(m)$	r^2	$kH(m-1/2)$	r^2	$kHC(m-1/3)$	r^2	B	A
C1	0.9720	0.064	0.9779	15.692	0.957	0.018	0.9727	0.967	14.235
C2	0.9038	0.070	0.9395	16.558	0.9234	0.019	0.9055	1.058	16.841
C3	0.9005	0.067	0.9328	16.221	0.9133	0.018	0.9009	1.027	16.098
C4	0.9062	0.068	0.9368	16.281	0.9163	0.018	0.9065	1.023	15.753
C5	0.9033	0.068	0.9365	16.270	0.9139	0.018	0.9035	1.020	15.622
C6	0.9240	0.068	0.9537	16.291	0.9289	0.019	0.9240	1.007	14.953
C7	0.9162	0.077	0.9656	17.217	0.9216	0.021	0.9162	1.006	13.206
C8	0.9638	0.085	0.9942	17.807	0.9482	0.023	0.9639	0.986	11.289
C9	0.9864	0.083	0.9875	17.409	0.9279	0.022	0.9948	0.899	9.194
C10	0.9741	0.089	0.9699	17.819	0.9126	0.023	0.9817	0.903	8.759

calipers, we measured the thickness and diameter of ten clopidogrel brands, finding standard deviations within $\pm 5\%$ deviation, as shown in Table 2. For film-coated tablets, disintegration time is crucial, ensuring uniformity across batches and consistent bioavailability¹⁷. In our study, disintegration times ranged from 2 to 8 minutes, well below the USP standard of 15 minutes. The friability test assessed how well tablets withstand mechanical shocks and attrition during production, packaging, and shipping. USP recommends friability less than 1%, and our study revealed friability between 0.02% to 0.3%^{18,19}. The assay test, evaluating API content in pharmaceutical tablets, showed that all Clopidogrel brands met the

on USP Pharmacopeia. Various kinetic models, including first order, Higuchi, Hixon Crowell, and Weibull models, were applied to the Clopidogrel dissolution data²¹. First-order kinetics leverages the concepts of absorption and elimination to describe the behaviour of a molecular entity²². The Higuchi model, suitable for porous systems, elucidates the drug release mechanism from tablets²³. The Hixon Crowell model describes changes in surface area and diameter of tablets during drug release²¹. The Weibull kinetic model presents different types of drug release behaviours and is useful for contrasting release patterns of various matrix systems²⁴. To measure the similarity between dissolution profiles, factors f_1 and f_2 were employed.

The Limits of Difference factor (f_1) ranges between 0 and 15, while the Similarity factor (f_2) ranges between 50 and 100. In this study, f_1 and f_2 values were calculated using the C7 reference standard, selected based on drug release. All brands showed f_1 and f_2 values within the acceptable limits, as presented in Table 4. Equations 2, 3, 4, 5, and 6 were utilized to compute model-dependent techniques, including First order, Higuchi model, Hixon Crowell model, and Weibull model, using a dissolution data solver and add-in programme in Microsoft Excel 2016. All kinetic models were evaluated based on the r^2 statistic for tablets designated C1–C10, as detailed in Table 5.

CONCLUSION

This comprehensive study aimed to assess the pharmaceutical quality and dissolution behaviour of various brands of 75 mg Clopidogrel tablets available in the local market of Karachi, Pakistan. The investigation covered a range of pharmacopoeial and non-pharmacopoeial tests, including weight variation, hardness, friability, disintegration time, thickness, diameter, assay, and dissolution studies. The tablets demonstrated satisfactory weight uniformity, mechanical strength, and resistance to friability, meeting pharmacopoeial standards. Disintegration times were well within the specified limits, ensuring prompt drug release in the gastrointestinal tract. The assay results indicated that the tablets contained the active pharmaceutical ingredient within the acceptable range, confirming the potency of the formulations. The dissolution studies, conducted in 0.1N HCl at various time intervals, revealed consistent drug release profiles for all brands. At the 30-minute mark, the drug release from all tested brands was within the USP-specified limits (not less than 80% in 30 minutes), ranging from 90.9% to 99.8%. Additionally, various kinetic models, including first order, Higuchi, Hixon Crowell, and Weibull models, were applied to the dissolution data, providing insights into the drug release mechanism but it was observed that clopidogrel release mechanism follows first order kinetics and Hixon Crowell cube root law. Furthermore, model-independent approaches, utilizing factors f_1 and f_2 , demonstrated similarity between dissolution profiles, reinforcing the quality and bioequivalence of the tested formulations. The values of f_1 and f_2 , obtained with the reference standard C7, fell within the acceptable ranges. This research successfully integrated pharmacopoeial and non-pharmacopoeial assessments, dissolution studies, and kinetic modeling to comprehensively evaluate the quality and performance of different Clopidogrel formulations. The results affirm the suitability of these formulations for therapeutic use and contribute valuable data for regulatory compliance and pharmaceutical

quality assurance¹⁹. The methodology employed, including the application of various kinetic models, enhances our understanding of drug release mechanisms, supporting further advancements in pharmaceutical research and development.

Conflict of interest: The authors declare no conflict of interest.

Authors' Contribution: HA and KZA: worked on Conceptualization, Financial support and guidance, HB: worked on Introduction, AS: worked on Methodology, WR: Worked on Results and NM: Worked on Discussion.

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Post-Operative Sensitivity in Posterior Composite Resin Restorations Using Etch and Rinse Versus Self-Etch Adhesive Systems

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and Muhammad Usman Khattak⁴

ABSTRACT

Objective: The purpose of performing this study was to weigh up the frequency of post-operative sensitivity (POS) using two-step etch and rinse against one step self-etch adhesives.

Methodology: This study was carried out in the Operative Dentistry's department, Fatima Memorial Hospital College of Medicine and Dentistry, Lahore and it was completed in 18 months from February 2020 to August 2021. A total of 60 patients presenting with carious lesions in maxillary and mandibular posterior teeth excluding third molars were included. Patients were divided into groups of two comprising of 30 patients each. Restoration of group A was performed using Excite (Ivoclar Vivadent, Schaan, Liechtenstein) in etch and rinse technique and restoration of group B was performed using Xeno III (Dentsply, Ballaigues, Suïça) in self-etch adhesive technique.

Result: The outcome of this study showed no significant difference in the frequency of POS in etch and rinse vs self-etch adhesive groups based on gender, age groups and educational status.

Conclusion: Composite restoration done using self-etch adhesive has similar results in terms of POS in comparison to restorations done using etch and rinse adhesive.

Key Words: Dental adhesives, post operative sensitivity, resin composite, restoration

How to cite: Keen HA, Ehsan S, Abbas Z, Qureshi HN, Khattak MU. Post-operative sensitivity in posterior composite resin restorations using etch and rinse versus self-etch adhesive systems. Ann Jinnah Sindh Med Uni. 2023;9(2):56-60

DOI 10.46663/ajsmu.v9i2.56-60

INTRODUCTION

People go to dental practitioner for pain relief and it is confirmed that the complaint of tooth sensitivity is felt differently at different times with differing stimuli and intensities. Restorative treatment is performed to get rid of signs and symptoms of reversible pulpitis from patient's teeth. If the previous sensitivity has not resolved or has resulted in a new POS, it gets distressing for the patient and the dentist. Treatment with lower prevalence of symptoms postoperatively is considered as treatment of choice.

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Posterior composites have become a widely known filling material replacing amalgam¹ due to three main reasons like demand for tooth-colored fillings, patients' concerns regarding mercury toxicity² and Minamata Agreement³. Apart from various reasons for failing posterior composites, POS is a usual phenomenon. POS may show a steady decrease over time or may persist for longer. The factors responsible for POS are not clear. It can be due to many factors few of them are shrinkage occurring after polymerization, gaps at the margins, below average adhesion, inadequate polymerization, unfavorable configuration factor (C-Factor) and remaining dentinal thickness (RDT), pre-operative dental factors, such as cracks⁴. The mechanism of persistent POS is that polymerization shrinkage forms a gap under the restoration which is then filled with dentinal fluid. When a stimulus whether hot or cold is applied to a tooth, fluid expansion or contraction forms gap and the fluid movement in the tubules causes POS⁵. Pulp is packed with sensory afferents that are involved in pain perception, whereas dentin has lesser amount of innervation but is very prone to sensitivity⁶. It is still doubtful if the fluid movement excites the nerve ending directly within the superficial pulp or

inner ends of tubules, or if the odontoblasts have a part in transduction mechanism.

Patients that are supposed to receive posterior composite resin restoration are usually those with caries or have signs and symptoms of reversible pulpitis. As the cavity depth increases, the permeability of dentin changes too and makes the tooth prone to POS. New adhesives have been constantly introduced that bond enamel and dentin effectively but even then, POS remains a major distress for the dentist⁷. Two types of adhesive applications are there according to whether or not prior etching with acid is needed. Self-etch adhesives are advantageous when compared with adhesives that require acid etching as an additional step. The primer has acid in it, so no rinsing is required. Thinner hybrid layer is formed, but the etched area is formed by the adhesive, thus minimizing hydrolysis of hybrid layer and hence, decreasing the chances of POS.

Few studies found no difference in POS between both adhesive systems. Rest of the studies inferred that, in cavities with reduced RDT, the use of self-etching adhesives was successful in reducing POS in comparison to total-etch adhesive systems⁸. Swift et al concluded that, after 6 months of placement, 17% of the patients suffered from POS following the use of etch and rinse group in comparison to 10% in self-etch adhesive group but it decreased with time eventually producing no significant difference. In order to duplicate this study in Pakistani population, a pilot study consisting of 30 patients in each group was carried out in our department from 2018-2019, that showed sensitivity incidence of 35% in etch and rinse and 7.1% in self-etch group. Therefore, in the light of pilot study conducted in the population of Pakistan and the rest of literature, this study has been planned to compare etch and rinse and self-etch in relation to POS frequency in Pakistani population. The results of this study will add to the literature the only adhesive strategy that is less prone to POS in relation to composite restorations, so as to provide the most predictable and pain free treatment to the patient.

METHODOLOGY

It was a randomized control trial. Sixty patients were selected as sample size using OpenEpi software. The Institutional Review Board of FMH approved this study by certifying it with IRB certificate No. FMH-03-2019-IRB-586-M. Patients were recruited at the outpatient department. Informed consent was taken from the patients. Thirty were in Group A (Two step etch and rinse) and other 30 were selected for group B (one step self-etch) with confidence level, power of test and level of significance to be 95%, 80% and 5% respectively, taking expected frequency of sensitivity

as 35% in etch and rinse and 7.1% in self etch group (cohort study). The technique applied was non-probability consecutive sampling.

Inclusion criteria was male and female patients within 18-50 years in age with posterior teeth having class 2 cavities with supra gingival margins and no preoperative signs and symptoms.

Exclusion criteria was those with previously restored teeth, deep caries with significant signs and symptoms like spontaneous pain and periapical infection and patients who do not give informed consent.

Random division of patients was done to eliminate bias consisting of 30 patients in each group using random number tables. Group A was assigned etch and rinse adhesive whereas group B was assigned self-etch. The procedures were done under isolation of rubber dam. Patients were informed to report on follow up calls after 24 hours, 15 days, and 30 days. Patients' response was graded on Visual Analogue Scale (VAS). Patient is supposed to have POS if he/she presents with pain of moderate or severe intensity (>4 on VAS), when evaluated at the end of 24 hours, 15 days, and one month. The patients were asked if they felt any sensitivity on taking hot or cold drinks/food, spontaneous pain, or pain on mastication.

Small round diamond bur was used to remove class 2 carious lesions followed by excavation. The cavity was later isolated and dried. Group A cavities were applied with two steps etch and rinse adhesives and group B cavities were applied one step self-etch adhesives. For Group A, class 2 cavities were acid etched with phosphoric acid for 15 second and then washed out for 10 second. The tooth surface was dried using triple air syringe. Excite (Ivoclar Vivadent, Schaan, Liechtenstein) was applied. Then, it was light cured for 10 seconds. Incremental technique of composite placement using Ivoclar Vivadent was applied. Group B class 2 cavities were applied with multiple coats of self-etchant using Xeno III (Dentsply, Ballaigues, Suíça), left in place for 20 seconds and air dried. Light curing was done for 10 second and finished with incremental technique of composite placement with Ivoclar Vivadent. Carbide finishing burs were used to perform minor finishing followed by polishing to achieve final luster.

The data was examined using SPSS-22.0. For descriptive analysis, means and standard deviation were calculated for the variables of quantitative nature which were age and pain at one month. To compare mean pain score, we used repeated measures ANOVA at 24 hours and one month. To assess the pain between two groups, independent sample T test was used to measure percentage of patients that have POS. Data

was stratified for age, gender, and educational status. Post stratification T test was used taking p-value <0.05 as significant.

RESULTS

Table 1 shows pain responses of patients with etch and rinse, with 80.0% patients presented with no pain after 24 hours, 83.3% with no pain at 15 days and 90.0% patients with no pain after 30 days. Moderate pain was observed in 10.0%, 13.3% and 10.0% of patients at 24 hours, 15 days and 30 days respectively. Whereas, unbearable pain was observed in 10.0%, 3.33% and 0.00 patients at 24 hours, 15 days and 30 days respectively.

Table 1: Pain Response of Subjects to Etch and Rinse

Pain responses	24 hours	15 days	30 days
No pain	24 80.0%	25 83.3%	27 90.0%
Moderate pain	3 10.0%	4 13.3%	3 10.0%
Unbearable pain	3 10.0%	1 3.33%	0 0.00

Table 2 shows pain responses of patients with self-etch adhesives, with 80.0% patients presented with no pain after 24 hours, 83.3% with no pain at 15 days and 90.0% patients with no pain after 30 days. Moderate pain was observed in 16.6%, 16.6% and 10.0% of patients at 24 hours, 15 days and 30 days respectively. Whereas, unbearable pain was observed in 3.33%, 0.00 and 0.00 patients at 24 hours, 15 days and 30 days respectively.

Table 2: Pain Response of Subjects to Self-etch

Pain Responses	24 hours	15 days	30 days
No Pain	24 80%	25 83.3%	27 90%
Moderate Pain	5 16.6%	5 16.6%	3 10%
Unbearable Pain	1 3.33%	0 0.00	0 0.00

Table 3 shows association of gender distribution POS and technique used. Statistically, there was no notable variation in POS based on technique used in male group with p value 1.00 and female group with p value 1.00.

Table 3: Association of Gender Distribution, Post-Operative Sensitivity and Technique Used

Gender				Technique		Total	P value
				Etch and Rinse	Self-etch		
Male	POS	Yes	Count	1	1	2	1.00
			% of Total	3.3%	3.3%	6.7%	
	No	Count	14	14	28		
			% of Total	46.7%	46.7%	93.3%	
Female	POS	Yes	Count	2	2	4	1.00
			% of Total	6.7%	6.7%	13.3%	
	No	Count	13	13	26		
			% of Total	43.3%	43.3%	86.7%	

* p-value is significant at <0.05

Table 4 shows association of age distribution, POS and technique used. Statistically, there was no notable variation in POS based on technique used in 18-30 age group with p value 0.935, 31-40 age group with p value 0.952 and 41-50 age group with p value 1.00.

Table 4: Association of Age Distribution, Post-Operative Sensitivity and Technique Used

Age				Technique		Total	P value
				Etch and Rinse	Self-etch		
18-30	POS	Yes	Count	1	1	2	0.935
			% of Total	5.9%	5.9%	11.8%	
	No	Count	7	7	8	15	
			% of Total	41.2%	47.1%	88.2%	
31-40	POS	Yes	Count	1	1	2	0.952
			% of Total	4.3%	4.3%	8.7%	
	No	Count	11	10	21		
			% of Total	47.8%	43.5%	91.3%	
41-50	POS	Yes	Count	1	1	2	1.00
			% of Total	5.0%	5.0%	10.0%	
	No	Count	9	9	18		
			% of Total	45.0%	45.0%	90.0%	

* p-value is significant at <0.05

Table 5 shows association of educational status, POS and technique used. Statistically, there was no notable variation in POS based on technique used in primary group with p value 0.704, middle group with p value 0.615 and matric and above group with p value 0.815.

Table 5: Association of Educational Status, Post-Operative Sensitivity and Technique Used

Educational Status				Technique		Total	P value
				Etch and Rinse	Self-etch		
Illiterate	POS	No	Count	3	3	6	-
			% of Total	50%	50%	100%	
Primary	POS	Yes	Count	1	0	1	0.704
			% of Total	16.7%	0	16.7%	
	No	Count	4	1	5		
			% of Total	66.7%	16.7%	83.3%	
Middle	POS	Yes	Count	1	2	3	0.615
			% of Total	5.3%	10.5%	15.8%	
	No	Count	8	8	16		
			% of Total	42.1%	42.1%	84.2%	
Matric and Above	POS	Yes	Count	1	1	2	0.815
			% of Total	3.4%	3.4%	6.9%	
	No	Count	12	15	27		
			% of Total	41.4%	51.7%	93.1%	

* p-value is significant at <0.05

Table 6 shows frequency of POS in etch and rinse versus self-etch with 5.0% patients having POS in each group and 45.0% patients having no POS in each group.

Table 6: Frequency of Post-Operative Sensitivity in Etch and Rinse Versus Self-etch

Technique		Post Operative Sensitivity		Total
		Yes	No	
Etch and Rinse	Count	3	27	30
	% of Total	5%	45%	50%
Self-etch	Count	3	27	30
	% of Total	5%	45%	50%

* p-value is significant at <0.05

DISCUSSION

This study aimed at comparing the frequency of POS in posterior composite resin restoration utilizing two steps etch and rinse and self-etch adhesives. A total of 60 patients, out of which 5% of the patients in whom etch and rinse was used had POS, 45% of the patients who received etch and rinse adhesive did not have POS. Same was the response with self-etch adhesives with 5.0% of patients having POS and 45% of the patients had no POS. Statistically, no difference was found in POS based on technique used (p value 1.00).

The outcome showed the occurrence of POS in etch and rinse and self-etch adhesives had no significant difference during both immediate post operative period and 30 days post operative. Previous studies showed conflicting results regarding the frequency of POS in etch and rinse and self-etch technique.

A study of randomized control trial nature was done to compare POS in etch and rinse versus self-etch which concluded that the adhesive did not influence the occurrence of POS⁹. Another study done by Scotti N et al showed that the use of a three-step etch-and-rinse versus a two-step self-etch adhesive did not significantly affect the POS experienced by the patient¹⁰. In both adhesive groups, an increase in POS was observed on the day immediately after the restoration placement. But, in both groups, POS decreased significantly after 1 week which showed the initial POS was due to pulpal insult and operative procedure. Auschill et al evaluated POS after adhesive treatments and the different stimuli causing it. Depth of the cavity was the one factor to have effect on the occurrence of POS and adhesive systems did not affect it. A study conducted by Muhammad Amin in 2018 showed there seemed more sensitivity initially in self-etch group but after a week no notable difference in POS was found between etch and rinse group and self-etch group¹¹. Whereas, another study conducted by Ali et al¹² showed that etch and rinse resulted in less POS as compared to self-etch adhesive. Somewhat similar result was shown in a study done by Ajmal et al. where class 5 cavities were restored using self-etch and etch and rinse adhesives. POS was reduced during the first 24 hours in self-etch group but no notable variation was elicited on other assessments¹³.

Few of the variables that were thought to affect POS, were age, gender and education status which were considered in this study but were found to be statistically insignificant. The educational status may be a significant predictor of postoperative pain due to various reasons, including the poor understanding of the preoperative

information, the level of anxiety and depression caused by that and the suboptimal request and use of analgesia. POS in composite is thought to be influenced by many factors like anesthesia use, rubber dam isolation, cavity design, adhesive used and clinical cavity depth in many clinical trials. The results of a lot of researches do not support that self-etch being better than etch and rinse in terms of POS or vice versa. With the exception of cavity depth, none of the other parameters had a prominent effect on occurrence of POS.

To the best of our knowledge, comparison of frequency of etch and rinse and self-etch adhesives based on gender, age & educational status was done in our study for the first time in Pakistan. It would be beneficial to conduct similar studies on posterior teeth with different cavity designs and cavity depths. Further studies are also needed to assess POS with regard to restorative technique, operator experience which would provide dentists and patients with better choices to reduce incidence of POS. The limitations of this study are that only posterior teeth were chosen and class 2 cavity design was assessed, other cavity designs and teeth type also influence POS.

CONCLUSION

The present study concluded, no significant difference in frequency of POS in posterior composites using etch and rinse versus self-etch adhesive.

Conflict of interest: The authors declared no conflict of interest.

Authors' Contribution: HAK: Conceived idea, designed study, collected data, and drafted manuscript; SE: Conceptualization and guidance; HNQ: Contributed to data collection, analysis, interpretation, and result compilation; ZA: Contributed to result interpretation and critically revised the manuscript; MUK: Assisted in manuscript writing and proofreading.

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CASE REPORT

Difficulties in Diagnosis of Neurological Manifestations of Wilson Disease

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ABSTRACT

An uncommon disease due to altered copper metabolism, Wilson disease primarily involves the basal ganglia and liver, and affects males and females equally. Diagnosis of Wilson disease may be difficult, requiring blood and urine tests, and may include a liver biopsy. Copper chelation with penicillamine or trientine, oral zinc, and a low copper diet are recommended therapies.

We report the case of a 32-year old man who presented with 6-month history of gradual onset, progressively worsening history of aphasia with loss of comprehension, inappropriate vocalizations, increased aggression, and disturbed sleep. The patient had been exhibiting speech abnormalities, wherein his speech patterns and utterances became socially inappropriate and unrelated to the context, making it difficult for him to understand and communicate effectively.

A differential diagnosis including auto-immune and infectious encephalitis, neurodegenerative disorders like Creutzfeldt-Jakob disease (CJD) and Wilson disease was made. MRI Scan Brain (with FLAIR) showed T2WI hyper-intense signals in bilateral basal ganglia, brain atrophy, hyper-intense signals in periventricular, cortical, and sub-cortical regions. CSF analysis showed TLC 74 cells/uL with 90% lymphocytes, RBC 0 cells/uL, Protein 62 mg/dl and Glucose 69 mg/dl with no organisms on Giemsa stain and AFB stain microscopy. GeneXpert-PCR for MTB was also negative. His blood, urinary and spinal fluid cultures did not grow any organism growth. Serum ceruloplasmin level was normal at 23 mg/dl. CSF autoimmune profile was negative. However, his 24-hour urinary copper was raised at 1201.8 ug (normal value 20-40 ug). Diagnosis of Wilson disease was made and he was started on penicillamine and zinc sulfate.

Key Words: Ceruloplasmin, copper, MRI brain, penicillamine, wilson disease

How to cite: Ghoauri MS, Abbas F, Butt NI, Rasheed MB, Malik AM, Sadaf S. Difficulties in diagnosis of neurological manifestations of wilson disease. Ann Jinnah Sindh Med Uni. 2023;9(2):61-63

DOI 10.46663/ajsmu.v9i2.61-63

INTRODUCTION

Primarily affecting the liver and basal ganglia in the brain due to altered copper metabolism, Wilson disease is inherited through autosomal recessive fashion¹. The genetic defect in Wilson disease is localized at the long arm of chromosome 13 (13q) which alters copper transporting ATP gene. Affecting females and males equally, age of presentation usually ranges from four

to forty years. Liver dysfunction is seen in the first decade of life in most Wilson disease patients. Hepatic symptoms include jaundice, vomiting, ascites, weakness, pedal edema, and skin itching. Neurologic and psychiatric features are more frequent in third and fourth decade of life. Neurological symptoms include speech abnormalities, tremors, personality changes, muscle stiffness, depression, anxiety and hallucinations^{2,3}.

If not diagnosed timely and treated promptly, Wilson disease can be fatal. Diagnosis of Wilson disease may be difficult, requiring blood and urine tests, and may include a liver biopsy. Genetic testing to screen family members of those affected has also been advised. Chelation of copper with penicillamine or trientine is the mainstay therapy in Wilson disease but may take 3-6 months to start working⁴. Oral zinc competes with copper for absorption at metallic ion transporter and is also prescribed. A low copper diet is advised with avoidance of chocolate, liver, mushrooms, shellfish, dried fruit and nuts. In neurological disease,

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physiotherapy and occupational therapy are also recommended.

Case Presentation

A previously healthy 32-year old gentleman presented with 6-month history of gradual onset, progressively worsening history of dysphasia, dysarthria, inappropriate vocalizations, drooling of saliva, increased aggression, and disturbed sleep. The patient had been exhibiting speech abnormalities, wherein his speech patterns and utterances became socially inappropriate and unrelated to the context, making it difficult for him to communicate effectively. There was a history of five episodes of generalized tonic-clonic fits in the last three weeks. Each fit occurred for 3-5 minutes in duration and was associated with urinary incontinence, frothing of saliva, and post-ictal confusion remaining for approximately up to 15 minutes. No history of trauma, fever, headache, dizziness, oral or genital ulcers, respiratory infections, sinusitis, skin rashes, joint pains, dysphagia, or hearing loss was recorded.

A shopkeeper by profession, he was married with two children and did not smoke or use illicit drugs. He was unable to work in the last four months due to his illness. There was no significant past history or family history of any similar disorder. On examination, he was fit-free having normal vital signs but no jaundice, pallor, clubbing or flapping tremors. On neurological examination, there was dysphasia and dysarthria with intact comprehension. Pupils were equally round and reactive to light with normal extraocular muscle movements. No nystagmus was found and fundoscopy revealed normal optic disc with no signs of papilledema. Slit-lamp examination did not reveal Kayser-Fleischer rings. There was drooling of saliva but no facial asymmetry with normal midline tongue and uvula. Signs of neck rigidity (Kernig and Brudzinski) were negative. There was generalized muscle rigidity but no focal sensory, motor, or cerebellar neurological deficit. There was flexor response to plantar reflex bilaterally and deep tendon reflexes were normal in all four limbs. Neither any viscera nor ascites were present clinically on abdominal examination. Respiratory and precordial examinations were normal.

A differential diagnosis included auto-immune and infectious encephalitis, neurodegenerative disorders like Creutzfeldt-Jakob disease (CJD) and Wilson disease. On investigation, CBC was normal TLC with normal ESR and CRP. His RFTs, LFTs and urinalysis were within normal parameters.

As shown in Figure 1, MRI Scan Brain (with FLAIR) showed T2WI hyper-intense signals in bilateral basal ganglia, brain atrophy, hyper-intense signals in periventricular, cortical, and sub-cortical regions. A

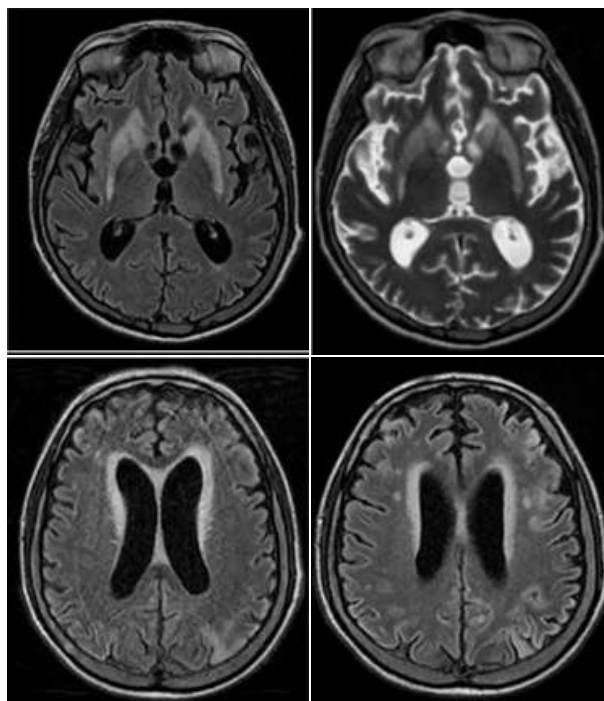


Fig. 1: MRI Scan Brain (with FLAIR) showing T2WI hyper-intense signals in bilateral basal ganglia, brain atrophy, hyper-intense signals in periventricular, cortical, and sub-cortical regions

lumbar puncture for CSF analysis was done. CSF analysis showed TLC 74 cells/uL with 90% lymphocytes, RBC 0 cells/uL, Protein 62 mg/dl and Glucose 69 mg/dl with no organisms on Giemsa stain and AFB stain microscopy. GeneXpert-PCR for MTB was also negative.

His blood, urinary and spinal fluid cultures did not grow any organism growth. Serologies for HBV, HCV, HIV and syphilis were negative. Serum ceruloplasmin level was normal at 23 mg/dl. His echocardiography, chest X-ray, and abdomen ultrasound scan were within normal parameters. After sending CSF for autoimmune work-up, he was started on intravenous methylprednisolone 1000mg/day on lines of autoimmune encephalitis. There was no clinical improvement.

CSF autoimmune profile (including anti-NMDA receptor antibodies, anti-CASPR2 antibodies, anti-glutamate receptor antibodies, anti-DPPX antibodies and anti-GABAb receptor antibodies) was negative. However, his 24-hour urinary copper was raised at 1201.8 ug (normal value 20-40 ug). He was diagnosed as having Wilson disease and started on penicillamine and oral zinc sulfate. Additionally, physiotherapy and occupational rehabilitation were also planned. On follow up at three months, he was tolerating medicines without any adverse effects. His dysarthria, dysphasia and improper vocalizations had improved partially while aggression and drooling had settled completely.

DISCUSSION

In 1912, Wilson first described clinical presentation of 12 patients with neurological manifestations of Wilson Disease including drooling, movement disorders, dysarthria and psychiatric symptoms. Up to 50% patients with neurologic symptoms have co-existing liver cirrhosis. Neurologic manifestations usually develop as a consequence of untreated disease, treatment failure, poor compliance with copper chelation therapy or in patients with misdiagnosed liver disease and in patients with clinically silent hepatic stage⁵.

MRI scan of brain is the most widely used neuro-imaging modality to aid in diagnosis of Wilson disease and rule out other causes. More than 90% patients of Wilson disease with neurological manifestations have MRI findings including symmetric T2WI hyper-intensities in the deep grey matter nuclei, basal ganglia, putamen, caudate nucleus, anterolateral thalamic nuclei, mesencephalic and pontine white matter^{6,7}. It should be noted that these T2WI hyper-intense lesions may be reversible with copper chelation therapy and reflect edema and demyelination due to copper toxicity⁸. Ultimately, brain atrophy may develop and may be irreversible. Atrophy is seen in 30-45% of newly diagnosed Wilson disease patients with neurological manifestations and appears to be more common in men. Partial improvement in atrophy with copper chelation has been reported⁹.

No single unanimously reliable non-invasive investigation is available to diagnose Wilson disease. Levels of serum ceruloplasmin, serum copper and urinary copper excreted during a 24-hour period are used to aid diagnosis. The gold standard for diagnosis is a liver biopsy⁴. Hepatic impairment, Kayser-Fleischer ring and abnormal serum ceruloplasmin level are frequently seen in Wilson disease but are not present in all the cases⁴. In the present case, elevated 24-hour urinary copper alongwith MRI brain findings were used to establish diagnosis of Wilson disease.

The present case highlights the difficulties in diagnosis of Wilson disease. Our patient presented with neuropsychiatric complaints in the fourth decade of life. There was no current or past history of liver involvement. At the time of assessment, there was no jaundice, Kayser-Fleischer rings, hepatosplenomegaly or ascites. His LFTs and serum ceruloplasmin level were also normal. MRI Scan Brain (with FLAIR) showed T2WI hyper-intense signals in bilateral basal ganglia, brain atrophy, hyper-intense signals in periventricular, cortical, and sub-cortical regions. However the 24-hour urinary copper levels were markedly elevated and helped to establish the diagnosis.

In conclusion, high clinical suspicion is necessary to diagnose Wilson disease so that timely diagnosis and

adequate treatment may be instituted to improve prognosis in these patients.

Conflict of interest: The authors declare no conflict of interest.

Authors' Contribution: NIB, MSAG and SS: Conceived and designed the study. NIB, MBR, and MMA: Did the initial literature research. MSAG and FA: Did the data collection, assembly and patient assessment. NIB, MBR and SS: Manuscript writing. MSAG, FA and MMA: Did the final critical review and corrections.

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LETTER TO EDITOR

Oral Microbiology—A Need of Time

Aman Ashar¹ and Mehwash Kashif¹

How to cite: Ashar A, Kashif M. Oral microbiology—a need of time. *Ann Jinnah Sindh Med Uni*. 2023;9(2):64
DOI 10.46663/ajsmu.v9i2.64

Dear Editor,

Dentistry is a specialized field of healthcare that is concerned with recognizing, preventing, and treating conditions that affect the teeth, gums, oral cavity, and tissues that support them¹.

It has many subdivisions like Orthodontics, Oral Maxillofacial Surgery, Oral Pathology, Oral Medicine, Prosthodontics, etc. The domain of oral Pathology, often known as oral and maxillofacial pathology or head and neck pathology, is to focus on illnesses of the mouth and the tissues that are connected with it². Therefore, it is the branch of Dentistry linked with Pathology.

Oral Microbiology, which comes under the domain of Oral Pathology, deals with the identification of microbiota causing oral illness and their interaction with normal flora of the mouth. As many oral diseases are the outcome of the interplay between complex microbes, knowledge and expertise in oral microbiology will facilitate the process of diagnosis and treatment so the importance of the subject cannot be neglected. Worldwide, many countries are offering postgraduate programmes like MDS, MSc, and PhDs for students interested to continue their studies in this field. The advancements in oral microbiology helps to enable a deeper comprehension of the ecological struggle between the local microbial flora and the periodontal, cariogenic and other infections of the oral cavity. In addition, it will serve as a springboard for the development of more focused and effective therapy strategies for treating dental and periodontal disease³. Unfortunately, in Pakistan, some subjects of dentistry are neglected like gerodontology, forensic odontology, pediatric dentistry, including oral microbiology. It is still a rudimentary subspecialty in Pakistan and is not being taught at undergraduate and postgraduate levels. Owing to the present dull situation regarding this

subject in Pakistan, there is a lack of opportunities for further studies in oral microbiology.

Therefore, it is recommended that Pakistan Medical and Dental Council and Higher Education Commission should focus on the incorporation of Oral Microbiology and other subspecialties in the curriculum and postgraduate programmes should be launched to broaden the horizon of this specialty.

Authors' Contribution: AA & MK: worked on Concept, design, critical review and final version of the manuscript.

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