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Editorial

Ghostwriting and Guest Authorship: Unveiling the Mask of Deception in Medical Research

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Every day, medical editors are shaping and producing documents that convey critical scientific information, leading to better outcomes and healthier lives. As the medical community strives for excellence in research and publication, two persistent concerns threaten the very foundation of our scholarly endeavors: ghost writing and guest authorship.[1]These practices, often shrouded in secrecy, undermine the integrity of medical research, compromise the credibility of our journals, and potentially harm patients.[2]

Medical ghostwriting is a new term. The ghostwriters in medicine are medical writers used by pharmaceutical companies or contract research organizations and medical communication agencies that serve the industry.[2,3] Some medical writers are employed by the industry or its service agencies; others are self-employed and work under contract. Ghostwriting is also considered to be a form of plagiarism, unethical behavior which could even go as far as to cause health problems for the population.[3]

Ghost writing, where individuals not listed as authors contribute substantially to manuscripts, is a pervasive problem. This lack of transparency obscures the true origins of research, making it difficult to discern conflicts of interest and assess the validity of findings.[4]Articles written by medical writers are published in medical journals. These articles can influence doctors and policy makers in their decisions that affect health. Therefore, the articles have marketing potential and there is a feeling that articles associated with manufacturers of pharmaceuticals make exaggerated promises and omit information that might disadvantage their products.[5]

Guest authorship another issue, where prominent researchers lend their names to papers without meaningful contribution, is equally troubling. This practice not only misrepresents the true authors but also perpetuates a culture of academic dishonesty. Reputed and renowned scientists, who have not participated in the conduct of the study or in the manuscript preparation, are enrolled to allow their names to be mentioned as authors. This phenomenon is harmful not only because it suppresses the contribution of ghost-authors but also because the guest "authors" bestow underserved credibility for a paper [6]

To address these concerns, we propose the following:

1. Journals should require detailed contributor ship statements, outlining specific contributions.
2. Authors must declare all potential conflicts of interest and funding sources.
3. Institutions should establish clear policies on authorship and contributor ship.
4. Researchers should adhere to guidelines set forth by organizations like the International Committee of Medical Journal Editors.

As editors, researchers, and clinicians, we must uphold the highest standards of integrity and transparency. By acknowledging and addressing ghost writing and guest authorship, we can ensure the credibility and reliability of medical research, ultimately benefiting patients and advancing our field.

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Preventive Role of Punica Granatum Peel and Seed Extract in Murine Model of Peptic Ulcer: A Histopathological Perspective

Mariyam Iftikhar Piracha,[1] Saadia Shahzad Alam,[2] Sadaf Jabbar,[3] Osheen Sajjad,[4] Maryam Rashid,[5] Neelofer Yousaf,[6]

Abstract

Objectives: To histologically assess prophylactic antiulcer effect of Punica granatum (Anar) peel and seed. To compare stomach histological aspects of Punica granatum peel and seed with standard drug pantoprazole in diclofenac induced murine model of peptic ulcer.

Material and Methods: This experimental animal study was conducted in post graduate medical institute (PGMI) Lahore, after approval from Institutional Review Board (IRB) of Federal post graduate medical institute (FPGMI). Eighty one male rats were segregated into 9 groups having 9 rats each. Control groups; G-1 (healthy control) and G-2 (disease control) were given only distilled water. Pantoprazole and Punica granatum peel (PGPE) and seed (PGSE) extracts were given orally once daily to the treatment groups (3-9) for 15 days as follow:G-3: pantoprazole 60mg/kg/d, G-4:PGPE 100mg/kg/d, G-5 PGSE 500mg/kg/d, G-6: PGPE 50mg/kg/d±PGSE 250mg/kg/d, G-7:Pantoprazole 30mg/kg/d±PGPE 50mg/kg/d, G-8:Pantoprazole 30mg/kg/d±PGSE 250mg/kg/d, G-9:Pantoprazole 30mg/kg/d±PGPE 50mg/kg/d±PGSE 250mg/kg/d. Groups 2-9 were then given 100mg/kg diclofenac orally on day 17 for induction of peptic ulcer. Histopathological analysis of stomach was done at the end of the study.

Results: The gastric mucosa of all the animals in healthy control group was intact and had a continuous epithelium. Disease control showed severe mucosal damage grade 3 involving entire mucosa, grade 2 involving 2/3rd of mucosa and grade 1 superficial erosion injury. The difference between mucosal findings of all groups was highly significant (p-value= 0.000). None of the treatment groups showed grade 3 mucosal damage.

Conclusion: Best results were observed in the group where Pantoprazole 30mg/kg/d ± PGPE 50mg/kg/d ± PGSE 250mg/kg/d combination was used.

Keywords: pomegranate, peptic ulcer disease, pantoprazole, diclofenac

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Introduction

Peptic ulcer, considered as the most common gastrointestinal disease causes severe complications such as bleeding, perforation, and may also lead to death if it is accompanied by other morbidities.[1] Though anti-inflammatory drugs, including nonsteroidal anti-inflammatory drugs (NSAIDs) such as Diclofenac and Piroxicam. These agents are widely used as analgesics and have been reported to cause gastric ulcers, ulcer perforation, gastric and duodenal bleeding, and ulcer death. Prostaglandin analogues like, proton pump inhibitors (PPIs) are used to treat peptic ulcer.[2] The disease management approaches have been markedly changed by the PPIs introduction. PPIs has become a pillar of medical therapy for peptic ulcer-related gastrointestinal bleeding.[3] It has been revealed that reactive oxygen species (ROS),

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especially the hydroxyl radical and superoxide anion, play an important role in the pathogenesis of acute experimental gastric lesions induced by NSAIDs. Cyclo-oxygenase isozymes in the gastric mucosa promote mucus production by the continuous production of prostaglandins and the inhibitions of cyclooxygenase and prostaglandin synthesis by NSAIDs could disrupt the intrinsic ability of the mucosa to prevent the injuries induced by exogenous and endogenous factors.[4]

Medicinal plants and herbal recipes have played substantial role in the management and cure of various diseases. Punica granatum (Pomegranate) peel and seed extracts have anti-inflammatory and antioxidants which have shown their significant protective effect against neuro-inflammation,[5] hepatotoxicity, renal damage,[6] cardio-vascular dysfunction,[7] metabolic syndromes,[8] and auto-immune diseases.[9] Several natural products showed that pomegranate possesses antiulcerogenic activity by their predominant effects on mucosal defensive factors.[10] The prophylactic antiulcer effect of PG peel and seed extracts (PGPE, PGSE) individually and in combination and in comparison, to Pantoprazole, were investigated against diclofenac induced gastric ulcer in murine model. Moreover, no previous research existed to compare the stomach histological aspects with PGPE and PGSE in comparison to the standard drug pantoprazole.

Materials and Methods

This experimental animal study was conducted in post graduate medical institute (PGMI) Lahore, after approval from Institutional Review Board (IRB) No. F-39/NHRC/Admin/IRB/88 daated:09-08-2016 of Federal Post Graduate Medical Institute (FPGMI). It took 17 days for the completion of the experiment. Eighty-one healthy male albino rats weighing130-170 grams were purchased from University of Veterinary and Animal Sciences (UVAS) Lahore, and kept in the polypropylene cages in standard housing and lighting conditions; 60-70% humidity, 25-270C, 12/12-hour light/dark cycle. The albino rats were randomly assigned to 9 groups (G-1 to G-9) having 9 rats each by lottery method. The rats were acclimatized to the new environment for one week before starting the experiment. Pantoprazole tablets (40mg) and Diclofenac tablets (50mg) were bought from Servaid Pharmacy, Lahore, Fresh PG fruits were purchased from local fruit market of Lahore. The identification, verification and extract preparation of the extracts were done in Applied Chemistry and Research Centre at Pakistan Council of Scientific and Industrial Research (PCSIR) Laboratories Complex, Lahore. Fruits were washed thrice with distilled water and peeled manually. One kilo peel and seeds were shade dried and finely ground in the grinder. Separate extraction was carried out for the peel and seeds with 80% methanol in a Soxhlet apparatus for 4hrs, and further concentration was performed at 40°C under controlled reduced pressure using a rotary vacuum evaporator. The extracts were collected in capped bottles and stored 12 at 4°C till further use. After acclimatization the animals in the healthy (G-1) and disease control groups(G-2) were given only 0.5ml distilled water orally daily for 15 days and the remaining seven groups were given prophylactic oral treatment (extracts and pantoprazole tablets dissolved in distilled water once daily for 15 days as per group designation detailed below.

Group 3:(standard group) Pantoprazole 60mg/kg/d
Group 4: PG peel extract (PGPE) 100mg/kg/d
Group 5: PG seed extract (PGSE) 500mg/kg/d
Group 6: PGPE + PGSE (50+250mg/kg/d)
Group 7: Pantoprazole + PGPE (30+50mg/kg/d)
Group 8: Pantoprazole + PGSE (30+250mg/kg/d)
Group 9: Pantoprazole + PGPE + PGSE (30+50+250 mg/ kg/d)

Animals of groups 2-9 were then fasted for 24 hrs (day 16) and given Diclofenac 100mg/kg orally in a single dose on the 17th day. After 4 hours the rats were anesthetized using chloroform and then surgically sacrificed for histological evaluation of the stomach. Stomach tissues were fixed in 10% formalin in properly labeled containers. The tissue was processed using a tissue processor, paraffin blocks were made for the processed tissue and about 5-µm thick sections were cut using a rotary microtome. The sections were then stained with hematoxylin and eosin for examination under light microscope. Slides were examined by the histopathologist in Fatima Jinnah Medical University (FJMU) Lahore, who was blinded to the study. The following stomach histopathological parameters were evaluated.

Grades:

Grade 0 = No ulceration
Grade 1 = Superficial erosion
Grade 2 = Ulceration involving 2/3rd of mucosa
Grade 3 = Ulceration involving entire mucosa
Inflammation: Present/ Absent
Hemorrhage: Present/ Absent

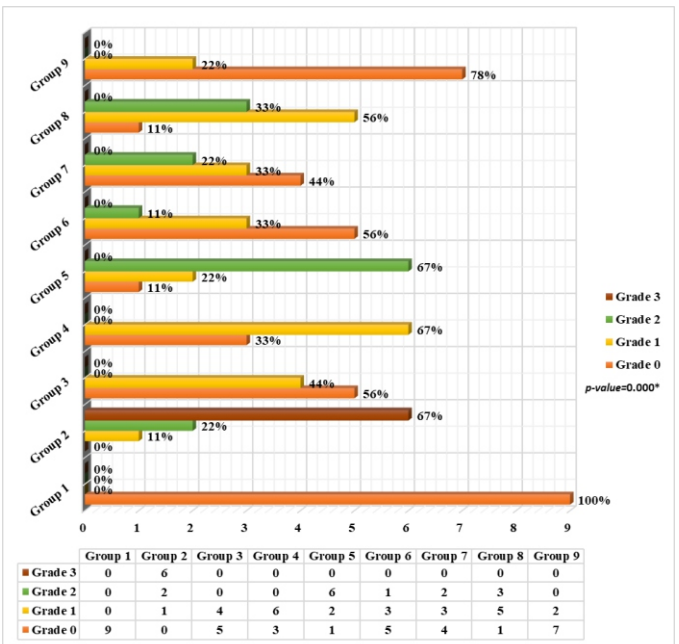
The data was entered and analyzed using SPSS version 23.0. Data for qualitative variables; grades, inflamma-

tion, mucosal hemorrhage was described by using frequency and percentages for each group. Comparison among groups was made by using Chi-square test. P-value of < 0.05 was considered statistically significant and <0.01 highly significant.

Results

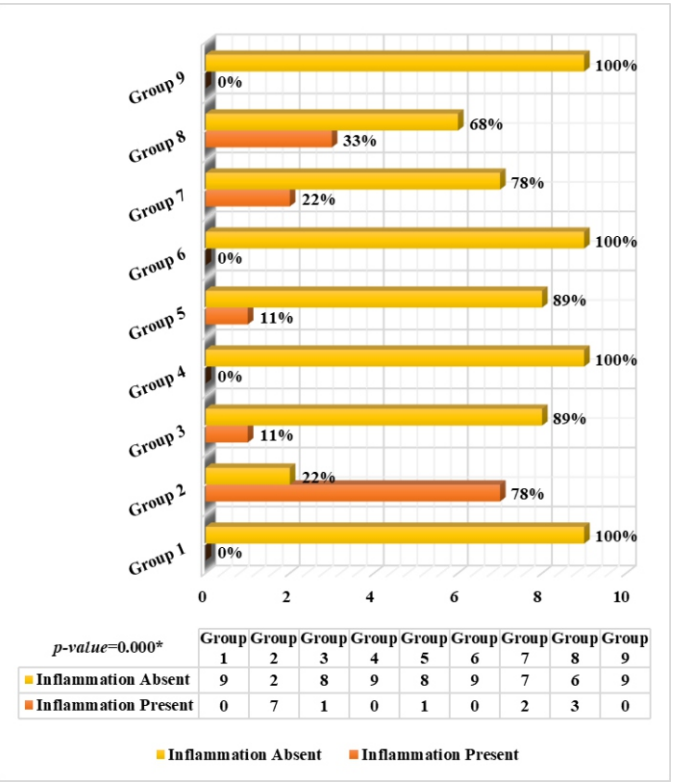
Out of 81, 46 (56.8%) rats had grade 1 to grade 3 changes. Chi square test revealed that there is an association between grades and groups. All the rats of group 1 were normal. In group 9, 7 (77.8%) rats and in group 3 & 6, 5(55.6%) rats were normal.

Out of 81, 14(17.3%) rats had inflammatory changes in their stomach. Chi square test revealed that there is an association between inflammation and groups. No inflammation was present in group 1, 4, 6 and 9. In group 2,7 (77.8%) rats, in group 3,1 (11.1%), in group 7, 2(22.2%) and in group 8 only 3(33.3%) rats had inflammation. Out of 81, 3 (3.7%) rats showed hemorrhage. Chi square test revealed that there is an association between hemorrhage and groups. No hemorrhage was observed in all groups except group 2, 3(33.3%) rats showed hemorrhage.



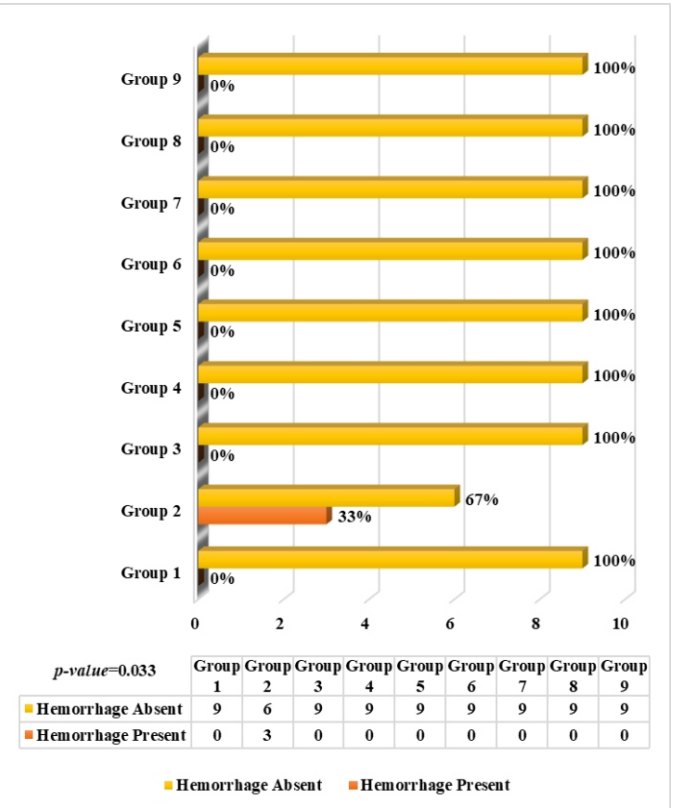
	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8	Group 9
Grade 3	0	6	0	0	0	0	0	0	0
Grade 2	0	2	0	0	6	1	2	3	0
Grade 1	0	1	4	6	2	3	3	5	2
Grade 0	9	0	5	3	1	5	4	1	7

Figure 1: *Graphical representation of histological grading of stomach.*



p-value=0.000*	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8	Group 9
Inflammation Absent	9	2	8	9	8	9	7	6	9
Inflammation Present	0	7	1	0	1	0	2	3	0

Figure 2: *Comparison of Inflammation among groups.*



p-value=0.033	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8	Group 9
Hemorrhage Absent	9	6	9	9	9	9	9	9	9
Hemorrhage Present	0	3	0	0	0	0	0	0	0

Figure 3: *Comparison of Haemorrhage among groups*

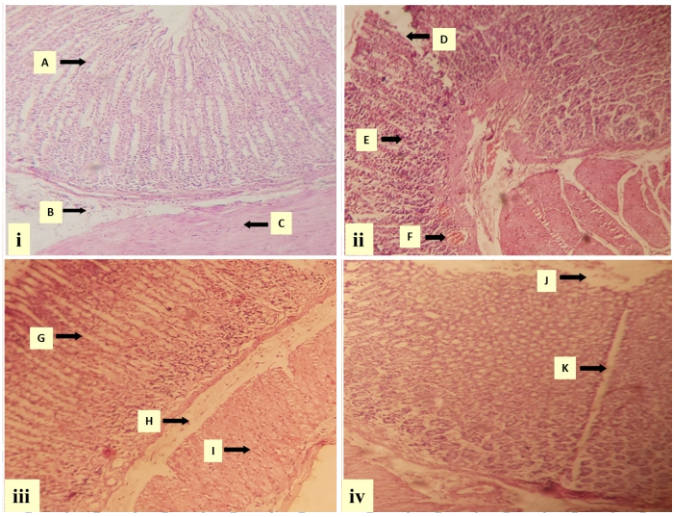


Figure 4: *i: Group 1 showing normal stomach histology; intact epithelium, no inflammation and hemorrhage (10X). A) Mucosa, B) Sub-mucosa, C) Muscularis mucosa. ii: Group 2 showing grade 3 mucosal damage, neutrophilic infiltrates and hemorrhage (10X). D) Ulcer involving the entire length of mucosa, E) Neutro-philic infiltrates, F) Hemorrhage. Iii: Group 3 showing normal stomach histology; intact epithelium, no infla-mmation and hemorrhage (10X). G) Mucosa, H) Sub-mucosa, I) Muscularis mucosa. Group 4 showing grade 1 mucosal damage (10X). J) Erosion, K) Artefact*

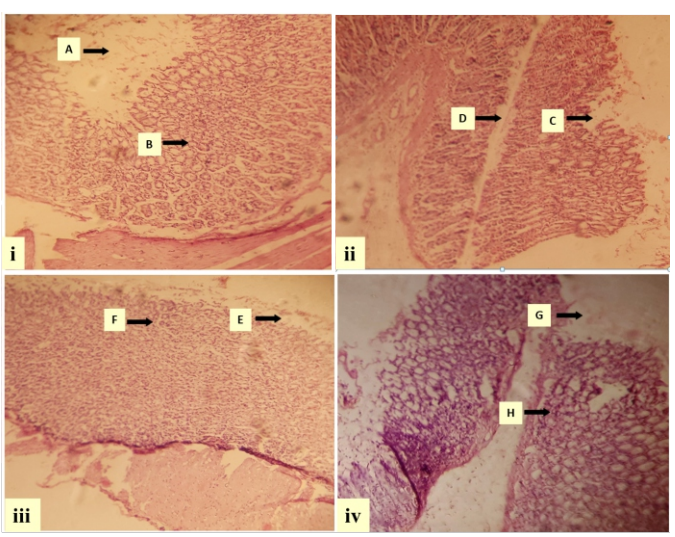


Figure 5: *Group 5 given PGSE 500mg/kg/d showing grade 2 mucosal damage and neutrophilic infiltration. A)ulceration of 1/3rd of mucosa, B) Neutrophilic infil-trates. Group 6 given combination of PGPE 50mg/kg/d & PGSE 250mg/kg/d showing grade 1 mucosal damage (10X). C) Erosion, D) Artefact. Group 7 given combi-nation of Pantoprazole 30mg/kg/d & PGPE 50mg/kg/d showing grade 1 mucosal damage and neutrophilic infiltration (10X). E) Erosion, F) Neutrophilic infiltra-tion. Group 8 given combination of Pantoprazole 30mg/kg/d & PGSE 250mg/kg/d showing grade 2 mucosal damage and inflammation (10X). G) Ulcer involving 1/3rd of mucosa, H) Neutrophilic infiltration.*

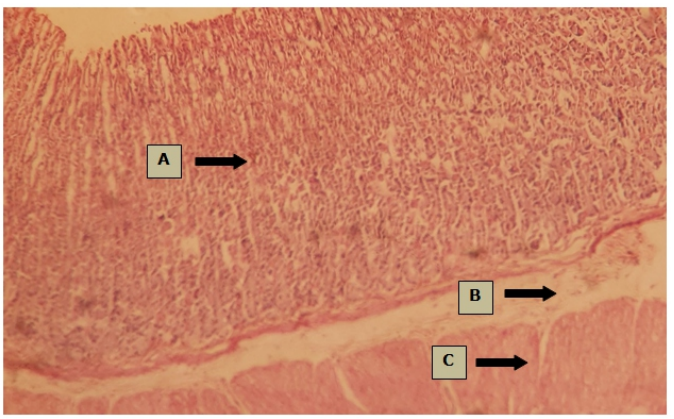


Figure 6: *Group 9 given combination of Pantoprazole 30mg/kg/d, PGPE 50mg/kg/d & PGSE 250mg/kg/d showing normal stomach histology (10X). A) Mucosa. B) Sub-mucosa, C) Muscularis mucosa*

Discussion

Peptic ulcer can be delineated as the presence of a deep destruction of the stomach lining or mucosa and/or duodenum, reaching beyond the muscularis mucosa, specifically to the muscle layer owing to the environmental gastric acid synthesis.[11] Furthermore, nonsteroidal anti-inflammatory drugs (NSAIDs) like diclofenac, have widely been used as therapeutic agents for many of the chronic illnesses but have been reported as one of the major causes of drug-induced gastrointestinal (GI) including peptic ulcer (PU) or bleeding.[12] Other risk factors for PUD are cigarette smoking, alcohol intake, advancing age and stress. Occasionally it may be idiopathic.[13] Punica granatum (Pomegranate) is a miraculous fruit being used in alternative medicine for treatment of various diseases since ancient time. Pomegranate fruit, peels and seeds have shown anti-ulcer effects in murine models due to their strong gastroprotective and antioxidant activity attributed to presence of phenolic compounds, flavonoids, tannins and anthocyanins. These are the main group of antioxidant phytochemicals known for their free radical scavenging activities.[14] The prophylactic antiulcer effect of PG peel and seed extracts (PGPE, PGSE) individually and in combination in comparison to Pantoprazole, a standard

antiulcer drug (PPI) in the murine diclofenac induced gastric ulcer model were investigated. Moreover, no previous research existed to compare the stomach histological aspects with PGPE and PGSE in comparison to the standard drug pantoprazole against diclofenac. The gastric mucosa of all the animals in healthy control group was intact and had a continuous epithelium. Disease control showed severe mucosal damage grade 3 involving entire mucosa, grade-2 involving 2/3rd of mucosa and grade 1 superficial erosion injury. These results further confirmed ulcerogenic potential of diclofenac which is a result of several mechanisms: local irritant activity due to its acidic nature, prostaglandin synthesis inhibition, NO synthesis inhibition[15] and increased oxidative stress due to formation of free radicals and ROS; O2-, OH and H2O2 which damage lipids, proteins and DNA. Additionally, lipid peroxidation resulting from free radicals, damages the cell and decreases the integrity of mucosal epithelial cells, producing epithelial disruption.[16]

The difference between mucosal findings of all groups was highly significant (P-value= 0.000). None of the treatment groups showed grade 3 mucosal damage. Best results were seen in group 9 (Pantoprazole 30mg/kg/d + PGPE 50mg/kg/d + PGSE 250mg/kg/d) having 77.8% rats with normal mucosa and only 22.2% showed grade 1 mucosal damage (gastric epithelium was not completely intact). These results were even better than the standard group in which 44.4% animals showed grade 1 damage.

This showed a unique, multipronged, preventive antiulcer activity of pantoprazole and PG peel and seed extracts against diclofenac seen by decreasing HCl production, increasing NO levels and mucus secretion,[17] precipitating proteins and forming a mucosal protective layer[18], overcoming the oxidative stress by scavenging ROS and increasing endogenous antioxidants; GSH, CAT and SOD.[19] This effect may be due to the presence of phytochemicals, especially ellagic acid, ellagitannins, punicalagin and flavonoids; anthocyanins and proanthocyanidin.[20]

Inflammation was not seen in gastric histology of all the animals of healthy control group whereas, neutrophilic infiltration was present in one third of the rats of gastric mucosa of disease control group. Diclofenac triggers neutrophil adherence to gastric microvascular endothelium, damaging the gastric mucosa releasing pro-inflammatory cytokines; tumor necrosis factor alpha (TNF-α) and interleukin-1 beta (IL-1β) resulting in acute inflammation presented as mucosal neutrophilic infiltration.[21]

There was significant difference in the presence of inflammation between disease and treatment groups (P-value=0.000). In groups 3 (Pantoprazole 60mg/kg/d), 7(Pantoprazole 30mg/kg/d + PGPE 50mg/kg/d) and 8(Pantoprazole 30mg/kg/d + PGSE 250mg/kg/d) 11.1%, 22.2% and 33.3% rats showed inflammation respectively. Whereas, neutrophil infiltration was absent in all the animals of group 4 (PGPE 100mg/kg/d), 6 (PGPE 50mg/kg/d + PGSE 250mg/kg/d) and 9 (Pantoprazole 30mg/kg/d+PGPE 50mg/kg/d+PGSE 250mg/kg/d) which demonstrates the strong anti-inflammatory effect of PG peel and seeds due to the presence of polyphenols like ellagic acid, gallic acid and ellagitannins mitigating inflammation by suppressing the pro-inflammatory cytokines (TNF-α and IL-1β).[22] Another research showed a potent anti-inflammatory activity of Punica granatum peel extract due to ellagic acid in Carrageenan-induced paw edema model, proving its ability to reduce acute inflammation.[23] Only the diclofenac treated disease control group showed hemorrhage in one third of rats primarily by interfering with the house keeping effects of prostaglandins resulting in decreased mucosal protection[24] and secondarily due to microvascular injury caused by neutrophil and oxygen free radical.[25]

Conclusion

The obtained findings revealed that pretreatment with a combination of pantoprazole, PGPE and PGSE significantly strengthened gastric mucosa, ameliorated the gastric mucosal damage and inflammation caused by diclofenac. Another noteworthy aspect was that hemorrhage was prevented in all groups including those given the drug and extracts individually, which showed that hemorrhage can be prevented by blocking any step in the formation of peptic ulcer whether acid secretion, mucosal damage or inflammation.

Conflict of interest: None

Funding Source: None

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SSA: Conceptualization of Project
SJ: Data Collection
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AA: Writing of Manuscript

Elevated Neutrophil-To-Lymphocyte Ratio as a Predictive Tool for Laryngeal Squamous Cell Carcinoma

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Abstract

Objective: To determine the mean value of Neutrophil-to-Lymphocyte Ratio (NLR) in laryngeal squamous cell carcinoma, premalignant laryngeal lesions, and benign laryngeal lesions.

Material and Methods: A cross-sectional study spanning over 6 months 12th July 2016 to 11th January 2017 at Department of ENT (Ear, Nose and Throat), Benazir Bhutto Hospital (BBH), Rawalpindi. A total of 30 patients with age 15-60 years having hoarseness of voice were included via convenience sampling for the study. Patients with vocal cord paralysis, acute infection, cardiovascular disease and unfit for GA (General anesthesia) were excluded. Complete Blood count with peripheral smear was done for NLR (Neutrophil-To-Lymphocyte Ratio). Informed consent for direct laryngoscope (DL) with biopsy under GA was obtained. Patient was prepared for GA. DL (Direct Laryngoscopy) was done under GA and sample was sent in formalin to the Department of Pathology Benazir Bhutto Hospital for histopathology.

Results: The mean age was 43.37±10.40 years. Out of these 30 patients, 20(66.67%) were male and 10 (33.33%) were females with male to female ratio of 2:1. Mean neutrophil-to-lymphocytic ratio was 2.79 ± 0.82. Mean value of NLR in laryngeal squamous cell carcinoma, premalignant laryngeal lesions and benign laryngeal lesions was 3.80 ± 0.26, 2.42±0.09 and 2.04 ± 0.13 respectively.

Conclusion: This study concluded that NLR can help to predict the likelihood of laryngeal squamous cell carcinoma in a patient presenting with hoarseness of voice and any laryngeal lesion on indirect laryngoscopy prior to direct laryngoscopy and biopsy.

Keywords: Hoarseness of Voice, Laryngeal Squamous Cell Carcinoma, Neutrophil-to-Lymphocytic Ratio.

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Introduction

Head and neck cancer (HNCA) refers to a heterogeneous group of primary cancers involving upper aerodigestive tract. They rank sixth among the most common cancers worldwide.[1] It is estimated that 6,400,000 new cases of HNCA and 350,000 deaths occur each year worldwide.[2] The etiology of head and neck cancers is unclear like all other cancers. However, several risk factors are associated like alcohol consumption and tobacco use.[3]

Hoarseness of voice is the commonest symptom related to laryngeal pathology that can be a laryngeal carcinoma or any benign laryngeal lesions. Efforts are still made to find a reliable marker for prediction of laryngeal squamous cell carcinoma (LSCC) from benign laryngeal lesion (BLL) and premalignant laryngeal lesions (PLL). The neutrophil-to-lymphocyte ratio (NLR) is a marker that shows the systemic inflammatory responses which also occur in any malignant condition.[4] Elevated neutrophil-to-lymphocyte ratio is an important diagnostic and

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prognostic biomarker in several carcinomas like it is independent diagnostic predictor in hepatocellular carcinoma.[5] In addition, preoperative high NLR is a significant diagnostic predictor of distinction of breast cancer from benign proliferative breast disease and differentiates between benign and malignant thyroid disorder.[6] Few studies linked elevated NLR to Head and Neck carcinomas, thus NLR of more than 5 in non-metastatic stages was set as predictive indicator of mortality[4]. Accor-ding to Rassouli et al[9] systemic inflammatory marker such as NLR are the independent predictors of recurrence in head and neck squamous cell carcinoma. Early diagnosis can lead to a good prognosis in laryngeal carcinoma in upto one third of the malignant cancers.[7] Survival mainly depends on tumor stage, patient age, tumor location, cervical lymph node invasion, and a variety of other histopathological prognostic parameters. Glottic tumors are typically detected at an earlier stage than supraglottic tumors, which can impact treatment options and prognosis.[8] The aim of our study was to determine NLR can be used as an indicator for differentiating LSCC from PLL and BLL in our population. This shall prove useful in low socioeconomic setup as NLR is an inexpensive is widely available marker. It can be used prior to direct laryngoscopy and biopsy to predict the likelihood of laryngeal squamous cell carcinoma in a patient presenting with hoarseness of voice and any laryngeal lesion on indirect laryngoscopy.

Material and Methods

A Cross-sectional study was conducted at Department of ENT, Benzair Bhutto Hospital (BBH) Rawalpindi from 12th July 2016 to 11th January 2017 after formal ethical approval. A total of 45 patients were admitted to the ENT ward during this period. Out of these 30 patients having age 15 to 60 years presented with hoarseness of voice were included in the study. Patients with mass/lesion in the larynx, supraglottic, and glottis regions, Transglottic growth, hyperemia and thickening of vocal cords, poloidal/papillomatous growth, cystic or nodular swelling and reddish mass in larynx were included for the study. Patients with vocal cord paralysis, acute infection, extra-laryngeal malignancy, history of cardiovascular disease or any condition that made the patent unfit for GA were excluded.

Demographic data including age, sex and type of lesion were recorded on structured proforma. Data was entered and analyzed by using SPSS.v.20.0. Mean & Standard Deviation was calculated for quantitative variables (age and NLR). Frequency & percentages were calculated for qualitative variable like gender, residence (rural/urban) and diabetes mellitus status (controlled/uncontrolled). Effect modifier like age, gender and type of lesion were controlled through stratification. Post stratification Student 't' test was applied and p-value ≤0.05 was taken as significant.

Results

The mean age of the patients in the study was 43.37 ± 10.40 years as represented in **(Table-1),** whereas Distri-bution of patients according to type of lesion is shown in **(Table-2),**. Out of 30 patients, 20 (66.67%) were male and 10 (33.33%) were females with male to female ratio of 2:1 **(Fig-I).** Mean value of NLR in laryngeal squamous cell carcinoma, premalignant laryngeal lesions and benign laryngeal lesions is depicted in **(Table-3),** and is found to be statistically significant among groups of different types of lesions. Stratification of mean NLR with respect to age groups and gender are indicated in **(Table-4,5)**.

Figure 1: *Distribution of patients according to Gender (n=30).*

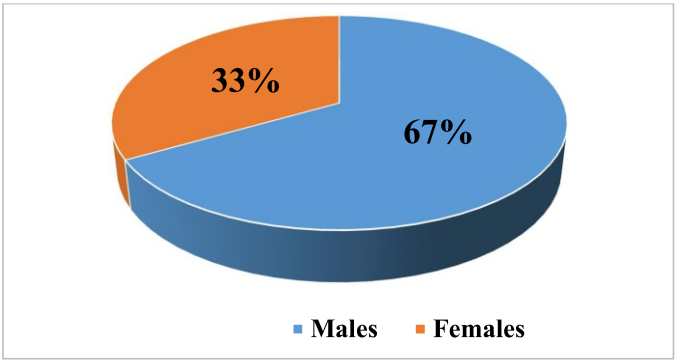

Table 1: *Distribution of patients according to Age (n=30)*

Age (in years)	No. of Patients	Percentage (%)
15-40	13	43.33
41-60	17	56.67
Total	30	100.0

**Mean ± SD = 43.37 ± 10.40 years

Table 2: *Distribution of patients according to type of lesion (n=30)*

Type of lesion	No. of Patients	%age
Benign	11	36.67
Pre-malignant	08	26.67
Malignant	11	36.67

Table 3: *Mean value of NLR in laryngeal squamous cell carcinoma, premalignant laryngeal lesions and benign laryngeal lesions*

Type of lesion	Neutrophil-to-lymphocytic ratio (NLR)		P-value
	Mean	SD	
Benign	2.04	0.13	0.0001
Pre-malignant	2.42	0.09	
Malignant	3.80	0.26	

Table-4: *Stratification of Mean NLR with respect to age groups*

Age groups (years)	Neutrophil-to-lymphocytic ratio (NLR)		P-value
	Mean	SD	
15-40	2.93	0.80	0.407
41-60	2.68	0.83	

Table-5: *Stratification of Mean NLR with respect to age groups*

Gender	Neutrophil-to-lymphocytic ratio (NLR)		P-value
	Mean	SD	
Male	2.82	0.82	0.783
Female	2.73	0.86	

Discusion

Larynx cancer is one of the most common cancers of head and neck. In 2012, the estimated new cases with laryngeal carcinoma were 157,000 and cancer related deaths was 83,000 worldwide.[9] The NLR and derived neutrophil–lymphocyte ratio (dNLR) has recently gained popularity as systemic inflammatory response biomarkers.[10] Neutrophil–lymphocyte ratio principally determines the systemic inflammation, particularly in chronic inflammatory diseases.[11] Several clinical studies have demonstrated that a high NLR value is associated with poor prognosis and survival in several cancers such as nasopharyngeal, gastrointestinal, lung and renal cancers.[12,13] In addition, dNLR, a modified form of NLR, is also used to demonstrate the systemic inflammation and prognosis in a variety of cancers such as gastrointestinal and breast cancers.[14] The pathophysiological relationship between high NLR or dNLR values and poor prognosis still remains unclear. It is hypothesized that it may be related to a decrease in the number of lymphocytes and increase in neutrophil counts in patients with cancer.[15] In fact, lymphocytes are responsible for anticancer immunity response, and CD8(+) T cells specifically control the tumor activity by apoptosis and cytotoxic effect. Therefore, lymphocyte counts are inversely correlated with severity of cancer.[16,17] Cancer-related inflammation may also lead to an increase in number of neutrophils.[18] Furthermore, cytokines, which are produced by cancerous cells, may trigger the migration of neutrophils from blood to tumor microenvironment; thus, neutrophils may stimulate the tumor growth and angiogenesis by vascular endothelial growth factor, IL-8 and matrix metalloproteinase.[19,20]

Age range in my study was from 15 to 60 years with mean age of 43.37 ± 10.40 years. Majority of the patients i.e. 17 (56.67%) were between 41 to 60 years of age. Out of these 30 patients, 20 (66.67%) were male and 10(33.33%) were females with male to female ratio of 2:1. In my study, mean value of NLR in laryngeal squamous cell carcinoma, premalignant laryngeal lesions and benign laryngeal lesions was 3.80 ± 0.26, 2.42 ± 0.09 and 2.04 ± 0.13 respectively.[21]

Recent study by Haddad CR et al5 concluded that in advanced Head and Neck cancer patients without metastasis, an NLR=5 was a prognostic indicator for mortality. According to Rassouli et al[22] systemic inflammatory marker such as NLR are the independent predictors of recurrence in head and neck squamous cell carcinoma as the group with NLR <4.2 that was only 7%. Moreover, the study investigating NLR in LSCS, premalignant laryngeal lesions and benign laryngeal lesions showed that mean NLR of the BLL, PLL and the LSCC groups were 2.12 ± 0.86, 2.32±0.68 and 3.46±1.51, respectfully.[23] So the NLR can be used to predict the LSCC from PLL and benign lesions.

The NLR is now routinely measured as part of the cancer work-up, as it is easily calculated from the white blood cell count and is universally available. However, the clinical relevance of the NLR is complicated because it represents a combination of factors related to both inflammation and host immunity. Recent studies have confirmed a link between the local inflammatory microenvironment that is favorable for tumor growth and metastasis of a tumor, and systemic responses induced by the tumor. Moreover, lymphocytopenia indicates a generalized state of immunodepression.[24]

These may be the possible mechanisms for decreased survival in patients with LSCC so the recognition of the NLR as a key component of tumor growth is important when using cancer therapies to decrease laryngeal carcinoma cell proliferation and metastasis in patients.

Conclusion

This study concluded that the mean value of neutrophil-to-lymphocytic ratio in laryngeal squamous cell carcinoma was higher than premalignant laryngeal lesions and benign laryngeal lesions subsequently. It is suggested to use this inexpensive test can be used prior to direct laryngoscopy and biopsy to predict the likelihood of laryngeal squamous cell carcinoma in a patient presenting with hoarseness of voice and any laryngeal lesion on indirect laryngoscopy.

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AA: Conceptualization of Project

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SI, FH: Statistical Analysis

M: Drafting, Revision

AA: Writing of Manuscript

Investigating Maternal and Fetal Outcomes in Burn Injury Cases During Pregnancy: Insights from a Major Burn Center

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Abstract

Objective: To determine the maternal and fetal outcomes of burn during pregnancy at a burn centre in a lower-middle-income country.

Material and Methods: This study was Retrospective and data was collected for 1 year. This retrospective study was conducted at the burn centre from 1st October 2022 to 30th September 2023 after obtaining ethical approval, and investigated outcomes in burned pregnant women. The data were extracted from patient records, supplemented by family contacts for missing information. A structured questionnaire systematically gathered essential data, including demographics (age, gestational age, parity, history of epilepsy), burn characteristics (depth of burn, intention and mode of burn, inhalational injury, TBSA involved, involvement of abdomen, pretreatment, blood transfusion, duration of hospital stay and surgical intervention), and maternal (discharged/expired) and fetal (IUD/Alive) outcomes.

Results: Out of twenty pregnant burn victims, 8 (40%) patients expired and 12 (60%) were discharged. Fetal outcome in terms of IUD and alive was 45% and 55%, respectively. When results were compared among discharged and expired groups using chi-square, it was statistically significant for gestational age, time of presentation, TBSA, inhalational injury, abdomen involvement and mode of burn ($p < 0.05\%$).

Conclusion: Burn incidents in pregnant women significantly adversely impact fetal and maternal well-being, especially in major burns.

Keywords: Burn, pregnancy, feto-maternal, outcome, flame burn, mortality.

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Introduction

Burn is a public health challenge worldwide, leaving long-lasting physical and psychological issues.[1]

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Approximately 90% of burn injuries take place in developing countries.[2] Burns are 4th most prevalent cause of injury, 5-12% of all global injuries and around four million females are affected annually.[3,4] Burns in pregnancy are linked to many complications including abortion, intrauterine death, preterm labour, stillbirth, and maternal mortality and morbidity.[5] The severity of the burn injury and the total surface area affected directly correlate with the feto-maternal outcomes.[6] These complications arise due to hypervolemia, pulmonary injury, septicaemia, and a catabolic state. There are different modes of burns, including flame burns, scald, chemical, and contact burns.[7] Majority of the chemical burns are due to assault or accidental.[8] According to one local study conducted, the analysis revealed that no statistically significant difference was found

between accidental and non-accidental unintentional and intentional burn injuries, except for the site involved (p-value 0.004), with the predominant cause of death being flame burns (81%).[9] Burns can be superficial, deep or mixed type.[10] According to another local study conducted in Islamabad shared results that accidental burns occurred in 79%, Kitchens were the most common accident site for females (27%), predominantly affecting housewives (35%), and Inhalational injury was observed in 23% of patients. On average, males had a total body surface area burned of 27.4%, while females had a higher average of 39.5% and among female patients mortality was 16%.[11] The acute management of burn injuries during pregnancy is of paramount importance, with a sharing burden on both surgeons and obstetricians, and the intensive care unit team with effort on preventing hypovolemia, hypoxia, and hypotension to minimise the risk of complications such as fluid and electrolyte imbalance, infection, and systemic inflammatory response syndrome. Additionally, ensuring adequate nutrition, implementing physiotherapy, and providing psychological support are crucial adjuncts to comprehensive care.[12]

Our study seeks to comprehensively investigate the demographic and burn-related characteristics of pregnant women experiencing burn injuries, aiming to identify key factors influencing maternal and fetal outcomes.

Material and Methods

After getting ethical approval from the institutional review board, this retrospective study is conducted at the Tertiary Care Burn Center. All burned pregnant women during the specified period from 1st October 2022 to 30th September 2023 were included without employing any exclusion criteria. After taking approval IRB No. 117/ED/JBRSC Dated 20-08-2022. Data was extracted from files, and in instances of incomplete data, we contacted the patients' families to obtain the missing information. All essential data, patient age, residence, parity, gestational age, total body surface area affected, burn intention, inhalational injury, mode of burn, involve-ment of abdomen, depth of burn, need for blood transfusion, surgical intervention, maternal and fetal outcome were systematically gathered from the patient's records utilizing a structured questionnaire. Data were analysed using SPSS 26, outcomes, and the characteristics of burn were calculated in terms of frequency and percentages. A comparison between discharged and expired was done using chi-square with p-value <0.05 taken as significant.

Results

Table 1 shows factors affecting maternal outcomes and their comparison among discharged and expired. Out of twenty pregnant burn victims, 8 (40%) patients expired and 12(60%) were discharged. 30% of patients were <25 years and 70% > 25 years of age, and mortality was (6 out of 8) 75% among the >25 years age group as compared to <25 years of age. 60% of patients had

Table 1: *Maternal Outcome*

Characteristics		Dis-charged	Expired	Total	P
Age	<25y	4	2	6(30%)	0.6
	≥25	8	6	14(70%)	
Gestational age	<28	10	2	12(60%)	0.009
	≥28	2	6	8(40%)	
Duration of presentation	<24	9	2	11(55%)	0.02
	≥24	3	6	9(45%)	
TBSA	<30	10	2	12(60%)	0.005
	≥30	2	6	8(40%)	
Inhalational injury	Yes	1	5	6(30%)	0.009
	No	11	3	14(70%)	
Abdomen involvement	Yes	1	6	7(35%)	0.002
	No	11	2	13(65%)	
Intention	A	11	5	16(80%)	0.2
	H	1	2	3(15%)	
	S	0	1	1(5%)	
Thickness	Superficial	1	0	1(5%)	
	Deep	1	2	3(15%)	0.4
	Mixed	10	6	16(80%)	
Mode of burn	Flame	9	8	17(85%)	
	Others	3	0	3(15%)	0.09
Blood transfusion	Yes	1	4	5(25%)	0.03
	No	11	4	15(75%)	
Surgical Management	Yes	2	3	5(25%)	0.28
	No	10	5	15(75%)	
Stay	< 7days	4	3	7(35%)	0.8
	≥7days	8	5	13(65%)	
Parity	< 2	3	1	4(20%)	0.4
	>2	9	7	16(80%)	
Epilepsy	Yes	1	1	2(10%)	0.7
	No	11	7	18(90%)	
Pretreatment	Yes	1	3	16(80%)	0.1
	No	11	5	4(20%)	

(A Accidental, H Homicidal, S Suicidal)

a gestational age (GA) of < 28 weeks at the time of presentation. 55% of patients were presented within 24 hrs. after the burn, however, 45% took >24 hours, and only 20% had a history of pretreatment before coming to the hospital. Mostly intention of burn was accidental (80%) followed by homicidal (15%) and suicidal (5%). Only 2 out of 20 patients had a history of epilepsy.

30% had inhalation injury and 65% had involvement of gravid abdomen. 80% of patients had mix-thickness burns. Most burns were due to flames (85%), followed by others i.e., scald and chemical burns (15%). The percentage of total body surface area (TBSA) burned was categorised as <30% and >30%, showing percentages of 60% and 40% with high mortality of 75% calculated among TBSA >30% and only 25% survived, which is statistically significant at p 0.005. The duration of stay in the hospital was classified into 2 categories <7 days (35%) and >7 days (65%). 75% of patients were managed conservatively and 25% had surgery (fasciotomy/ escharotomy/excision plus superficial thickness skin grafting) while 75% were managed conservatively and 25% of patients were transfused with blood. When results were compared among discharged and expired groups using chi-square, it was statistically significant for gestational age, time of presentation, TBSA, inhalational injury, abdomen involvement and mode of burn (p <0.05%). However, results were not significant for the history of epilepsy, surgical interventions, blood transfusion, intention of burn, age, parity, the thickness of burn, duration of hospital stay and history of pretreatment before coming to the burn center. Fetal outcome in terms of IUD and alive was noted showing percentages

Table 2: *Fetal Outcome*

FETAL OUTCOME	N	PERCENTAGE
IUD	9	45%
ALIVE	11	55%

of 45% and 55% respectively (Table 2). Among IUD's all patients had spontaneous vaginal expulsion except one who had a C-section.

Discussion

We conducted this study to find out fetal and maternal outcomes due to burn and the important factors associated with the outcomes. In our review, flame was found to be the most common cause of burns 85%, followed by scalds and chemical burns. Supporting our results, many previous reviews also stated flame burn as the commonest mode among adults 50-70%.[13,14,15] contrary to this some studies were in favour of scald as the most common cause.[16] Intention of burn was accidental (80%) followed by homicidal (15%) and then suicidal (5%). Also supported by a previous study that calculated accidental (unintentional) intention of burn among 79%-85% study population.[11,17,18]

Results were not significant statistically for history of epilepsy, surgical interventions, blood transfusion, intention of burn, age, thickness of burn, duration of hospital stay and history of pretreatment before coming to Burn Center. First aid is necessary for burn patients before hospital admission but there is a lack of adequate knowledge on first aid and the rationale of their use, people use unsuitable substances on burn wounds all over the world.[19,20] The Majority of patients presented within 24 hours of burn, however high mortality was calculated among those with delayed presentation that is greater than 24 hours. 35% of patients had involvement of gravid abdomen and 65% did not, results were significant with P <0.05. There was also high mortality among multiparous and having a gestational age of greater than 28 weeks and results were significant statistically, contrary to a previous study where the trimester of pregnancy was unrelated to maternal and fetal outcomes.[21]

When the duration of stay was compared, those with >7 days had high mortality as compared to those having less than 7 days stay. An important factor was the presence of inhalational injury among 30% of patients, with associated mortality calculated as 62.5% and significant with P 0.009. 60% had < 30% TBSA involved, 40% of patients had more than 30% area involved, and TBSA burned also affected overall mortality with a high expiry rate among the TBSA >30% group, these results were supported by previous studies.[21] Burn increases capillary permeability and third space loss that results in hypovolemia and decline in blood pressure, ultimately causing placental insufficiency and fetal ischemia, with the additional presence of inhalational injury causing further hypoxia in mother and fetus, thus increasing mortality.[22] In our study, in terms of feto-maternal outcomes 40% of pregnant females expired while 60% were discharged and 45% had IUDs while 55% alive issues. This mortality calculated for our study population was lower as compared to other studies among pregnant females.[21] One study had better feto-maternal 18.8% and 12%, respectively and one study mortality was 43% which is close to our expiry percentage of 40%.[23, 24, 25] One

local study on management and outcome of mother and fetus after burn also concluded that TBSA, burn thickness, gestational age, time of presentation after burn, presence of inhalational injury and affect the outcomes.[26] Raising awareness about the dangers of burn injuries in pregnant women is a critical step in reducing these high maternal mortality rates. Education on burn prevention, coupled with the implementation of immediate and appropriate medical responses, can significantly improve outcomes for both mothers and their unborn children. As healthcare professionals, we must advocate for increased resources, training, and public awareness campaigns to address this pressing issue.[27]

Our study has certain limitations, retrospective, single-centered with a small sample size, data collected from the medical records, and we have not followed discharged patients to look for long-term feto-maternal outcomes. Given the limited existing literature on burns in pregnancy, there is a clear need for additional studies that not only explore factors influencing outcomes but also incorporate long-term follow-up assessments concerning wound healing in patients.

Conclusion

We concluded that burns during pregnancy significantly increase maternal and fetal mortality. Gestational age, time of presentation, TBSA, inhalational injury, abdomen involvement and mode of burn have significant effects on maternal and fetal outcomes.

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Authors Contribution

BA, FAK: Conceptualization of Project
RER, MAY: Data Collection
AW: Literature Search
MAY, MZ: Statistical Analysis
BA, FAK, RER: Drafting, Revision
BA, FAK, YS: Writing of Manuscript

Effect of Moringa Oleifera Leaves on Hepatocytes Glycogen Content After Hepato-toxicity with Bisphenol-A in Albino Rat

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Abstract

Objective: To reveal the effects of Moringa Oleifera leaves extract on depleted glycogen stores after hepatotoxicity with Bisphenol-A in albino rats by using PAS staining.

Material and Methods: This experiment was carried out at Post Graduate Medical Institute, 32 adult rats were procured and distributed into 4 groups A, B, C and D. The study duration was 6 weeks. Group A was control and was given corn oil only. Group B, received BPA, 50mg/kg/bw. Group C and D received BPA 50mg/kg along with MoLE 250mg/kg and 500mg/kg. Liver was removed, fixed and slides were prepared by using PAS stain. The number of positively PAS stained (magenta colour) liver cells/40 cells were counted from every group and their mean was compared among groups. The statistical analysis was carried out by applying SPSS 21.

Result: The mean value of PAS positive cells in group B was lowest (14.25±4.27). However, in groups A and D, mean was calculated as 27.6±4.7 and 24.25±5.39, respectively.

Conclusion: Administration of MoLE (250mg/kg & 500mg/kg) restored the depleted glycogen content in cells, which was due to the toxic effect of BPA. The number of cells rises with increasing the dose of MoLE.

Keywords: MoLE: Moringa Oleifera Leave extract, BPA: Bisphenol-A, PAS: Periodic Acid Schiff stain.

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Introduction

The compound called Bisphenol A (BPA) is defined as a synthetic high production monomer utilized in polycarbonate plastics and the resins of epoxy in consumer products. This compound is used to bottles made of plastic, plastic food containers, warm receipts,[1] (Mourad and Khadrawy, 2012). Epoxy resins containing BPA are used to coat the inside of food and beverages metallic cans (Carwile et al., 2009).[2] Exposure to high temperature, the presence of acidic food or drinks in containers or cans may result in leaching of BPA into food. Thus the exposure of human beings is mainly through the use of contaminated food and drinks packed in containers with BPA. The liver assumes an essential part in the human body; it is responsible for blending glucose, plasma proteins, clotting factors, and urea; storing glycogen, fat,[3] and nutrients; regulating amino acids; and creating bile for metabolizing fat.[4]

Therapeutic plants have been investigated as a characteristic solution for liver diseases.[5] Moringa oleifera is an excellent source of phenolics and flavonoids comprise various pharmacological activities.[6] Different concentrates from restorative plants showing high hepatoprotective action in creature models.[7] Dynamic parts in restorative plants, like phenolic, and flavonoid compounds, display antioxidant and anti-inflammatory properties that assist with blocking lipid peroxidation

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and abatement the oxidative stress marker malondialdehyde (MDA) and hepatic enzymes,[8] Moringaoleifera (has a place with family Moringaceae) is a fast-growing tree with the tripinnate leaves structure as and is dispersed in African and Asian nations. In Pakistan, Moringa oleifera is known as "Suhannjana" and is adored as the "Miracle tree" for its restorative properties.[9] The leaf powder of M. oleifera is plentiful in calcium, iron, protein, starches, copper, and nutrients, iron, vitamin A, and L-ascorbic acid.[10] M. oleifera is demonstrated to be used in the treatment of various diseases, ascites, viral diseases (e.g., flu infection), bacterial contaminations, and various kinds of boil. Its leaves are eminent for their hepatoprotective, anti-inflammatory, antihypertensive, and antimicrobial exercises and lessen hyperglycemia and dyslipidemia.[11] Wealthy in antioxidants, for example, superoxide dismutase, catalase, phenols, flavonoids, and carotenoids, Moringa oleifera leaves display powerful hepatoprotective potential[12] and restores Glutathione. Treatment with Moringa oleifera leaves relieves liver harm and advance recovery, conceivably credited to its safeguarding effects on the plasmalemma and proteins.[13] Its hepatoprotective capacities have been widely considered with different hepatotoxicants, including antitubercular drugs and diclofenac sodium, yielding impressive outcomes.[14] This recommends the capability of Moringa oleifera as a characteristic remedial specialist for liver-related messes, inferable from its multi-layered pharmacological profile and significant protective effects against hepatotoxicity.[15]

Material and Methods

This experiment was carried out at Post Graduate Medical Institute, 32 adult rats were procured and distributed into 4 groups A, B, C and D. The study duration was 6 weeks. After taking approval from Ethical Committee No 5999-6000/PGMI Dated 10-07-2014. Thirty two adult albino rats of either sex, weighing (170-200g) were procured Post Graduate Medical Insti-tute Males and females were kept in separate cages. They were kept at temperature of 28.0±2.0ºC under 12hr light/dark cycles and were given rat diet and water ad libitum. After seven days, using lottery method, rats were divided into four equal groups. A, B, C and D, each comprising of 8 rats. They were put in respective labe-lled cages. BPA was procured from Daejung –Korea. Moringa leaves were obtained from the gardenof Uni-versity of the Punjab, Lahore, Pakistan. The leaves were authenticated by Professor Abdul Nasir Khalid, Department of Botany, from that same University. BPA and MoLE were dissolved in corn oil. Dose was freshly prepared on daily basis and was given through oral gavage. Animals were sacrificed at end of the 7th week and liver was dissected out. It was fixed with formalin, slides were made, labeled according to the rat number and group and stained with Periodic Acid Schiff Stain. The PAS stain demonstrates glycogen content and produce a bright pink or magenta colour. Two slides of each rat were observed from every group and number of positive PAS stained cells (magenta colour)/40 cells in each slide were counted .Their mean was taken and was compared among groups. The data was evaluated by applying SPSS 21. The quantitative data (PAS positive cells) was presented in the form of Mean± standard deviation (S.D). ANOVA was applied to determine the statistical differences among groups. For comparison among groups, Post Hoc Tuckey was applied.

Results

The mean value of PAS positive cells for group A, B, C and D are given in Table 1. The group B had of the lowest mean of 14.25±4.27 while group A has highest mean value of 27.6±4.7. ANOVA revealed markedvariations among four groups with p-value <0.001. Photomicrograph of the liver from group A, The Post Huc-Tockey test revealed that the group B and C when compared to group A had p-values <0.001 and 0.280 respectively. Group C had high value as compared to group B and the difference was significant with p-value 0.009. (Table- 2).

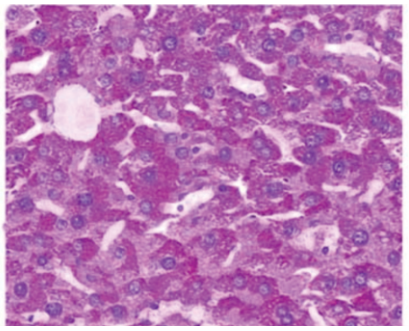

Figure 1a: *Magenta granules seen in cytoplasm of hepatocytes, indicates the presence of glycogen.*

Table 1: *Mean value of PAS positive cells in various animals groups.*

Parameters	Groups				P-value
	A Mean ±SD	B Mean ±SD	C Mean ±SD	D Mean ±SD	
Number of PAS positive cells/40 cells	27.6±4.7	14.25±4.27	23.00±5.68	24.25±5.39	<0.001

Table 2: *Mean value of PAS positive cells in various animals groups by applying Post HucTockey test.*

Groups	Group Comparisons	Mean Difference Between groups	Std. Error	P-value
Group A	Group B	**(A-B)** 13.38	2.52	<0.001
	Group C	**(A-C)** 4.63	2.52	0.280
	Group D	**(A-D)** 3.38	2.52	0.547
Group B	Group C	**(B-C)** -8.75	2.52	0.009
	Group D	**(B-D)** -10.00	2.52	0.002
Group C	Group D	**(C-D)** -1.25	2.52	0.059

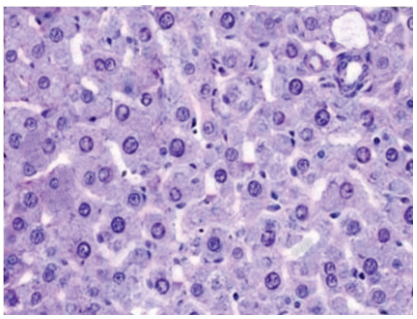

Figure 1b: *Subjecting the same preparation to PASD staining technique, which envisaged digestion of glycogen by Diastase. X400.*

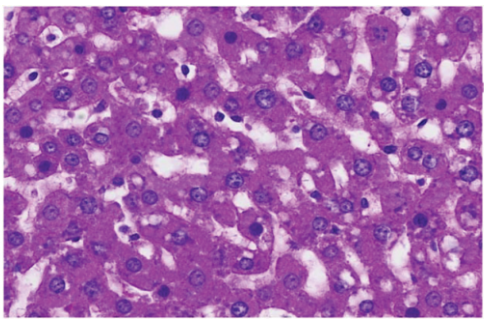

Figure 2: *Photomicrograph of liver from group B. PAS stain showing that hepatocytes cytoplasm was depleted of glycogen. PAS stain. X400.*

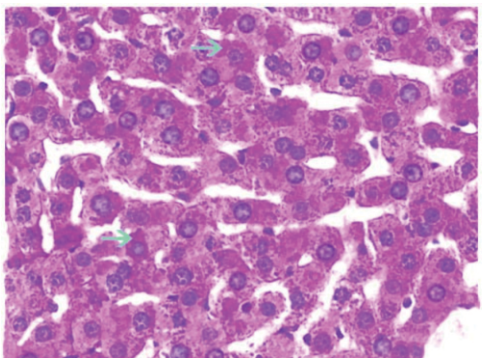

Figure 3: *Photomicrograph from group D. Magenta granules (green arrow) seen in hepatocytesindicated the presence of glycogen. PAS staining shows presence of glycogen. PAS stain. X400.*

Discussion

BPA is a compound widely used in plastic manufacturing and for coating the inside of the cans, thus it is found in many plastic products, bottles, food and beverage cans. Liver is the major organ which is affected following an oral exposure to BPA. BPA disturbs the normal function of liver by generating ROS leading to oxidative stress. This oxidative stress lead to lipid peroxidation and causes damage to hepatocytes and normal liver architecture. Moringa oleiferaleaves have preventive and curative properties for many liver diseases. The leaves of Mo plant possess high nutritional value and are good source of antioxidants. In current study, we observed that the liver section from group A when stained by using the PAS technique, revea-led strong magenta colour in the cytoplasm of hepato-cytes, which indicated the presence of glycogen **(Fig.1a)** We calculated the number of these positively PAS stained (magenta colour) cells from every group and compared their mean among groups by using Anova **(Table.1)**. The variation in batches was remarkable (p-value <0.001). The group B had lower mean value which proved that the number of cells with prominent PAS staining were decreased, thus pointed towards depleted glycogen content in hepatocytes **(Fig-2)**. Mean value of PAS positive cells in various animals groups by applying Post HucTockey test is shown in **Table.2**. This result matches with a study done by Ahmed et al., 2015, in which PAS staining revealed a decrease in glycogen content after administration of BPA. Ahmed et al., 2015, Exposure to BPA reduces glycogen content by decreasing the glycogen synthesis and promoting glycolysis.[16] After administration of MoLE in group C and D the glycogen content was improved. A research by Ndong

et al., 2007[17] showed decreased glycogen stores in liver cells due to the effect of anemia, but this glycogen was increased after treatment with Mo leaves. Omotoso et al., 2015, reported that in lead treated group there was a decrease in hepatocytes glycogen deposits, which were improved with Mo leaves.[18] It is presumed that the flavonoids in Mo leaves are strong inhibitors of an enzyme glycogen phosphorylase, which can break down glycogen. It was proven in studies that in diabetic rats, administration of Mo extract improved glycogen synthesis as it enhances glycogen synthase activity.[19] Another study manifested improvement in muscle and hepatic glycogen levels after administration of Mo leaves to the rats.[20]

In current experiment, BPA at dose of 50mg/kg caused decreased glycogen content These harmful effects were presumably due to oxidative stress and impaired enzymes activity Moreover this damage was ameliorated by co-administration of MoLE, as proved on the basis of histological and biochemical grounds. MoLEsignicantly improved the glycogen content in hepatocytes and these effects were more evident in group D, in which 500mg of moringa extract was given, as compared to group "C" which had 250mg of moringa extract. The healing effects of these leaves were more as we increased the dose. It was evident that the protective effects of Mo leaves were dose dependent; the liver damage restored more towards normal with increasing the dose of MoLE. This was accredited to the improved SOD, GSH and catalase activities after administration of leaves extract.[21]

Conclusion

Moringa oleifera leaves have protective effect on liver. It restored the depleted glycogen content in rats after administration of BPA. The effect of Moringa rises as dose of Moringa is increased. The powder of the leaves can also be used as a nutrient product to improve the diet. These results highlight that MoLE could be regarded as a source of natural antioxidants, thus its use in different medicinal fields should be encouraged.

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Authors Contribution

AS: Conceptualization of Project
NH: Data Collection
AW: Literature Search
NN: Statistical Analysis
FI: Drafting, Revision
AH: Writing of Manuscript

Efficacy of Racecadotril in Acute Gastroenteritis in Children at Tertiary Care Hospital

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Abstract

Objective: To compare the mean reduction in number of stools in racecadotril group with placebo group in the management of acute gastroenteritis in children with some dehydration.

Material and Methods: It was Quasi experimental study conducted in the Department of Pediatric Medicine, Children Hospital and ICH, Pakistan. Duration was of this study six months from 01/08/2022 to 31/01/2023. A total of 60 patients presented with acute gastroenteritis were divided to 2 groups. Group A oral rececadotril in addition to standard WHO treatment for acute gastroenteritis and Group B only standard WHO treatment along with 5ml of distilled water in the form of placebo. After 3 days both the groups were contacted and asked about the number of stools. Drug was considered efficacious if the number of stools is ≤2 per day after three days.

Results: In our Mean No. of stools at baseline in Group A was 7.001.26 in Group B 7.30±1.09, p-value = 0.328, mean No. of stools after treatment in Group A was 2.47±0.77 and in Group B 4.63±0.67, p-value = 0.0001. Comparison of mean reduction in number of stools after treatment in Group A was 4.53±1.14 and in Group B 2.67±1.03, p-value=0.0001. Conclusion: Racecadotril is more effective when compared with placebo group in the management of acute gastroenteritis in children with some dehydration.

Keywords: acute gastroenteritis, children, management, racecadotril, effective.

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Introduction

Diarrhea typically refers to having three or more unusually loose or watery bowel movements in a day.[1] Acute gastroenteritis, as described by the American Academy of Pediatrics, is a type of diarrhea that comes on suddenly and may include other symptoms like nausea, vomiting, fever, and stomach pain.[2] An estimated 525,000 children under the age of five worldwide pass away from diarrheal illness each year.[3]

Acute diarrhea is infection of gastrointestinal tract caused by variety of viruses, bacteria and protozoa. Rota virus is being the most common cause of acute diarrhea in well nourished breastfed infants and children under the age of five years.[4] Treatment of acute gastroenteritis with ORS is the most effective and recommended intervention by the WHO for prevention and management of dehydration. Annually, 11 million child deaths occur, of which two thirds are avoidable due to the widespread use of zinc supplements and oral rehydration salts (ORS) for the treatment of diarrhoea.[5]

The use of Zinc has shown significant reduction in the duration and severity of diarrhea and is the essential component in the treatment of gastroenteritis according to the WHO recommendations.6 Racecadotril is a new addition for the treatment of acute gastroenteritis. Racecadotril (acetorphan) is an inhibitor of enkephalinase (endorphin-metabolizing enzyme neutral endopeptidase).[7] It is the specific inhibitor of the neutral endopeptidase present on the epithelium of kidney and small intestine.[8]

Rececadotril reduces the hypersecretion of water and electrolytes in the intestinal lumen by preventing the degradation of endogenous enkephalins.[4] In randomized double blind control trials rececadotril has proven effi-

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cacious than placebo in acute diarrhea in adolescents and adults.[9] A study was conducted by Sultana et al[5] on 100 children in 2020. Study was randomized controlled trial and rececadotril group showed significant reduction in stool frequency than the placebo group (11.95± 2.41 Vs 14.85±1.95, p=0.0001) on third day of admission. Diarrhea is major of cause of mortality, second to pneumonia in under 5 children and is common cause of hospitalizations due to dehydration and electrolytes imbalances. If the results of this study will be favorable, the drug will definitely help in reducing under 5 mortalities, malnutrition and need for hospitalizations due to diarrheal illness. Moreover, this study will also help to fulfill the gap in literature.

Material And Methods

A Quasi experimental designed, during the period from 01/08/2022 to 31/01/2023, the study was conducted at the Pediatric Medicine Department of Children hospital and ICH, involving 60 patients (30 in each group). After taking approval from IRB No. Ref No. 187-89 dated 07-10-2021. These patients were selected based on specific inclusion criteria: they pre-sented with acute gastroenteritis and some dehydration, were of either gender, and aged between 6 months and 14 years. Excluded were those with malnutrition, immu-nocompromised status, osmotic diarrhea, severe dehy-dration, bloody diarrhea, chronic diarrhea, or those with severe dehydration. Following approval from the ethical committee of Children Hospital and Institute of Child Health, Faisalabad, informed consent was obtained from guardians. Patient demographics (age, gender, weight, height) were recorded, along with their contact details. A thorough medical history was taken, and a standard physical examination was conducted upon admission, including assessing dehydration status. Patients were then randomly assigned to two groups using a blinded envelope system: Group A received oral racecadotril in addition to standard WHO treatment for acute gastroenteritis, while Group B received standard WHO treatment along with 5ml of distilled water as a placebo. After three days of intervention, both groups were contacted by phone to inquire about their stool frequency. The intervention was considered successful if the stool frequency was ≤2 per day after three days. Data were input and analyzed using SPSS version 23. Mean and standard deviation were computed for quantitative variables such as age and stool frequency. An independent sample t-test was utilized to compare stool frequency between the two groups. Effect modifiers like age, gender, weight, and disease duration were controlled by stratifying the data, followed by post-stratification independent sample t-tests. A p-value of ≤0.05 was deemed statistically significant.

Results

Our study observed that 63.33% of patients in Group A and 53.33% in Group B were aged between 1-7 years, while 36.67% in Group A and 46.67% in Group B were aged between 8-14 years. The mean age was 7.13±3.03 years in Group A and 7.40±2.71 years in Group B. Regarding gender distribution, 56.7% of patients in Group A and 53.3% in Group B were male, while 43.3% in Group A and 46.7% in Group B were female. The mean duration of the disease was 1.23±0.43 days in Group A and 1.20±0.41 days in Group B, with a non-significant p-value of 0.759. The table presents a comprehensive comparison between Group A and Group B concerning the number of stools before and after treatment, as well as the mean reduction in stool frequency post-treatment. At baseline, there was no statistically significant difference in the mean number of stools between the two groups, with Group A having a mean of 7.00 stools and Group B having a mean of 7.30 stools, both with relatively similar standard deviations. After treatment, however, notable distinctions emerged. Group A exhibited a substantial reduction in stool frequency, with a mean of 2.47 stools post-treatment, whereas Group B showed a less pronounced decrease, with a mean of 4.63 stools. This discrepancy in post-treatment stool frequency was highly significant, as indicated by the p-value of 0.0001. Furthermore, analyzing the mean reduction in stool frequency after treatment revealed a stark contrast between the groups. Group A demonstrated a substantial mean reduction of 4.53 stools, while Group B exhibited a lesser mean reduction of 2.67 stools. This difference was also highly significant, underscoring the efficacy of the intervention in Group A compared to the control group.

Table 1: *Comparison of two groups regarding number of stools*

	Group A(n=30)		Group B(n=30)		P value
	Mean	SD	Mean	SD	
At baseline	7.00	1.26	7.30	1.09	**0.328**
After treatment	2.47	0.77	4.63	0.67	0.0001
Mean reduction after treatment	4.53	1.14	2.67	1.03	0.0001

Discussion

Each year, around 2 billion cases of acute diarrhea affect children, primarily in underdeveloped countries, according to the World Health Organization. This disease accounts for about 18% of deaths among children under five years old. Globally, diarrheal illnesses are a leading cause of both illness and death in children. Dehydration results from the loss of water and electrolytes due to intestinal issues like malabsorption and excessive secretion. The main treatment approach for children with watery diarrhea is to administer oral rehydration solutions to replace the ongoing fluid losses. Oral rehydration therapy remains the primary and recommended treatment for acute diarrhea in children, according to the World Health Organization. While this approach has significantly reduced morbidity and mortality rates associated with diarrhea, it has limited impact on reducing stool volume or frequency.

Recognizing this limitation, the World Health Organization has proposed the use of pharmacological treatments alongside rehydration therapy, provided these medications are proven safe and effective for pediatric patients. Racecadotril is one such medication that works by inhibiting intestinal hypersecretion without significantly affecting motility. Studies have shown that oral racecadotril is safe and effective in treating acute watery diarrhea in both children and adults. This study aims to evaluate the efficacy of racecadotril as an additional treatment, which could potentially contribute to reducing mortality rates, malnutrition, and the need for hospitalization among children under five years old due to diarrheal illnesses. Additionally, this research may help fill gaps in existing literature regarding the management of acute diarrhea in pediatric patients.

In our study, Group A had a mean age of 7.13±3.03 years, while Group B had a mean age of 7.40±2.71 years. In terms of gender distribution, 56.7% of Group A and 53.3% of Group B were male, with 43.3% and 46.7% being female, respectively. The mean duration of disease was similar between Group A (1.23±0.43 days) and Group B (1.20±0.41 days), with a non-significant p-value of 0.759. At baseline, the mean number of stools was 7.00±1.26 in Group A and 7.30±1.09 in Group B, with a p-value of 0.328. After treatment, Group A showed a mean of 2.47±0.77 stools, significantly lower than Group B's 4.63±0.67 stools (p-value=0.0001). The mean reduction in stool frequency after treatment was 4.53±1.14 in Group A and 2.67±1.03 in Group B, also significant at p=0.0001.

Sultana et al[5] conducted a randomized controlled study on 100 children in 2020, finding a significant reduction in stool frequency in the control group compared to the placebo group (11.95±2.41 vs. 14.85±1.95, p=0.0001) on the third day of admission, supporting our findings on racecadotril's efficacy.

Quantitative analysis of racecadotril's effect in placebo-controlled and add-on studies has been performed previously, with meta-analysis focusing on placebo control studies due to heterogeneity in active controls. Nine studies reported a reduction in mean stool number on the second day post-racecadotril administration, contrasting with our study's significant reduction after 3 days. Two larger studies found no statistically significant difference, yet meta-analysis demonstrated racecadotril's benefit in reducing stool frequency.

Numerous studies assessed racecadotril's efficacy based on various criteria, including the national diarrhea prevention and treatment commission's parameters in China.[13] Meta-analyses[14] consistently showed racecadotril's benefits in terms of efficacy, whether categorized by improvement, cure rates, or global efficacy scales.

Considering this evidence, racecadotril emerges as an efficacious and well-tolerated treatment for reducing stool frequency compared to placebo, as supported by multiple studies.

Considering all these evidence we come to conclusion that rececadotril is efficacious and more tolerable then placebo in reducing mean number of stools.[15]

Conclusion

We concluded that the mean reduction in number of stools was significantly lower in racecadotril group when compared with placebo group in the management of acute gastroenteritis in children with some dehydration.

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Authors Contribution

ZMA: Conceptualization of Project
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Scalpel Vs Diathermy Skin Incision in Maxillo-facial Surgery, A Randomized Controlled Trial

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Abstract

Objective: To compare the outcomes of scalpel versus diathermy skin incision in maxillofacial surgery.

Material and Methods: This Study design was randomized controlled trial and place and duration of study was CMH Medical College, Lahore from 1st July to 30th December 2023. A total of 138 patients planned to undergo maxillofacial surgery were included in the study. Patients were divided randomly through paper lottery method into "scalpel group" and "diathermy group" containing equal number of patients. Post-operatively, patients in both groups were assessed at one month follow up for the wound outcomes. Data was analyzed using SPSS 20.

Results: Mean age was 52.65±17.19 years. There were 96 (69.56%) male and 42 (30.44%) female patients. Mean operative time, mean blood loss and mean pain VAS at day 3 was significantly less in diathermy group, ($p<0.001$). Frequency of wound infection at day 14 follow up in scalpel group was 6 (8.69%) while in "diathermy group" it was 4 (5.79%), ($p=0.511$). In scalpel group, frequency of patients reporting good cosmesis was significantly higher as compared to diathermy group, ($p=0.001$).

Conclusion: In maxillofacial surgeries, diathermy use is a safe and effective alternative as compared to scalpel for making skin incision.

Keywords: Diathermy, Maxillofacial surgery, Outcome, Scalpel.

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Introduction

Among many diverse healthcare problems addressed by the relatively new field of "maxillofacial surgery" are diseases related to gums & teeth, abnormalities of the face, problems with the temporomandibular joint (TMJ), injury to face secondary to trauma and malignancies of the head and neck.[1] Like any other field, medical care based on evidence depends on drawing from the latest scientific findings while also taking the preferences of patients and clinicians' experience into account when making decisions about treatment to improve patients' satisfaction.[2,3] The most important aspect of a surgery, particularly performed on the face, is the cosmetic disfigurement that can potentially occur secondary to adverse wound and scar outcome that is a major concern for these patients adding to the actual fear of the surgery imposing major impact on life quality of the patients undergoing maxillofacial surgery.[4]

One factor that has major influence on the outcome of surgery is the method used to make the incision at the operative site. With advancement in the field of surgery, conventional instruments are continuously being replaced by electro-surgical ones with the preliminary aim of improving surgical outcomes.[5] On the other hand, diathermy also has adverse effects associated with it including increased incidence of infection of the surgical wound, excessive scarring and poor tensile strength of healed wound that has somewhat curtailed its use on a larger scale.[6] In addition, due to mechanism used by diathermy involving physically burning the operative

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tissue, there is an obvious risk of getting skin burns which is a major concern,[7] particularly in maxillofacial surgeries. Major advantage, however, of diathermy is that not only it reduces the time duration of the surgery but also makes it easier to achieve hemostasis and reduce the blood loss related to the surgery.[8]

When it comes to previous literature regarding wound outcomes with scalpel versus diathermy induced skin incision, there has been quite controversial results with studies reporting no difference in the wound outcomes 8 while other reporting diathermy to provide significantly better patient outcomes compared to scalpel made skin incision.[9] Furthermore, in the field of maxillofacial surgery not much literature is available in this regard. Therefore, this study was conducted with the aim of comparing outcomes of "scalpel" versus "diathermy" skin incision in maxillofacial surgery.

Material and Methods

This randomized controlled trial was conducted at CMH Medical College, Lahore from 1st July to 30th December 2023 after obtaining approval from the ethical review board of "CMH Medical College, Lahore" (ERB#:05/ERC/CMH/LMC). IRCT registration number: IRCT20240101060587N1. Sample size was calculated using WHO sample size calculator for two means using following formula:[9]

For calculation following parameters were used:

$$n = \frac{\left\{ z_{1-\alpha/2}\sqrt{2\bar{P}(1-\bar{P})} + z_{1-\beta}\sqrt{P_1(1-P_1)+P_2(1-P_2)} \right\}}{(P_1-P_2)^2}$$

- Level of significance = 10%
- Power = 80%
- Anticipated frequency of good cosmesis in scalpel group = 86.67% [11]
- Anticipated frequency of good cosmesis in diathermy group = 69.23% [11]

This gave a sample size of 138 (69 in each group). Adult patients who had the age more than eighteen years, males and females with "oral squamous cell carcinoma (OSCC)" or "pleomorphic adenoma of the parotid gland" were included in this study. Patients with any other type of malignancy, previous history of treatment for either OSCC or pleomorphic adenoma, pre-existing scar on operating site, conditions that can affect wound healing (like diabetes, immunosuppression, connective tissue diseases or steroid intake) and those undergoing chemotherapy or radiotherapy were excluded from the study.

Patients were selected through "non-probability consecutive sampling" technique. A written consent which was signed by the study participants was made an essential pre-requisite. Baseline characteristics including age (in years), gender (male/female), side of face incision was made (left/right) and indication for surgery (OSCC / pleomorphic adenoma) were documented. Patients were given explicit pre-procedural information regarding the instrument used to perform skin incision. Before making any incision, site of surgery was marked by a marker to define the field of surgery. After this, paper lottery were made with half having "scalpel" written on them while half having "diathermy" written on them. Drawing of lottery was performed by research team member blind to what had been written on the paper. Based on this patients were divided randomly into "scalpel group" or group A and "diathermy group" or group B. In group A (scalpel group), skin incision was made using a standard scalpel number 20 while in group B (diathermy group), this was done using surgical diathermy machine set on cutting mode giving 500kHz current. All the procedures were performed by same team of surgeons to minimize operator bias. In all the patients, after surgery was completed, skin was approximated using interrupted "silk" sutures 3/0. Intraoperative parameters including operative time and blood loss (measured by weighing gauze pieces with 1gram of weight equaling 1ml of blood loss) were documented. After surgery, standard post-operative care with injection co-amoxiclav (Augmentin ®) 1.2 grams eight hourly and injection metronidazole (Flagyl ®) 500mg eight hourly. Patients were kept admitted for 3 days and before discharge, pain assessment was made using "pain visual analogue scale (VAS)" scored 0-10; 0 being no pain and 10 being worst pain ever. Patients were asked to follow up at day 14 after procedure to assess for wound infection. In case of infection, extended antibiotic course was offered to the patients. Finally at 1 month follow up, cosmetic outcome was assessed based on cosmesis VAS (0-10) labelled as "good" if cosmesis VAS was ≥ 7, "satisfactory" if score was 4-6 and "bad" if < 4.

"Data was analyzed by using Statistical Package for Social Sciences (SPSS) 20. Quantitative data was represented using mean ± standard deviation (SD). Qualitative data was represented by using percentage and frequency. Operative time and blood loss were compared between

groups using Student t-test while frequency of wound infection and cosmetic outcome was compared between groups using Chi-square test. A p-value of ≤ 0.05 was considered as statistically significant".

Results

In this study, a total of 138 patients (69 in each group) were included. Mean age of study participants was 52.65±17.19 years. There were 96 (69.56%) male patients while 42(30.44%) patients were females. In 71 (51.45%) patients, skin incision was made on left side of face while in 67 (48.55%) patients it was made on right side of face. 83 (60.15%) of the patients had surgery for "OSCC" while 55 (39.85%) patients were operated for removal of "pleomorphic adenoma" of parotid gland. Comparison of baseline characteristics between groups is given below in **(Table-I)**. Mean operative time in "scalpel group" was 103.24 ± 6.78 minutes while in "diathermy group" it was 91.02±7.62 minutes, ($p < 0.001$). Mean blood loss in "scalpel group" was 286.86±24.95 ml while in "diathermy group" it was 232.63±24.38 ml, ($p<0.001$). Mean pain VAS at day 3 in "scalpel group" was 4.42 ± 1.06 while in "diathermy group", it was 2.78±1.02, ($p < 0.001$). Frequency of wound infection at day 14 follow up in "scalpel group" was 6 (8.69%) while in "diathermy group" it was 4 (5.79%), ($p= 0.511$). Cosmetic outcome and aforementioned data is given below in **(Table-2)**:

Table 1: *Comparison of baseline characteristics between groups (n = 138)*

Parameters	Scalpel group (A) (n = 69)	Diathermy group (B) (n = 69)	p-value
Mean age	53.47 ± 17.11 years	51.84 ± 17.36 years	0.578
Gender			
Male	49 (71.01%)	47 (68.12%)	0.711
Female	20 (28.99%)	22 (31.88%)	
Side of face operated			
Left	35 (50.72%)	36 (52.17%)	0.865
Right	34 (49.28%)	33 (47.83%)	
Indication of surgery			
OSCC	39 (56.52%)	44 (63.77%)	0.385
Pleomorphic adenoma	30 (43.48%)	25 (36.23%)	

Discussion

Amongst various surgical pathologies of face and oral cavity, "oral squamous cell carcinoma (OSCC)" and "pleomorphic adenoma of the parotid gland" are two most major and challenging indications necessitating maxillofacial surgery.[12,13] Maxillofacial surgery is relatively newer surgical field that is undergoing the process of evolution and one of the most important aspect of this particular field of surgery is the cosmesis since scar tissue on the face can significantly impact quality of life of the patients.[14,15] In general surgery patients, diathermy has increasingly been replacing the conventional method of giving skin incision with the use of scalpel since it has the tendency to achieve effective hemostasis while inflicting the incision thus reducing the operative blood loss.[16,17] The conventional surgery with use of scalpel results in longer operative time [23]. However, due to its tendency to cause burn injuries and ugly looking scars,[18] its use in maxillofacial surgery is not widely studied which prompted the conductance of present study.

Table 2: *Comparison of outcomes between study groups (n = 138)*

Outcomes	Scalpel group (A) (n = 69)	Diathermy group (B) (n = 69)	p-value
Mean operative time	103.24 ± 6.78 minutes	91.02 ± 7.62 minutes	< 0.001
Mean blood loss	286.86 ± 24.95 ml	232.63 ± 24.38 ml	< 0.001
Mean pain VAS at day 3	4.42 ± 1.06	2.78 ± 1.02	< 0.001
Wound infectionat day 14	6 (8.69%)	4 (5.79%)	0.511
Cosmetic outcome at day 30			
Good	47 (68.12%)	27 (39.13%)	0.001
Satisfactory	17 (24.64%)	25 (36.23%)	
Bad	5 (7.24%)	17 (24.64%)	

In present study, average age of patients was 53 years with clear male predominance which corresponds with the fact that malignancies of the oral cavity, in particular the OSCC is much more common in older men who have the age more than 40 years.[19] No statistical difference was observed between study groups in terms of baseline characteristics ($p > 0.05$). In terms of mean operative time and blood loss during operation, use of diathermy significantly reduced the duration of procedure as well as mean blood loss which was consistent with the finding of a meta-analysis in which nine studies were analyzed which showed that as compared to scalpel, use of electric diathermy resulted in significant reduction of these parameters ($p < 0.05$).[20] On the other hand, Kumar et al.[11] reported no significant difference between scalpel and diathermy groups in terms of mean operative time when used in maxillofacial surgery. In terms of mean pain score, diathermy use was associated with

significantly reduced post-op pain at day 3 which was consistent with the findings of a study conducted by Panni et al.[21] In terms of frequency of post-op wound infection, although scalpel use was associated with higher frequency of wound infection but the difference between study groups was not of statistical significance ($p = 0.511$). This was similar to findings of Kumar et al.[11] but was not congruent with the findings of Panni et al.[21] who found that frequency of wound infection was significantly higher with scalpel use ($p = 0.046$). In terms of cosmetic outcome, use of scalpel provided significantly better cosmesis with higher frequency of patients reporting good cosmesis ($p = 0.001$). This was not congruent with the findings of Kumar et al.[11] who found no significant difference between scalpel and diathermy made skin incision in maxillofacial surgery as well as another study done in Pakistan but on urethra.[22] Based on present study, it is evident that diathermy provides a clear advantage over the use of scalpel to make skin incision for maxillofacial surgeries in terms of reduced operative time, lesser blood loss, lower seve-rity of pain and lesser wound infection rates. However, for better cosmesis, scalpel is a better option as compared to diathermy. Due to such promising results it is reco-mmended that diathermy can safely replace use of scalpel for making skin incisions in maxillofacial surgery but special care should be taken while performing such surgeries to ensure best possible cosmetic outcomes.

Conclusion

In conclusion, diathermy inflicted skin incision in maxillofacial surgeries is better than scalpel in terms of reduced operative time, lesser blood loss, lower severity of pain and lesser wound infection rates. However, for better cosmesis, scalpel use is much better option as compared to diathermy.

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Authors Contribution

VSK, AAC: Conceptualization of Project
VSK, AAC, HJM: Data Collection
VSK, AAC, HMJM, FQ, AIU, AKM: Literature Search
VSK, AAC, FQ, AIU: Statistical Analysis
VSK, AAC, HMJM, FQ, AKM: Drafting, Revision
VSK, HMJM, AIU: Writing of Manuscript

Histopathological Overview of Common Prostatic Lesions

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Abstract

Objective: To determine the frequency of different prostate lesions in our population regarding age and to grade carcinoma prostate according to the Modified Gleason Grading System.

Material and Methods: A 3-year retrospective study of prostate biopsies received at the Pathology Department, Central Park Teaching Hospital /Central Park Medical College, Lahore from 1st January 2021 to 31st December 2023. Previous records were reviewed regarding age, type of surgical specimens, diagnosis and Gleason grading in prostate cancer cases. Statistical analysis using SPSS version 21 was utilized for calculations.

Results: Out of 260 prostate biopsies, 251(96.53%) specimens were transurethral resection of prostate (TURP), 6(2.30%) cases were core needle biopsies (CNB) and 3 (1.15%) cases were radical prostatectomy (RP) specimens. Benign cases constituted of 230 (88.46%) cases which included 200 (86.95%) cases of benign prostate hyperplasia (BPH) and 30 (13.04%) cases of BPH with associated prostatitis. Malignancy was reported in 30 (11.03%) cases. Patient's age ranged between 40-95 years with cases in the age group of 61-70 years constituting of 100 (38.46%) cases. These included 89 (38.69%) cases of BPH and 11 (36.60%) malignant cases. Maximum number of malignant cases were seen in age >70 years constituting of 13(43.33%) cases. Ten (33.33%) cases of carcinoma prostate had Gleason grade 5 and 8 (26.66%) cases had Gleason grade 4. Perineural invasion was noted in 24 (80%) cases.

Conclusion: All prostatic lesions predominate in the older age group. Benign lesions outnumber malignant lesions by a ratio of almost 8:1 with the commonest biopsy being TURP. Majority of prostate cancers had high Gleason grade groups of 4 & 5.

Keywords: Benign prostatic hyperplasia, prostatitis, carcinoma prostate, Gleason grading system.

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Introduction

The prostate gland is a male genital organ located at the base of the urinary bladder. It is involved in three main pathological processes, mainly benign prostatic hyperplasia (BPH), carcinoma prostate and prostatitis.[1] The main culprit of these lesions appears to be high levels of the male hormone testosterone, some cytokines and local growth factors.[2] BPH is the commonest pathological entity characterized by benign enlargement of the prostate which histologically manifests as hyperplasia of both the stromal and epithelial components of its transitional zone.[3] Prostatitis is an inflammatory lesion which may be acute, chronic and granulomatous. It is usually associated with BPH in approximately 10 to 15 percent of cases.[4]

Carcinoma prostate is a significant public health issue ranking as the 2nd most common malignancy in elderly men after carcinoma lung and is the 5th most common cause of cancer related deaths in men in the United States[5]. Worldwide in 2020, over 1.4 million new cases

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and 381,000 deaths were attributed to carcinoma prostate, making it the most common cancer among men and it is estimated that by 2030, the number of new cases and deaths due to this malignancy will increase substantially.[6,7] There is a marked epidemiological variation in the incidence rates of carcinoma prostate related to advanced age, genetic and familial predisposition associated with dietary and environmental influences. African-American men have the highest incidence rates and the most aggressive types of carcinoma prostate.[8] In Pakis-tan, carcinoma prostate ranks amongst the top 10 common cancers and the 3rd most common genitourinary tract cancer in males.[9] The Gleason grading system is the single most reliable and significant prognostic indicator of carcinoma pros-tate based exclusively on the histological architectural pattern of the tumor as seen under the 4X objective of the light microscope.[10,11] It was devised by Dr Donald Gleason in 1966 and based on the architectural pattern of the glands by adding the most common and the second most common pattern.[12] A new Gleason grade group ranging from 1 to 5 was later introduced by Epstein and his team in 2014. This new system has the added beneficial impact on the patient's psychological awareness regarding his disease and the available choices of different treatment modalities.[11]Patients with prostate lesions frequently present with non-specific symptoms of increased urinary frequency, urinary retention, nocturia and rarely hematuria[13]. Diag-nosis of prostate lesions requires estimation of elevated serum PSA levels, digital rectal examination (DRE) and ultrasound. However, the ultimate gold standard for diagnosis is a tissue biopsy. Due to its anatomic proximity to the rectum, biopsy can be easily obtained via the transrectal route or the perineal route.[8]

The purpose of this study is to determine the nature of prostatic lesions in our population regarding frequency, age and Gleason grade group in histologically confirmed cases of carcinoma prostate.

Material and Methods

This was a 3-year retrospective study commencing from January 2021 to December 2023, conducted at the Histopathology section of Department of Pathology at Central Park Teaching Hospital/Central Park Medical College, Lahore, Pakistan. It was approved by the Ethical Review Committee of CPMC (Letter No: CPMC / IRB- No / 1454, Dated: 27/02/2024).

Previous records of these cases were retrieved from computer data and biopsy record registers maintained at the Histopathology section. During this 3-year period, 260 prostate biopsies comprising mainly of trans urethral resection of prostate (TURP), few core needle biopsies (CNB) and radical prostatectomy (RP) specimens were included in this study. The retrieved histopathology slides were reviewed by 2 consultants to confirm the diagnosis. Autolyzed and inadequate biopsy specimens were excluded from the present study. Available clinical data regarding patient's age, clinical history, clinical suspicion, type of surgical procedure and final diagnosis was incorporated in a proforma. Prognostic pathological parameters regarding prostatic carcinoma including Gleason score with grade groups, perineural invasion (PNI), extra prostatic extension (EPE) and tumor load were noted. Grading of prostate carcinoma was done according to the modified Gleason grading system as shown in **(Table-I)**.[14] The obtained data was analyzed using Statistical Package for Social Sciences (SPSS) version 21. Frequency and percentages were calculated for the number of cases, age, benign and malignant categories, Gleason's grading and perineural invasion.

Results

Out of a total of 260 prostate biopsies received during a 3 year period commencing from 1st January 2021 to 31st December 2023, 251 (96.53%) cases were TURP specimens, 06 (02.30%) cases were CNB specimens and 3 (01.15%) cases were RP specimens. Out of 260 prostate specimens, 230 (88.46%) cases were classified as benign lesions which included 200 (86.95%) cases of BPH and 30 (13.04%) cases of BPH associated with prostatitis (Figure 1 & 2). Cases of prostatitis were segregated as 15 cases of chronic prostatitis, 4 cases each of follicular prostatitis and acute prostatitis, 3 cases of granulomatous prostatitis, 2 cases of abscess formation and 1 case of infarction. Malignancy was reported in 30(11.3%) prostate biopsies of which 28 (93.33%) cases were adenocarcinomas, 01 (3.33%) case was poorly differentiated carcinoma based on immunohistochemical stains (IHC) and 01 (3.33%) case was a metastatic urothelial carcinoma invading the prostate. In the present study, patients age ranged between 40-95 years. Age distribution ranges are depicted in Table-2. Maximum number of cases were seen in age group of 61-70 years constituting of 100 (38.46%) cases. These included 89 (38.69%) benign

cases and 11 (36.60%) malignant cases. However maximum number of malig-nant cases were seen in age >70 years constituting of 13 (43.33%) cases. Mean age was 68.9 and median age was 70 years. The updated Gleason grading system was applied for grade determination of prostate cancer cases as shown in Table-3. Maximum number of cases were seen in grade group 5 comprising of 10 (33.33%) cases followed by grade group 4 having 8 (26.66%) cases. A grade of 2 and 3 was assigned to 3 (10%) and 5 (16.66%) cases respectively, while 2 (6.6%) cases had grade group 1 on morphology **(Figure 3, 4 & 5)**. Perineural invasion was observed in 24 (80%) cases and extraprostatic extension was seen in 3 (10%) cases with high Gleason scores of grades of 4 and 5 (Figure 6). Majority of cases had high tumor load in the range of 80-95%.

Table 1: *The New Contemporary Prostate Cancer "ISUP Modified Gleason Grading System* [14]

New Grading System Morphologic Patterns and Grade Group Pattern Composition	
Grade Group	**Pattern Definition**
Grade Group 1 (Gleason score ≤6)	Only individual, discrete, well-formed glands
Grade Group 2 (Gleason score 3 + 4 = 7)	Predominantly well-formed glands with a lesser component of poorly formed/fused/cribriform glands
Grade Group 3 (Gleason score (4+3=7)	Predominantly poorly formed/fused/cribriform glands with a lesser component of well-formed glands[a]
Grade Group 4 (Gleason scores 8)	Only poorly formed/fused/cribriform glands or predominantly well-formed glands with a lesser component lacking glands[b] or predominantly lacking glands with a lesser component of well-formed glands[b]
Grade Group 5 (Gleason score 9-10	Lacks gland formation/necrosis with or without poorly formed/fused/cribriform glands[a]

[a] For cases with more than 95% poorly formed/fused/cribriform glands or lack of glands on a needle core or at radical prostatectomy, the component of less than 5% well-formed glands is not factored into the grade.
[b] Poorly formed/fused/cribriform glands can also be a more minor component.

Table 2: *Age Distribution of Prostate Lesions (n=260)*

Age in Years	Benign Cases 230 (88.46%)	Malignant Cases 30 (11.53%)	Total No. of Cases 260 (100%)
≤50	6 (2.60%)	00	6 (2.30%)
51-60	50 (21.73%)	06 (20%)	56 (21.53%)
61-70	89 (38.69%)	11 (36.6%)	100 (38.46%)
>70	85 (36.95%)	13 (43.33%)	98 (37.69%)
Ratio of benign to malignant cases = 7.66:1			

Table 3: *Categorization of Prostate Cancer Cases with Gleason scoring and grading*

Gleason Grade Group	Total No. of Cases (n=30)	Frequency Percentage %
Grade Group 1 (GS=3+3=6)	02	6.6%
Grade Group 2 (GS=3+4=7)	03	10%
Grade Group 3 (GS=4+3=7)	05	16.66%
Grade Group 4 (GS=4+4, 3+5,5+3=8)	08	26.66%
Grade Group 5 (GS=4+5, 5+4=9), (GS=5+5=10)	10	33.33%
Poorly Differentiated Carcinoma	01	3.33%
Metastatic Urothelial Carcinoma	01	3.33%

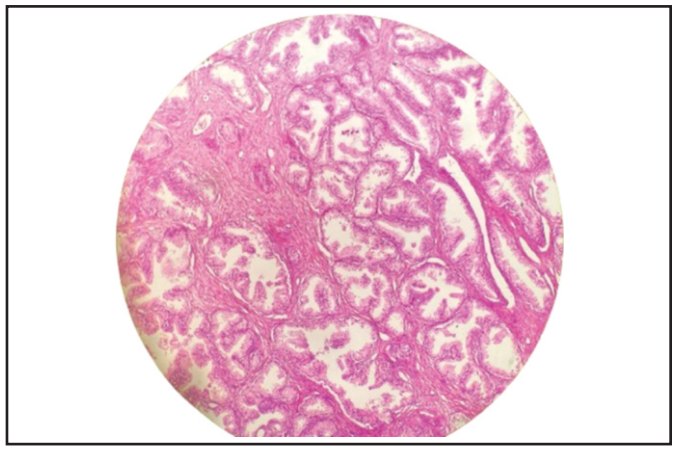

Fig-1: *Benign Prostate Hyperplasia: Benign glandular component showing increased number of glands with papillary infoldings and an intact basal layer.*

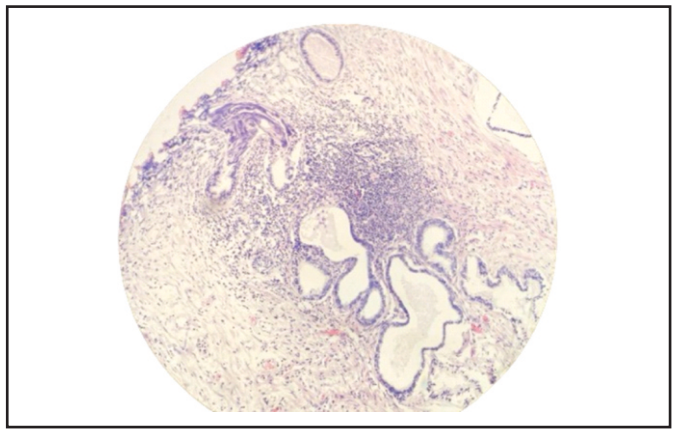

Fig-2: *Chronic Prostatitis:Stroma showing dense*

aggregates of Lympho-plasmacytic cells surrounding dilated prostatic acini.

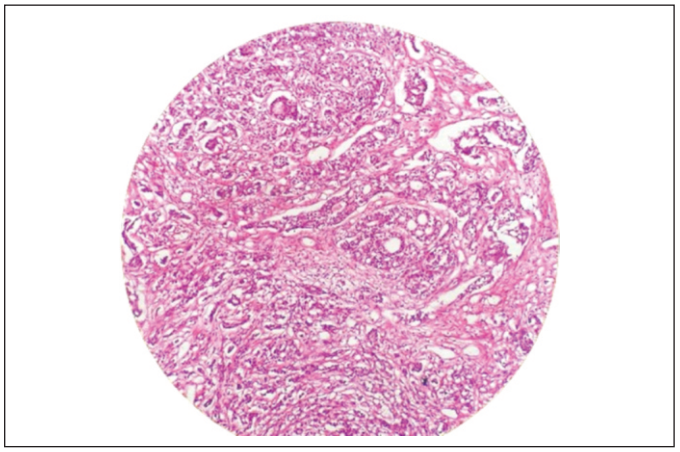

Fig-3: *Carcinoma Prostate: Gleason Grade group 2 (3+4=7) showing a mixture of well-formed and poorly formed glands.*

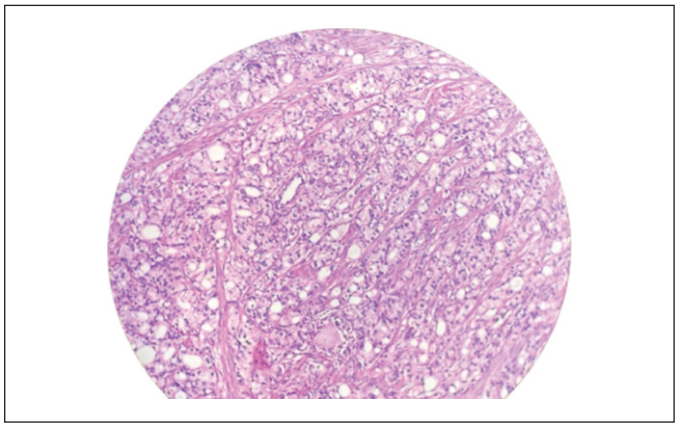

Fig-4: *Carcinoma Prostate: Closely packed small neoplastic glands with pale cytoplasm and hyperchromatic nuclei. Gleason Grade Group* 3(4+3=7).

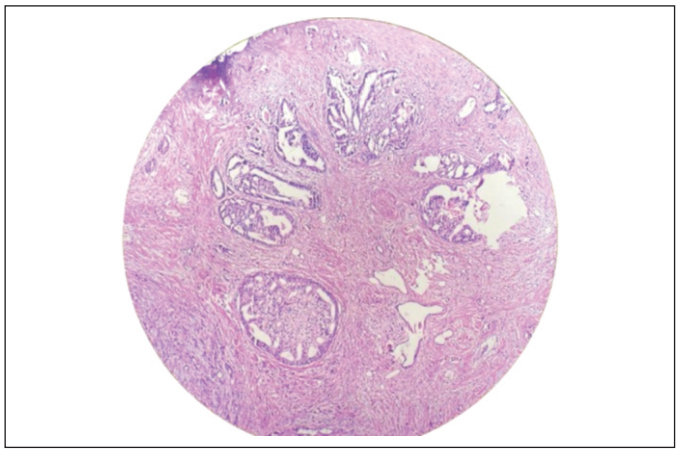

Fig-5: *Carcinoma Prostate showing cribriform morphology of the neoplastic glands Gleason Grade group 4 (4+4=8).*

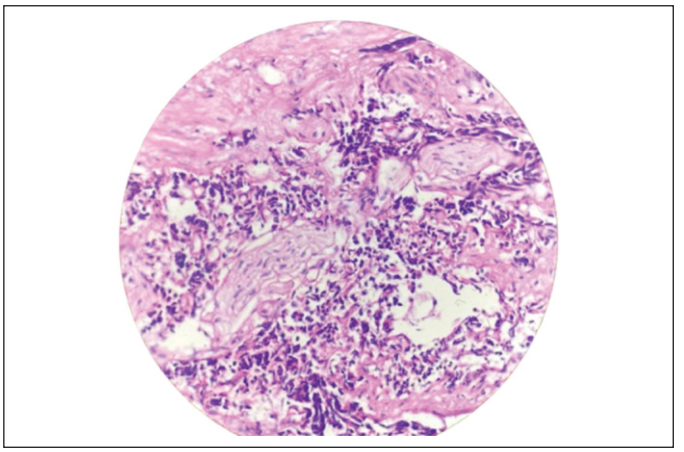

Fig-6: *Perineural invasion: Two nerves circumferentially surrounded by neoplastic cells.*

Discussion

Prostate biopsies comprise a significant proportion of surgical samples received in a running Histopathology laboratory. Among these, prostate cancer in the most commonly diagnosed malignancy in elderly men.[15] Some Asian countries like Lebanon, Kuwait, United Arab Emirates, Qatar and Japan have a high incidence of carcinoma prostate.[16] There is no country wide statistical data available to estimate the exact prevalence of carcinoma prostate in Pakistan as it is a low socioeconomic country with its healthcare system facing several challenges and setbacks. However, according to a large meta-analysis study by Sohail Akhtar and colleagues published in 2023 which included data from 11 articles between 2000 and 2023 from different geographical areas of Pakistan the pooled prevalence of prostate cancer was 5.20%.[17] According, to the Pakistan National Cancer Registry prostate cancer is the 2nd most common malignancy among males in Pakistan.[9]

In the present study, different types of prostate surgical biopsies included TURP, CNB and RP specimens. TURP specimens comprised of 251 (96.53%) cases, indicating that this is the commonest received prostate surgical specimen. A study conducted by Srinivasan and Wang in 2019 concluded that TURP is considered the gold standard treatment for surgical management of enlarged prostates.[18]

In the current study maximum number of 230 (88.46%)

cases were diagnosed as benign lesions which comprised 200 (86.95%) cases of BPH and 30 (13.04%) cases of BPH with associated prostatitis. Almost similar results were quoted by Satyasri and Sinha in 2018 who reported 279 (86.91%) cases of nodular hyperplasia and 117 (42.09%) cases with an associated element of prostatitis.[19] Another study by Sabalpara et al in 2019, quoted BPH as the sole benign entity with no case of prostatitis.[20] A recent study at King Edward Medical University, Lahore, Pakistan in 2021 showed 461 (73.30%) cases of BPH and 88 (14%) cases of BPH associated with prostatitis.[21] These results are in accordance with the present study at CPMC/CPTH. A study conducted by Sumaya et al in 2020 reported a maximum of 37 (41.1%) cases of prostate lesions in the age group of 61-70 years, followed by the 71-80 years age group with 23 (25.6%) cases 22. In the current study similar results are observed in the 61-70 years age group and 98 (37.69%) cases in >70 years age category.

As regards age group of carcinoma prostate in the present study, a maximum of 13 (43.33%) cases are noted in the age group >70 years followed by 11 (36.6%) cases in the age group of 61-70 years. This finding is in accordance with the study by Bhatta et al who observed that benign lesions are more common in the age group of 61-70 years and malignant cases predominate in the age group of 71-80 years 4. The Gleason grading system is the single most important and powerful prognostic predictor in carcinoma prostate. It plays a crucial role in prognosis, clinical management and therapeutic options.[11] In the present study 30 (11.53%) out of 260 cases were diagnosed as carcinoma prostate. These comprised of maximum cases in Gleason grade group 5 which included 10 (33.33%) cases followed by 8 (26.66%) in grade group 4. Collectively grade group 3,2 and 1 constituted of 5 (16.66%), 3 (10%) and 2 (6.6%) cases respectively. Similar results were observed in a study conducted in 2018 which also showed 37.30% cases in grade group 5 4. Another study by Shah et al reported a Gleason grade group 5 in 40% cases followed by 30% cases in grade group 4[23]. A study by Loeb et al in 2016 observed that only 1% of men had Gleason grade group 5 on biopsy whereas grade group 1 was the highest occurring grade comprising of 67%.[24] This observation is in contrast to the present study and many other similar studies. A high Gleason score and grade is associated with a poor prognosis.

Perineural invasion (PNI and lymphovascular invasion (LVI) are associated with a poor disease outcome.[25] PNI is associated with extra prostatic extension of tumor and ultimate recurrence. In the present study PNI was observed in 24 (80%) cases, majority of which showed high Gleason scores and grades.

Conclusion

All prostatic lesions predominate in the older age group. Benign lesions outnumber malignant lesions by a ratio of almost 8:1 with the commonest biopsy being TURP. Majority of prostate cancers had high Gleason grade groups of 4 & 5.

Conflict of Interest: *None*

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Authors Contribution

KB, MA: Conceptualization of Project
SN, NM: Data Collection
MA, ZR: Literature Search
ZR: Statistical Analysis
NM, ZMA: Drafting, Revision
KB, SN: Writing of Manuscript

Original Article

A Comprehensive Assessment of Oxidative Stress from Hematological Parameters in Alzheimer's Patients

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Abstract

Objective: To evaluate the relationship between oxidative stress and hematological parameters in patients with Alzheimer's disease.

Material and Methods: This was a cross-sectional study conducted at Liaquat University Hospital from May to July 2022 aimed at assessing Alzheimer's disease (MCI-AD) that causes mild cognitive impairment and also using NIA-AA diagnostic criteria in this study. The study included 300 participants, divided into case and control groups based on cognitive function and neuroimaging. Exclusions were made for individuals with any other additional brain pathology or mental illness that falsifies the study results. Peripheral blood samples were taken under standardized conditions for flow cytometry analysis and also for measuring reactive oxygen species (ROS) and various oxidative stress markers. Flow cytometry data was examined using FCS Express software.

Results: The correlation between oxidative stress and Alzheimer's disease is significant (P = 0.001), with odds ratios ranging from 1.8 to 2.2, sensitivity and specificity values of 0.50 and 0.6, and a robust likelihood ratio of 1.4.

Conclusion: This study underscores the potential relationship between Alzheimer's disease and oxidative stress.

Keywords: Alzheimer's disease, Antioxidants, Free radicals, Neurodegenerative disease, Oxidative stress

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Introduction

Alzheimer's disease is a neurodegenerative ailment that is widely widespread. It is defined by a loss of cognitive function caused by the deposits of beta-amyloid peptide and neurofibrillary tangles in memory and cognitive centers such as the cerebellar cortex and the hippocampal regions.[1] There are several mechanisms underlying the development of Alzheimer's disease, but the most potent one is oxidative stress, which modifies the structures of biomolecules such as proteins, lipids, and nucleic acids in the brain. These then build up within brain cells and cause the degeneration of neural cells[2]. Multiple research investigations provide substantial proof that the brain becomes particularly susceptible to oxidative stress because it uses oxidative processes at a fast pace and has inadequate antioxidant levels.[3-4]

For several oxidative activities, including respiration, the brain has substantial quantities of mitochondria. Utilizing mitochondrial respiration results in the production of superoxide's, which are free radicals that kill brain cells and are also linked to the pathogeneses of Alzheimer's disorders.[5] Many investigations have shown that mitochondrial fragmentation is another feature in patients with Alzheimer's disease. This occurs due to reduced expression of fission-related genes like Drp1 and Fis1, as well as elevated expression of fusion-

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related genes like Mfn1, Mfn2, and Opa1, which cause neuronal injury that causes mitochondrial fragmentation.[6-7]

Alzheimer diases is a highly prevalent disease that is widely spreading around the world, with an increasing rate of 117% in the last 26 years and keeps increasing havoc. It is still most common in Turkey and Brazil.[8] According to recent research, it is estimated that by the end of 2050, 152 million people around the world will be affected by Alzheimer's disease because of an increase in risk factors and the genetic composition of the population.[9]

Many therapies are developed to treat Alzheimer's disease either fully or partially as medical research advances. At the moment, cholinesterase inhibitors and rhivastigmine are used to treat AD symptoms, however not totally.[10-11] Although antioxidants are theoretically the greatest option for treating AD, their inability to pass the blood-brain barrier limits its applicability. However, because to advancements in nanotechnology, it is now feasible to treat AD by administering significant amounts of antioxidants effectively to the brain[12-13]. Mesenchymal stem cell therapy is additional excellent technique for treating AD owing to its anti-inflammatory properties, which lower the rate of neuronal cell damage.[14-15]

The aim of this research is to show relation between oxidative stress and development of Alzehmeirs disease.

Material and Methods

This was a cross sectional study which was conducted in Out Patient Department of Neurology in association with Department of radiology of Liaquat University Hospital. This study was conducted from May 2022 to July 2022 after approval from ethical review committee. The National Institute on Aging-Alzheimer's Association (NIA-AA) has recently revised its MCI-AD diagnostic criteria, which were used in this study. According to its criteria, the diagnosis of MCI-AD (Mild Cognitive Impairment due to Alzheimer's disease) is based on cognitive assessment, CSF biomarkers, and structural neuroimaging, which include computed axial tomography (CAT) and nuclear magnetic resonance (NMR). This study consists of 300 subjects who were divided into two groups: a control group and a case group. In the control group, 200 subjects were included, including all people without any cognitive impairment (normal function and cognition as confirmed by comprehensive neuropsychological testing) and also showing negative neuroimaging (CAT (Computed Axial Tomography) and NMR (Nuclear Magnetic Resonance)). On the other side, the selection criteria for the case group included 100 people with any cognitive impairment revealed in neuropsychological tests (CDR and altered RBANS-DM) but no impairment in daily routine activities, and they were also positive for neuroimaging). Individuals who failed to meet all the requirements specified for each group or who had any additional brain pathology including high grade vascular Sub- corticoid brain, hydrocephalous or any other additional brain deformity or abnormality that was determined by Neuroimaging were not allowed to participate in this study, and patients with significant mental illness, moderate to severe dementia , significant sensory system impairment or had history of any brain disorder that falsified this investigation were also excluded. To mitigate potential confounding variables, peripheral blood samples were taken under standardized circumstances following an overnight fast. Flow cytometry was used to quantify the amount of ROS, with a FACSCanto II (Becton Dickinson, BD, CA, USA) device being used to evaluate the results. The levels of Nitric oxide, Superoxide anion, hydrogen peroxide and peroxunitrite/ hydroxyl radical were measured by monitoring variations in the median fluorescence intensity (MFI) generated by diaminofluorescein (DAF), hydroxyphenyl fluorescein (HPF), dichlorofluorescein (DCF), and dihydroethidine (DHE), respectively. 106 cells were incubated for 180 minutes (for DAF) or 30 minutes (in case of DCF, HPF and DHE) at 37°C in the dark with 10 μmol/L of HPF, 160 mmol/L of DHE, 2 μmol/L of DAF and 20 mmol/L of DCF. The samples were then washed, reconstituted in PBS, and maintained cold until flow cytometry was able to collect 10,000 events. After that, the data was examined using FCS Express software.

The normal values of oxides in CSF and Blood plasma are:

1. NO (Nitric Oxide) (CSF 0.2-0.4μM, plasma 0.1-0.5 μM)
2. O2 – (Superoxide Anion) (CSF0.02-0.02 μM, Plasma 0.01-0.1 μM)
3. ONOO (Peroxunitrite) (CSF 0.02-0.1 μM, Plasma 0.01-0.05)
4. OH (Hydroxyl Radical) (CSF0.002-0.02 μM, Plasma 0.001-0.01 μM)

Results

Tabel-1 compares oxide biomarker levels between a 100-person Control group and a 200-person Case group. Notably, the Case group, particularly individuals with elevated levels, showed significantly higher levels of Nitric Oxide (NO), Peroxynitrite (ONOO), Superoxide (O2), and Hydroxide (OH) compared to the Control group. Statistical significance (P values ranging from 0.001 to 0.04) emphasizes these differences. Elevated NO levels in the Case group demonstrated a strong association (Odds Ratio: 2.2, 95% CI: 1.1 to 1.5), with similar associations found for ONOO, O2, and OH. Sensitivity/Specificity ratios of 0.5/0.6 and Likelihood Ratios ranging from 1.313 to 1.5 .

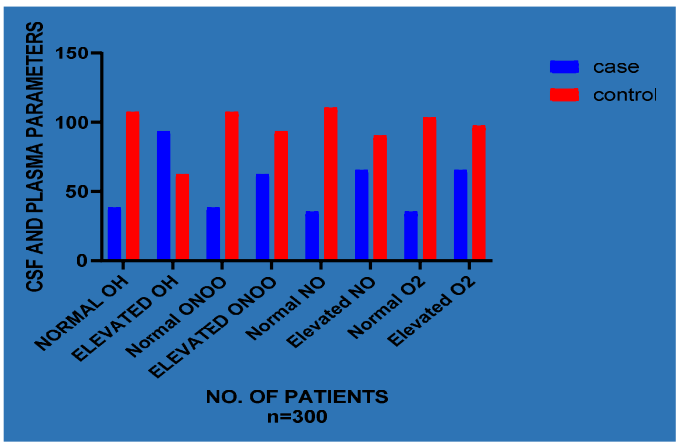

Figure 1: *Compares the different Levels of Oxides in Plasma and CSF between the Control Group and the Case Group*

Discussion

Among people over the age of 65, Alzheimer's disease is one of the most prevalent diseases associated with dementia. It causes neurodegeneration of neuronal cells in the brain by amassing beta-amyloid aggregates and tau protein tangles.16 The main contributory factor invol-ved in many brain neurodegenerative disorders is oxi-dative stress. It happens when a variety of superoxide or oxide species, such as OH, ONOO, O2, and NO, start to build up inside the neuronal cells of the brain. Many studies have shown that mitochondrial malfunction, inflammation, or elevated metal levels that interfere with electron transport chain activity cause their levels to become abnormally high, causing neurodegenera-tion.17 Our study results concur with the findings of Andra et al. that mitochondrial damage leads to the accumulation of amyloid bodies in neurons by elevating the levels of oxides, which increases the risk of develo-ping alzehmeirs disease in the elderly population.18 The study results of Elena Temango et al. also show that people with elevated levels of free radicals in their neu-ronal cells have a higher risk of developing alzehmeirs disease than a normal population.19 Carmen Peña-Bautista et al.'s study supports our findings, which indicate that Alzheimer patients' plasma and CSF had higher levels of oxides—such as OH and ONOO—than those of the normal control group. This indicates a strong correlation between oxidative stress and Alzheimer's disease, with an Odd ratio of more than 1 to 2.2.20

Alzheimer's disease is currently treated with many approved medications, such as memantine, rhivastig-mine, galantamine, and donepezil, that inhibit an enzyme called acetylcholinesterase, which increases the levels of acetylcholine in the brain and improves cognitive functions. Another therapy called monoclonal antibodies includes Aducanumab, which directly removes excess amyloid beta from the brain and lessens the buildup of neuritic plaques in the brain. Recent advancements in the field of nanotechnology also help to treat Alzheimer's disease to a great extent by directly delivering the anti-oxidants to the brain and lessening the development and complications of Alzheimer's disease.21-22-23

The present discourse posits that oxidative stress is a critical factor in the pathogenesis of Alzheimer's disease, and that the mitigation of this illness is contingent upon the amounts of oxides present in neural cells.

Table 1: *Association of serum / CSF levels of Oxides in normal versus Alzehmeirs patients.*

Biomar kers	Control with normal levels of oxides	Control with elevated levels of oxides	Case with normal levels of oxides	Case with elevated levels of oxides	Total	P value	ODD Ratio	95%CI	Sensitivity /specifity	Like hood ratio
NO	110	90	35	65	300	0.001	2.2	1.1 to 1.5	0.5/0.6	1.5
ONOO	107	93	38	62	300	0.01	1.877	1.04 to 1.45	0.5/0.6	1.408
O2	103	97	37	62	300	0.007	1.247	1.06/1.46	0.5/0.6	1.471
OH	105	95	40	60	300	0.04	1.65	1.00 to 1.39	0.5/0.6	1.313

Conclusion

This study underscores the significant implications of oxidative stress in the progression of Alzheimer's disease.

Conflict of Interest: *None*

Funding Source: *None*

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Authors Contribution

SFU: Conceptualization of Project
SS: Data Collection
HRC: Literature Search
KW: Statistical Analysis
AHS: Drafting, Revision
SB: Writing of Manuscript

Comparative Cardioprotection Analysis Of Phoenix Dactilyfera. Ajwa Dates (an Ace Inhibitor) with Captopril , In Clozapine Generated Cardiotoxic Rat Model

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Abstract

Objective: To compare the cardioprotective effects of Ajwa dates fruit and pit extracts with Captopril in Clozapine induced cardiotoxic rats.

Material and Methods: Place and duration of study at Postgraduate Medical Institute Lahore for 21 days. In an experimental study, forty-eight rats were randomly divided into six groups of eight rats per group. Group 1 was healthy Control group. Myocarditis was induced in all other groups by Clozapine injection. Group 2 was diseased control group while groups 3 – 6 were given Captopril and Ajwa fruit and pit extracts respectively. After 21 days, blood samples were collected from heart and serum was used for further analysis. Tissue samples were collected by homogenizing hearts in phosphate buffer and supernatant used for biochemical testing. Oxidative stress parameters, CK-MB, GSH, GSH-Px, LDH and MDA were studied in all groups.

Results: LDH and CK-MB values of group 6, having captopril 5mg and ajwa fruit and pit both extract, was closest to the control group. Similarly GSH, GSH-Px and MDA values in group 6 were closest to the control group. P-Value came out to be significant for both comparisons hence ensuring the significance of the results.

Conclusion: Captopril and Ajwa dates have strong cardioprotective and antioxidant effects. Patients who are experiencing adverse effects, may benefit from the combination of Ajwa dates and low-dose captopril.

Keywords: LDH (Lactate dehydrogenase), CK-MB (Creatinine kinase), GSH,GSH-Px (Glutathione peroxidase), MDA (Malondialdehyde)

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Introduction

Clozapine has been extensively employed as a pharmacotherapeutic agent for managing psychosis over several decades. Its noteworthy efficacy, particularly in addressing refractory cases of schizophrenia, has been a subject of considerable recognition. A study has revealed its superior effectiveness compared to alternative antipsychotic medications in the context of treating resistant forms of schizophrenia.[1] While being a globally utilized pharmaceutical, it is imperative to acknowledge that clozapine is not without significant adverse effects. Agranulocytosis stands out as a prevalent adverse reaction associated with the drug, and additionally, myocarditis has been identified as a serious and noteworthy adverse effect in certain patient populations. These adverse events necessitate careful consideration and monitoring in clinical practice.[2]

In a recent research study, findings indicated a notable occurrence of cardiomyopathy, with an incidence rate ranging from 3-4%. These observations were specifi-

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cally documented in a middle-aged female patient who was hospitalized for the management of myocarditis, an adverse event that manifested during the course of her clozapine treatment.[3]

Ajwa dates are renowned for their nutritional prowess. Revered for their myriad health benefits, these dates offer a multitude of advantages for human well-being on a significant scale.[4] Ajwa dates exert positive influences on hormone levels, red blood cells (RBCs), platelets, and hemoglobin (Hb). Functioning as antioxidants, they play a role in antihypertensive, anti-inflammatory, and anticancer activities. Moreover, they contribute to the prevention of allergies, diabetes, diarrheal diseases, and exhibit antibacterial properties, serving as effective agents against cancer.[5] Also at cellular and chemical level, Ajwa dates has been considered very beneficial in many medical conditions.[6] In accordance with research findings, Ajwa dates have demonstrated efficacy in lowering cholesterol levels, managing diabetes, exhibiting anticancer properties as previously discussed, and contributing to the control of cardiovascular diseases.[7] Extensive examination and practical observation have revealed that date seeds possess numerous beneficial properties. Specifically, on a cellular level, these seeds augment antioxidant activity by enhancing the functionality of relevant enzymes.[8] These attributes serve as a safeguard against the oxidative stress associated with myocardial injury. Additionally, a study involving pediatric cancer patients has substantiated that the consumption of Ajwa dates enhances their treatment efficacy and overall outcomes.[9] Furthermore, it is well-established that captopril is an extensively utilized medication for hypertension management, effectively alleviating the strain on cardiac tissue. This pharmaceutical agent belongs to the angiotensin-converting enzyme (ACE) inhibitors, a prominent class of antihypertensive drugs.[10]

Moreover, findings from a study conducted on diabetic rats demonstrated the drug's efficacy in efficiently reducing the apoptosis of myocardial cells induced by diabetes. The medication enhances the function of the renin-angiotensin system, thereby effectively improving ventricular function. The consequential impact of this drug holds a noteworthy position in the therapeutic approach to managing myocardial workload.[11] Despite the extensive positive effects of captopril in addressing cardiac issues, it is essential to acknowledge the presence of a range of adverse effects that impact various aspects of human health. Certain studies have indicated that it may contribute to fibrosis in the heart and lung tissues following a myocardial infarction.[12]

Several well-known adverse effects associated with captopril and related drugs include: paroxysmal cough occurring in approximately 10% of patients, proteinuria in almost 1% of patients, inadequate renal function in nearly 0.2% of patients, neutropenia/agranulocytosis, pruritic rash in about 0.7% of patients, altered taste or loss of taste in approximately 2-4% of patients, and, importantly, anaphylactic and allergic reactions observed in a majority of patients.[(13)] The objective of our study is to to explore the protective effects of various extracts of Phoenix dactylifera (AJWA DATES) on Clozapine's cardiotoxicities in rats.

Material and Method s

An experimental case control study. Place of study was Postgraduate Medical Institute Lahore. After taking the IRB approval No. F-39/NHU/Admin/IRB dated 06-11-2014. Sample size was 48 rats. Study duration was 21 days and Study subjects Forty eight rats were randomly divided into six groups with eight rats in each group. Sample size was Forty eight and Data Collection procedure. Forty eight rats were randomly divided into six groups with eight rats in each group. Group 1 was healthy Control group. Myocarditis was induced in all other groups by Clozapine injection intraperitoneally. Group 2 was diseased control group while groups 3 – 6 were given Captopril and Ajwa fruit and pits extracts respectively. After 21 days, blood samples were collected from heart and serum was used for further analysis. Tissue samples were collected by homogenizing hearts in phosphate buffer and supernatant used for biochemical testing. Myocardial damage was determined by Oxidative stress parameters LDH, CK-MB, MDA, GSH, GSH-Px were studied in all groups.

Table 1: *A tabular description of various animal groups and the diet/ treatment they were given.*

Groups	Diet/ treatment given
Group 1(healthy control)	Standard Laboratory diet
Group2(diseased control)	Clozapine Only
Group3	CLZ & CAP 5mg
Group4	CLZ & CAP 10mg
Group5	CLZ & Aq. ADFE + Aq. ADPE
Group6	CLZ & CAP 5mg & Aq. ADFE + Aq. ADPE

Mean	Control	CLZ	CLZ & CAP 5mg	CLZ & CAP 10mg	CLZ & aq. ADFE + aq. ADPE	CLZ & CAP 5mg & aq. ADFE + aq. ADPE	p-value
LDH(IU/L)	205.63±11.08	312.63±10.54	215.38±17.31	214.13±9.95	211.00±9.62	212.00±10.32	<0.001
	196.36-214.89	303.81-321.44	200.90-229.85	205.81-222.44	202.96-219.04	203.37-220.63	
CK-MB(IU/L)	240.25±10.24	332.13±3.76	252.75±8.07	206.50±7.46	242.38±16.77	252.25±7.96	<0.001
	231.69-248.81	328.98-335.27	246.01-259.49	200.26-212.74	228.36 -256.39	245.60-258.90	
MDA(µmol/g protein)	321.13±5.92	433.88±14.96	324.88±7.14	321.13±5.92	324.88±7.14	322.88±6.85	<0.001
	316.18-326.07	421.37-446.38	318.91-330.84	316.18-326.07	318.91-330.84	317.14-328.61	
GSH value (nmol/g protein)	34.75±1.67	17.63±1.19	31.13±1.13	34.25±1.83	31.50±1.51	33.75±1.49	<0.001
	33.35-36.15	16.63-18.62	30.18-32.07	32.72-35.78	30.24-32.76	32.51-34.99	
GSH-Px(IU/g protein)	27.50±1.20	16.13±1.73	24.88±2.90	26.00±3.02	26.00±2.62	26.25±2.61	<0.001
	26.50-28.50	14.68-17.57	22.45-27.30	23.47-28.53	23.81-28.19	24.07-28.43	

Data was analysed by SPSS Version 20.0. Comparison was performed between various groups using Tukey's test. P-value of 0.05 was kept as reference and the results were compared to check for the significance of the outcome.

Results

The study was conducted on 48 rats divided into 6 groups (8 rats/group). Group 1 was the healthy control group. Group 2 was diseased control group, it was given CLZ only. Group 3 and 4 were given CLZ and Captopril 5 and 10 mg respectively. Group 5 was given CLZ and Aq. ADFE & Aq. ADPE. While Group 6 was given CLZ and Captopril 5mg plus Aq. ADFE and Aq. ADPE.

The overall difference among groups was found significant with $p<0.001$. In group-wise comparison, it was noted that group 2 had significantly higher LDH levels as compared to all other groups with $p<0.05$. All groups other than group 2 had significantly lower levels as compared to other groups. The group 3, 4, 5 and 6 had no significant difference from each other. For CK-MB, the overall difference was found significant with p-value <0.001. In group-wise comparison, it was observed that the group 1 had significantly lower CK-MB levels. The group 3 had insignificant difference with group 4, while 2, 5 and 6 had significantly higher levels of CK-MB as compared to group 4. Group 3 had no significant difference from group 5 and 6 but had significantly higher levels as compared to group 1. The group 5 and 6 had no difference from each other.

The mean MDA levels were noted and found highest were also noted and found to be and highest for CLZ injected group 2.433.88umol/g protein (p-value <0.001). The values of groups 3 and 5 were comparable to each other and closer to control group (324.88 umol/g). The value of group 4 was the closest to control group (321.13 umol/g). Value of group 6 was found closer to group 1 (322.88 umol/g). The GSH value was in reverse order in comparison to previous variables and mean value was highest for group 1 and lowest for group 2, and were recorded to be 17.6±1.2 and 34.8±1.7 respectively. Again group 4 had relatively closer mean values to group 1. Group 3 had significantly lower levels than group 4. After overall significance the group wise comparison revealed that group 4 had no significant difference from group 1 while all other groups had significantly lower values as compared to group 1.

The GSH-Px levels had almost similar results as for GSH. The table and figure below show the mean levels of GSH-Px levels for all groups. The overall difference was significant and in group-wise comparison. The group 1 had significantly higher values than all. Group 2 had significantly low levels, while the group 3-6 had no significant difference from group 1. The mean values of biochemical parameters of aforementioned groups (Table-1) are illustrated by bar charts. The overall difference among groups was found significant with $p < 0.001$. After 21 days of injections of Clozapine, group 2

(I) Group	(J) Group	LDH (IU/L)	CK-MB (IU/L)	MDA (µmol/g protein)	GSH value (nmol/g protein)	GSH value (nmol/g protein)
Control	CLZ	0.000	0.000	0.000	0.000	0.000
	CLZ & CAP 5mg	0.567	0.138	0.951	0.000	0.280
	CLZ & CAP 10mg	0.701	0.000	1.000	0.984	0.819
	CLZ & aq. ADFE + aq. ADPE	0.941	0.998	0.951	0.001	0.819
	CLZ & CAP 5mg & aq. ADFE + aq. ADPE	0.885	0.169	0.998	0.760	0.906
CLZ	CLZ & CAP 5mg	0.000	0.000	0.000	0.000	0.000
	CLZ & CAP 10mg	0.000	0.000	0.000	0.000	0.000
	CLZ & aq. ADFE + aq. ADPE	0.000	0.000	0.000	0.000	0.000
	CLZ & CAP 5mg & aq. ADFE + aq. ADPE	0.000	0.000	0.000	0.000	0.000
CLZ & CAP 5mg	CLZ & CAP 10mg	1.000	0.000	0.951	0.002	0.938
	CLZ & aq. ADFE + aq. ADPE	0.975	0.306	1.000	0.996	0.938
	CLZ & CAP 5mg & aq. ADFE + aq. ADPE	0.992	1.000	0.997	0.012	0.867
CLZ & CAP 10mg	CLZ & aq. ADFE + aq. ADPE	0.995	0.000	0.951	0.008	1.000
	CLZ & CAP 5mg & aq. ADFE + aq. ADPE	0.999	0.000	0.998	0.984	1.000
CLZ & aq. ADFE + aq. ADPE	CLZ & CAP 5mg & aq. ADFE + aq. ADPE	1.000	0.359	0.997	0.046	1.000

(Diseased control) had highest levels of LDH and CK-MB isoenzymes, 312.6±10.5 and (332.13IU/L) respectively as compared to Groups 1,3,4, 5, 6 having (P-value < 0.001). *Tukey's test*

Discussion

Comparative cardio-protection analysis of Phoenix dactylifera (Ajwa dates), often hailed for its potential cardiovascular benefits, against captopril, a well-known ACE inhibitor, in a clozapine-generated cardiotoxic rat model presents an intriguing avenue for exploration.[14]

In a clozapine-induced cardiotoxic rat model, the table displays mean values and confidence intervals for a range of biochemical markers suggestive of heart injury and oxidative stress across treatment groups. Treatment options include combination therapies, clozapine (CLZ), clozapine with aqueous extract of Ajwa dates (ADFE) and Ajwa date pits (ADPE), clozapine with captopril (CLZ & CAP), and control. P-values show the statistical significance of differences between groups. Particularly in cardiac tissue, LDH is a sign of cellular injury. The increased LDH levels in the CLZ group as opposed to the control group point to myocardial damage brought on by clozapine. LDH levels were considerably lower in the captopril-treated group than in the CLZ group after both 5 mg and 10 mg of captopril, as well as ADFE and ADPE, suggesting possible cardioprotective effects. LDH levels were significantly reduced by the combined therapy as well, indicating possible synergistic effects.

Phoenix dactylifera, particularly Ajwa dates, is rich in bioactive compounds like polyphenols, flavonoids, and antioxidants, which have demonstrated potential in promoting cardiovascular health. These compounds exhibit anti-inflammatory, antioxidant, and vasodilatory properties, which could mitigate the adverse effects of cardiotoxicity induced by clozapine.[15]

An enzyme called CK-MB, which is frequently employed as a diagnostic for myocardial infarction, is released from injured myocardial cells. As with LDH, increased CK-MB levels in the CLZ group indicate clozapine-induced myocardial injury. When compared to CLZ alone, treatment with captopril—especially at a dose of 10 mg—as well as ADFE and ADPE dramatically decreased CK-MB levels, suggesting cardioprotective benefits. The combo therapy significantly decreased the levels of CK-MB as well.

On the other hand, captopril, a conventional ACE inhibitor, is widely prescribed for managing hypertension and heart failure. Its mechanism of action involves inhibiting the angiotensin-converting enzyme, thereby reducing the production of angiotensin II and promoting vasodilation, ultimately alleviating cardiac workload and improving cardiac function.[16] Lipid peroxidation

and oxidative stress are indicated by MDA. The clozapine toxicity-induced increase in oxidative stress in cardiac tissue is indicated by the considerably higher MDA levels in the CLZ group as compared to the control. MDA levels were significantly reduced after treatment with captopril, ADFE, and ADPE, indicating potential antioxidant effects. MDA levels were significantly reduced by the combined therapy as well, suggesting possible synergistic or additive antioxidant effects.

While GSH-Px is an enzyme that catalyzes the reduction of hydrogen peroxide and organic hydroperoxides by GSH, GSH itself is a significant antioxidant. The CLZ group's significantly lower GSH and GSH-Px levels in comparison to the control group suggest compromised antioxidant defense systems in the context of clozapine-induced cardiotoxicity. GSH and GSH-Px levels were significantly elevated after treatment with captopril, ADFE, and ADPE, indicating a possible restoration of antioxidant ability. GSH and GSH-Px levels were also significantly elevated by the combo therapy. In a clozapine-generated cardiotoxic rat model, where clozapine-induced myocardial damage is mimicked, evaluating the efficacy of Ajwa dates versus captopril offers valuable insights into their comparative cardioprotective effects. Parameters such as cardiac biomarkers (troponins, creatine kinase), histopathological changes in cardiac tissue, oxidative stress markers, and cardiac function assessments (echocardiography, ECG) can be monitored to gauge the extent of cardio-protection provided by each intervention.[17]

Ajwa dates, with their rich array of bioactive compounds, may exert cardioprotective effects by scavenging free radicals, reducing oxidative stress, modulating inflammatory pathways, and enhancing endothelial function. These mechanisms could potentially mitigate clozapine-induced cardiotoxicity by attenuating oxidative damage, inflammation, and apoptotic pathways in cardiac tissue.[18] Overall, the comparative cardio-protection analysis of Ajwa dates and captopril in a clozapine-generated cardiotoxic rat model holds promise for uncovering novel therapeutic interventions for managing drug-induced cardiovascular complications, while also shedding light on the mechanisms underlying the cardioprotective effects of natural dietary constituents versus conventional pharmacotherapy.[19]

Conclusion

The results of this comparative study indicate that in a rat model of cardiotoxicity induced by clozapine, captopril and Ajwa dates, especially in the form of aqueous extracts, have strong cardioprotective and antioxidant effects. Additionally, combination therapy including Ajwa dates and captopril shows synergistic benefits that may improve antioxidant activity and cardio-protection. These findings encourage future research into Ajwa dates as a natural adjunct therapy for the treatment of drug-induced cardiotoxicity, possibly providing a more secure and comprehensive method of managing cardiovascular health. Patients whose tolerance to larger doses of captopril is being limited by adverse effects may benefit from the combination of Ajwa dates and low-dose captopril.

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Authors Contribution

FK: Conceptualization of Project
MFA: Data Collection
QTA: Literature Search
WA: Statistical Analysis
II: Drafting, Revision
SSA: Writing of Manuscript

Frequency of Idiopathic Membranous Nephropathy (IMN) in Different Age Groups in Nephrology Department of Private Hospital

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Abstract

Objective: To see the age distribution of patients of idiopathic membranous nephropathy in general population of Pakistan and prevalence of PLA2R positivity in the cases of idiopathic membranous nephropathy in our population.

Material and Methods: The research was done at the Department of Morbid Anatomy and Histopathology; Post Graduate Medical Institution and division of Nephrology, Fatima Memorial Hospital, Shadman Lahore and division of Nephrology, Fatima Memorial Hospital, Shadman Lahore. The duration of study was from September 2018 to December 2019. It was a descriptive cross-sectional study. A total of 83 patients diagnosed with idiopathic membranous nephropathy (iMN) based on clinical history and light microscopy were included in our study.

Results: The mean age was 36.91 ±12.97years. The majority of the patients 54.09% (45) were from age 21 to 40 years.

Conclusion: The iMN is commonly seen in 35 years to 45 years of age group

Keywords: idiopathic membranous nephropathy, glomerulonephritis, nephrotic syndrome

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Introduction

Membranous nephropathy (MN) is one of the main reasons of nephrotic syndrome occurs in adult population in whole world.[1] One-third of patients lose progress to end-stage disease of kidney.[2] The severity of kidney disease is directly related to the degree of proteinuria.[3,4] Studies in Pakistan indicate that membranous nephropathy is the second most common reason for nephrotic syndrome. In one local study MN was 23.50%,[5] and a study conducted in the UK, revealed a prevalence of 26.9%.[6] The term MN indicates early histologic changes recorded in light microscopy: Subepithelial deposition of immune complex at the glomerular basement membrane leading to decreased or less cellular penetration.[7] It has two variants on the bases of its etiology. These types are primary or idiopathic Membranous nephropathy(iMN) and secondary (sMN).[8] The latter develops due to a variety of medical conditions, including autoimmune system disease, infections, neoplasia, and drugs.[9] Our study was done to observe the age distribution of iMN in our series of patients diagnosed with MGN.

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Material and Methods

It was a cross-sectional descriptive study. The research was done at the Department of Morbid Anatomy and Histopathology, Post Graduate Medical Institution and division of Nephrology, Fatima Memorial Hospital, Shadman Lahore and division of Nephrology, Fatima Memorial Hospital, Shadman Lahore. After the taken

approval from Ethical Committee No 8229-31 dated 16-06-2017. The duration of study was from September 2018 to December 2019. It used a non-probability convenience sampling technique. A total of n = 83 biopsies/ paraffin-embedded blocks of patients with membranous glomerulopathy were inclu-ded. The sample size was calculated by following for-mula keeping confidence level equal to 95% and margin of error equal to 10%.

$$n = \frac{Z^2_{1-a/2}\, P(1-P)}{d^2}$$

- $Z^2_{1-a/2}$ = for 95% confidence level = 1.96
- α = level of significance = 5%
- P = Prevalence of membranous glomerulopathy in population
- d = Margin of error = 10%
- n = Sample Size = 83

Results

Renal biopsy tissues from 83 patients of iMN, were included in the study. The age group ranged was from 14 to 80 years, The mean age was 36.91 ±12.97years (Table: 1). The majority of the patients 54.09% (45) were from age 21 to 40 years (Table:2). In the age group >50 we had 14 patients (16.8%).

Table 1: *Age distribution in cases of Idiopathic Membranous Nephropathy*

Age group	Frequency (n)	Percentage (%)
<20	06	7.2
21-30	25	30
31-40	20	24.09
41-50	18	21.6
51-60	12	14.4
>60	02	2.4
Total	83	100

**Mean age 36.97+-12.97 years*

Discussion

iMN mainly affects the people of the young age group. The patients included in our study were more than 13 years of age with the mean age of 36.91 years ± 12.95 (median 35, mode 35). Various studies have been done from different centers around the world that showed the correlation of age in the development of iMN. The study from University of Chicago tried to see the pattern of development of iMN and they concluded that iMN is present frequently in people with the age more than 40 years. They also highlighted that iMN prevalence in children is less, if it is found in patients less than 13 years of age, then mostly hepatitis B virus infection is the underlying cause, or they may have hypersensitivity to thyroid gland.[10] The study from Manchester, United Kingdom, mean age of the development of iMN was in the 4th to 5th decade.[9] Another study which was also done in Manchester, UK, showed mean age 52.6 ± 13.6.[11] Similar study from China revealed that the mean age of the patients of iMN was 40.8 ± 14.6 year.[12] The data from a tertiary care unit of India revealed that patients of iMN had mean age of 41.5 years while in the comparison of the study done by Lasern ,in which mean age of iMN patient was 57.5 years.[13] Mean age in our patietns with iMN was 36.91 years ± 12.95 (median 35, mode 35), close to Asian and Chinese patients and clearly different tan the western population. A different genetic makeup in our population or environmental factors especially pollution may be the responsible factor for the early expression of iMN. The genetic makeup and environmental factors in our country are comparable with that of China and India. A study conducted in China concluded that the environmental factor is one of the main reasons for early development of kidney disease. Particulate matter, which is the unit of measurement of pollution in atmosphere, is high in China and of Particulate matter resembles the calculation of Particulate matter of of Japan and India.[14] This study also quoted the values of particulate matter in UK and USA which is very less than that which occurs in China, japan and India.[15] This study gave the concept that cytokines which are released by the irritation of the respiratory epithelium due to environmental pollution, cause development of iMN.[16,17]

Conclusion

Majority of patients suffering from iMN in Pakistan are between the ages of 35-45 years of age.

Conflict of Interest: *None*

Funding Source: *None*

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Authors Contribution

AJ: Conceptualization of Project
SZ: Data Collection
MMR: Literature Search
MF: Statistical Analysis
UI: Drafting, Revision
IH: Writing of Manuscript

Exploring the Association Between Body Mass Index (BMI) and Fasting Blood Sugar: Implications for Early Intervention

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Abstract

Objective: This study aims to investigate the correlation between body mass index (BMI) and fasting blood sugar (FBS) levels among individuals in Lahore, Pakistan.

Material and Methods: This prospective research study was carried out from May to June 2023, involving 150 participants aged 21 to 60. Height, weight, and fasting plasma glucose levels were recorded, and BMI was determined using the formula: weight (kg) / (height (m) X height (m)). The relationship between BMI and FBS was explored using statistical methods.

Results: Participants with a BMI over 25 showed a higher prevalence of FBS over 100 mg/dl compared to those with a BMI under 25. The study demonstrated a clear correlation between BMI and FBS levels ($p < 0.05$). Among individuals with a BMI under 25, 60 out of 75 had FBS levels below 100 mg/dl, highlighting the potential impact of a lower BMI on optimal blood sugar levels.

Conclusion: This study confirms a significant association between BMI and fasting blood sugar levels, highlighting the importance of weight management for optimal health and blood sugar regulation. Regular monitoring of BMI and FBS levels is essential for identifying potential risk factors and implementing preventive measures.

Keywords: BMI, FBS, Type 2 Diabetics Mellitus, Obesity, Pakistan

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Introduction

Understanding the correlation between body mass index and fasting levels of blood sugar is crucial for assessing an individual's health and identifying potential health risks. By examining the relationship between these two indicators, we can gain valuable insights into the overall well-being and potential health conditions of an individual.[1] Numerous investigations have been carried out to find the connection between fasting blood sugar levels and body mass index.[1,2]

The body mass index (BMI) and fasting blood sugar (FBS) are two important health indicators that can provide valuable information about a person's overall health and potential risk for different health conditions. Basal metabolic rate (BMI), which divides an individual's weight in kilos by the square of their height in meters, is used to classify people into various weight categories. Studies have indicated a relationship between fasting blood sugar levels and body mass index.[3,4] It has been observed that individuals with higher BMI tend to have higher fasting blood sugar levels.[5] This relationship implies that being overweight, especially in the form of adipose tissue, may increase the risk of developing insulin resistance and having poor glucose metabolism, which raises blood sugar levels.[6] To completely comprehend the connection between BMI and fasting blood glucose (FBG) level more investigations are required.[5] Nonetheless, many studies have shown that obesity,

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which is frequently manifested by a high BMI, can raise the chance of insulin resistance and type 2 diabetes.[7] The finding that obese patients' insulin receptor numbers can return to normal if their insulin levels are lowered implies that insulin resistance plays a secondary role in the alterations in insulin receptor numbers rather than being the fundamental cause.[5] Individuals with higher BMI may have decreased insulin sensitivity, leading to elevated fasting blood sugar levels. In addition to the correlation between BMI and FBS levels, research has also highlighted the role of abdominal obesity in influencing blood glucose regulation.[7] Maintaining ideal blood sugar management requires maintaining a healthy body weight through a balanced diet and consistent exercise. Individuals with high BMI should focus on adopting modified lifestyle habits, including a balanced and nutritious diet, regular exercise, and weight management plans.[8] By managing body weight and reducing excess adipose tissue, individuals can potentially improve insulin sensitivity and blood sugar regulation[9]. Type 2 diabetes often develops gradually in obese adults due to poor glucose metabolism and insulin resistance. because of a confluence of lifestyle factors, including inactivity poor diet, and genetics. FBS less than 100 mg/dl is regarded as normal, while FBS between 100 and 125 mg/dl and Hb A1C 5.7%–6.4% are classified as impaired.[6] Fasting glucose during which the body becomes less responsive to insulin, leading to elevated blood sugar levels.[10] and the link between obesity and impaired glucose metabolism is well-established. Obesity, particularly abdominal obesity, contributes to an increase in insulin resistance and decreased insulin sensitivity, resulting in high fasting blood sugar levels and an increased risk of developing type 2 diabetes.[11] Obesity has a great impact on glycemic control and overall metabolic health. Adipose tissue, especially visceral fat, secretes many pro-inflammatory cytokines and adipokines, which contribute to chronic low-grade inflammation and insulin resistance.[12] This, in turn, disrupts glucose homeostasis and leads to elevated fasting blood sugar levels. Studies have also shown that dyslipidemia "a condition marked by increased triglycerides and lower levels of high-density lipoprotein cholesterol" is frequently present in obese persons. These lipid abnormalities further increase insulin resistance and the potential for the development of hyperglycemia.[13]

The American Diabetes Association stated that obesity, especially abdominal obesity as demonstrated by a high BMI and waist circumference, is one of the risk factors for diabetes. Significant risk factors for the development of type 2 diabetes also include poor glucose metabolism, insulin resistance, and decreased insulin sensitivity due to increased body weight.[14,15]

Individuals with higher BMI need to prioritize weight management through the adaptation of a healthy lifestyle, including a complete and balanced diet and regular exercise. They may be able to lower their chance of getting diabetes, control blood sugar levels, and enhance their sensitivity to insulin by doing this.[16] Regular monitoring of BMI and fasting blood sugar levels can help in identifying potential risk factors early and taking proactive steps to maintain optimal health.

It's crucial to remember that the correlation between BMI and FBG levels underscores the significance of maintaining a healthy BMI for optimal blood sugar regulation.[11] By making informed decisions and taking necessary measures, individuals can work towards improving their overall well-being and reducing their chance of developing type 2 diabetes.

Material and Methods

This prospective study was carried out from May to June 2023 at different medical college Hospitals in Lahore, Pakistan. Regularly visiting healthy patients of the hospital for check-ups were selected to be a part of this study with authorization from the Institutional Ethics Committee No 684/ERC/CMH/LMC dated 27-05-2023. Psychiatric, diabetic, pediatric, and pregnant participants. Those receiving treatment for cardiovascular disorders and diabetes mellitus were not included in the study group. There were one hundred and fifty people in the study group, ages 21 to 60. The subjects' height, weight, sex, and age were noted after they gave their informed consent. 50 guys in the study group had a BMI of 25–29 kg/m^2, while the control group had a BMI of 18–24 kg/m^2. Both height and weight were reported to be the closest 0.5 cm and 0.5 kg, respectively. Fasting (8–12 hours, followed by an overnight fast).

Venipunctures of the median cubital vein were used to obtain venous blood samples, which were then centrifuged to separate the plasma. The Glucose Oxidase technique was used to estimate the fasting plasma glucose levels using an ERBA-Transasia fully automated analyzer[17]. Body mass index can serve as a predictor of fasting blood sugar levels. Higher BMI values are

associated with an increased risk of elevated fasting blood sugar levels.

BMI was determined by using the following formula:

BMI = weight (kg) / (height (m) X height (m))

Once we have calculated BMI, we can interpret the results using the following categories:

- Underweight: BMI less than 18.5
- Normal weight: BMI 18.5–24.9
- Overweight: BMI 25–29.9
- Obesity: BMI 30 or higher

It is a statistical measurement of an individual's weight scaled by height. It serves as a straightforward method of dividing sedentary people with average body composition into groups based on the percentage of body fat they contain.

Results

The study had 150 participants who met the inclusion and exclusion criteria were included in the study. They appeared to be in good health. forty of the individuals were not allowed to participate in the trial because their fasting blood glucose levels fell within the range of diabetes. Out of the total 150 members, 81 (54%) were women and 69 (46%) were men. Participants in the study ranged in age from twenty-one to sixty years. The average age of the males and females was 37.98 ± 10.51 and 36.4 ± 10.11 years, respectively.

The T-test is done in pairs. Figer 2 indicates that 40/50 participants with a BMI less than 25 kg/m2 had an FBS of less than 100 mg/dl. 38/50 Subjects with a BMI greater than 25 kg/m^2 had an FBS greater than 100 mg/dl. (Figer-3) There is a significant p-value. The study demonstrates a clear correlation between BMI and FBS.

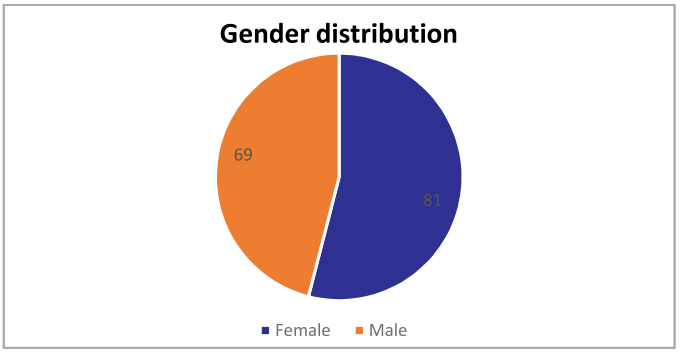

Figure 1: *Gender distribution of the study population*

Table 1: *Age-specific FBS and BMI*

Age group	Mean BMI	Mean FBS (mg/dl)
21-30 years	20.25	78.37
31-40 years	24.57	92.05
41-50 years	24.22	93.02
51-60 years	27.15	89.73

Table 2: *Comparing of BMI and FBS*

BMI Kg/m^2	FBS<100 mg/dl	FBS>100 mg/dl	Total
BMI<25	60	15	75
BMI>25	18	57	75
Total	78	72	150

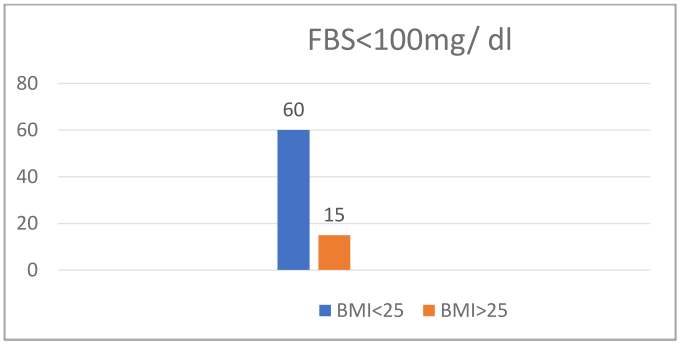

Figure-2: *BMI versus FBS*

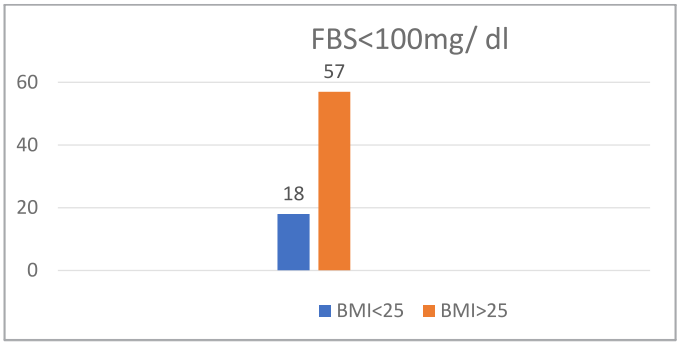

Figure-3: *BMI versus FBS*

Discussion

After recognizing the substantial impact of BMI on fasting blood sugar levels, it is crucial to explore the specific relationship between BMI categories and fasting blood sugar levels 2. According to the data provided, there is a clear connection between BMI and fasting blood sugar levels. The data indicates that individuals with a BMI over 25 have a higher prevalence of FBS over 100 mg/dl compared to those with a BMI under 25. The data implies that maintaining a healthy BMI under 25 could contribute to better blood sugar management. 60 out of 75 individuals with a BMI under 25 have FBS levels below 100 mg/dl, highlighting the potential

impact of a lower BMI on optimal blood sugar levels. Individuals who are overweight or obese are categorized at a higher risk for elevated fasting blood sugar levels, indicating impaired glycemic control.[14] Regular monitoring of BMI and fasting blood sugar levels is crucial for identifying potential risk factors and taking necessary steps to maintain optimal health.[18] Additionally, the relationship between BMI and fasting blood sugar levels highlights the importance of weight management and maintaining a healthy BMI to improve glycemic control. In conclusion, there is a significant association in BMI and fasting levels of blood sugar.[15]

Pregnancy-related high blood glucose levels have been regularly linked to BMI, suggesting a connection between BMI and fasting blood sugar.[18] In this study, we find, a positive correlation was found between BMI and fasting blood glucose levels 1. A different longitudinal study discovered a substantial correlation between early adulthood BMI trajectories and diabetes later in life, indicating that young adulthood is a critical time for the onset of diabetes.[12] An additional cross-sectional study conducted on adult Chinese participants revealed a correlation between diabetes and impaired glucose metabolism and BMI and waist circumference, with central obesity being more significant[2]. Additionally, a descriptive study found that fasting serum glucose was significantly increased in obese individuals, indicating a potential risk for cardiovascular and metabolic diseases[13]. These findings suggest that there is an association between BMI and FBS, highlighting the importance of early intervention and prevention strategies for individuals with high BMI to control glucose levels and lower the risk of developing diabetes.

Conversely, some participants with a BMI over 25, 57 out of 75 have FBS levels above 100 mg/dl. This demonstrates a higher prevalence of elevated fasting blood sugar levels in this BMI category. These findings emphasize the significance of weight management and sustaining a healthy BMI for better blood sugar control. Individuals with a BMI over 25 may benefit from lifestyle adjustments aimed at reducing excess body weight to enhance glycemic control and decrease the risk of developing conditions such as type 2 diabetes.[19]

Current research suggests adipose tissue and monocytes release the adipokine resistin. It gets its name from its capacity to obstruct or hinder the action of insulin. It was suggested as a connection between diabetes and obesity. Obese individuals' adipose tissue secretes more pro-inflammatory cytokines, glycerol, hormones, non-esterified fatty acids, and other chemicals that aid in the emergence of resistance to insulin.[11]

The purpose of our research is to confirm that elevated body weight and fasting levels of blood sugar are positively correlated. In the BMI <25 control group, most individuals displayed less than 100 mg/dl of fasting blood sugar (Graph 1). Most participants in the study group with BMIs had FBS levels above 100 mg/dl (Graph 2). Since patients with diabetes mellitus and cardiovascular disorders were not included in our study, other factors, such as the impact of stress in everyday life, should be taken into account as potential causes of the elevated FBS that results in prediabetes. It is well known that stress hormones raise blood sugar levels. Stress, both mental and physical, raises these hormones, which raises blood sugar levels.[12]

Conclusion

The correlation between BMI and fasting blood sugar levels is substantial and underscores the importance of individuals being mindful of their BMI and taking proactive measures to attain and sustain a healthy weight for optimal health and blood sugar regulation. Regular physical activity is also crucial for enhancing insulin sensitivity and glucose uptake, thereby assisting in combating insulin resistance, which can lead to long-term health complications.[7]

Conflict of interests: *None*

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Authors Contribution

HNL: Conceptualization of Project
AF: Data Collection
SI: Literature Search
MI: Statistical Analysis
RA: Drafting, Revision
MR: Writing of Manuscript

Frequency and Antimicrobial Susceptibility Pattern of Urinary Tract Infections in Children Having Cerebral Palsy

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Abstract

Objective: This study aimed to ascertain the frequency of UTIs, identify causative organisms, and assess their antimicrobial susceptibility among CP children at Children's Hospital, Lahore.

Material and Methods: This study was conducted over six months from February to August 2022, this was a cross-sectional study. The study was conducted at the Children's Hospital, Lahore (CHL). 125 CP children (aged 2-12 years, both genders) were evaluated for urinary tract issues. Isolated organisms underwent susceptibility testing against common antibiotics.

Results: CP children (mean age: 6.3±3.1 years) exhibited varying CP subtypes: spastic diplegia (35.2%), spastic hemiplegia (26.4%), spastic quadriplegia (22.4%), and dyskinetic CP (16.0%). UTIs were diagnosed in 34.4% of cases, predominantly caused by E. coli (53.5%), streptococcus fecalis (20.9%), Proteus Mirablis (14.0%), and Klebsiella (11.6%). Notably, E. coli, Proteus, and Klebsiella displayed 100% sensitivity to ciprofloxacin, ceftriaxone, cotrimoxazole, and gentamicin. However, susceptibility of streptococcus fecalis varied, being 100% sensitive to both ceftriaxone and ciprofloxacin, while exhibiting 55.6% sensitivity to cotrimoxazole and 66.7% to gentamicin.

Conclusion: A significant proportion of CP children exhibited UTIs primarily caused by E. coli and streptococcus fecalis, both fully susceptible to ceftriaxone and ciprofloxacin. These findings advocate for routine UTI screenings in CP children, emphasizing the preference for ceftriaxone and ciprofloxacin in managing positive cases.

Keywords: Cerebral palsy, urinary tract infection, antimicrobial susceptibility.

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Introduction

Cerebral palsy (CP) is a group of disorder related to movement and posture, leading to limitation of activity due to the non-progressive injury to immature developing brain of fetus or infant.[1] CP can result from any injury or damage to the brain during the prenatal, perinatal or postnatal period of life.[2] Its prevalence is around 2 to 3 children per 1,000 live births and is considered to be the most common cause of childhood disabilities.[1,3,4] Cerebral palsy can not only lead to reduced life expectancy, but it is also a very commonly cause of morbidity in children, which may include menial disabilities, recurrent respiratory infections or aspiration pneumonias, epilepsy, vision or hearing defects and infections of urinary tract.[5,6]

Urinary tract infection (UTI) is common among children having cerebral palsy with prevalence of around 8.5 to 56.7%.7 The factors leading to increased chances of UTI among CP patients include delayed attainment of bowel and bladder control, neuromotor dysfunctions, low mental capabilities including difficulty in communicating the desire to void, bowel and bladder dysfunc-

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tions.[7] The repeated urinary tract infections can lead to vesicoureteral reflex and upper urinary tract damage. Therefore, it is pivotal to diagnose urinary tract infections in pediatric population to prevent any long-term morbidity or mortality related to undiagnosed urinary tract infections.[8] A previous Nigerian study suggests a high prevalence (38.5%) of UTI among CP patients, especially among ones having severe immobility. The major organism causing UTI were reported to be E. coli (45%), Streptococcus fecal is (20%) while Proteus and Klebsiella were 10% each. All the isolated organisms were 100% resistant to amoxiclave, cotrimoxazole, nitrofurantoin, and nalidixic acid, while sensitivity to quinolones and ceftriaxone was 100%.[9] In a study conducted by Ryaktiimbo, a frequency of UTI in CP children was 13.1%. The major organism causing UTI were reported to be E. coli (58.3%) and P. mirablis (23.1%), both had low sensitivity to ampicillin (28.5%) and cotrimoxazole (28.5%) while they were sensitive to ciprofloxacin and ceftriaxone (both 100%).[7]

Although many studies in other countries showed a correlation between both frequency and antimicrobial susceptibility pattern of UTI with CP child, but there has been only one previous such study conducted in Pakistan at Hayatabad Medical complex, Peshawar suggesting increased frequency of UTI (32.7%) in children having CP but it did not report antimicrobial susceptibility pattern[(10)]. Now a day, one of the major problems faced by Pakistani physicians is lack of any locally published data on antimicrobial susceptibility pattern, which results in injudicious use of antibiotics. Therefore, this research was conducted to determine the frequency as well antimicrobial susceptibility pattern of UTI among cerebral palsy patients at the Children's Hospital, Lahore to guide establishment of evidence based clinical management of UTI among CP children.

Material and Methods

It was an observational cross-sectional study was conducted at Children's Hospital, Lahore (CHL)., over 6 months from February 2022 to August 2022. After the taking approval from CPSP/REU/PED-2020-075-5868 2nd Feb. 2022. The sample size of 125 cases was calculated with 6% margin of error and 95% confidence level while taking expected frequency of UTI among children with CP as 13.1%.[7] Patients were selected by non-probability technique of consecutive sampling. The children having age 2 to 12 years, both genders presenting with Cerebral palsy with different functional impairment according to European Classification were included in the study. The children who had used any antibiotic within 2 weeks of presentation to hospital or having menstruation, vaginal or penile discharge at presentation or ones having diabetes mellitus (BSR ≥ 200mg/dl), chronic heart disease (medical record) or hypertension (BP ≥ 140/90mmHg) were excluded from the study. The UTI was labelled if there was detection of ≥ 100,000 colony forming units per mL of pathogen organism cultured from specimen obtained. The antibiotic susceptibility pattern was determined as reaction of the most commonly isolated organisms i.e. E. coli, Proteus Mirablis, Streptococcus fecalis and Klebsiella to the antibiotics by CLSI criteria (Ceftriaxone: MIC=64µg/mL, Zone diameter ≤ 13mm resistant and ≥ 18 mm sensitive, Gentamicin: MIC=2µg/mL, Zone diameter ≤ 12mm resistant and ≥ 15 mm sensitive, Cotrimoxazole: MIC=2µg/mL, Zone diameter ≤ 10mm resistant and ≥ 16 mm sensitive, Amikacin: MIC=32µg/mL, Zone diameter ≤ 14mm resistant and ≥ 17 mm sensitive, Ciprofloxacin: M1C= 15µg/mL, Zone diameter ≤ 16mm resistant and ≥ 20 mm sensitive and Ampicillin: MIC=16µg/mL, Zone diameter ≤ 13mm resistant and ≥ 17 mm sensitive)

After ethical committee of the College of Physicians and Surgeon of Pakistan approved the study synopsis, 125 patients visiting the CHL were selected, who fulfilled the inclusion and exclusion criteria. After obtaining informed consent in written, a detailed history was taken (name, age, gender and weight, functional impairment of cerebral palsy and duration of symptoms). A urine sample was taken via aseptic technique in a sterile container. All samples were sent to the laboratory for complete urine analysis and antibiotic susceptibility. Reports were assessed and if bacterium and pus cells detected on urine sample, then urinary tract infection was labelled as per the operational definition. In patients with positive urinary tract infection, organisms and antibiotic susceptibility was checked by using minimal inhibitory concentration method as described in operational definition. All the data was noted and recorded on the pre-designed proforma. All the urine analyses were done from the same hospital laboratory and important confounding factors were controlled by exclusion. The data was then entered into and analyzed through SPSS version 22.0. The numerical variables i.e., weight, age and duration of symptoms were presented as mean ±SD. The categorical variables i.e., the type of CP, gender, presence of UTI, organism and antimicrobial

susceptibility pattern were presented as frequency and percentage. To address effect modifiers, data was stratified for age, weight, gender, duration of symptoms and type of CP. Post-stratification, Fisher's exact test/ chi-square was applied to compare the presence of UTI, organisms and antimicrobial susceptibility pattern in stratified groups taking P-value of ≤0.05 as significant.

Results

The mean age of the participants was 6.3±3.1 years where 2 year was minimum age and maximum age was 12 years. Majority (n=72, 57.6%) of the children were aged ≤ 5 years with 31 (24.8%) children aged between 6-10 years and 22 (17.6%) children aged above 10 years. There were 82 (65.6%) boys and 43 (34.4%) girls and male to female ratio was 1.9:1. The commonest type of CP among participant was spastic diplegia (n=44, 35.2%) followed by spastic hemiplegia in 33 (26.4%) **(Table 1).** The UTI was diagnosed in 43 (34.4%) children with cerebral palsy. There was no statistically significant difference in the frequency of UTI across various sub-groups of children based on age (p=0.956), gender (p=0.934), weight (p=0.976) and type of CP (p=0.998) as shown in Table 1. E. coli was the most frequent causa-tive organism and was isolated in 23 (53.5%) cases of UTI followed by streptococcus fecalis in 9 (20.9%) cases, Proteus Mirablis in 6 (14.0%) cases and Klebsiella in 5 (11.6%) cases as shown in **(Table 2)**. None of the organism showed sensitivity to amikacin and ampicillin. E. coli, Klebsiella and Proteus were 100% sensitive to ciprofloxacin, ceftriaxone, cotrimoxazole and genta-micin.

Table 1: *Characteristics of participants and comparison of urinary tract infection across various subgroups of children with cerebral palsy.*

Characteristics	n	UTI n (%)	P-value
Age			
• ≤5 years	72	24 (33.3%)	0.956
• 6-10 years	31	11 (35.5%)	
• >10 years	22	8 (36.4%)	
Gender			
• Boy	82	28 (34.1%)	0.934
• Girl	43	15 (34.9%)	
Type			
• Spastic Hemiplegia	33	11 (33.3%)	0.998
• Spastic Diplegia	44	15 (34.1%)	
• Spastic Quadriplegia	28	10 (35.7%)	
• Athetoid/Dyskinetic	20	7 (35.0%)	

Table 2: *Frequency of Various Organisms Isolated in Children with Urinary Tract Infection and Cerebral Palsy.*

Causative Organism	Frequency (n)	Percent (%)
E. coli	23	53.5 %
Proteus Mirablis	6	14.0 %
Streptococcus Fecalis	9	20.9 %
Klebsiella	5	11.6 %
Total	43	100.0 %

Table 3: *Susceptibility Pattern of Various Isolates to Common Antibiotics*

Antibiotic	E. Coli	Proteus M.	Strepto-coccus F.	Klebsiella
Ciprofloxacin	23(100.0%)	6(100.0%)	9 (100.0%)	5(100.0%)
Ceftriaxone	23(100.0%)	6(100.0%)	9 (100.0%)	5(100.0%)
Cotrimoxazole	23(100.0%)	6(100.0%)	5 (55.6%)	5(100.0%)
Gentamicin	23(100.0%)	6(100.0%)	6 (66.7%)	5(100.0%)
Amikacin	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Ampicillin	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)

However, susceptibility of streptococcus fecalis varied, being 100% sensitive to both ceftriaxone and ciprofloxacin, while exhibiting 55.6% sensitivity to cotrimoxazole and 66.7% to gentamicin as shown in **(Table 3).**

Discussion

In children with cerebral palsy, there is disturbed immune function which is multifactorial in origin and leads to increased risk of infection that has been identified as a significant contributor of early death.[3,4,11] A few recent studies suggested that a considerable proportion of CP children had undiagnosed urinary tract infection which required appropriate management depending upon causative organism and its antimicrobial susceptibility.[7,9,10] Recurrent febrile UTIs have been associated with upper urinary tract deterioration in children having CP and therefore necessitate proper urological evaluation.[8,9] However, the available evidence on the frequency of UTI, underlying organism and its antimicrobial susceptibility was limited and varied among studies which called for the present study.

In our study, the mean age was 6.3±3.1 years. Mahmood et al. (2019) reported similar mean age of 6.8±2.3 years at the time of diagnosis of cerebral palsy in children presenting at National Institute of Rehabilitation Medicine (NIRM) Islamabad.[12] Roheen et al. (2019) observed similar mean age of 6.6±9.9 years among such CP children.[13] Khandaker et al. (2018) reported mean age of

7.7±4.6 years in Bangladesh[14] while Shrestha et al. (2017) observed it to be 8.3±2.9 years in Nepalese children with cerebral palsy.15 Al-Hammad et al. (2015) reported similar mean age of 6.7±2.7 years in Saudi such children.[16] We observed that there was male (1.9:1) predominance among CP child. In a similar local study, Iqbal et al. (2019) observed similar male to female ratio (1.9:1) among CP children presenting at Jinnah Hospital Gujranwala.[17] Whereas, Khandaker et al. (2018) observed it to be 1.8:1 in Bangladesh.14 And Shrestha et al. (2017) found it to be 1.6:1 in Nepal.[15] Our finding is also similar to that Awan et al. (2019) who also reported male to female ratio of 1.9:1 among CP children in KSA.[18] We observed that majority of the patients had spastic diplegia (n=44, 35.2%) followed by spastic hemiplegia in 33(26.4%) cases and spastic quadriplegia in 28 (22.4%) cases.[20] (16.0%) children had athetoid/dyskinetic cerebral palsy. Ghazal et al. (2019) reported similar frequency of spastic diplegia (35.8%), spastic hemiplegia (27.4%), spastic quadriplegia (18.8%) and athetoid/dyskinetic CP (23.0%) among children presenting at the CHL.[19] Yalçinkaya et al. (2014) observed similar frequency of spastic diplegia, spastic hemiplegia, spastic quadriplegia and athetoid CP and reported it to be 31.5%, 31.4%, 25.3% and 11.8% respectively in Turkish such children.[20] In a similar Iranian study, Inaloo et al. (2016) documented comparable frequency of spastic diplegia (35.0%), spastic hemiplegia (25.0%), spastic quadriplegia (23.0%) and athetoid/dyskinetic CP(17.0%) among such children.[21] Similar frequency of spastic diplegia, spastic hemiplegia, spastic quadriplegia and athetoid CP has been observed by Al-Hammad et al. (2015) who reported it to be 40.3%, 31.8%, 13.8% and 14.1% respectively in KSA.[16] Whereas in a study conducted at Children's Hospital Lahore, involving 100 patients with cerebral palsy (CP), the mean age was 4.090±1.672 years compared to 6.3±3.1 years in our study. Though in that study around 2/3rd patients were male like our study but the most common type of CP was spastic quadriplegic (79%) variety.[22] In the present study, UTI was diagnosed in 34.4% children with cerebral palsy. E. coli was isolated in 53.5% cases followed by streptococcus fecalis (20.9%), Proteus Mirablis (14.0%) and Klebsiella (11.6%). E. coli, Klebsiella and proteus were 100% sensitive to ciprofloxacin, ceftriaxone, cotrimoxazole and gentamicin. The susceptibility of streptococcus fecalis varied, being 100% sensitive to both ceftriaxone and ciprofloxacin, while exhibiting 55.6% sensitivity to cotrimoxazole and 66.7% to gentamicin.

Our observation is comparable with a similar local study conducted at Sir Ganga Ram Hospital, Lahore where Anwar et al. (2020) observed UTI among 34.0% of children having cerebral palsy. However, the author didn't investigate causative organism or its antimicrobial susceptibility.[23] A comparable frequency of 33.8% for UTI has been reported by Kanta et al. (2021) in Bangladeshi children with cerebral palsy. Yet again, the author didn't investigate causative organism or its antimicrobial susceptibility.[24]

Similar frequency of urinary tract infection among CP children has been observed by Anigilaje et al. (2013) who reported it to be 38.5% in Nigeria. The author also observed comparable frequency of E. coli (45.0%), streptococcus fecalis (20.0%), Proteus Mirablis (10.0%) and Klebsiella (10.0%) among such children. They also reported that these isolates were 100% sensitive to ceftriaxone and ciprofloxacin.[9]

In a recent Indian study, Chate et al. (2021) observed urinary tract infection in 32.3% of children with cerebral palsy. The author reported comparable frequency of E. coli (53.8%), streptococcus fecalis (23.1%), Proteus Mirablis (15.4%) and Klebsiella (7.7%) among such children. They too observed that these microbes were 100% sensitive to ciprofloxacin and ceftriaxone. Streptococcus fecalis in their series was 50.0% sensitive to cotrimoxazole and 75.0% sensitive to gentamicin.[25]

The present study is first of its kind in local population giving insight into type and susceptibility pattern of organisms causing UTI in CP children. This adds to the limited previously published evidence on the topic. The strengths of our study is large sample size of 125 cases along with strict exclusion criteria. Our data was also stratified to address various effect modifiers. In the current study, we observed that a substantial proportion of CP children had urinary tract infection with E. coli and streptococcus fecalis as the frequent causative organism. We also observed that these microbes were 100% susceptible to ceftriaxone and ciprofloxacin which are cheap and routinely available antibiotics at government as well as private setups. In the light of this evidence, we advocate that in future practice CP children should be screened for UTI and these antibiotics should be preferred in the management of positive cases. This is of prime importance as children having repeated UTI have risk of developing renal damage and long term complications and need to be investigated properly to manage underlying cause.[26] The cross-sectional study design was one of the limitation of our study

as we didn't follow these children to determine the response to treatment and long term outcome which could have further helped us in the management planning of such cases. Such a study is of vital importance and is strongly favoured in future clinical research.

Conclusion

A significant proportion of CP children exhibited UTIs primarily caused by E. coli and streptococcus fecalis, both fully susceptible to ceftriaxone and ciprofloxacin. These findings advocate for routine UTI screenings in CP children, emphasizing the preference for ceftriaxone and ciprofloxacin in managing positive cases.

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Authors Contribution

WR: Conceptualization of Project
MS: Data Collection
ZRA: Literature Search

MS: Statistical Analysis

ZRA: Drafting, Revision
ZRA, WR: Writing of Manuscript

Minimizing the Hurdle of Pain During Administration of Local Anesthesia Using Bupivacaine in Large Operative Field

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Abstract

Objective: To study use of our technique and anesthetic solution in minimizing the discomfort and pain of administration of anesthetic solution without complications.

Material and Methods: This study was conducted in Prime Care Hospital from June 2022- June 2023. In this case series, we present 100 cases who underwent minor plastic surgery and general surgical procedures using our anesthetic ZA solution containing 0.5% bupivacaine (20 cc) , 1 % xylocaine (20cc) , NAHCO3 (3 cc) and epinephrine (1 cc) diluted in 250 cc Normal Saline. We injected solution in the sub-cutaneous plane with four quadrant fan shape technique via spinal needle (26G). We noted the information acquire from the patients on questionnaire form about the pain score during administration of solution along with validation from the patient for continuation of this technique on other patients along with length of analgesia.

Results: Among 100 patients, 65 were males and 35 were females. Our minimum calculated pain score during administration was 1 maximum was 5, mean was 3, per operatively was 0 , post operatively was 0,remained 0 upto 8 hours after operation. Our calculated duration of action was 9 hours and maximum duration was 14 hours after which patient was given oral pain killers. It is further noted that the pain tolerance was more in age group 25-35 years and less among age group 15-25 years patients. Acceptability of procedure was 87%. No complications were noted throughout the procedure.

Conclusion: Our anesthetic solution is cost effective, patient friendly , provides long duration of anesthesia with minimizing need of multiple pricks and re-adminstration. Hence, reducing pain experience along with validation from the patient . it requires minimum experience , equipment with high success rate.

Keywords: pain during multiple pricks, minor surgeries, Xylocaine, Bupivacaine, NAHCO3, Adrenalin

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Introduction

Surgery has entered in the new era after the invention of local anesthesia .Since then, medical professionals, researchers have long been engaged in efforts to invent new anesthetic drugs or to increase the duration of action of local anesthetics to reduce the pain during administration local anesthesia. Procaine was invented by Alfred Einhorn in 1905. The Swedish chemists' Nils Löfgren and Bengt Lundqvist invented lidocaine in 1943. Bupivacaine was discovered in 1957.[1]

Lidocaine is a fast-acting local anesthetic with a time of onset of between 1 and 5 min and a rather short duration of action of 1–2 h.[2] Whereas, Bupivacaine has a slower onset of 10–15 min but a longer duration of up to 8 h.[2] In combination, they can provide fast, long-lasting regional pain blocking.[2] Local Anesthesia reversibly inhibit nerve transmission by binding voltage-gated sodium channel in the nerve plasma membrane.[3] In the effort to minimize pain because of local anaes

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thesia use, several strategies have been employed to achieve this objective, including the development of extended-release local anesthetics,[4] the implementation of physical tour-niquet techniques,[5] the concurrent administration of supplementary medications such as analgesics or vaso-constrictor drugs,[6,7] the invention of mechanical devices for continuous or intermittent delivery of local anesthe-tics,[8] administration of local anesthesia in surgical wounds after stitches[9] and the utilization of pharmaceutical drug delivery systems.[10] The term "wide-awake" denotes that the surgical procedure on the hand is executed with the patient being fully conscious.

Procedure selection criteria for local anesthesia include minor, quick procedures not demanding the patient to stay overnight with no need for the person to be unconscious. Injection technique is an important factor in achieving nearly pain-free experience for patients and ensuring effective anesthesia in the field. The superficial skin has the highest concentration of nerve endings, which branch repeatedly from larger nerve fibrils in the deeper dermis and subcutaneous fat.[11-12] Use of fine guage needle, limited use of same needle, perpendicular injection technique, slow pulsatile injection and initial deeper of injection are some of the recommendations to reduce the pain of anesthesia administration[13]. Topical anesthetic application, such as EMLA (Eutectic mixture of local anesthetics:lidocaine 2.5%-prilocaine 2.5%) cream applied 60 to 120 minutes prior to intra lesional anesthetic injection has been found in multiple studies to attenuate injection pain.[14]

In this study we share our experience and results regarding the use of our technique and anesthetic solution, a mixture of 0.5% bupivacaine (20 cc), 1 %xylocaine (20cc), $NAHCO_3$ (3 cc) and epinephrine (1 cc) diluted in 250 cc Normal Saline in variety of plastic surgical and other surgical day case procedures with long duration of analgesia minimizing the discomfort and pain of administration of anesthetic solution without complications. We named our solution as Zameer anesthetic solution (ZA solution).

Material and Methods

This case series of 100 patients was conducted in the department of plastic surgery in Prime Care Hospital, a private setup. Patients were selected and planned for variety of plastic surgical and other surgical procedures during the period of June 2022-June 2023. After the taking approval for ethical committee No Ref: PCH/1029. Detailed history and examination, all patients were informed about on going research. Informed consent were taken.

Patients selected for procedures were made sure to be fit in general health. Procedures performed were selected on criteria of being a day case, not needing general or spinal anesthesia. Thorough histories were taken regarding comorbidity, previous surgical procedures and allergic history to any specific drug. Selected patients undergoing procedures via our ZA solution administrating technique were counseled and offered informed consent with prior information regarding conversion of local anesthesia to general anesthesia or regional anesthesia, if needed.

Patients were positioned supine. Intravenous catheters were secured and intravenous Ringer lactate fluid was attached. Cardiac monitor and Pulse oximeter were attached. Emergency and anti-anaphylactic drugs were prepared. NSAIDs and acetaminophen were kept standby. Antibiotic was administrated. An anesthetist was on board ,if there is need of conversion from local anesthesia to regional or general anesthesia. Surgical site was prepared with Povidine-Iodine solution and standard protocol of sterilization was followed.

ZA Solution was administrated via our fan shaped administrating technique, in which we marked surgical field with gentian violet staying 6cm away from the boarder of main surgcial field along with 4 points marked at corner of the surgical field and 2 in the middle of surgical filed dividing the surgical field into 4 quadrants. ZA solution administration started at first given marked six points from proximal to distal along with the length of limb in a perpendicular pulsatile method without aspiration till the site become tumescent and elevated from the surrounding skin. 20 minutes were given for action of onset of the ZA solution. Then we administrated ZA solution in four quadrants through above mentioned six points with single prick without withdrawing spinal needle from the entry point, in fan shaped method again 15 mintues were given to the marked surgical field to let the solution become fully anesthesied the field. Patients were asked about pain according to numeric pain score (shown in table 1) at the end of administration and recommendation from the patient about the administration of this technique, on other patients. During the procedure patient was asked about the pain every 2 hourly per operative, post operative period till the pain starts on a questionnaire which is filled by doctor at

Table 1:

Rating	Pain Level
0	No Pain
1–3	Mild Pain (nagging, annoying, interfering little with ADLs)
4–6	Moderate Pain (interferes significantly with ADLs)
7–10	Severe Pain (disabling; unable to perform ADLs)

time of administration per operative, post operative up till 2 hour after which patient was discharged on oral antibiotics and analgesic with instruction, how to fill the questionnaire form till pain starts and return the form on first postoperative visit.

Results

The mean age of patients selected in this study was 25 years (range 20-35 years) with 65 males (65%) and 35 (35%) females. Procedures done with this technique are listed in Table 1. All patients well tolerated surgery, remained vitally stable and were discharged as day case. 87 patients recommended this technique to be used on other patients (Chart 1). Effects of our ZA solution lasted around 9 -14 hours. Our average pain score during administration was 2 to 4 mean 3, per operatively was 0 and post operatively was 0 (Table 2). Pain score at 4,6,8,10,12 and 14 hours were 0,0,0,1,2,3 respectively) (Chart 2). No procedure was converted to spinal or general anesthesia. No patient showed hypersensitivity reaction to our ZA solution.

Table 1: *Name of procedures performed with their no*

Sr. No	Procedure Name	Number of procedure done
1	large lipoma	4
2	scar revision	6
3	hair transplant	7
4	release of burn contractures on hand	6
5	multiple tendon repair	14
6	release of dupuytren's contracture	4
7	STSG	7
8	FTSG	16
9	Fat Grafting	4
10	Liposuction	6
11	Small flap coverage	4
12	Carpal tunnel syndrome	3
13	Gynecomastia	6
14	Breast Lump exision	3
15	Sebacoeus Cyst	10

Table 2: *Pain score measurement in females and males*

Pain Score during administration	Number of Female Age Range 15-35y	Number of Male Age Range 15-35y
1	6	17
2	11	18
3	14	21
4	2	6
5	2	3
6	0	0
7	0	0
8	0	0
9	0	0
10	0	0

Chart 1: *Validation Chart*

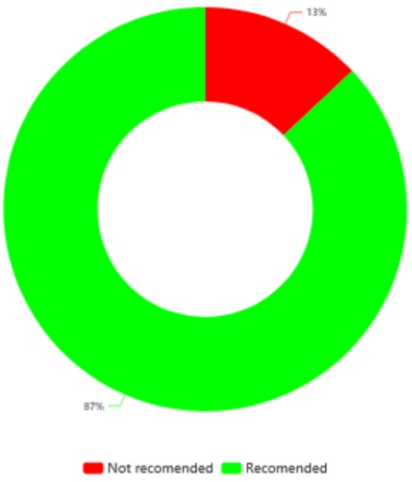
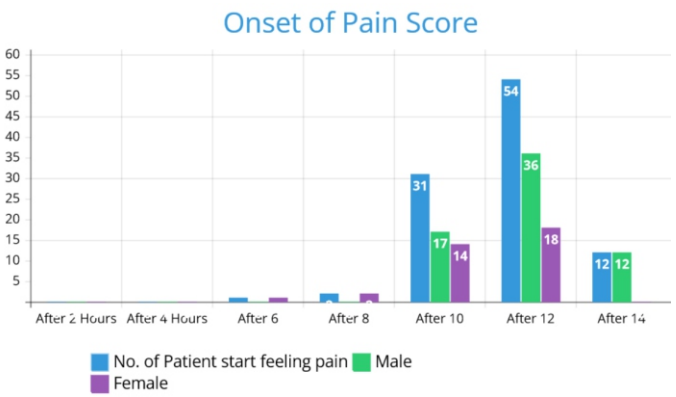


Chart 2: *Onset of pain score.*



Discussion

Nowadays many methods and techniques are being practiced to reduce per operative and post operative pain. Newer and better anesthetic drugs are being administrated. Wide awake surgeries alternatives are appealing to individuals who appreciate the concepts of abstaining from preoperative testing, avoiding tourniquet-induced discomfort, minimizing hospital stay durati-

on for surgi-cal procedures, maintaining the opportunity to commu-nicate with their surgeon throughout the operation, and eliminating the need for sedation that may impair cog-nitive clarity upon returning home post-surgery.[14,15]

Tumescent Solution contain Xylocain but not Bupivacain which does not provide longer period of pain control. WALANT technique is, now a days trend in hand surgery especially tendon transfer. In this technique we consider our ZA solution and technique would be more beneficial as it will provide pain relief for longer period and avoid re-administration of local anesthesia during long surgery. Our ZA solution is a mixture of 0.5% Bupivacaine (20 cc), 1 % Xylocaine (20cc), $NAHCO_3$ (3cc) and epinephrine (1 cc) diluted in 250 cc Normal Saline. Despite the use of bupivacaine as long acting anesthetic drug, adverse effects like hypersensitivity and cardio toxicity hold a fear in surgeons heart, that prevents its common use. Using a safe prescribed dose of 2.5mg/kg , offers a safe practice for bupivacain. Xylocain with epinephrine offers a dose of 7mg/kg, acting as a fast acting anesthetic drug. Epinephrine allows vasoconstriction thus a blood less surgery. NAHCO3 acts as a buffer allowing to minimize the pain of local anesthetic during its administration. We use tumescent technique while administrating in subcutaneous tissue around surgical field thats provide us a great benefit of administration of drug without calibrating dosage of our mixture as our mixture is very diluted. We offer a dilution of 250 ml normal saline, eliminating the risk of over dose of any, used drug. Patient always receive safe dosage as we prepared mixture in prescribed safe dosage and contained epinephrine that causes peripheral vasoconstriction hence decreased its systemic absorption. Moreover systemic absorption is also hampered because of hydro-static pressure of Tumescent technique which causes pressure on the nearby vasculature and collapse them. Most of the local surgical procedures usually are well away from major vessels, so that our solution can easily be utilized for skin and subcutaneous tissue surgery. Our technique is not applicable over fingers and appendages however ZA solution can be used in traditional way of regional blocks. Our mixture can be used in all types of surgical procedure among other specialties as well, limited to skin and subcutaneous tissue.

It's a well known fact that pricking with fine gauge needle causes less pain and to avoid the repeated pricking the needle must be long enough to anesthetize a significant area, for which, we used 26G Spinal needle which can be easily maneuvered without retrieving from the entry site avoiding re pricking hence decreasing pain. The cost of spinal needle is a twice as compared to other needle but the difference is of less than 1$. It is also very helpful in administrating solution in fan shape directions easily with another hand in large area. Its is also proven that perpendicular slow pulsatile administration of injection, and initial deeper injection helps in significant reduction of pain during administration of local anesthesia.[16] Among other benefits our technique of dividing surgical site into four quadrant and staying 6cm away from target surgical site during administration of our solution, provide good analgesia and vasoconstriction as it blocks the sensory nerve and cause vasoconstriction in a $6cm^2$ area. In tumor surgery and dirty wound hydro static effect also cause collapse of vessels along with adrenaline effect of vasoconstriction which in turn stops seedling of tumor and spread of infection in nearby or distant area. Our ZA solution along with our administration technique proved an essential factor in minimizing the pain in local anesthesia injection. The induction time required to do a surgery with our technique is about 10-15 minutes, which is comparable to regional or general anesthesia i.e. 10-15 minutes.[17] Our data showed that our technique with ZA solution resulted in low pain score. Average pain score during administration was means 3. None of the patient needed I/V analgesic. Intravenous access is not mandatory. We felt no use of any monitoring equipment, as all our patients remained vitally stable. As shown by our study, surgeons can operate in remote and impoverished areas, even with limited infrastructure. The preoperative phase is reduced, as patients have the flexibility to arrive just 30 minutes before the scheduled start time. A preoperative workup is not mandatory for except viral markers for safety of surgeons ,staff and theatre. The number of procedure cancellations and delays is minimized as there is no requirement for preoperative clearance from an anesthesiologist. Also use of ZA solution eliminates the need to apply tourniquet per operatively excluding the tourniquet complications and offering blood less surgery. Patient remains awake, pain free, can safely discharge as a day case and safely drive back to reach their destination.

Conclusion

We conclude that our ZA solution containing bupivacaine and solution administrating technique is safe cost effective, patient and poor system friendly.

It gives liberty to perform long surgery without re administration and can be practice without need of monitoring equipment.

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Authors Contribution

ZAM: Conceptualization of Project
AR: Data Collection
AA: Literature Search
HA: Statistical Analysis
GAN: Drafting, Revision
IA: Writing of Manuscript

Effects of Mesalazine and Coenzyme-Q10 on Colonic Histology in Rat Model of Ulcerative Colitis

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Abstract

Objective: To observe the preventive effects of mesalazine and Coenzyme-Q10 on colonic histology in dextran sulfate sodium induced rat model of Ulcerative Colitis.

Material and Methods: It was an animal experimental study conducted in the Department of Pharmacology, King Edward Medical University and University of Veterinary & Animal Sciences, Lahore. Forty eight 48 healthy male albino rats were divided into 6 groups. Groups A and B were taken as healthy and diseased control groups. Groups B, C, D, E, and F were given 4% Dextran Sulfate Sodium in drinking water for inducing ulcerative colitis. Concomitantly, the rats were treated as per group designation, with full or half doses of mesalazine (50, 100mg/kg/day) and Coenzyme-Q10 (30mg/kg/day). Animals were euthanized after the study and colonic tissue was examined for histopathological changes (extent of inflammation, extent of crypt damage, cryptitis, crypt abscesses, and basal cell plasmacytosis).

Results: There was a marked improvement in the crypt architecture as well as the inflammatory changes in the combined treatment group as compared to the groups given Mesalazine and Coenzyme-Q10 separately.

Conclusion: Combined treatment with Mesalazine and coenzyme-Q10 has a better protective effect on colon in ulcerative colitis than either of them used alone.

Keywords: ulcerative colitis, mesalazine, coenzyme-Q10, histopathological, dextran sulfate sodium

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Introduction

Ulcerative colitis (UC) is a major phenotype of inflammatory bowel disease (IBD). It is a common chronic disease characterized pathologically by intestinal inflammation and epithelial injury. It is a chronic remitting-relapsing disease and manifests by abdominal pain, diarrhea, blood in stool, pallor, fever, low energy, and weight loss.[1,2] Disease is usually mild but it can be life-threatening during severe attacks. Also, there is an increased risk of colorectal cancer.[3] Studies in recent years have identified a major role of both genetic and environmental factors in the pathogenesis of UC.[4] A combination of these risk factors seems to initiate alterations in epithelial barrier function thereby allowing the translocation of luminal antigens into the bowel wall. Subsequently, aberrant and excessive cytokine responses to such environmental triggers cause subclinical or acute mucosa inflammation in a genetically susceptible host. In patients that fail to resolve acute intestinal inflammation, chronic intestinal inflammation develops that is induced by the uncontrolled activation of the mucosal immune system. In particular, mucosal immune cells such as macrophages, T cells, and subsets of innate lymphoid cells seem to respond to microbial

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antigens by producing cytokines that can promote chronic inflammation of the gastrointestinal tract.[5,6] Previously, IBD was considered to be a Western disease but now, it has become a global issue.[7,8] Since 1990, the incidence has been rising in newly industrialized countries in Africa, Asia, and South America.[9] Yearly change in the incidence (specified as Annual Percentage Change) of ulcerative colitis is +14•9%.[10] From 2000 to 2010, the adjusted annual incidence rate for UC was 12.2 per 100,000 persons.[11] About 47,400 people died due to UC and Crohn's Disease together in 2015.[12] At present, no curative treatment is available that results in prolonged disease, long-term diarrhea, and recurrences that affect overall health and quality of life.[9]

Pharmacologic management aims to induce remissions and prevent UC exacerbation or acute flare. Various drug groups are used for this purpose with varying control of the disease. Sometimes, surgeries are also required. However, to date, no curative plan has been devised. The drugs used in general include sulfasalazine, aminosalicylates or 5ASA (e.g, mesalazine, olsalazine, basalazide), steroids in high doses (e.g, prednisolone, methylprednisolone, hydrocortisone, beclometasone) and other immunomodulators (e.g., azathioprine, 6-mercaptopurine, methotrexate). Antibiotics are also given if required.[13] Mesalazine is considered one of the most potent drugs used in UC. Its regular use is protective against mucosal damage and permeation. It is used in most UC patients to induce and maintain remissions. The precise mechanism of action of mesalazine is unknown but evidence has shown that it acts by targeting and inhibiting the COX-1 and COX-2 enzymes. It also inhibits the activation of various inflammatory cells and biomarkers and restores the pathophysiological balance of the disease to normal.[14,15] Co-Q10 is used to treat various disorders related primarily to suboptimal cellular energy metabolism and oxidative injury. Studies supporting the efficacy of Co-Q10 appear most promising for a variety of diseases, including UC.[16] The Dextran Sulfate sodium-induced UC model is one of the widely used models as it can be easily developed owing to the wide availability and effectiveness of DSS. Some researchers have suggested that DSS mainly affects the large intestine i.e. middle and distal third of the large intestine and resembles human disease both symptomatically as well as histologically.[17] The present study is designed to observe the role of mesalazine and Co-Q10 alone and in combination on colonic histology in UC and to hypothesize that the combination therapy has a better effect than each given separately.

Material and Methods

The study design was an animal experimental study. The study was conducted in the King Edward Medical University and University of Veterinary & Animal Sciences, Lahore. After taking the approval from ethnical committee with IRB No. 789/RC/KEMU dated 13-05-2019. Sampling Technique was Simple random sampling. Sample Size was forty-eight rats were divided into six groups by lottery method. Male Sprague-Dawley rats, weighing 180g to 220g. Rats showing signs of any disease. 48 adult healthy albino rats of male gender were purchased from and kept in the animal house UVAS (University of Veterinary and Animal Sciences), Lahore. Animals were divided randomly into 6 equal groups having 8 rats in each group. The rats were exposed to natural day and night cycles at room temperature of 22±2°C with 50±5% humidity throughout the experiment. They had free access to rat chow and water ad libitum. An interval of seven days was given to them to get acclimatized before the start of the experiment. The calculated dose for an individual rat, i.e. 100mg/kg/day of Mesalazine[18] and 30mg/kg/day of Co-Q10[19] were weighed and prepared in 4% methocel solution and 0.5% carboxymethylcellulose respectively. DSS was added to the drinking water of rats of groups B, C, D, E, and F. A 4% w/v solution was made by adding DSS (molecular weight 40-50kDa) in water and rats were given this solution to drink throughout the study.[20] Forty-eight rats were divided into six groups randomly by lottery method, with 8 rats in each group. These groups were labeled as A, B, C, D, E, and F. Rats in group A (normal control group) were fed with standard rat diet throughout the study period of 7 days. Rats in group B (disease control group) were fed with a normal diet but 4% w/v solution of DSS was given instead of water. Rats in group C were given oral mesalazine prepared in 4% methocel solution at a dose of 100mg/kg/day once daily, DSS in drinking water, and placebo vehicle (0.5% carboxymethylcellulose). The rats in group D were given oral Co-Q10 prepared in 0.5% carboxymethylcellulose at a dose of 30mg/kg/day once daily, DSS in drinking water, and placebo vehicle (4% methocel). The rats in group E were given mesalazine prepared in 4% methocel solution at dose of 50mg/kg/day orally once daily, Co-Q10 prepared in 0.5% carboxymethylcellulose at dose of 30mg/kg/day orally once daily and DSS in drinking water and the rats in group F were given

mesalazine prepared in 4% methocel solution at dose of 100mg/kg/day orally once daily, Co-Q10 prepared in 0.5% carboxymethylcellulose at dose of 30mg/kg/day orally once daily and DSS in drinking water. Twenty-four hours after the last dose administered the rats were sacrificed at the end of day 7. The colon of each rat was identified and dissected out and preserved in formalin separately. The lower colon was cut out and washed thoroughly with distilled water. A 3cm segment of the colon was separated and fixed in a 10% neutral-buffered formalin solution (pH 7.4). Processed colon tissues were fixed in paraffin wax and cut into 5um thick sections. Slides were prepared and stained with H&E stain to visualize the histological changes of inflammatory parameters (Extent of inflammation, Extent of crypt damage, Cryptitis, Crypt abscesses, Basal cell plasmacytosis). Light microscopic evaluation of DSS-induced damages to colonic mucosal and submucosal layers and response to mesalazine and Co-Q10 administration were scored as follows:

Extent of inflammation; 0 for no inflammatory changes, 1 for changes involving mucosa only, 2 for mucosal and submucosal involvement, 3 for mucosal, submucosal, and muscular layer involvement, and 4 for trans-mural changes. The extent of crypt damage was scored as; 0 for no damage, 1 for basal one-third crypt damage, 2 for basal two-third crypt damage, 3 for entire crypt damage, and 4 for crypt damage plus ulceration. Cryptitis was scored as; 0 for none, 1 for <25%, 2 for 25-50%, 3 for 50-75%, and 4 for >75%. Crypt abscesses were scored as; 0 for none, 1 for focal, and 2 for multi-focal. Basal cell plasmacytosis was scored as; 0 for none, 1 for focal, 2 for multi-focal, and 3 for diffuse.[18] Data was analyzed by using Statistical Package for Social Studies (SPSS) software for Windows (version 23.0) and Graph-Pad Prism (version 8). Histopathological changes were scored as numbers and expressed as percentages of changes in groups; the Chi-square test was used for evaluation. The significance of differences was measured through the Whitney U test. A P-value of less than 0.05 was considered significant.

Results

Inflammation was absent in all rats in Group A. In the disease group (Group B), 25% (02) rats had inflammation involving mucosa, submucosa, and muscular layer and 75% (06) rats had trans-mural extent of inflammation. In rats receiving mesalazine (Group C), 63% (05) rats had inflammation extended up to mucosa and 38% (03) rats had inflammation involving mucosa and submucosa. None of them had inflammation involving the muscular layer. In rats receiving Co-Q10 (Group D), 75% (06) rats had inflammation extended up to mucosa and submucosa and 25% (02) rats had inflammation involving mucosa, submucosa, and muscular layer. None had trans-mural involvement. In rats receiving a half dose of mesalazine along with Co-Q10 (Group E), 75% (06) rats had inflammation extended up to mucosa and submucosa and 25% (02) rats had inflammation involving mucosa, submucosa, and muscular layer. None had trans-mural involvement. While in rats receiving mesalazine in full dose along with Co-Q10 (Group F), 88% (07) rats had inflammation up to the mucosal layer and 13% (01) rats had inflammation involving mucosal and submucosal layers. None had inflammation involving the muscular layer. Comparison of the extent of inflammation among groups showed a significant difference with a p-value of .000. Crypt damage was absent in all rats in Group A. In the disease group (Group B), 25% (02) rats had damage of basal two-thirds of the crypts, and 75% (06) rats had entire crypt damage along with ulceration. In rats receiving mesalazine (Group C), 38% (03) rats had damage to basal one-third of crypts, and 63% (05) rats had damage of basal two-thirds of crypts. In rats receiving Co-Q10 (Group D), 100% (08) rats had damage of basal two-thirds of the crypts. In rats receiving a half dose of mesalazine along with Co-Q10 (Group E), 25% (02) rats had damage of basal one-third of crypts, and 75% (06) rats had damage of basal two-thirds of crypts. While in rats receiving mesalazine in full dose along with Co-Q10 (Group F), 100% (08) rats had damage of basal one-third of crypts. Comparison of the extent of crypt damage among groups showed a significant difference with a p-value of .000.

Cryptitis was absent in all rats in Group A. In disease group (Group B), 25% (02) rats had cyptitis that involved 25-50% of the crypt area, 38% (03) rats had cryptitis involving 50-75% of the crypt area, and 38% (03) rats had cryptitis involving >75% of crypt area. In rats receiving mesalazine (Group C), 100% (08) of rats had cyptitis that involved <25% of the crypt area. In rats receiving Co-Q10 (Group D), 75% (06) rats had cyptitis that involved <25% of the crypt area, and 25% (02) rats had cyptitis that involved 25-50% of the crypt area. In rats receiving a half dose of mesalazine along with Co-Q10 (Group E), 25% (02) rats had cyptitis that involved <25% of the crypt area, and 75% (06) rats had cryptitis involving 50% of the crypt area. While in rats receiving mesalazine in full dose along with Co-Q10 (Group F),

100% (08) rats had cyptitis that involved <25% of the crypt area. Comparison of cryptitis among groups showed a significant difference with a p-value of .000.

Absent in all rats in Group A. In the disease group (Group B), 100% (08) of rats had multi-focal crypt abscesses. In rats receiving mesalazine (Group C), 100% (08) of rats had focal crypt abscesses. In rats receiving Co-Q10 (Group D), 75% (06) rats had focal crypt abscesses and 25% (02) rats had multi-focal crypt abscesses. In rats receiving a half dose of mesalazine along with Co-Q10 (Group E), 25% (02) rats had focal crypt abscesses and 75% (06) rats had multi-focal crypt abscesses. While in rats receiving mesalazine in full dose along with Co-Q10 (Group F), 100% (08) rats had focal crypt abscesses. Comparison of crypt abscesses among groups showed a significant difference with a p-value of .000.

seen in any rat in Group A. In the disease group (Group B), 100% (08) of rats had diffuse basal cell plasmacytosis. In rats receiving mesalazine (Group C), 63% (05) rats had focal basal cell plasmacytosis and 38% (03) rats had multifocal basal cell plasmacytosis. In rats receiving Co-Q10 (Group D), 75% (06) rats had multifocal basal cell plasmacytosis and 25% (02) rats had diffuse basal cell plasmacytosis. In rats receiving a half dose of mesalazine along with Co-Q10 (Group E), 88% (07) rats had multifocal basal cell plasmacytosis and 13% (01) rats had diffuse basal cell plasmacytosis. While in rats receiving mesalazine in full dose along with Co-Q10 (Group F), 100% (08) rats had focal basal cell plasmacytosis. Comparison of basal cell plasmacytosis among groups showed a significant difference with a p-value of .000.

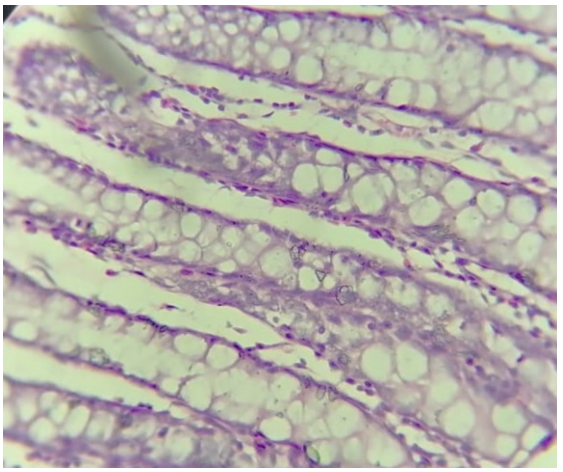

Figure 1: *Picture showing normal histology of the colon. Normal crypt architecture with no inflammation seen in group A (H&E 40X)*

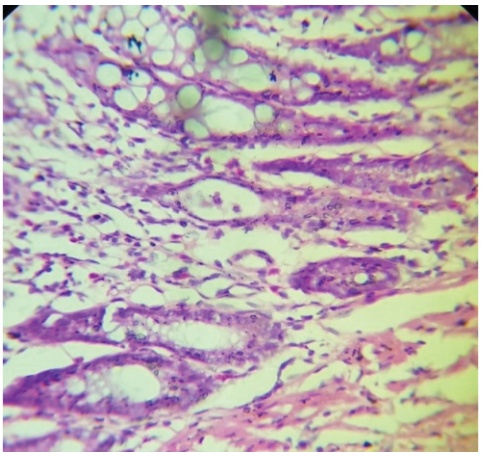

Figure 2: *Figure shows significant crypt abscesses and total loss of crypt architecture in group B. Significant inflammation can be seen with cryptitis (H&E 40X)*

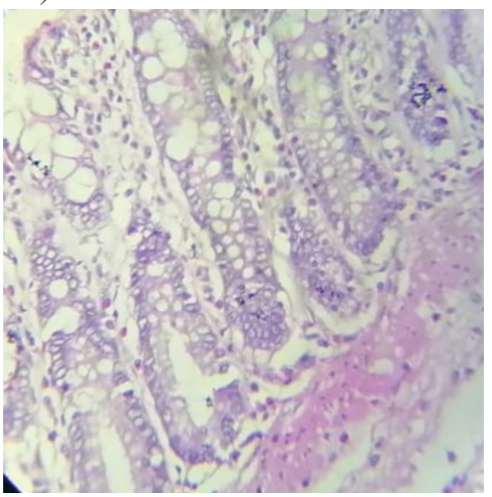

Figure 3: *Figure showing basal cell plasmacytosis in group B (H&E 40X)*

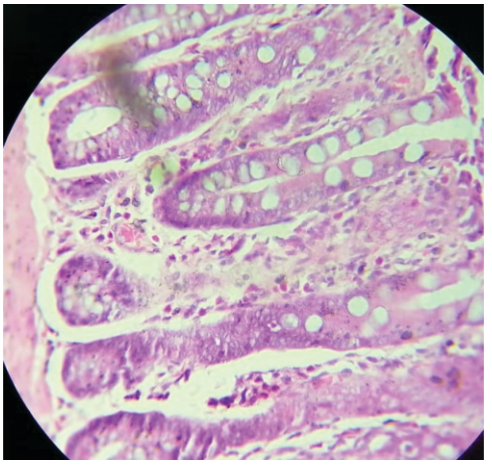

Figure 4: *Figure shows normal crypt architecture, and mild inflammation in the group treated with mesalazine (Group C). No cryptitis or crypt abscess was*

seen. (H&E 20X)

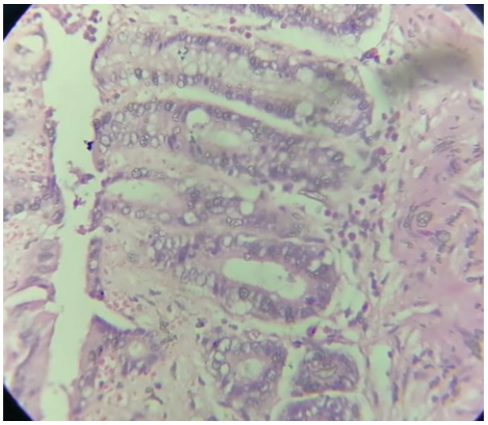

Figure 5: *Figure shows mild cryptitis and inflammation in the group treated with Co-Q10 (Group D). No crypt abscesses were seen. (H&E 20X)*

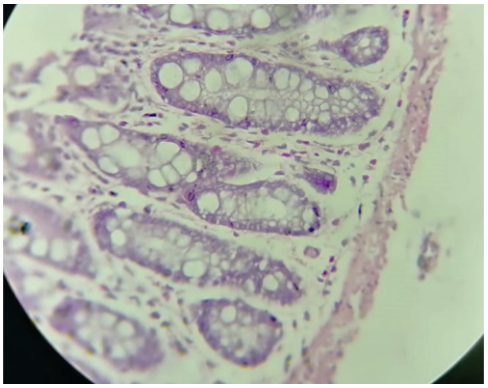

Figure 6: *Figure shows mild cryptitis, inflammation, and focal crypt abscesses in group E. (H&E 10X)*

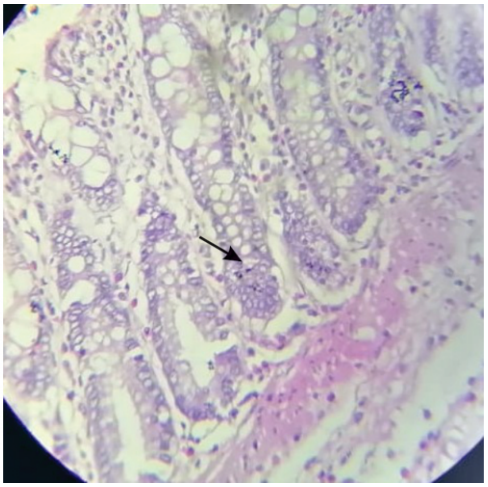

Figure 7: *The figure shows normal crypt architecture, no inflammation, no cryptitis, and no crypt abscesses seen in the group treated with a combination of mesalazine and Co-Q10 (Group F) (H&E 20X)*

Discussion

Histopathological study with light and deca-head microscopy revealed some major changes in inflammatory changes and architecture of crypts. DSS led to a complete distortion of the crypt architecture. It caused severe inflammatory changes, cryptitis, and basal cell plasmacytosis which led to degeneration of the crypts and produced focal and dense crypt abscesses. Similar findings were reported in the research works using the rat model of DSS-induced UC.[18,21] After the administration of Mesalazine for 7 days, there was some reversal of these histopathological changes in the colonic sections of the rats of group C. The extent of crypt abscesses, cryptitis, and inflammation was decreased. A significant restoration of crypt architecture was seen. Such results were seen in a study where Mesalazine was used to treat DSS-induced UC in rats.[18] Administration of Co-Q10 for 7 days in rats of group D, inflammation was reduced and crypt architecture restoration was seen to some extent. Crypt abscesses were settled to a significant extent. Similar results were seen in a study where Co-Q10 was given for UC.[16] Administration of half dose of Mesalazine and full dose of Co-Q10 for 7 days, in rats of group E, reduced inflammation and restored crypt architecture to some extent. Full doses of both Mesalazine and Co-Q10 when administered together completely settled down the inflammation, cryptitis, crypt abscesses, and basal cell plasmacytosis and completely restored the crypt architecture. No study to date has suggested the potentiation of therapeutic effects of Mesalazine with administration of Co-Q10.

Conclusion

This study has demonstrated that the combined administration of Mesalazine and Co-Q10 has exerted a stronger effect on restoring colonic histology to normal in the UC rat model as compared to both of these drugs given alone.

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Funding Source: *None*

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Authors Contribution

HP: Conceptualization of Project

SH: Data Collection

AF: Literature Search

KSA: Statistical Analysis

MM: Drafting, Revision

MFJ: Writing of Manuscript

Persistence of COVID-19 Among Private Medical College Students Even after Immunization

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Abstract

Objective: To ascertain the frequency of COVID-19 among Private Medical College Lahore students despite vaccination aimed at immunity against evolving variants of concern, thus safeguarding against potential future epidemics globally.

Material and Methods: The study was conducted at the Central Park Medical College among students of different years of medicine using a cross-sectional quantitative approach and circulating e-questionnaire to collect information about COVID occurring before and after vaccination, the number of booster shots given, type of vaccine administered. The gathered data was examined using the ANOVA technique. It was conducted from March 2022 to August 2022.

Results: There were 402 participants in the study. The results showed that immunization in its entirety was very successful. Students who received the first booster dose had fewer COVID-19 cases, and those who received the second shot had even greater success in lowering the number of cases.

Conclusion: To sum up, COVID-19 is still very contagious. This study helps to explain related symptoms and offers insightful information on breakthrough instances that occur after vaccination. The findings highlight how crucial it is to inform the public on social distancing. Governments can leverage these findings to formulate strategies and interventions to mitigate potential future pandemics.

Keywords: Covid -19, vaccine, Virus SARS Covid

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Introduction

The novel coronavirus known as coronavirus 2 (SARS-CoV-2) first surfaced in Wuhan, China, in late 2019. It is often diagnosed as coronavirus disease-2019 (COVID-19), a severe acute respiratory illness. After the virus began to spread outside of China, the WHO deemed COVID-19 to be a pandemic on March 11, 2020. According to Yu Shi, on March 15, 2020, SARS-CoV-2 quickly spread to 34 Chinese provinces and cities; infections were reported in 144 nations, territories, and places on five continents (World Health Organization, 2020). The Islamic Republic of Pakistan, which likewise had the greatest death toll in the middle of May 2022, also declared an emergency.

Many individuals who were impacted by COVID-19 experienced various mental and physical health problems. Frailty, alcohol-related conditions, cardiovascular risk factors (smoking-related conditions, hypertension, diabetes mellitus, dyslipidemia, and obesity-related conditions), cardiovascular diseases (heart failure, coronary heart disease, cardiac rhythm disorder, valvular heart disease, occlusive peripheral arterial disease, stroke, and pulmonary embolism), chronic respiratory conditions, dialysis, having a kidney transplant, liver failure, active cancer, depression, psychosis, dementia, epilepsy, Parkinson's disease, inflammatory bowel disease, rheu-

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matoid arthritis, and ankylosing spondylitis) were found to be associated with severe COVID-19 outcomes (Kim Bouillon, 2021).

The research of vaccines had quickened as several nations battled new illnesses brought on by COVID-19. Immunization against the virus and prevention of its spread is achieved through vaccination, which is a vital and economical measure (Fiolet, 2021). More than three billion COVID-19 doses had been given globally by the middle of 2021, and 24% of the world's population had received at least one dose of the vaccine. Around 40 million doses of the COVID-19 vaccine were being given out daily globally by that point. By the middle of 2021, the following nations had vaccinated at least 50% of their citizens against COVID-19: the United States, France, Italy, Spain, Chile, Uruguay, Israel, Bahrain, Hungary, and the United Kingdom. By the end of June 2021, however, just 1% of individuals in low-income nations had received a dosage of the COVID-19 vaccination. Just 53 million vaccination doses have been given in Africa [Duduzile and Charles, 2021]. According to Adeel Siddique, there are more than 6 million and 20 million fully and partially vaccinated individuals in Pakistan (out of a total population of about 220 million). By the final week of July 2021, the total number of doses given out daily at approved COVID-19 immunization locations had risen to approximately 600,000–900,000 doses. In 2021, Michael et al.

The global COVID-19 epidemic is still far from under control, and both prevention and control of the outbreak continue to present formidable obstacles. Since there is currently no known cure for COVID-19, the most practical and affordable ways to contain the pandemic are to encourage vaccination campaigns and foster herd immunity. On the other hand, the recipients' level of neutralizing antibodies declines with time, and the vaccine's protective effectiveness progressively wanes. It is yet unclear if booster vaccinations are required to increase the immune system's defenses against illness. We compiled the available information on the efficacy and durability of COVID-19 vaccinations in this work.

We discovered that using a booster immunization approach is essential. Still, not every participant needs a booster shot six months following the first vaccination. High-risk populations, such as the elderly and those with immunodeficiency, should receive priority treatment. Compared to homologous immunizations, a heterologous booster can strengthen immune protection and elicit stronger immunological responses. Nevertheless, additional empirical evidence and clinical investigations are required to confirm the safety of heterologous vaccination approaches. (Meng and Associates, 2022).

The prevalence of COVID-19 was examined for the first time in Pakistan although numerous cases were still reported there even after receiving the full immunization.

Material and Methods

This study involved 402 students in total. The professorial group created and approved the Standardized Questionnaire (Acharya 2010) from the month of March-August in the year 2022. The IRB registration No was CPMC/IRB-No/1381-A. Age, gender, marital status, travel history, times of coronavirus, year of vaccination, COVID occurring before and after vaccination, number of booster shots given, type of vaccine used, and corona symptoms were among the ten factors that were taken into consideration when formulating the questions. The students were contacted through both manual and online means. 'Yes' and 'No' responses to the items were used as a measurement system. To compare the pupils with and without corona, a bar chart was created. Data on the durability of corona following vaccination after one and two booster injections was analyzed by ANOVA.

Results

Demographic statistics included age, gender, times of coronavirus, COVID occurring before and after vaccination, number of booster shots given, and type of vaccine administered in students of Central Park Medical College, Lahore. This research includes 402 participants, with the majority being females at 237 (58.9%) and the remaining being males at 165 (41.1%). Most participants fall within the age range of 20 to 23. Our Results, by One-way ANOVA, indicated a highly significant effect of vaccination (DF=2, F=532.30, p=0.001). The effect of vaccination was found to be highly effective for being completely vaccinated (Mean=138.67, SD=3.21). The application of 1st booster shot to the students was found to decrease the corona cases.

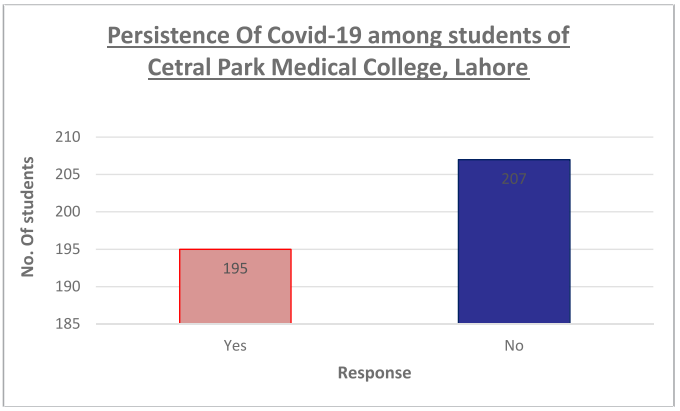

Figure 1: *Graph showing students having corona after vaccination*

One-way ANOVA showed highly significant results for the effect of vaccination (DF =2, F = 532.30, p= 0.001). The effect of vaccination was found to be highly effective for being completely vaccinated (Mean = 138.67, SD=3.21). The application of 1st booster shot to the students was found to decrease the corona cases (Mean= 138.67, SD=3.21) while the application of 2nd booster shots was highly significant. (Mean= 105.0, SD=5.00)

Table 1 and 2: *Effect of vaccination and booster shots on the students of corona vaccination*

Sr. No	Group	Mean±SD
1	A	138.67a± 3.21
2	B	105.0b±5.00
3	C	29.67c±4.16

DF	F	P
2	532.3	0.001

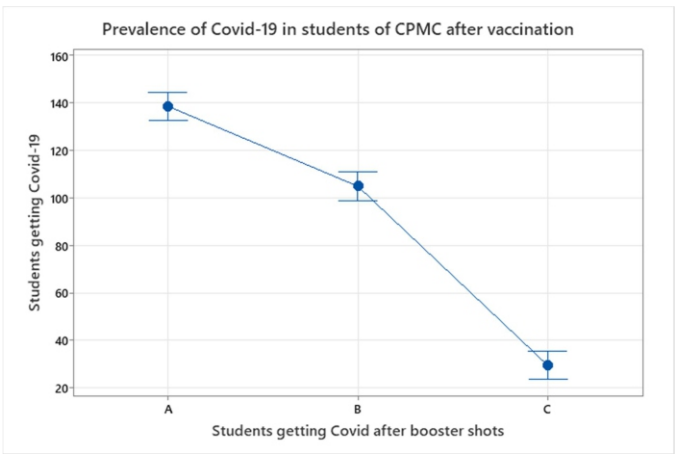

Figure 2: *Comparison between the people having corona after 1st and 2nd shots*

The graph illustrates that the COVID-19 prevalence among CPMC students was lower after the 2nd booster shot when compared to the prevalence after 1st booster shot.

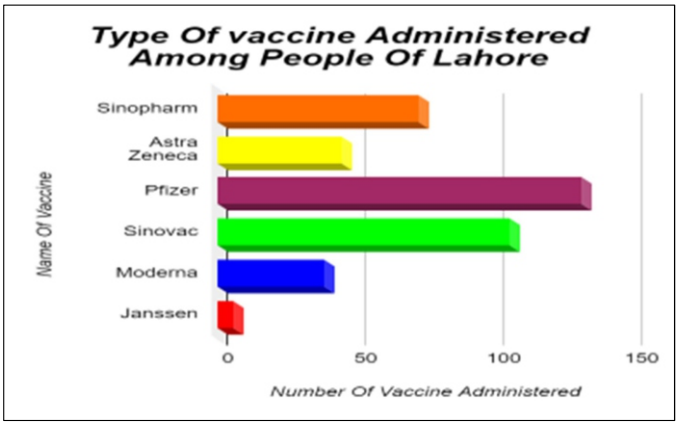

Figure 3: Effect of different Types of vaccines administered to students of CPMC

Every vaccination we tested produced incredibly meaningful results. After Pfizer was shown to be a very efficient vaccine, Sinovac was also shown to be effective. Sinopharm also demonstrated very encouraging outcomes in this area. When compared to Moderna, Astra Zeneca demonstrated more noteworthy outcomes, although Janssen was less successful in this area.

Discussion

This study represents the inaugural investigation into the prevalence of COVID-19 among CPMC Students post-vaccination. Our findings as in figure 1 underscore the profound impact of complete vaccination among CPMC students, a feat largely attributable to the proactive vaccination campaign spearheaded by the Government of Pakistan, which not only encouraged but also provided free vaccination services across the nation (Gias et al 2023).

Against the backdrop of the COVID-19 pandemic, vaccination emerges as the paramount strategy for mitigating the spread of this perilous disease, leveraging available vaccines within healthcare systems globally[7]. Notably, Cihan's 2021 study reported that 41.8% of the US, 2.3% of Asia, 17% of Europe, 0.6% of Africa, 8.8% of South America, and 5.6% of the globalpopulation would be fully vaccinated against COVID-19.

(Khosa et al., 2023)Figure 2 illustrates the remarkable efficacy of vaccines in preventing COVID-19 (Patel et al. 2022) emphasize vaccination as the most effective

defense against viral diseases, reducing disease severity and transmission.

The utilization of booster shots has yielded significant outcomes. In the US, health professionals advocate for revaccination six months post-second dose for Pfizer BioNTech or Moderna recipients. Conversely, since April 2021, EU administrations have deliberated on accelerating booster vaccinations. In France, citizens aged 18 and above are eligible for booster shots five months post-second dose, with penalties for those failing to comply by mid-December 2021

Israel's proactive approach has resulted in over 40% of citizens receiving a third dose, as evidenced by a Lancet study indicating an 81% increase in effectiveness against mortality post-third dose. Despite successful vaccination campaigns, rising SARS-CoV-2 infections are attributed to the Delta variant's higher infectivity and reduced immunity post-earlier vaccination. Early evidence suggests mRNA vaccine's third dose efficacy in mitigating severe COVID-19 outcomes

The persistence of COVID-19 prevalence post-vaccination can be attributed to several factors. Firstly, no COVID-19 vaccine offers 100% protection (Thompson et al. 2021), with estimated adjusted vaccine effectiveness against SARS-CoV-2 infection at 91% and 81% for full and partial vaccination, respectively. Secondly, vaccine efficacy varies across different COVID-19 variants, as demonstrated by Fiolet (2021) and Zeng (2022), with booster vaccination proving more effective against Delta and Omicron variants

Our research confirms positive outcomes for all vaccines studied. Pfizer's notably high efficacy may be due to study biases favoring younger demographics as elderly individuals are less likely to participate in online surveys.

Further research is imperative to explore booster doses, heterologous vaccination, dosing intervals, vaccine breakthrough infections, and the duration of vaccine-induced immunity against emerging variants of concern.

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Authors Contribution

FNT: Conceptualization of Project
HT: Data Collection
ZT: Literature Search
RS: Statistical Analysis
SM: Drafting, Revision
AK: Writing of Manuscript

Career Decision Making, A Question for Young Medical Undergraduates: A Study at Government Medical College

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Abstract

Objective: To know the causes/problems of medical graduates which comes during selection of their sub-specialty after completion of their MBBS/BDS graduation course.

Material and Method: This study done at Government Medical College of Pakistan from April 2023 to June 2023. Two groups were made and a questionnaire based data collected. The data later was analyzed.

Results: A total of 100 students participated in first group of this study including 17 in year I, (out of 30) 08 in year II, (out of 30) 09 in year III (out of 25) and 06 in year IV (out of 15). The final year was not included as they were scattered in various wards. The age range of the respondents was 18-20 years. 45.1% participants were single. 47 male and 53 female students participated.

Conclusion: Professional and personal life dynamics as well as urge for and concern in the effort to choose sub-specialty was tremendous.

Keywords: Career, Medical Graduate

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Introduction

Learning during under graduation as medical student confers good mental decision making strength to medical students. This empowers the health department to adjust this technical force according to the needs of provincial population.[1] Health Department Government of Balochistan has reported that many specialists in certain fields are needed while new medical graduates require selected career counseling.[2] The Medical graduates in Balochistan (mostly rural) needs career counselling at undergraduate level, as most of the medical students in the field feel very tense during service provisioning after getting medical degree and job.[3] It is quite vague that how medical students precedent various factors during selecting a medical speciality after graduation.[4] Medical students in the field of health profession have extreme level of anxiety, depression and distress while comparing to non-medical professionals. A successful medical professional requires to prove medical field a prestigious one during practice.[5] Upon graduation students directly link money in choosing specialty, have greater earning probabilities in addition to life style.[6] High earning potential during medical practices always matters in selecting future sub specialty after completion of under graduate studies, says students.[7] Its unclear that during subspecialty decision making by fresh medical doctor; any consultant, postgraduate, family member, can play role in career decision making or speciality selection.[8] Making career decision during studies is quite difficult to plan. The parent and society pressure in addition to children for best outcome often matter to reduce this stress. This may boost to develop psycho-

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logical illness while making any stressful career decision making.[9] This decision can assist the administration of potentials at under graduate level, increases career gratification and selection of doctors according to the needs of population.[10] The aim of this study is to find those aspects which were most relevant in guiding speciality choice amid medical students from Bolan Medical College Quetta Pakistan. The role of medical institute or health administration during student's study for selection of subspecialty or career counselling can also be sorted out in this study.

Material and Methods

Approval from research ethical board has been taken before initiation of this study. Various number of medical students from first to fourth year mbbs/bds have been taken randomly from Bolan Medical College Quetta. Written and implied consent taken from all participants. After approval from ethical committee IRB No 34/2023. This study was conducted from April 2023 to June 2023. Separate focus groups made for students of each year. Demographic information collected from students. Data was collected and analyzed in iterative manner, using constant comparative analysis based on grounded theory approach. Three investigators (ZK, MM, MZ) coded freely and data analysis done with generation of designs and themes regarding study. In second stage of study a survey conducted used data from focused student groups to make a questionnaire.

Table 1: *Basic Demographic Description*

	Demographic Information	
1	**Age n (%)**	
	18-20	50 (90)
	21-23	7 (8)
	24-26	1(2)
2	**Year of Study n (%)**	
	Year I	17 (30)
	Year II	8 (30)
	Year III	9 (25)
	Year IV	6 (15)
3	**Gender n (%)**	
	Male	24 (47)
	Female	31 (53)
4	**Marital status**	
	Single	41 (90)
	Married	3 (10)
5	**Postgraduation preference**	
	Yes	51
	No	11
	Don't know	38

Various ques-tions were asked in survey from medical students directly. The data collected into MS Excel and analyzed later. Descriptive statistics described as mean + SD. Propor-tions explained in percentages. The methodological triangulation form generated the second dataset by the survey which compare the findings from focus group.

Results

A total of hundred students participated in this study. Most of them did not responded at all. The respondents involved were seventeen in first year, eight in second year, nine in third year and six in fourth year. Many efforts were made to conduct a focus group among students offering many sitting sessions. Very poor response rate were noted due to lack of student reply interest. The age of the respondents were 18-20, male were 47% and 53% were female. Maximum number (17) of participants were from first year. 45.1% participants were single. 51% were aiming to do postgraduation after completion of graduation. They all want to stay in the main city and not to move in periphery. Here six major notions appeared: personal life dynamics; professional life influences; desire/interests; altering interests, previous experiences and mentorship (Fig 1).

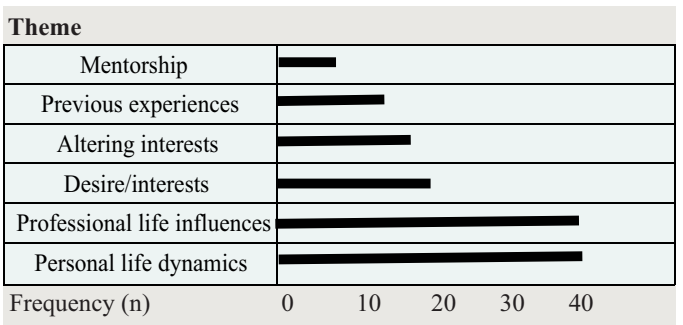

Figure 1. *Frequency of theme occurrence*

Within these primary themes additional 12 subthemes were identified.

Table 2. Amongst four subthemes within professional life influences (40) comprised flexibility (21), job market (10), income (8) and research prospects (1):

The four subthemes within personal life dynamics (36) included lifestyle (12), family commitments (9), and maternity leave/pregnancy (5):

- "Lifestyle would be a big question. I think that is one of the factors which splits people as to whether they need to continue with family medicine or surgery like field which is more time consuming."

- "I am interested in emergency medicine and I am sure work load would affect the quality of time with the family."
- I have to live in city, I can't live anywhere else. I lived in Quetta for my 1st year of undergrad.
- For women, we know that we are the ones getting pregnant, we are probably thinking more about what would come after that residency.
- At the end of the day, I think I made more of an emotional decision. I decided on something I really liked….."
- I find it a little overwhelming. When you go in you are bombarded with so much information.

The hidden curriculum and the subthemes of prestige and stigma emerged with concerns being voiced around family medicine in particular:

- So, when someone asks you what are you thinking of going into, neurologists' sounds really cool, but family doctor doesn't sound that cool.
- With regard to family medicine, there is the feeling that you should be striving for more.

Ninety students involved in this second study phase **(Table-3)**. Though students from all four years were invited to involve in the study, the majority were from year two (n=55). 20 students from year one (n=20). 10 students from year three (n=10), 5 students from year four (n=5). More than half number of students were female 48 (53%). More than two third of the students were single. Engaged or married were 20. Few students had got any other university degree. Majority of students came from middle class family 54%. Out of 90 participants 10 had any immediate family member who was physician.

Table 3: *Demographic Information for participants.*

Demographic Information n=90	
Age (mean ± SD,)	25 ± 2.0 (20-25)
Year 2 (n %)	55 (61)
Male/female %	53/47
Relationship %	
Single students' %	78%
Engaged or married	22%
Have any other degree too	04
Economic status (middle class)	54%

Before beginning in medical school only one third students had some idea of which speciality they wanted to follow and the same ratio changed their mind in selection. Reasons student explained why change speciality comprised experiences in hospital services (45%), medical college syllabus (20%), mentorship (30%) and others. Experience of career selection, 55% of students change the ideas during medical schooling, 50% students expressed it depends on anxiety & stress during work load and 48% students excitedly selected the field more

Table 2: *Primary themes and Sub-themes*

Themes/subthemes	Frequency	Example
Professional Life Influences (40)		
Flexibility	21	"Flexibility that is opportunity to do things other than just clinical practice."
Job Market	10	"One for me is job offers at the end of it all"
Income	10	"For me it was medical school and about the time that I won't be making money in 4year of medical school & residency."
Research prospects	01	"If I see interesting research in a specialty….I can see whatever part of this research is coming to fruition and be a part of that."
Personal life dynamics (36)		
Life style	13	"Lifestyle and what you want outside of medicine is big factor."
Family Commitments	09	"I am starting in my thirties, and if I go into a long residency, then I won't really be earning income until I am in my forties, and I have a family.'
Maternity Leave/Pregnancy	05	It's different for women. Women are more affected by the length of the program.
Interests	03	Making sure you are going to like what you want to do forever
Altering Interests	06	I have not had exposureto all of the specialties yet but I find my interests have changed very quickly from the day when I began medical.
Previous experiences(13)		
Mentorship	07	One of the big motivators towards the specialties I have been interested in has been the mentors who have been in my life.

than once & changed repeatedly.

Students had asked to mark any five reasons (1 to 5, 1 as most & 5 as least important) in speciality selection guide (Figure 2). Passion the most common factor (n=80), lifestyle (n=75), flexibility (n=60), opportunities of employment (n=60), family concerns (n=55), income (n=60), location (n=40), mentorship (n=35), respect (n=10). Specialists were seen differently by society agreed by 60% participants. Number of students expressed that specialists are more precious than family medicine, "its general view in medicine to look down on general practitioners….valued the surgical practitioners." Personal possessions like mentors (40%), clinical market (60%), and experiences (50%) were most guided selection of speciality including websites by (35%). Students counselling never helped the students in selection of career and this is used less commonly by students.

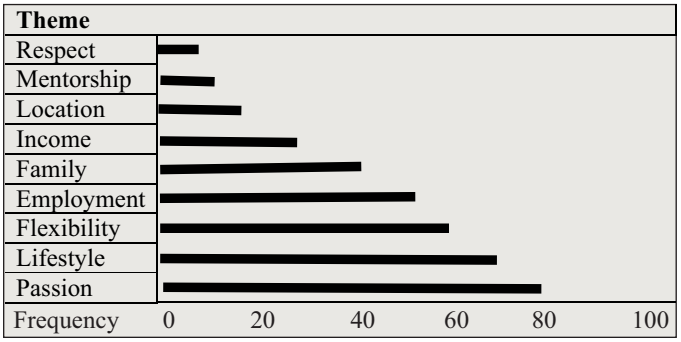

Figure 2. *Factors urging choice of specialty*

Discussion

This study confers special focus on important factors deciding on a speciality to follow. Here medical students envisaged professional and personal life factors (flexibility & lifestyle) with interest remained most vital. This was expressed in both phases of this study that passion remained most important factor which attracts respondents for field choice. The choices of students may get change simultaneously just because the choice of initial years of study did not solid one and repeatedly gets altered depending on interests & more competitions. This may be to some extent due to having more time to detect their interests lie after experiencing a good time of clinical training with seniors. The student's career selection obviously depends on their lifestyle and passion.[11] Certain other studies also found same tendency for students to decide their specialties with maximum lifestyle control.[12] The observations of limited education principles were also remarkable. Few students think that family medicine is underrated compared to other specialitities.[13] This observation has been previously explained in the literature with students reporting that some speciality preceptors criticize family medicine.[14] Wide experience to specialists through both clinical experience and class room experience at tertiary level care may influence students insights.[15] Many personal experiences of mentorship, staff and internet search of global demands appear to be the most helpful to students during decision making process.[16] This is also noticed here that students are also concerned over debt interests.[17]

Conclusion

Professional and personal life dynamics as well as urge for and concern in the effort of a specialty seem to be extremely significant deliberations for medical students when determining which specialty to follow.

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Authors Contribution

ZK: Conceptualization of Project
AZK, ARZK: Data Collection
MZ: Literature Search
GSS: Statistical Analysis
MM: Drafting, Revision
GSS: Writing of Manuscript

Genomic Variations in the Dengue Virus Non-Structural Protein 4A

Saira Mushtaq,[1] Rameesha Shafiq,[2] Sarwat Jahan,[3] Farah Naz Tahir,[4] Zahid Mahmood,[5] Afshan Zareen Bilal [6]

Abstract

Objective: To identify the most common geographic specific mutations in the Dengue Virus Non-Structural Protein 4A circulating in Faisalabad, Pakistan.

Material and Methods: This research was conducted at Institute of Molecular Biology and Biotechnology, The University of Lahore, Aziz Fatimah Hospital and Allied Hospital Faisalabad during the dengue outbreak of 2022. About 120 DENV isolates were selected from the laboratories of tertiary care hospitals of Faisalabad and Lahore for analysis of sequencing of the whole genome. Only 23 samples were sequenced after viral isolation, quantification, and cDNA synthesis.

Results: A total of 88 different types of mutations with different frequencies in all domains have been detected in NS4A proteins. Those mutations which presented with the most frequency were Q19L, I89M, and A93V (n=6) each R76K (n=5), V2I (n=4), and G38E (n=3).

Conclusion: Future DENV vaccination development research will be especially profited by the mutations found in the current study. Genomic epidemiology during each DENV outbreak in various regions is essential for improving public health and creating new regulations for outbreaks in the future.

Keywords: DENV; genome; mutations; Pakistan; Non-Structural proteins; NS4A.

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Introduction

Dengue is a viral ailment propagated by mosquitoes that is characterized by its acute and recurring nature. It is brought about by the dengue virus (DENV) and is commonly seen in tropical regions. In the last five to six decades, the incidence of dengue has risen by a factor of thirty. Presently, around 390 million individuals globally acquire dengue fever every year.[1] National Institute of Health (NIH) Islamabad, reported the number of dengue fever in Pakistan to be 22,938 in 2017, over 3,200 cases in 2018, 24,547 patients in 2019, and 3,442 cases in 2020.[2]

Dengue viruses (DENV), belonging to the Flaviviridae family and Flavivirus genus,[3] induce a dengue infection that manifests as high fever, joint and muscle pain, vomiting, exhaustion, myalgia, skin rash, hemorrhagic episodes, abdominal discomfort, and circulatory shock. DENV viruses are enveloped RNA viruses with a single-stranded genome. The open reading frame (ORF) of the DENV genome, which spans approximately 11 kilobases and is flanked by the 5' and 3' untranslated regions, is encoded. The open reading frame (ORF) encodes a solitary polyprotein, which is further segmented into 7 non-structural proteins (NS-1, NS-2, NS-2B, NS-3, NS-4, NS-4B, and NS-5) and 3 structural proteins (C: capsid, M: membrane, and E: envelope).[4]

NS4A is an integral membrane protein of DENV and play many roles in its replication and relations with its

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host. It is a 127aa protein with 16kDa molecular weight. Among the seven domains the upper three domains (aa3-11, 14-31, and 41-45) as shown in Figure 1 are present on the membrane surface. Three domains are membrane embedded and one is cytosol domain (aa80-96). The 2K sequence NS4A is present at C-terminus. Through protease cleavage this 2K is separate from NS4A serving as a signal peptide to translocate NS4B to ER lumen of cell[5].

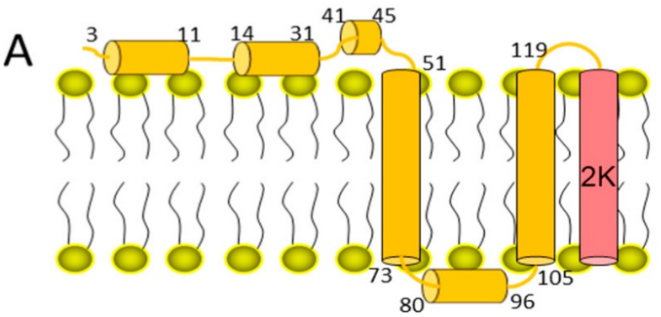


Fig-1: *Domain organization of NS4A.*

The first two domains are present at the membrane surface and one above the surface. Three are embedded in the membrane and one is inside systole. Out of the transmembrane domains (TMDs), it was found that the first TMD, which is made up of 48 amino acids, helps make an amphipathic coil that helps oligomerization happen[6]. The NS4A protein is an important part of the replication complex that is attached to the endoplasmic reticulum membrane[7]. If the amino acid sequences in NS4A are changed it loses its function.

Material and Methods

This research was conducted at Institute of Molecular Biology and Biotechnology, The University of Lahore, Aziz Fatimah Hospital and Allied Hospital Faisalabad during the dengue outbreak of 2022. After taking the IRB approval Ref: No. IEC/272-23 dated 12-09-2023, the temporal sampling method was applied while selecting patients from the dengue wards of both hospitals, and they were con-sented in writing. It was determined that the patients had dengue infection based on the results of a positive polymerase chain reaction (PCR) test for DENV, a positive NS1-antigen test, or positive IgM antibodies for DENV. On Performa, the results of clinical exami-nations, laboratory tests, and other diagnostic proce-dures were documented along with the clinical history and examination findings. Confirmed patients with dengue fever older than 13 years and of both sexes. The study did not include participants with comorbidities like hepatitis, chronic liver illness, typhoid fever, or malaria. Additionally excluded were patients who had dengue shock syndrome (DSS).

120 Blood samples were collected from the dengue patients within 7 days of the onset of symptoms and centrifuged and stored. The GeneJET viral DNA/RNA purification kit (Cat no. K0821) was used to get viral RNA directly from the serum of DENV-positive patients. The extracted RNA was quantified by performing PCR and gel electrophoresis. DENV WGS sequencing was carried out on selected samples on an Ion 510 chip. Data was uploaded to Torrent Suite Server 4.10 once the prepared chip was put onto the Ion XL 5 sequencer for sequencing. EpiData Analysis, a software program developed by the WHO, was used to calculate and summarize the mutation frequencies. Excel analysis was used to check the data for flaws. The viral DNA/RNA purification kit (Cat no. K0821) was used to extract RNA from the samples. After extraction, the RNA was quantified using PCR and gel electrophoresis. DENV WGS sequencing was then performed on selected samples using an Ion 510 chip. Once the chip was prepared and placed onto the Ion XL 5 sequencer, data was uploaded to Torrent Suite Server 4.10 for analysis. To calculate and summarize the mutation frequencies, EpiData Analysis, a software program developed by the WHO, was used. Excel analysis was used to check the data for flaws.

Table 1: *Frequency of mutations in NS4A protein of DENV.*

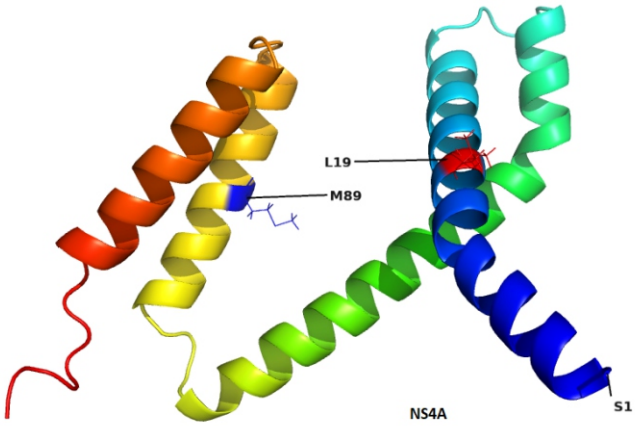
*MUT	*S.14	S.15	S.16	S.17	S.18	S.19	S20	S21	S22	S23	S24	S31	*Freq
V2I	P			P					P			P	4
L8S	P												1
G11E									P				1
Q19L	P			P					P	P	P	P	6
V29I										P			1
G38E	P								P	P			3
G38R										P			1

G38V			P					P		2
D50E								P		1
R76K	P		P			P	P		P	5
V88S								P		1
I89M	P		P		P	P	P		P	6
I89S								P		1
I89V		P								1
A90E								P		1
A93R								P		1
A93V	P		P		P	P	P		P	6
A106G								P	P	2
V2I	P		P			P			P	4
L8S	P									1
G11E						P				1
Q19L	P		P			P	P	P	P	6
G38E	P					P	P			3
G38R							P			1
G38V			P					P		2
D50E								P		1
T51R								P		1
I52K								P		1
I52M						P				1
I52V						P				1
E53G						P				1
E53W								P		1
L55G								P		1
L55M			P							1
M56W								P		1
L57N								P		1
A59W								P		1
I61K								P		1
A62H								P		1
V63F								P		1
T65Q								P		1
G66H								P		1
V68S								P		1
T69P								P		1
F71N								P		1
F72K								P		1
L73T								P		1
S74N								P		1
R76K	P		P			P	P		P	5
I89M	P		P		P	P	P		P	6
I89S								P		1
A93V	P		P		P	P	P		P	6
L95T								P		1
A106G								P	P	2
R127A								P		1
R127G		P								1

Results

After quantification 23 of 120 blood samples were sequenced. A total of 88 different types of mutations with different frequencies in all domains have been detected in NS4A proteins. Those mutations which presented with the most frequency were Q19L, I89M, and A93V (n=6) each R76K (n=5), V2I (n=4), and G38E (n=3). A total of 21 mutations T51R, I52K, I52M, I52V, E53G, E53W, L55G, L55M, M56W, L57N, A59W I61K, A62H, V63F, T65Q, G66H, V68S, T69P, F71N, F72K, and L73T have been detected in the fourth domain of NS4A with different frequencies as shown in **(Table-1).**

The fifth cytosol domain has 18 mutations with different frequencies. A total of 13 mutations have been detected in the sixth membrane embedded domain with 1 and 2 frequencies each. The last K domain has five mutations



at C-terminal regions.

Figure 2. Structure of DENV NS4A protein and location of most common mutations.

In figure 2 Mutation which are very common have been labeled with red (L19) and blue (M89). The NS4A contain three alpha helix inter wingle with each other. Two mutations at position L8S and G11E, have been detected in first surface domain of NS4A. Similar to the first domain, the second domain also harbored two mutations at positions Q19L and V29I respectively while the third smallest domain among all has no mutation.

In **(Table-2)** the effect of Different Mutations is shown on the protein function. In DENV1 serotypes, mutation G38E repeated in 3 samples and A106G in 2 samples affects the protein function. Similarly, in DENV2 sero-types Mutation E122G and R127G were repeated in 1 sample each is affecting protein function.

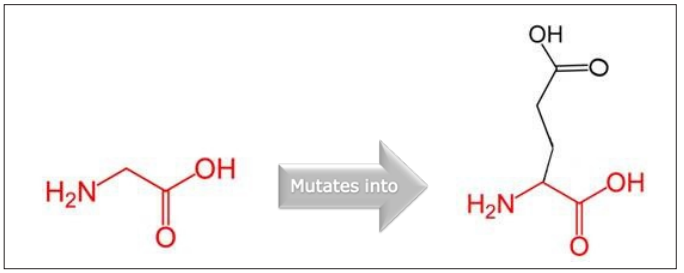


Figure 3: *Schematic structures of the original Glycine (left) into a Glutamic acid (right) amino acid at position 38*

We are taking the mutation G38E into further consideration because of its high frequency and its ability to affect NS4A protein function.

Discussion

In our study a total of 88 different types of mutations with different frequencies in all domains have been detected in NS4A proteins. NS4A protein has seven

Table 2: *Predictions About Mutations on NS4A protein Function.*

Serotype	Mutation	Frequency	Median Sequence Conservation	Sequences Represented At This Position	Protein Function	Prediction Score
DENV1	Q19L	6	3.04	18	Tolerated	0.24
	I89M	6	3.04	33	Tolerated	0.52
	A93V	6	3.04	33	Tolerated	0.34
	R76K	5	3.04	33	Tolerated	1.00
	V2I	4	3.67	33	Tolerated	1.00
	G38E	3	3.04	33	Affect protein function	0.00
	A106G	2	3.04	33	Affect protein function	0.01
DENV2	E122G	1	2.99	29	Affect protein function	0.00
	R127G	1	2.99	29	Affect protein function	0.00

domains. Out of which first two domains are present at the membrane surface and one above the surface. Three are embedded in membrane and one inside systole. In a study it was found that the N-terminal 49 residues of NS4A are found in the cytoplasm, where the N-terminal NS3–4A cleavage site is where the viral protease processes them[6]. Out of the trans membrane domains (TMDs), it was found that the first TMD, which is made up of 48 amino acids, helps make an amphipathic coil that helps oligomerization happen[7]. The NS4A protein is an important part of the replication complex that is attached to the endoplasmic reticulum membrane[8]. If the amino acid sequences in NS4A are changed it loses its function.

Membrane associated NS4A is linked to another protein NS4B by 23aa long conserved signal peptide. Previous study showed that mutations in NS4A (Leu48Ala, Thr54Ala, and Leu60Ala) influences the NS4A interactions with NS4B, abolishing the viral replication while mutations Phe71Ala and Gly75Ala has no effect on interaction of NS4A-NS4B on replication, highlighting the importance of mutations on NS4A-NS4B interaction and replication[9]. Therefore, blocking the interaction of NS4A-NS4B is a good antiviral strategy. In clinical setup dengue virus is diagnosed with clinical presentation but there are different parameters liver function test, serum proteins and viral markers for its diagnosis.[10,11]

Little hydrophobic protein NS4A (16 kDa) is ineffectively caught on, and it is still hazy what part it plays within the viral replication cycle. Kunjin infection (KUNV) NS4A is watched to limit to the assumed destinations of RNA replication and polyprotein preparing and interaction between NS4A and NS.[1] is essential for RNA to replicate proposing that flavivirus NS4A is included in a few steps of viral RNA intensification, conceivably by securing replicase components to intracellular layers[12]. Four anticipated transmembrane portions (pTMSs) make up the minor necessary layer protein known as NS4A. Even though pTMS4, too known as the 2k part, isn't a component of the developed NS4A, when it is cleaved from the developed NS4A and it will acts as a signal peptide for the NS4B localization within the ER12. The first 48 N-terminal set of NS4A serve a basic part in viral RNA's replication, as appeared by the L6E and M10E transformations which crush the both hydrophilic as well as hydrophobic nature of the N-terminal of NS4A. Moreover, when embedded as single changes, these changes had a comparable effect, NS4A interatomic positively with exceedingly bent liposomes, as which has been already illustrated. These two-point changes radically disable this interaction, as appeared by CD spectroscopy[13,14]. NS4A has also been directly linked to the formation of DENV virus replication organelles (vRO) using a method that doesn't depend on replication[15]. It is likely that the placement of NS4A, NS4B, or the NS4A-2K-4B precursor within the membrane leaflet makes it easier for the membrane to bend, which is needed for vRO formation[16].

Knowledge about certain areas of NS4A protein might help in ascertaining the sites where drugs can works against it. A study conducted showed that Helix α4 and the PEPEKQR sequence are good places for drugs to target in NS4A because they are needed for NS4A – 2K cleavage and NS4A–NS4B association, respectively[15].

Conclusion:

The sequencing techniques used in the molecular investigation of DENV's whole genome offer a clear picture of the diversity of the virus in terms of mutations that arise in the many DENV genotype targets. During the course of this analysis, we found that the DENV NS4A protein included a number of different alterations. The results of experiments reveal that mutations can have an effect not only on a virus's ability to replicate but also on its severity, its ability to penetrate a host cell, and its ability to disseminate. On the basis of this genetic heterogeneity, diagnostic procedures and markers can be developed, which may in the future lead to improvements in the treatment of DENV fever.

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Authors Contribution

SM: Conceptualization of Project
RS: Data Collection
SJ: Literature Search
FNT: Statistical Analysis
ZM: Drafting, Revision
AZB: Writing of Manuscript

Assessmentof Patient's Satisfaction Regarding Labor and Delivery Services at Tertiary Care Hospital

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Abstract

Objective: To assess the patient's satisfaction regarding labor and delivery services at tertiary care hospital.

Material and Methods: This hospital based cross sectional study was conducted in postnatal ward of Bahawal Victoria Hospital Bahawalpur. Data was collected using preformed, structured questionnaire from 326 women. Data was entered and analyzed using SPSS version 21. Frequencies and percentages were calculated for qualitative variables and dimensions of satisfaction. Chi-square test was applied to assess the statistical relation between the defined dependent and independent variables. P value < 0.05 was considered as significant.

Results: The proportion of women having very good level of satisfaction was 27.3% while 17.2% had satisfactory level of satisfaction, 34.7% had privacy issues, 57% waited too long to see the doctor. Satisfaction of the respondents was not significantly associated with age of the respondents (p= 0.383) Variables including mother's education (p = 0.008), monthly family income (p = 0.021), family type (p = 0.000) and parity status (p = 0.000) were statistically associated with women's satisfaction.

Conclusion: Healthcare professionals need to understand the expectations the mothers have and provide care that is consistent with those expectations. Health system should be devised to increase maternal satisfaction with health organizations and provide maternal-friendly services.

Key words: Patient satisfaction, obstetric health care, labor, intrapartum care, service quality.

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Introduction

Satisfaction is one of the most frequently reported outcome measures for quality of care and enhanced satisfaction has been identified as a goal for improvement in health care. Women's satisfaction with maternity services specially care during labor and child birth has become increasingly important to health care providers, administrators as well as policy makers (Faruquie et al., 2016). Maternal mortality rate in Pakistan is 276 per 100,000 live births while the share of women delivering at a health facility is 48.2% (Kanwal et al., 2019). Almost two hundred fifty thousand maternal death occurred globally in year 2011 and just 23% of the countries contributed into the 80% of the deaths including Pakistan, India, Ethiopia and Nigeria(Getenet et al., 2018).

Patient satisfaction has now become a fundamental part of facility based management strategies around the world and is the most frequently reported outcome measure for quality of care. The World Health Organization (WHO) place great emphasis on this aspect of patient satisfaction, as it is means of secondary prevention of maternal mortality(WHO, 2019). The mothers who are highly satisfied are more likely to follow the recommendations and prescriptions of the health care provider (Tesfaye et al., 2016) .

A study conducted in Ethiopia revealed that the percentage of completely satisfied women with the services provided to them during their facility visits ranges from

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2.4% to 21%, while among those mothers who were not satisfied the main cause of dissatisfaction was incomplete information related to prescribed medicines and procedures were not adequately explained to them by the health care staff (Wambua et al., 2015). The poorest source of dissatisfaction which was reported by majority i.e. 82% of the study participants was pain control (Atiya, 2016) .

A study which was conducted in India depicted that majority of the women (with 68.7% vaginal birth and 69.2% cesarean birth) were satisfied in general with provision of labor and delivery services. Among the mothers, those who had vaginal birth were least satisfied as they mentioned they were had faced problems in meeting their newborn child, while those who underwent cesarean births were dissatisfied with inadequate postpartum care(Jha et al., 2017).

During the assessment of obstetric care, the influencing factors for client satisfaction include provider's civil attitude, availability of medical drugs while culturally improper care, discourteous services and absence of moral support discourage patients from accessing obstetric care. Providing support to patient along with reassurance is favorable and affect a mother's evaluation of quality of services (Geberu et al., 2019)(Sun et al., 2017). In the past few decades, health care has evolved from service focused to patient centric with more focus on outcomes, and patient satisfaction has been selected as a critical metric to evaluate the service quality in public as well as private sector. In our country, Pakistan, health care organizations have been more focused towards client and efforts are being made but evidence is lacking on patient's perspective (Jha et al., 2019).

The present study was conducted with the aim to evaluate the way in which health services are being delivered with the eyes of consumers so that the gap areas could be identified, and focus could be brought towards deficient areas which ultimately lead to improvement of quality of care.

Material and Methods

This was cross sectional analytical study carried out during the period of June 2019 to December 2019 after taking ethical No. 987/DME/QAMC, Bahawalpur from institutional ethical review committee. The calculated sample size for the study is 326 at 95% confidence level, 5% margin of error and 68.7%[6] anticipated population proportion (satisfaction with services after vaginal delivery). The women of reproductive age (15-45 years) were included in the study with gestational age between 37-41 weeks and those who have had given vaginal birth 6 hours before or caesarean delivery 24 hours before. A preformed and structured questionnaire was used for data collection. The questionnaire incorporates two domains. The first domain comprises of sociodemographic profile while the other include the study variables. The participants were explained the purpose of the study and items of questionnaire were discussed where needed. Total 16 questions were asked to measure satisfaction of the respondents with the services. Response of each question was measured on 5 point Likert Scale, Strongly disagree = 1 , disagree = 2, uncertain = 3 , agree = 4, strongly agree = 5. Composite score of each respondent ranges from 16 – 80. Level of satisfaction on the basis of composite score was divided into three categories. The score > or equal to 65 was categorized as very good, the score with the range of 50-64 was categorized as satisfactory and score of < or equal to 35 was taken as poor.Data was collected from the mothers after obtaining consent. The data collection took about three weeks. Data was entered and analyzed by using statistical package for social sciences (SPSS) version 21.Chi square test was applied to assess the statistical relation between the defined dependent and independent variables. P-value < 0.05 was considered as significant.

Results

Sociodemographic characteristics:

The mean age of respondents was 26.48±6.25 years. The age distribution of the respondents (n=326) showed that the highest proportion of women were between the ages of 21-25 years (35.6%), followed by 26-30 years (25.8%), 15-20 years (16.9%) and 31-35 years (15.9%). Regarding the educational status, 23.9% women were unable to read and write while 45.7% had primary schoo-

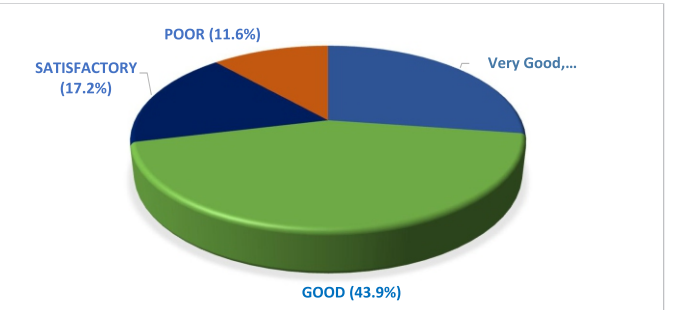

Figure 1: *Patient's satisfaction with Obstetric Services.*

ling. Only 20.8% had secondary education and 9.6% had above secondary education. The monthly income of 83.5% of the families was found to be below RS. 50,000. Majority of the respondents 45.7% were living in the extended family. As far as the parity status was concerned, 29.4% women were primigravida, 49.7% were multipara and 20.9% were grand multipara. As for mode of delivery, the data revealed that 47.8% of the respondents had spontaneous vaginal delivery, 25.1% had instrumental delivery. While 17.6% of the women delivered baby by elective caesarean section and 9.5% of the women underwent emergency caesarean.**(Table 1)** The proportion of respondents whose satisfaction level with obstetric services falling in category "good" was found to be 43.9%, followed by "very good" 27.3%, satisfactory (17.2%) and "poor" 11.6% shown in **(Fig-1)**. Of all the satisfaction dimensions, respondent's satisfaction related to privacy in the ward (34.70%), information about medications (34.60%) and the time spent by health care staff with the patient (38.00%) were

Table 1: *Socio-demographic characteristics of the study subjects.*

Variable	Response category	Frequency	Percentage
Age (in years)	15-20	55	16.9%
	21-25	116	35.6%
	26-30	84	25.8%
	31-35	52	15.9%
	≥36	19	05.8%
Women's education	Illiterate	78	23.9%
	Primary	149	45.7%
	Secondary	68	20.8%
	Above secondary	31	09.6%
Monthly income of the respondent	Below 50,000	272	83.5%
	≥50,000	54	16.5%
Family type of the respondent	Nuclear	78	23.9%
	Extended	149	45.7%
	Polygamous	68	20.8%
Parity of the respondent	Primigravida	96	29.4%
	Multipara	162	49.7%
	Grand multipara	68	20.9%
Mode of delivery in respondent	Spontaneous vaginal	156	47.8%
	Instrumental	82	25.1%
	Elective caesarean	57	17.6%

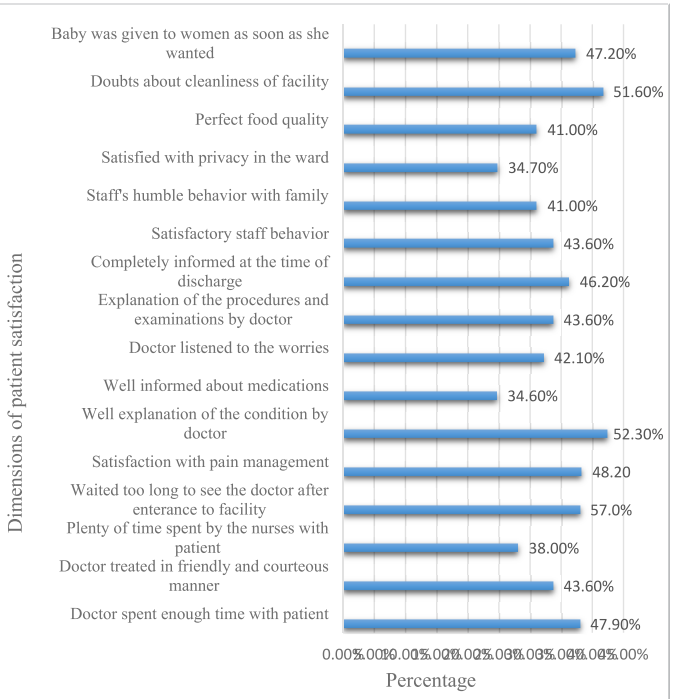

Figure 2: *Proportion of women with dimensions of satisfaction.*

Table 2: *Socio-demographic characteristics associated with women's satisfaction with obstetric services.*

Sociodemographic characteristics	Satisfaction with services				X^2 value	P value
	V.Good	Good	Satisfactory	Poor		
Age						
16-20	16	22	10	07		
21-25	26	60	18	12		
26-30	20	33	18	13	12.806	0.383
31-35	19	23	07	03		
36-40	08	05	03	03		
Women's education						
Unable to read&write	22	40	10	06		
Primary	41	60	23	25	22.24	0.008
Secondary	12	30	20	06		
Above secondary	14	13	03	01		
Monthly income						
Below 50,000	75	125	39	33	09.709	0.021
≥50,000	14	18	17	05		
Family type						
Nuclear	21	39	07	29		
Extended	48	75	38	01	56.441	0.0000
Polygamous	20	29	11	08		
Parity						
Primigravida	33	41	11	04		
Multipara	42	63	24	30	26.86	0.000
Grand Multipara	14	39	21	04		
Mode of delivery						
Spontaneous vaginal	36	77	24	19		
Instrumental	24	28	17	13	11.75	0.227
Elective caesarean	19	27	07	04		
Emergency caesarean	10	11	08	02		

found to be first least three values shown in **(Fig-2)**. Women's level of satisfaction with obstetric services was significantly associated with education level of the respondents (p = 0.008), family income (p = 0.021), family type (p = 0.0000), parity status of the women (p = 0.0000) while there is no association between the age of the women and the mode of delivery with levels of satisfaction with services (p = 0.383, p = 0.227 respectively) shown in **(Table 2)**.

Discussion

This paper presents a study that estimated the level of women's satisfaction with labor and delivery services provided at Bahwal Victoria Hospital, Bahawalpur. In our study, overall level of satisfaction with intrapartum care was good as majority(43.9%) of the respondents were in category good. While 27.3% of the respondents were completely satisfied. These findings were found to be similar to results of a survey conducted in Sudan where 47.5% of the patients were satisfied with the quality of intrapartum services (Gitobu et al., 2018). However, the findings are less than other studies conducted in Amhara region, Serbian hospital and Brazil where mother's satisfaction level with maternal and child care was found to be 61.9%, 81% and 67% respectively (Takács et al., 2015)(Ali Mohamed Ahmed Alawad et al., 2015).The reason of this difference might be attributed to the different study settings and population background. The overall satisfaction score of the present study is in concurrence with other studies conducted in Nepal where intrapartum satisfaction is 56% (Paudel et al., 2015).

The study measured patient satisfaction using 16 items of maternal satisfaction. Among the study respondents, 52% were satisfied with cleanliness of the facility which is concurrent with findings of a survey showing that 54.7% of the mothers responded with either 4 or 5 the points (satisfaction score, while higher than a study conducted in Sudan which showed 47.5% satisfaction percentage among the study respondents (Atiya, 2016). A study conducted in Lady Reading Hospital Peshawar illustrated that only 20% of the women said that cleanliness of the facility is satisfactory. This indicate that healthcare organizations must take applicable measures to address this issue. The results of present study depicted that privacy issues were not adequately handled as only 34.7% of the respondents were completely satisfied with privacy in the wards. These are in concurrence with findings of a survey, assessing level of maternal satisfaction with free services in Kenyan public health facilities, which showed that only 39.6% of the women were satisfied with the level of privacy in wards and 23.3% responded negatively (Gitobu et al., 2018).

In our study, the satisfaction with labor and delivery services was found to be significantly associated with educational level of the respondents (p=0.008). Comparatively, studies conducted in Ethiopia and Serbia portrayed that higher the educational status was negatively associated with maternal satisfaction (Woldeyohanes et al., 2015) (Dewana et al., 2016). However, results of our study are similar to another survey which depicted that women with no formal education were more satisfied when compared to those who had basic secondary level of education(Panth & Kafle, 2018). The difference could be attributed to higher level of expectations of mother as well as awareness with the provision of health services in facilities (Dewana et al., 2016)(Asrat et al., 2018).

Our study showed that satisfaction with labor and delivery services was found to be significantly associated with the parity of the respondents (p=0.0000). This is similar to a study which showed that patient satisfaction increases with number of children, but contrast with another survey which showed no statistical association between maternal satisfaction and obstetric characteristics despite of the fact that multiparous mothers were over 2 times more likely to be contented and satisfied with labor and delivery services when compared to primiparous (Nuri et al., 2019) (Takács et al., 2015).

Conclusion

Healthcare professionals need to understand the expectations the mothers have and provide care that is consistent with those expectations. Health system should be devised to increase maternal satisfaction with health organizations and provide maternal-friendly services.

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Authors Contribution

AA: Conceptualization of Project
HA: Data Collection
NB: Literature Search
AF: Statistical Analysis
NA: Drafting, Revision
AM: Writing of Manuscript

Vitamin D Supplementation Reduce Serum Inflammatory Biomarker (TNF-α) Levels in Male Albino Mice on High Fat Diet

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Abstract

Objective: The objective of this study was to determine whether vitamin D supplementation affects the levels of inflammatory biomarker (TNF- alpha) in mice (male albino) or not?

Material and Methods: This was a Quasi experimental study. Sample size was 90. Consecutive, nonprobability sampling technique was used. Mice were randomly divided into three groups, each group containing 30 mice. This study was carried out for 6 weeks. Normal diet was administered to mice of group A. High fat diet was administered to group B mice. High fat diet & vitamin D supplementation were given to group C mice. Vitamin D was administered to group C mice through oral gavage (100ng/kg/day) for 6 weeks. Terminal blood sampling technique was used to collect blood sample. ELISA technique was used to determine levels of TNF-α (Tumor necrosis factor alpha). SPSS version 20 was used for analysis of data.

Results: Group B mice had significantly raised serum TNF-α levels as compared to group A mice. Group C mice had significantly reduced levels of tumor necrosis factor-alpha as compared to mice of group B and mice of group A.

Conclusion: Vitamin D supplementation might be beneficial by reducing levels of serum TNF-α levels in mice on high fat diet.

Keywords: Vitamin D, High fat diet, Hyperlipidemia, TNF-α.

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Introduction

High fat diet induces inflammation throughout the body like brain (in hypothalamus by blunting insulin sensitivity and leptin pathway), liver, fat cells, intestine and skeletal muscles. HFD alters gut microbiota and decreases its diversity. It increases bacteroides and decreases fermicutes in the gut. This shift of gut microbiota activates toll like receptors (TLR) signaling pathway, so, there is increased permeability of intestinal epithelium to bacterial endotoxins like lipopolysaccharides (LPS) which are then delivered to circulation.[1] Dietary free fatty acids also activate intestinal epithelial calls. Lipopolysaccharides (LPS), free fatty acid (FFA) stimulate intestinal cells and lead to the production of TNF-α. All these substances (LPS, FFA, inflammatory cytokines) are delivered through circulation to various parts of body, thus inducing inflammation there.[1] These substances activate toll like receptors (TLR) in macrophages and convert them to M1 type macrophages, which release inflammatory cytokines like TNF-α, IL-12 and IL-6.[1] Activated macrophages reach adipose tissues, muscles, blood vessels and pancreas and induce inflammation there. Increased CD8+-T cells in adipose

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tissues lead to macrophages accumulation there, so adipose tissue don't store lipids because of HFD induced stress rather lipids are accumulated in the peripheral tissues. This ectopic accumulation of lipids leads to increased expression of inflammatory mediators and macrophage recruitment there thus further promoting inflammation.[1] T cells ($CD4^+$ and $CD8^+$) are abundant in obese adipose tissues. $CD4^+$T (cluster of differentiation 4^+T cells) cells are destined to become Th1 and Th17 cells which are proinflammatory.[2] Adipose tissue Th2 cells attenuate inflammation though they are less in number.[3] New studies have identified a hematopoietic stem cell (HSC) derived cell called circulating fibroblast precursors (CFP) which have increased expression of CD45 and discoidin domain receptor 2 (DDR2). DDR2 has a role in the activation of immune cells. $CD45^+$ $DDR2^+$ cells reside inflammatory cells like adipocytes. They elicit increased production of inflammatory cytokines like interferon gamma (INF-γ), IL-6 and TNF-α by $CD4^+$ T cells.[2]

Expansion of adipose tissue results in hypoxia due to increased distance between adipose tissue and blood vessels. This hypoxia induces the expression of a factor (HIF-1, hypoxia inducible factor-1). HIF-1 is responsible for negative effects of obesity. Hypoxia leads to cell death and fibrosis. Macrophages infiltrate to eat up dead cells. There is abundance of M1 type macrophages which lead to production of TNF-α and IL-6.[3] Vitamin D binds with vitamin D receptors in nucleus. Vitamin D and VDR bind with retinoid X receptors. This whole complex induces or suppresses the activation of various genes on DNA.4 Vitamin D has an immune modulatory & anti-inflammatory role too[5,6]. Many immune cells express vitamin D receptors (VDR). Vitamin D increases regulatory T cell production and polarizes the differentiation of T-helper cells into type-2 helper cells rather than Th1 and Th17 types.7 Helper-2 cells have anti-inflammatory properties while helper-1 cells are pro-inflammatory. Dendritic cells modify antigen and present it to T cells. T lymphocytes become either inflammatory or anti- inflammatory cells depending upon the cytokines secreted by dendritic cells. Vitamin D converts dendritic cells (DC) to tolerant dendritic cells which produce anti-inflammatory cytokines (IL-10) rather than inflammatory ones (TNF-α, INF-γ). Metabolic reprogramming by switching towards glycolysis and PI3K/Akt/mTOR pathway (phosphatidylinositol-3-kinase/protein kinase B/mammalian target of rapamycin) are necessary for the production of tolerant dendritic cells. Indole amine 2,3 dioxygenase (IDO) induction on dendritic cells is also necessary for the production of tolerant dendritic cells. All these mechanisms are regulated by vitamin D.[8] Vitamin D promotes polarization of macrophages to M2 phenotype (anti-inflammatory phenotype which produces IL-10) rather than M1 phenotype. These M1 type macrophages release inflammatory cytokines like IL-6, IL-12, TNF-α and differentiate Th cells to Th1 and Th17 subtypes. In macrophages, nuclear factor kappa B (NFκB) up regulates miR-155 which inhibits suppressors of cytokine signaling (SOCS) proteins to induce inflammation. Vitamin D activates thioestrase superfamily member 4 gene (THEM4) and results in the inhibition of NF-κB signaling pathway to activate suppressor of cytokine signaling (SOCS) and reduces inflammation by decreasing the production of various inflammatory cytokines.[8] (Fig-1) Vitamin D is required for keeping CD8+ T cells quiescent. Thus, decreased activation of CD8+ T lymphocytes leads to decreased production of inflammatory cytokines (TNF-α and INF-γ).[8] (Fig-2)

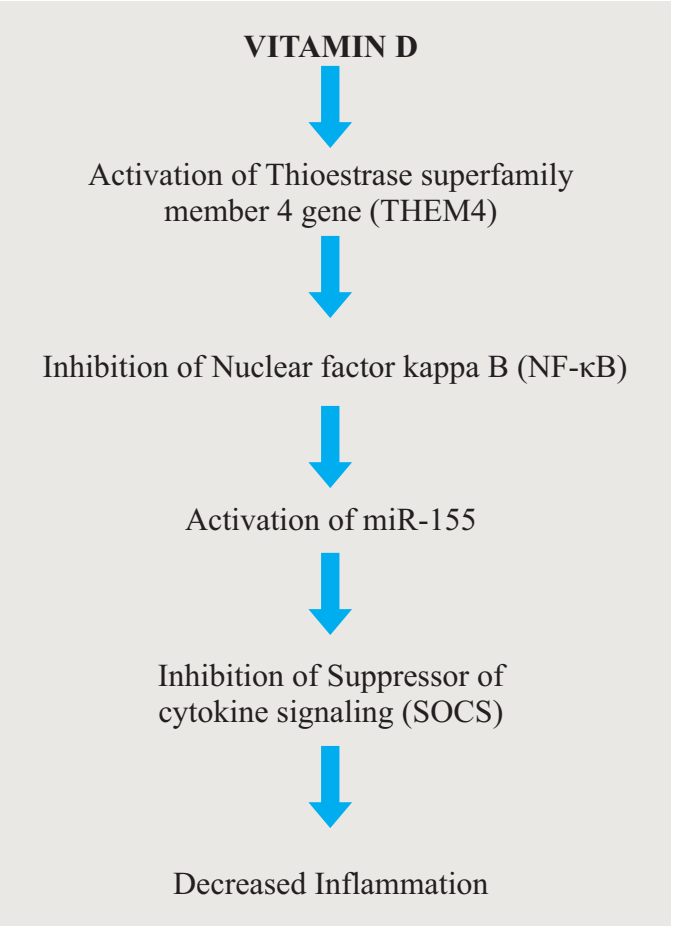

Figure 1: *Anti- inflammatory role of vitamin D*[8]

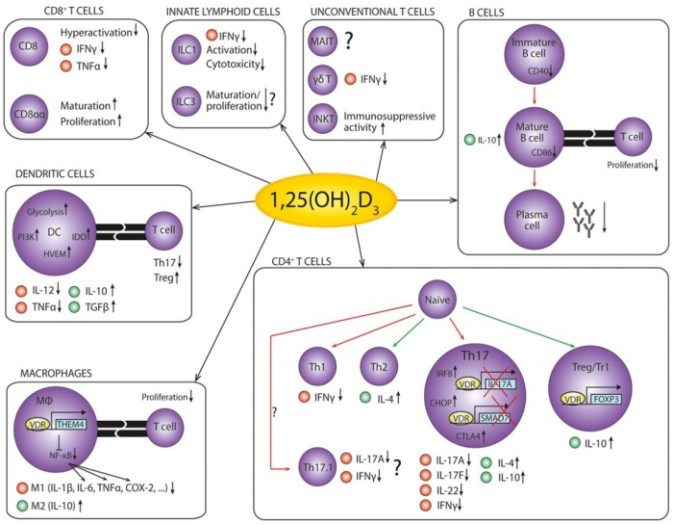

Fig-2: *Immune modulation by vitamin D*[8]

- *red dots are for down regulation*
- *green dots are for up regulation*

Material and Methods

It was a Quasi experimental study conducted at Department of Physiology Akhtar Saeed Medical and Dental College (AMDC), Lahore from February 2021 to November 2021. After taking approval from Ethical Committee IRB No 207 dated 22-11-2017. Non probability consecutive sampling technique was used. University of Veterinary and Animal Sciences, Lahore provided 90 male albino mice. Mice were 8-10 weeks of age. One week was given for acclimatization. Lottery method was used to divide mice into 3 groups randomly.

Group A (Normal diet group, n=30) mice were raised on normal diet for 6 weeks. (11% kcal from fat)

Group B (High fat diet group, n=30) Mice were administered high fat diet9 for a duration of 6 weeks. (44% kcal from fat)

Group C (high fat diet plus vitamin D group, n=30) mice were administered high fat diet and vitamin D for a duration of 6 weeks.

100ng/kg/day[10,11] was the dose of vitamin D. It was administered through oral gavage tube for 6 weeks. Weight was recorded once every week to calculate the weekly dose of vitamin D.

Bood samples were collected by terminal cardiac puncture method. Blood samples were centrifuged to separate the serum and were kept at -20°C until ELISA technique was applied.

SPSS version 20 was used for analysis of data. Mean values and standard deviations were recorded. One way ANOVA was used to compare the results of three groups. Post hoc Tukey's test was used to see the significance of difference amongst three groups.

Results were scribed as mean ± SD.

p value ≤ 0.005 was taken significant.

p value ≤ 0.001 was taken highly significant.

Results

Group B mice had significantly raised serum TNF-α levels as compared to group A mice. Group C mice had a significant reduction in the levels of tumor necrosis factor alpha as compared to group B and group A mice. (Table-1&2)

Table 1: *Serum TNF-α levels of normal diet group (A), high fat diet group (B) and high fat diet plus vitamin D group(C)-One way ANOVA*

Parameter	Group A (normal diet) (number=30)	Group B (high fat diet) (number=30)	Group C (high fat diet +vitamin D) (number=30)	p value
Serum TNF _α (pg/ml)	23.55±2.39	46.11±7.45	18.14±4.84	0.000 **

*** p value ≤ 0.001 was taken highly significant.*

Table 2: *Serum TNF-α levels among normal diet group (A), high fat diet group (B) and high fat diet plus vitamin group (C)- Post hoc Tukey's test*

Parameter	Comparison of the groups		p value
Serum tumor necrosis factor alpha (pg/ml)	A	B	0.000**
	B	C	0.000**
	C	A	0.000**

*** p value ≤0.001 was taken highly significant.*

Discussion

Vitamin D has anti-inflammatory effect. In one study, vitamin D reduced cardiac hypertrophy by decreasing TNF-α expression in rats' heart and blocking NF-KB/P65 signalling pathway.[12]

El-Boshy et al[13] showed that vitamin D reduced inflammation throughout the body and it had anti oxidative effects too in para-cetamol toxicity in rats. They showed that paracetamol toxicity caused markedly elevated levels of hepatorenal markers and increased level of inflammatory biomarkers including TNF-α, whereas anti-inflammatory and anti-oxidative biomarkers were reduced significantly. Vitamin D, when taken prophylactically or therapeutically reduced inflammation. Results were more marked in prophylactic vitamin D

group than therapeutic vitamin D group. In our study, we used high fat diet model to induce inflammation and vitamin D was used to reduce fat induced inflammation. High fat diet elevated TNF-α levels while vitamin D reduced it.

In another study[14], macrophages were treated with vitamin D and then exposed to lipopolysaccharide (LPS) challenge. RNA expression of VDR, tumor necrosis factor-alpha and another nuclear factor (NF-κB) in macrophages was reduced in vitamin D treated unstimulated cells as well as vitamin D treated LPS stimulated cells. TNF-α production was also reduced in both groups. This study showed that vitamin D had anti-inflammatory action both in normal and pro-inflammatory conditions by reducing TNF-α production by macrophages and decreased expression of signaling proteins.[14]

Dadaei et al[15] showed that vitamin D supplementation in inflammatory bowel disease (IBD) patients for 12 weeks reduced serum TNF-α levels but results were not significant statistically. Oliveira Brito et al[16] demonstrated that vitamin D had a beneficial effect in reducing inflammation induced by monocytes in chronic kidney disease patients. They incubated monocytes with or without vitamin D for 24 hours, then monocytes were treated with serum of healthy or uremic patients. In uremic monocyte pool treated with vitamin D, expression of toll like receptor (TLR4), monocyte chemo attractant protein (MCP-1) and cathelicidin protein levels were decreased. Uremic pool had increased levels of TNF-α and other inflammatory cytokines but vitamin D in the presence of uremia did not affect TNF-α levels. There was no difference in the activity of nuclear factor kappa B (NF-κB) in both groups. Vitamin D decreased TLR4 expression but it had no effect on tumor necrosis factor-alpha levels or NF-κB levels probably due to a very short duration of incubation period (24 hours). In another study, WD obese rats had increased weight, BMI and high levels of TNF- α and leptin. Vitamin D supplementation reduced body weight, BMI, TNF- α and leptin levels.[17]

Conclusion

Vitamin D supplementation may prove beneficial by lowering levels of inflammatory cytokine TNF-α in male albino mice on high fat diet.

Conflict of Interest *None*

Funding Source *None*

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Authors Contribution

CN: Conceptualization of Project
CN: Data Collection
MS: Literature Search
MN: Statistical Analysis
NN: Drafting, Revision
ZH: Writing of Manuscript

Assessment of Changes in Dentoskeletal Parameters Among Class-II Subjects Following Treatment with the Herbst Appliance: A Longitudinal Study

Khurram Shahzad,[1] Omair Anjum,[2] Muhammad Farhan Khan,[3] Khurram Nadeem,[4] Asad Mahmood,[5] Junaid Israr Ahmed Khan[6]

Abstract

Objective: The purpose of this longitudinal study was to investigate dentoskeletal parameter changes in Class II patients after treatment with the Herbst appliance, therefore offering insights into treatment efficacy and results

Material and Methods: A prospective observational design was employed at the Orthodontic Department of Children's Hospital and Institute of Child Health & Avicenna dental College, Lahore in Lahore, Fifty patients who met the inclusion criteria completed pre-treatment assessments, Herbst appliance therapy, and post-treatment evaluations. The data collection includes demographic information, baseline dentoskeletal parameters, and post-treatment measures.

Results: The study included 50 cases, with 34% (n=17) aged 11-12 years and 66% (n=33) aged 13-14 years. Pre-treatment values for SNA were 82.08±0.97, SNB was 75.12±1.32, and IMPA was 95.82±2.32. Post-treatment, SNA decreased to 80.8±1.16, SNB increased to 76.34±1.44, and IMPA rose to 100.62±2.83. Mean differences post-treatment were -1.28±0.61 for SNA, 1.22±0.55 for SNB, and 4.8±1.31 for IMPA, with p-values of 0.07, 0.01, and 0.01 respectively, indicating significant mandibular advancement and lower incisor inclination.

Conclusion: Herbst appliance therapy significantly improved dentoskeletal parameters in skeletal Class II patients, particularly enhancing mandibular position and dental aesthetics. These findings underscore the importance of tailored treatment strategies for optimal orthodontic outcomes.

Keywords: class II malocclusion, dentoskeletal parameters, functional appliance, herbst appliance

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Introduction

Functional appliances are considered to be a very efficient orthodontic therapy, especially for skeletal Class II malocclusions with mandibular retrognathism.[1] Because of their skeletal structure, these malocclusions present difficulties, sometimes demanding orthognathic surgery after the completion of growth, to correct substantial skeletal deformities.[2] Functional appliances like the Herbst appliance offer potential opportunities for dentoskeletal improvements without surgical intervention.[3] Recent research has focused on experiments conducted on mandibular protrusion in developing animals, yielding insights into condylar growth stimulation and glenoid fossa remodeling.[4] The Herbst appliance, known for its effectiveness in Class II malocclusion treatment, has attracted attention for its ability to cause dentoskeletal changes in young adults. Fixed

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functional appliances [FFA], such as the Herbst, require less active patient engagement than removable functional appliances, which rely largely on patient compliance.[5] Furthermore, Class II division 1 malocclusion, the most common sub-classification of malocclusion,[6] has been addressed using a variety of therapeutic approaches. Extraction of the upper first permanent molars has been proposed as a possible therapeutic option, especially in situations of extensive molar damage or previous extractions. Studies have found considerable improvements in the PAR index using this treatment plan, demonstrating its usefulness in obtaining ideal dental results.[7] The Herbst appliance is extensively used in orthodontics for Class II correction, providing benefits like relatively shorter treatment periods and a lower reliance on patient compliance.[5] Its effects include both skeletal and dental changes dental, such as anterior protrusion of the lower dental arch and distalization of the upper arch.[8] However, a variety of skeletal changes are observed among people, which are influenced by characteristics such as gender and treatment duration. One prominent disadvantage of the Herbst therapy is the proclination of the lower incisors, which is caused by the stresses imposed by the appliance.[9] Despite this, the Herbst appliance has therapeutic advantages over other functional appliances, such as continuous 24-hour therapy and no interference with speech.[10] In current dentofacial orthopedics, Herbst appliance therapy followed by a conventional fixed appliance treatment phase, has proven to be an effective strategy for treating severe Class II malocclusions.[11] The first phase, the Herbst appliance phase accomplishes Class II correction, while the second phase of fixed appliance concentrates on ultimate tooth alignment, yielding total treatment results.[12] The current study assesses the dentoskeletal effects of Herbst appliance therapy in skeletal Class II, Class II division 1, mandibular retrognathic individuals. This study aims to provide clinicians with additional data for future differential treatment planning by combining insights from previous research and clinical observations, thereby contributing to the refinement of orthodontic strategies for Class II malocclusions and potentially reducing reliance on surgical interventions.

Material and Methods

This prospective observational study, carried out at the Orthodontic Department of Children's Hospital and Institute of Child Health & Avicenna dental College, Lahore in Lahore, Pakistan from October 2016 till March 2017. IRB No was CPSP/REU/DSG-2014-075-1438 dated 11-06-2016. The sample size was 50 patients, which were carefully calculated to assure statistical significance. This calculation was based on a 95% confidence level and 80% power of test, with an expected mean change in SNA (sagittal relationship of the maxilla to the cranial base) in the study participants of -1.35±1.113. The sample selection procedure was carefully planned to ensure the study's reliability. Non-probability sequential sampling was used, which ensured a methodical app-roach to participant inclusion. Inclusion criteria were carefully defined to capture a specific subset of patients: those with a Skeletal Class II relationship with an ANB angle greater than 4° and an SNB angle less than 78°, aged 11 to 14 years, presenting with an overjet of at least 5 mm, and possessing a specific SN-MP angle range of 32°± 4°. Additionally, bilateral Class II molar and canine relations were required for inclusion. In contrast, patients who had previously received orthodontic treatment were excluded, as well as were those born with missing or removed permanent teeth, with the exception of third molars. Patients with syndromes or skeletal abnormalities were also excluded to ensure that the research cohort was homogenous and devoid of confounding characteristics that may bias the results. The data collection began with the identification of 50 suitable children from the orthodontic clinic at The Children's Hospital in Lahore. The informed consent process was carried out, ensuring that participants were fully informed about their participation in the study and the processes involved. The Institutional Review Board provided ethical approval to ensure that the research was conducted in accordance with the highest ethical standards. Each participant's basic demographic information, such as name, age, residence, and contact information, was thoroughly documented to guarantee proper data recording. Baseline values for dentoskeletal parameters were rigorously gathered to lay the groundwork for pre-treatment evaluation. The Herbst appliance, especially Banded Design, was used during the active therapy period. A wax construction bite was recorded with the jaw in a forward posture, resulting in edge-to-edge incisors. When a normal or corrected overjet was achieved, indicating the finish of active therapy, post-treatment data were methodically gathered.

Results

This study included a total of 50 cases/patients that

satisfied strict inclusion/exclusion criteria in order to reveal the Dentoskeletal parameter changes in skeletal Class II patients treated with the Herbst appliance, offering critical insights into orthodontic care techniques. The age distribution study indicated a large proportion of patients aged 11-12 years (34%, n=17), emphasizing the need of early intervention in treating Class II malocclusions during the growth phase **(Table-1).** The remaining 66% (n=33) in the 13-14 age group highlight the possible diversity in treatment results dependent on

Table 1: *Age and Gender distribution (n=50)*

Variable	Categories	N (%)
Age	11-12	17 (34%)
	13-14	33 (66%)
	Mean Age (S.D)	12.54 (1.41)
Gender	Male	19 (38%)
	Female	31 (62%)

developmental stage. The gender distribution revealed a greater number of female participants (62%, n=31) than males (38%, n=19), indicating possible gender-related variations in orthodontic treatment responses, which have been seen in earlier study but require more exploration.

The mean changes in Dentoskeletal parameters pre- and post-treatment with the Herbst appliance indicated

Table 2: *Comparison of mean change in dentoskeletal parameters in class II patients treated by twin block (n=50)*

	Parameters	Pre-Treatment		Post Treatment		Difference		P value
		Mean	SD	Mean	SD	Mean	SD	
Pre-treatment	SNA	82.08	0.97	80.8	1.16	-1.28	0.61	0.07
	SNB	75.12	1.32	76.34	1.44	1.22	0.55	0.01
	IMPA	95.82	2.32	100.62	2.83	4.8	1.31	0.01

interesting results. Pre-treatment values for SNA (82.08 ± 0.97) and SNB (75.12±1.32) were within predicted ranges for Class II malocclusions, suggesting a baseline presentation in our study population. The pre-Treatment IMPA score (95.82±2.32), indicates incisor inclination, a key indicator in evaluating treatment success for mandibular advancement**(Table-2).**

Post-treatment evaluations revealed considerable changes. The decrease in SNA (80.8±1.16) post-treatment indicates a positive shift in maxillary position, which aligns with treatment aims to rectify Class II connections. Increased SNB (76.34±1.44) leads to better mandibular placement and facial harmony. The significant rise in IMPA post-treatment (100.62±2.83) suggests an increase in lower incisor inclination.

The mean difference analysis after treatment revealed complex findings. The SNA mean difference (-1.28 ± 0.61) was not statistically significant (p-value: 0.07), indicating minimal changes in maxillary position. However, the SNB mean difference (1.22±0.55) was highly significant (p-value: 0.01), indicating significant mandibular advancement, improving dental and facial aesthetics — an important outcome in Class II correction. The Herbst device increases lower incisor inclination, as evidenced by a substantial IMPA mean difference (4.8±1.31, p-value = 0.01).

Stratification by age and gender allowed for a more thorough study, finding possible moderators impacting

Table 3: *Stratification for data regarding mean difference after treatment with regards to age (n=100)*

	Parameters	Age: 11-12 years		P value	Age: 13-14 years		P value
		Mean	SD		Mean	SD	
Mean difference	SNA	-1.30	0.64	0.23	-1.24	0.56	0.10
	SNB	1.18	0.53	0.001	1.29	0.59	0.001
	IMPA	4.79	1.11	0.001	4.82	1.67	0.001

treatment results. The observed mean differences highlight the multidimensional nature of Class II correction, underlining the importance of individualized treatment approaches based on patient characteristics and developmental phases. These findings have practical relevance, helping orthodontists optimize treatment options for Class II malocclusions, particularly in young patients receiving Herbst appliance therapy **(Table-3).**

Discussion

Our study explores into the intricacies of dentoskeletal alterations caused by the Herbst appliance in Class II malocclusions, providing insight into its therapeutic effectiveness and implications for orthodontic treatment. One important issue investigated is the best time to begin functional appliance therapy. McNamara et al. (2020) highlight the need of timely therapy with the pubertal growth spurt to optimize therapeutic effects.[13] The gender and age-related distributions in our study were carefully adjusted to ensure a strong comparison between pre-treatment and post treatment measurements. According to Carneiro Chagas Tanus (2022), patients with smaller and retrusive mandibles react better to functional

appliance therapy, which is an important component in our treatment outcome study.[14] The methodology provides a targeted evaluation of the Herbst appliance's influence on dentoskeletal parameters, yielding useful insights into its therapeutic value for Class II malocclusion. Analysis of sagittal skeletal changes reveals intriguing findings on maxillary and mandibular changes caused by the Herbst device. While no substantial influence on maxillary development was observed, the appliance revealed considerable improvements in mandibular positioning, which were consistent with treatment goals for Class II correction. These findings are consistent with previous research demonstrating the Herbst appliance's usefulness in encouraging mandibular growth and repositioning, particularly in cases with mandibular retrusion.[15,16] The Herbst appliance's substantial improvements in mandibular dimensions contribute greatly to occlusal relationship and facial esthetics, strengthening its function as an effective treatment option for Class II malocclusions.[17] Moving on to dental changes caused by the Herbst device, this appliance focuses on occlusal relationships, molar position and incisor protrusion.[18] These alterations, while important for occlusal correction, also affect vertical dimensions and anterior-posterior tooth placements. Dental alterations, particularly in overjet and molar correction percentages, demonstrated a balanced role for skeletal and dental components in treatment results.[19] Herbst therapy resulted in significant distalization of upper molars and lower incisor protrusion, consistent with existing data on Herbst-induced dental changes.[20] The study's findings support previous literature on the Herbst appliance's influence on dental parameters and emphasize the importance of rigorous and careful monitoring of dental changes during therapy. Vertical skeletal changes, such as increased lower face heights and ramus elongation, demonstrate the Herbst appliance's overall impact on facial morphology and mandibular development.[21] These adjustments not only enhance aesthetics, but also help to stabilize occlusal relation and long-term treatment outcomes.

The study's strengths include its prospective observational design, which allows for long-term data collection and assessment of treatment results in a real-world clinical context. The addition of a well-defined sample size derived using statistical power and confidence levels improves the study's robustness. Furthermore, using consistent data collecting techniques and verified measuring tools improves the reliability and validity of the results. Furthermore, the stratification of data by age and gender aids in the management of potential confounding variables, adding to the study's scientific strength. However, there are certain Limitations to consider. The study's six-month length may not capture long-term therapy effects, particularly skeletal alterations that may continue to evolve after this time period. The use of non-probability sequential sampling may add selection bias, reducing the generalizability of the results to a larger sample. Furthermore, the study's emphasis on dentoskeletal characteristics may ignore other essential considerations, such as patient-reported results or potential therapeutic side effects. Future study might address these limitations by including longer follow-up periods, using randomization to select samples, and conducting a more detailed assessment of treatment results.

Conclusion

The study's findings provide important insights into the Herbst appliance's multifarious impact on dentoskeletal parameters in Class II malocclusions. This study makes a substantial contribution to our understanding of functional appliance treatments and their personalized applications in orthodontic practice by matching treatment scheduling with growth spurt stages and using a thorough approach.

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Funding source *None*

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KS: Conceptualization of Project
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Exploring the Factors Associated with Needle Stick Injuries Among Healthcare Workers at Tertiary Care Hospital

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Abstract

Objective: To determine the frequency of Needle stick injuries (NSIs) and their associated factors among health care workers (HCWs).

Material and Methods: A descriptive cross-sectional study was conducted among HCWs who were working at Nishtar Hospital Multan from 19-02-2023 to 1-12-2023. After approval from the institutional ethical committee, data was collected by face-to-face interviews with one hundred and ninety-five study participants selected through a non-probability convenient sampling technique. Data analysis, including descriptive and analytical statistics was carried out using SPSS Version 26.

Results: Out of 195 study participants, the frequency of NSI was 129 (66.2%), 187 (95.9%) had awareness of NSI. The most common factor responsible for NSI was work overload (26.7%). The majority of the participants (65.6%) did not attend any needle safety prevention workshop in the past six months. Practicing in medicine and surgery wards, not wearing a pair of gloves, the time of injury and not attending any workshop were statistically significant factors associated with NSI among HCWs.

Conclusion: In our study, the frequency of NSI was high among HCWs. It is critical to address this issue and train the HCWs by setting up regular training sessions for them due to their poor practices of safety devices and personal protective equipment, inadequate post-exposure response, underreporting response, and lack of awareness of these hazards.

Keywords: Needle stick injury, Risk factors, Healthcare workers, Tertiary care hospital.

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Introduction

In hospitals and other medical facilities, sharp injuries are common and can have serious consequences.[1] Needle stick injuries (NSIs) are inadvertent bodily piercings caused by contaminated instruments including needles, ampoules, and lancets used in healthcare settings.[2]

According to the World Health Organization, annually 3 million healthcare workers (HCWs) worldwide are estimated to be exposed to blood-borne viruses. This led to the development of HBV in 2 million, HCV and HIV in 900,000 and 170,000 HCWs respectively, with the vast majority of incidents (90%) occurring in developing nations.[3]

NSIs besides physical sufferings also cause psychological anguish, dread, tension, and worry in HCWs. This leads to an increase in absences from work and has a direct detrimental impact on health care services.[4] In addition to this, the cost of their medical care, blood tests, and lost workdays places a heavy burden on the health care system.[5] It has been estimated that the cost of each NSI case to the healthcare system is between 650 and 750 USD, including direct and indirect costs.[6] Many factors enhance the risk of needle stick injury.

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The majority of the time, healthcare staff's flagrant negligence and dangerous practices are the reasons for needle stick injuries. According to experts, no single safety policy can be relied upon to be effective in all circumstances.[2] Moreover the risk of infection to the employee following NSIs depends on the blood-borne pathogens implicated, the degree of the injury, the employee's immune system, and the use of appropriate and effective prophylaxis after injury.[7]

Currently, there is a paucity of data regarding the true estimates of needle stick injuries, making it difficult to assess the true scope of the issue in Pakistan. So far studies conducted in Pakistan show the variability of prevalence from 37.5% to 71.6%.[8,9,10] Therefore it is vital to comprehensively identify and understand all relevant factors that are playing a key role in its prevalence including those that may be undercounted or underestimated. NSI preventative guidelines and healthcare curricula will be more effectively structured if the gaps in knowledge and the differences between knowledge and practice are recognized. This study will help in identifying areas for practice improvement and keeping these variables in consideration can help in developing effective preventive strategies for healthcare workers.

Material and Methods

After institutional review board permission, this descriptive cross-sectional study was conducted in Nishtar hospital Multan. After the taking approval from ethical committee IRB No 4548 dated 25-02-2023. Our study population comprised doctors who were included in patient care and willing to parti-cipate working as house officers, postgraduate residents and consultants in the Medicine, Surgery, Gynecological & Obstetric and Pediatric departments. Those who were in administrative posts in these departments and not willing to participate were excluded from our study. The calculated sample size was 195 with a 95% confidence level, a margin of error d=7% and prevalence p=55%[11] which was calculated by the following formula $n=z^2\ pq/d^2$. Data was collected through non-probability convenient sampling technique. Confidentiality was guaranteed and informed verbal consent was obtained. A self-designed questionnaire with closed-ended questions was used for in-person interviews. The first part of the questionnaire comprised of participant's demographic information including age and gender and the next part includes questions related to the circumstances surrounding needle stick injuries, risk factors for NSI, prior knowledge from any training in preventing needle injuries, pre-exposure immunization status, post-exposure response: reporting needle stick injuries and following protocol in case of injury. Data was analyzed by using IBM SPSS statistics 26. The result was presented in the form of tables. A Chi-square test was performed to assess the association of factors with needle stick injuries. A p value of <0.05 was taken as statistically significant.

Results

In our study, the frequency of needle stick injury was 66.2%.The majority of the study participants were female. Most of the participants were in the age group 20 to 25 years. Half of the research participants were aware of universal precautionary guidelines and 95.9% of the participants were aware of needle stick injury. 61.5% of participants with a history of needle stick injury reported washing immediately with soap and water. Most common cause of needle stick injury was work overload and 66.6% of injuries occurred during the day time. The details of the factors are given in Table 1.

Table 1: *Characteristics of study participants*

Variables	Frequency	Percentages
Gender		
Male	83	42.6%
Female	112	57.4%
Age		
20-25 years	97	49.7%
26-30 years	72	36.9%
31-35 years	13	6.7%
36-40 years	7	3.6%
Above 40 years	6	3.1%
Category		
House Officer	113	57.9%
PGR	61	31.3%
Senior Resident	7	3.6%
Consultant	14	7.2%
Ward of Practice		
Medicine	72	36.9%
Surgery	56	28.7%
Obs and Gynae	38	19.5%
Paediatrics	29	14.9%
Aware Of needle stick injury		
Yes	187	95.9%
No	8	4.1%

Knowledge about universal precaution guidelines		
Yes	115	58.97%
No	80	41.02%
Fully vaccinated against Hepatitis B		
Yes	147	75.4%
No	48	24.6%
Years of work practice		
Less than 6 months	82	42.1%
1-5 years	89	45.6%
More than 5 years	24	12.3%
Attended any workshop about needle stick injury within months		
Yes	67	34.4%
No	128	65.6%
Needle stick injury from the previous 1 year		
Yes	129	66.2%
No	66	33.8%
Causative factor for needle stick injury (n=129)		
Recapping	48	37.20%
Workload	52	40.31%
Sleepiness /tiredness	7	5.42%
During surgery	20	8.75%
Blood test sampling	2	1.55%
After Needle Stick injury did you wash immediately with soap and water		
Yes	79	61.24%
No	50	38.7%
Time of Injury		
Day	86	66.6%
Night	43	33.3%
Nature Of injury		
Superficial	8	6.2%
Deep	121	62.1%
Did you receive medical attentio after needle stick injury		
Yes	42	32.55%
No	87	67.44%
Report needle stick injury to health authorities		
Yes	34	26.35%
No	95	73.64%

Table 2: *Association of factors with needle stick injury*

Factors	Needle stick injury		p-value
	Yes	No	
Age			
20-35 years	120	62	**0.700**
36-40 years and above	9	4	
Gender			
Male	60	23	**0.119**
Female	69	43	
Attended workshop about NSI			
Yes	38	29	**0.044**
No	91	37	
Ward of practice			
Medicine	52	20	
Surgery	29	27	**0.018**
Gynecological and obstetrics	24	14	
Paediatrics	24	5	
Time of injury			
Day	83	3	**0.000**
Night	46	1	
Wearing a pair of gloves			
Yes	37	29	**0.05**
No	92	37	

Further analysis after applying the chi-square test revealed that needle-stick injury was significantly associated with prior safety workshops on needle prevention and the type of ward where participants were practicing. Statistical significant association of needle stick injury with daytime and not a wearing pair of gloves was also noted (table- II)

Discussion

NSIs are a significant and ongoing source of exposure for healthcare workers to dangerous and occasionally fatal diseases. Our study highlighted that the overall frequency of needle stick injury among healthcare workers in the previous 12 months was 66.2%. The findings are parallel to the work conducted in teaching hospitals in Iraq (53.8%) and the research reported by Getaw (60.2%).[12,13] However our results were inconsistent with the study conducted by Zemene et al (11.57%) and in India (20.1%).[14,15] This variation could be explained by differences in how standard operating procedures are used, occupational health and safety systems, policy availability and execution, inadequate NSI management, and unsafe work settings.[11]

In the current research, two-thirds of study participants

were aware of needle stick injury and 43.6% of participants did not report needle stick injury to a higher authority, one of the studies carried out in Saudi Arabia revealed that 94.7% had knowledge of needle stick injury but 52.7% (48/91) did not report their injuries.[16] Another finding from Alsabaani and colleagues has also found that 47.3% % of healthcare workers did not report their injuries.[17]

Another important observation of our study is that work overload is the main risk factor for needle stick injury. This contrasts with the research conducted by Zemeene et al where sleep at work was reported as the most common risk factor.[14] Research conducted in another tertiary care hospital in Pakistan reported that injecting medicine and drawing blood (42%) was mostly a reason for needle stick injury.[18] The various risk factors for needle stick injuries among healthcare workers in different research studies can be attributed to variations in work settings, overloaded hospitals and access to appropriate safety equipment.

It was noted by our study that most of the participants (65.6%) with needle stick injuries did not attend any workshop in the last 12 months. The interventional study has found that sensitization for the prevention and management of NSIs are essential in preventing occupational hazards and contributed to a significant improvement in the level of knowledge and practice regarding needle stick and sharp injuries.[19] Studies conducted in Ethiopia also supported our work where participants who had no training on safety and health were more significantly associated with needle stick injury.[20,21]

In the present research, day time duty was significantly associated with needle stick injury. Likewise a study conducted by Kifah et al and in Iran reported that 68% and 97.2% of needle stick injury occur during the morning shift. Hadis and colleagues reported that the possible reason for the increasing prevalence of needle stick injury in the morning hours would be that the majority of patients found it feasible to visit hospitals during these hours .[22,23]

Our research indicated the majority of participants do not have habits of wearing a pair of gloves (66.53%). Mehdi et a in his study showed that the 79.3% of study participants stated that wearing gloves is not necessary.[24] This is comparable to the study conducted in Turkey where 36.9% of healthcare workers had not been wearing gloves at the time of the incident.[25] The variations in wearing gloves among healthcare workers in different settings before needle stick injuries may be influenced by factors such as the availability of gloves, compliance with safety protocols, and individual perceptions of risk.

Conclusion

In our study, it was found that NSI is still one of the major problems among health care providers. The most effective way to reduce its incidence is by implementing a thorough plan that tackles the institutional, behavioral, and device-related factors that influence the occurrence of NSIs. Developing an active surveillance system, assessing, and using needle devices with safety features are essential components of this effort and continuing training initiatives must be a part of any preventive strategies of hospitals.

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UA, MB: Data Collection

AA: Literature Search

AA: Statistical Analysis

BI: Drafting, Revision

RA: Writing of Manuscript

Exploring Burn Severity in Domestic Violence: A Cross-Sectional Study Assessing Medium Associations and Implications

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Abstract

Objective: To explore the relationship between the medium of burns and medical severity among victims of domestic violence. Specifically, we investigate the prevalence of burn mediums, categorize the severity of medical outcomes, and examine the association between the two factors.

Material and Methods: It was cross-sectional study and data was collected from 250 participants involved in domestic violence incidents, sourced from Accident & Emergency Department of Department of Mayo Hospital Lahore and filtered at the Medicolegal Clinic King Edward Medical University Lahore. Participants were categorized based on gender and age group. Medical severity was assessed based on skin reactions, while burn mediums encompassed scalds, dry/flame burns, electrocution, and chemical burns. Statistical analyses, including frequency distribution and association tests, were conducted to elucidate the relationship between burn mediums and medical severity.

Results: Scalds emerged as the most common burn medium, with a significant association observed between scalds and heightened medical severity ($p = 0.001$). The majority of participants exhibited severe medical outcomes, underscoring the profound impact of domestic violence on victims' physical well-being.

Conclusion: Our findings highlight the distinct implications of different burn mediums on medical severity in domestic violence incidents. Tailored interventions and preventive measures are imperative to mitigate the risk of severe injuries and enhance support for victims.

Keywords: Domestic Violence, Burns, Medical Severity, Scalds, Degree of Burns, Medicolegal

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Introduction

Domestic violence, including burns as a result of this; remains a pervasive issue worldwide, inflicting physical, psychological, and emotional harm upon its victims.[1] Among the myriad of methods employed by perpetrators, burns represent a particularly severe form of assault, leaving lasting scars both physically and mentally. As such, comprehending the relationship between the medium of burns and the resultant medical severity is paramount in addressing the multifaceted challenges posed by domestic violence.[2] Such incidents make a huge burden on medicolegal clinics resultantly involving law enforcement personals and legal framework.[3]

Medicolegal concern amid domestic violence embark on a comprehensive exploration aimed at unraveling the intricate association between the medium of burns and the ensuing medical severity among victims of

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domestic violence.[4] Our investigation delves into the diverse manifestations of burns, ranging from scalds and dry/flame burns to electrocution and chemical burns, meticulously scrutinizing their implications on the severity of injuries sustained by victims.[5]

The first aspect under scrutiny pertains to the medical severity of burns incurred by the victims. Through meticulous categorization, our analysis delineates the spectrum of medical severity, encompassing skin reactions ranging from redness to blistering and the dire need for skin grafts.[6] Such delineation enables a nuanced understanding of the extent of physical trauma inflicted upon the victims, facilitating tailored interventions and medical management strategies.[7]

The frequency distribution of medical severity underscores the profound impact of domestic violence on the physical well-being of victims.[8] Notably, the overwhelming majority of participants experience issues from skin blistering or raw, open wounds to the pervasive nature of severe injuries in such incidents. Still a significant proportion necessitate skin grafts, signify the gravity of the inflicted trauma and the imperative modes for prompt medical intervention.[9]

Domestic violence reported in different medicolegal settings in the country sheds light on the diverse mediums through which burns are inflicted in violence scenarios. Scalds emerge as the predominant cause, followed by dry/flame burns, electrocution, and chemical burns.[10] Such variation in mediums underscores the multifaceted nature of domestic violence, wherein perpetrators employ diverse methods to inflict harm upon their victims.[11]

Crucially, the association between the medium of burns and medical severity emerges as a focal point of our inquiry in this research project. Here we present a meticulous analysis, delineating the nexus between the medium of burns and the resultant medical severity. Usually, scalds emerge as a significant predictor of heightened medical severity along with electrical and chemical burns yet set aside the famous dry flame burns. Such findings needs to be illuminated to find out the distinct implications of different mediums of burns on the severity of injuries sustained, thereby informing targeted interventions and preventive measures.[12]

Our study endeavors to illuminate the complex interplay between the medium of burns and the ensuing medical severity in domestic violence incidents. By unraveling this intricate association, we aim to empower stakeholders with the insights necessary for informed decision-making, fostering effective interventions, and ultimately, mitigating the pervasive impact of domestic violence on victims' well-being.

Material and Methods

It was cross-sectional study and data was collected from 250 participants involved in domestic violence incidents, source from the Medicolegal Clinic Department of Mayo Hospital Lahore and filtered at the Medicolegal Clinic King Edward Medical University Lahore., following approval from the institute's ethical committee IRB No 4525 dated 14-12-2015. Data collection occurred between December 2017 and July 2018. Parti-cipants were categorized based on gender and age group, with 117 females and 133 males included in the analysis. Two key aspects were explored: medical severity and medium of burns. Medical severity was assessed based on skin reactions, categorized into various levels of severity. The medium of burns encompassed scalds, dry/flame burns, electrocution, and chemical burns. Statistical analysis, including frequency distribution and association tests, was conducted to elucidate the relationship between the medium of burns and medical severity, providing insights into the implications of different burn mediums on injury severity.

Results

Medical severity in cases of domestic violence refers to the extent of physical harm inflicted upon victims, particularly in relation to burns. It encompasses various skin reactions, ranging from redness and blistering to the need for skin grafts, reflecting the severity of the injuries sustained. Understanding medical severity is crucial in assessing the impact of domestic violence incidents on victims' physical well-being and guiding appropriate medical interventions.

Table 01 presents the frequency distribution of medical severity among the participants involved in domestic violence events. The clinical features examined include skin reactions following burns, categorized into different severity levels. From the table 01, it is evident that the majority of the participants (91.6%) experienced skin blisters, raw and open wounds following the domestic violence incidents. A smaller proportion of participants had skin that turned red (6.4%) while a few required skin grafts (1.2%). Only a couple of cases had undetermined skin depth (0.8%) among all the cases involved in the research project.

The medium of burns refers to the specific method or agent through which the burn injuries were inflicted. In the context of domestic violence, this can include various forms such as scalds (caused by hot liquids or steam), dry/flame burns (resulting from direct contact with fire or heat sources), electrocution (caused by electrical currents), and chemical burns (resulting from contact with corrosive substances). Understanding the medium of burns is essential in determining the nature and severity of injuries, as well as guiding appropriate medical treatment and preventive measures. Table 02 presents the frequency distribution of the medium of burns among the participants involved in domestic violence events. The data categorizes the burns based on the medium or cause of the injury. From the table, it's evident that scalds represent the most common medium of burns, accounting for 53.6% of cases. Dry or flame burns are the next most frequent at 36.8%, followed by electrocution at 6.8%, and chemical burns at 2.8%.

This distribution provides insight into the various causes of burns experienced by the participants, indicating the prevalence of scalds and dry/flame burns in domestic violence incidents, with smaller proportions attributed to electrocution and chemical burns. Table 03 presents an association analysis between the medium of burn and medical severity among the participants involved in domestic violence incidents. The table cross-tabulates the medium of burn (Scald, Dry, Electricity, Chemical) with the medical severity (Skin turned Red, Skin was blistered, Need Skin grafts, Undetermined). Additionally, it provides the total number of participants for each combination and the associated p-values. Among the 134 participants who suffered scalds, 9 had skin that turned red, 123 had blistered skin, and 2 required skin grafts. There were no cases of undetermined severity.

For the 92 participants who experienced dry/flame burns, 6 had skin that turned red, 85 had blistered skin, and 1 required skin grafts. There were no cases of undetermined severity.

Among the 17 participants affected by electricity, none had skin that turned red, 15 had blistered skin, and 2 required skin grafts. There were no cases of undetermined severity.

Of the 7 participants who suffered chemical burns, 1 had skin that turned red, 6 had blistered skin, and none required skin grafts. There were no cases of undetermined severity.

The p-value associated with the association between various mediums of burns and medical severity was calculated to be 0.001suggesting statistically a gross significant association between mediums and medical severity among domestic burns. The analysis indicates that the association of mediums of burns with that of medical severity are significantly associated risk factors responsible for burns.

Our analysis yielded significant insights into the association between the medium of burns and medical severity among victims of domestic violence. Firstly, the frequency distribution of medical severity revealed that the majority of participants (91.6%) experienced skin blistering or raw, open wounds following the incidents, indicating a prevalent pattern of severe injuries. A smaller proportion necessitated skin grafts (1.2%), underscoring the gravity of the inflicted trauma.

Finally, the distribution of burn mediums elucidated scalds as the most common cause (53.6%), followed by dry/flame burns (36.8%), electrocution (6.8%), and chemical burns (2.8%). Such variation highlights the

Table 1: *Frequency of Medical Severity*

Clinical Feature	Frequency	Percent
Skin Turned Red	16	6.4
Skin Blisters/ Raw and Open	229	91.6
Skin needing Grafts	3	1.2
Skin Depth Undetermined	2	0.8
Total	250	100.0

Table 2: *Frequency of Medium of Burns*

Medium of Burn	Frequency	Percent
Scalds	134	53.6
Dry/Flame Burns	92	36.8
Electrocution	17	6.8
Chemical Burns	7	2.8
Total	250	100.0

Table 3: *Association of the Medium of Burn with Medical Severity*

Medium of Burn	Medical Severity				Total (*n*=250)	P value
	Skin turned Red (*n*=16)	Skin was blistered (*n*=229)	Need Skin grafts (*n*=3)	Un-determined (*n*=2)		
Scald	9	123	2	0	134	0.001
Dry	6	85	1	0	92	
Electricity	0	15	0	2	17	
Chemical	1	6	0	0	7	

diverse methods employed by perpetrators in inflicting harm upon their victims. Furthermore, our analysis unveiled a significant association between the medium of burns and medical severity. Specifically, scalds emerged as a significant predictor of heightened medical severity, with a statistically significant association observed ($p = 0.001$). This finding underscores the distinct implications of different burn mediums on the severity of injuries sustained, warranting tailored interventions and preventive measures.

Discussion

The findings of our study shed light on the intricate relationship between the medium of burns and the resultant medical severity among victims of domestic violence. Through a comprehensive analysis of data collected from 250 participants, we have unveiled significant insights into the patterns and implications of burns inflicted in such incidents. In this discussion, we delve deeper into the implications of our findings, elucidate their relevance in the broader context of domestic violence, and outline avenues for future research and intervention.[13]

One of the key observations from our study is the prevalence of severe injuries among victims of domestic violence. The majority of participants exhibited skin blistering or raw, open wounds following the incidents, underscoring the profound impact of such assaults on the physical well-being of victims. This finding aligns with existing literature highlighting the grave consequences of domestic violence, particularly in cases involving burns, which often result in long-term physical and psychological sequelae.[14]

Moreover, our analysis delineated scalds as the most common medium of burns, followed by dry/flame burns, electrocution, and chemical burns. Such variation underscores the diverse methods employed by perpetrators in inflicting harm upon their victims, reflecting the multifaceted nature of domestic violence.[15] Notably, scalds emerged as a significant predictor of heightened medical severity, highlighting the distinct implications of different burn mediums on injury severity. This finding is consistent with previous research indicating the severity of scald injuries and the challenges associated with their management.[16]

The association between burn mediums and medical severity underscores the importance of tailored interventions and preventive measures in addressing the diverse needs of victims. Specifically, our findings suggest the imperative for prompt medical intervention in cases involving scald injuries, given their heightened severity. Additionally, interventions aimed at raising awareness and promoting preventive strategies, such as safe handling of hot liquids and education on fire safety, are crucial in mitigating the risk of burn injuries in domestic settings.[17]

Furthermore, our study underscores the need for a multidisciplinary approach in addressing the complex challenges posed by domestic violence. Effective interventions necessitate collaboration among healthcare professionals, law enforcement agencies, social service providers, and policymakers to ensure comprehensive support and protection for victims.[18] Moreover, initiatives aimed at addressing the root causes of domestic violence, including gender inequality, socioeconomic disparities, and cultural norms, are essential in fostering long-term prevention efforts.[19] While our study provides valuable insights into the association between burn mediums and medical severity in domestic violence incidents, several limitations warrant acknowledgment. Firstly, the retrospective nature of the data may have introduced bias or incomplete information, limiting the generalizability of our findings. Additionally, the sample size, while representative, may not capture the full spectrum of domestic violence cases, particularly those that go unreported or undocumented.[17,20,21]

Our study emphasizes the profound impact of domestic violence on the physical well-being of victims and highlights the intricate interplay between the medium of burns and resultant medical severity. By elucidating this complex relationship, we aim to inform targeted interventions and preventive strategies, ultimately striving towards the mitigation of the pervasive impact of domestic violence on individuals and communities. Future research endeavors should focus on longitudinal studies to further elucidate the long-term outcomes of burn injuries in domestic violence survivors and inform evidence-based interventions.[1]

Conclusion

In conclusion, our study provides a comprehensive examination of the association between the medium of burns and medical severity in domestic violence incidents. Through meticulous analysis of data from 250 participants, we have elucidated the profound impact of burns on the physical well-being of victims, with scalds emerging as a significant predictor of heightened

medical severity. These findings underscore the importance of tailored interventions and preventive measures to address the diverse needs of victims and mitigate the risk of severe injuries. Moving forward, a multidisciplinary approach, coupled with efforts to address the root causes of domestic violence, is essential in fostering long-term prevention and support for victims. By shedding light on this complex relationship, our study contributes to the broader discourse on domestic violence and informs evidence-based interventions aimed at enhancing the well-being and safety of individuals and communities affected by such incidents.

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Authors Contribution

RA: Conceptualization of Project
MH: Data Collection
SS: Literature Search
NH: Statistical Analysis
MAS: Drafting, Revision
RM: Writing of Manuscript

Efficacy of Sulbutiamine-a Fat Soluble Thiamine in the Management of Diabetic Symmetrical Peripheral Neuropathy

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Abstract

Objective: To evaluate the efficacy of sulbutiamine, a fat-soluble derivative of vitamin B1, in reducing pain intensity among diabetic patients with painful symmetrical peripheral neuropathy (DSPN).

Material And Methods: Our quasi-experimental study involved 320 diabetic patients with painful symmetrical peripheral neuropathy, selected through non-probability purposive sampling from the Institute of Endocrinology and Metabolism, Services Hospital Lahore, between March 1, 2023, and August 31, 2023. Participants were administered Sulbutiamine, a fat-soluble derivative of vitamin B1-thiamine, at a dosage of 200mg twice daily over six weeks. Follow-up assessments were conducted at 3 and 6 weeks, with efficacy evaluated based on a minimum two-point improvement in pain intensity using the Numeric Pain Rating Scale.

Results: Demographic analysis revealed an average patient age of 59.49 years, a BMI of 30.10 kg/m^2, and a slight male predominance (55.9%). The final efficacy of fat-soluble thiamine at the 6th week was 68.4%. Stratified analysis highlighted significant variations in efficacy at 3rd and 6th weeks, emphasizing the influence of variables such as age, comorbidities, and diabetes duration. Age, comorbidities, and diabetes duration exhibited strong statistical significance ($p < 0.05$), indicating their impact on treatment outcomes, while other variables demonstrated variable significance at different assessment points.

Conclusion: In conclusion, the administration of sulbutiamine, a fat-soluble form of vitamin B1, led to a noteworthy reduction in pain among diabetic individuals suffering from painful symmetrical peripheral neuropathy. This study underscores the potential of sulbutiamine as an effective intervention for managing DSPN, with implications for improving the well-being of diabetic patients.

Keywords: Diabetic Symmetrical Peripheral Neuropathy, Efficacy, Fat-soluble vitamin B-1

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Introduction

Diabetes mellitus represents a metabolic disorder characterized by dysregulated metabolic processes and elevated glucose levels stemming from inadequate insulin secretion and resistance to its action at target organs.[1] This disease manifests with both microvascular and macrovascular complications.[2] Among these complications, symmetrical peripheral neuropathy, particularly the painful variant, stands out as a deleterious microvascular consequence.[3] Diabetic neuropathy is a prevalent complication occurring in both types of Diabetes mellitus. Distal symmetric polyneuropathy is observed in one out of every four diabetic patients, with its prevalence escalating in tandem with disease progression; approximately 50% of individuals with diabetes for 25 years are predisposed to develop neuropathy.[4,5] The management of neuropathy entails the use of diverse pharmacological agents such as opioid-like analgesics, anticonvulsants, tricyclic antidepressants,

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and selective serotonin reuptake inhibitors. A myriad of drugs, including methylcobalamin, alpha-lipoic acid, vitamin B6, folate, L-arginine, biotin, among others, may be employed individually or in various combinations to alleviate altered or painful sensations and dysesthesia.[6,7] Several vitamins, including thiamine, play crucial roles in neuropathy management. Thiamine is integral to the transketolation process in the pentose phosphate shunting pathway and is involved in the initiation of nerve impulse propagation.[8,9]

Fat-soluble vitamin B1/thiamine, distinguished by its higher bioavailability compared to water-soluble thiamine, is deemed more efficacious for therapeutic purposes. Its utility lies in decelerating the progression of diabetic complications by augmenting intracellular levels of thiamine diphosphate, ultimately enhancing transketolase activity. This study aimed to ascertain the efficacy of fat-soluble form of vitamin B-1/ thiamine in the treatment of diabetic symmetrical peripheral neuropathy.

Material and Methods

The study adopted a quasi-experimental design, encompassing a sample of 320 diabetic patients selected through purposive non-probability sampling. Individuals with symmetrical painful peripheral neuropathy were specifically identified from the Institute of Endocrinology and Metabolism, Services Hospital Lahore. After the approval of IRB Committee Registration No.1026-A/22/SIMS dated 08-10-2022, the study spanned six months, commencing on March 1, 2023, and concluding on August 31, 2023. The sample size 320, determined with a confidence level of 95% and a margin of error of 5.5%, considered an anticipated efficacy of 47% for lipid-soluble variant of thiamine in managing diabetic peripheral painful neuropathy. The inclusion criteria comprised diabetics with symmetrical peripheral painful neuropathy complications within the age range of 18-60 years, encompassing both genders, exhibiting a baseline Numeric Pain Rating Scale (NRS) score of at least 3. Exclusion criteria involved factors such as blood macrocytosis, HbA1c value ≥ 9%, a history of vitamin B-1(thiamine) allergy, elevated renal function tests (creatinine level > 1.5mg/dl), and prior use of medications like analgesics, multivitamins, antidepressants, anti tuberculous therapy, and antiepileptics. Informed written consent was obtained, and eligible patients meeting the criteria were enrolled. The study procedure was thoroughly elucidated to all participants. Initial assessments involved the measurement of symptoms using the Numeric Pain Rating Scale, with a minimum score of 3 or higher. Patients were prescribed a fat-soluble vitamin B1/thiamine Sulbutiamine, in tablet form at a dosage of 200mg twice daily for a duration of 6 weeks. Follow-up visits were scheduled at 3 weeks and 6 weeks post the initial visit. Efficacy was gauged based on a criterion of at least a two-point improvement in pain from baseline on the Numeric Pain Rating Scale after six weeks. To mitigate bias, a singular researcher conducted assessments for all patients.

Table 1: *Demographic characteristics of population under study (n=320)*

Variables	Mean	Std. Deviation
Age (In completed years)	59.49	±11.27
Duration of Diabetes (years)	11.65	±7.95
BMI* (kg/m²)	30.14	±7.30
HbA1c (%) at baseline	6.84	±0.69
BSF** (mg/dl) at 3 weeks	123.9	±20.16
BSF (mg/dl) at 6 weeks	124.39	±20.51
Pain rating at baseline	6.38	±1.82
Pain rating at 3 weeks	5.81	±1.93
Pain rating at 6 weeks	4.61	±2.13
	Numbers	**Percentage %**
Gender		
Male	179	55.9 %
Female	141	44.1 %
Co morbidities		
Yes	110	34.0 %
No	210	65.6 %
Smoking		
Yes	56	17.5 %
No	264	82.5 %
Improvement at 3rd week		
No	251	78.4 %
Mild (2 points on PRS)	56	17.5 %
Moderate (≥ 2 points on PRS)	13	4.1%
Improvement at 6th week		
No	105	32.8 %
Mild (2 points on PRS***)	111	34.7 %
Moderate (≥ 2 points on l	104	32.5 %
Outcome		
Efficacy at 3rd week	69	21.5 %
Efficacy at 6th week	219	68.4 %

*Where; *BMI= Body mass index, **BSF=Blood sugar fasting, ***PRS=Pain rating scale*

Results

The results hold the promise of unravelling valuable insights into a novel therapeutic approach. This groundbreaking investigation seeks to illuminate the outcomes of administering fat-soluble thiamine, a potentially innovative intervention, in alleviating the symptoms of diabetic peripheral neuropathy. The results are poised to provide a comprehensive understanding of the impact of this treatment on pain management, and hence overall patient well-being. By delving into the quantitative outcomes and statistical analyses, the result section aims to contribute robust evidence that may shape future considerations for managing diabetic peripheral neuropathy, offering hope for improved quality of life for individuals affected by this prevalent and challenging complication of diabetes.

Table Ⅰ shows demographic characteristics of population under study, it explains several variables, listed along with their mean and standard deviation values. These variables include "Age, duration of diabetes, BMI, HbA1C, BSF at different time points gender distribution, improvement noted at 3rd and 6th week and lastly efficacy at 3rd and 6th week. The mean and standard deviation values for each of these variables are provided in the table. Based on the table, some relevant results that can be inferred include the average age of the patients being around 59.49 years, the average BMI being 30.10 kg/m^2. Male (55.9%) participants were slightly more than females, Final efficacy (at 6th week) reported is 68.4%. Table II presents the stratified analysis of the relationship between Efficacy and various variables. It can be inferred from bivariate analysis that Outcome of using Thiamine in patients with painful neuropathy are affected by various factors,

Table 2: *Stratification of Efficacy at 3rd & 6th week with different confounding variables. (n=320)*

Variable	Sub Groups	Efficacy at 6 weeks		P-Value	Efficacy at 6 weeks		P-Value
		Yes	No		Yes	No	
Age	18-40 Years	4(16.7%)	20(83.3%)		18(75.0%)	6(25.0%)	
	41-60 Years	34(23.8%)	109(76.2%)	0.03*	82(57.3%)	61(42.7%)	0.002**
	61-80 Years	31(19.2%)	112(80.8%)		119(77.5%)	34(22.5%)	
Obesity	Non-Obese (BMI (kg/m²): 18-29.9)	25(17%)	122(83.0%)	0.06	101(68.7%)	46(31.3%)	0.92
	Obese (BMI (kg/m²): ≥30)	44(25.4%)	129(74.6%)		118(68.2%)	55(31.8%)	
Gender	Male	34(19.0%)	14.5(19%)		120(67%)	59(33.0%)	
	Female	35(24.8%)	106(75.2%)	0.20	99(70.2%)	42(29.9%)	0.54
Smoking	Smoker	6(10.7%)	50(89.3%)		36(64.3%)	20(35.7%)	
	Non-Smoker	63(23.69%)	201(76.1%)	0.03*	183(69.3%)	81(30.7%)	0.46
Comorbidities	Co-morbidity present	12(10.9%)	98(89.1%)		66(60.0%)	44(40.0%)	0.01*
	No comorbidity	57(27.1%)	153(72.9%)	0.001**	153(72.9%)	57(27.1%)	
Duration of Diabetes (years)	1-15 year	61(27%)	165(73%)		159(70.4%)	67(29.6%)	
	16-30 year	8(9.0 %)	81(91%)	0.001**	60(67.4%)	29(32.6%)	0.004**
	≥30 year	0(0.0%)	5(100%)		0(0.0%)	5(100%)	
HbA1c (%)	<5.7	23(19.3%)	96(80.7%)		91(76.5%)	28(23.5%)	
	5.7-6.4	24(22.2%)	84(77.8%)	0.73	66(61.1%)	42(38.9%)	0.041*
	≥ 6.5	22(23.7%)	71(76.3%)		62(66.7%)	31(33.3%)	
Pain Raing (points on scale)	1-5	21(17.2%)	101(82.8%)		79(64.8%)	43(35.2%)	
	6-10	48(24.2%)	150(75.8%)	0.13	140(70.7%)	58(29.3%)	0.26

Footnotes: *Statistical significance for difference in proportions are calculated using Pearson's Chi-Squared test. P less than 0.05 was considered statistically significant*
**P value Significant at < 0.05, ** significant at < 0.01*
Abbreviations: *p stands for probability of rejecting null hypothesis when it is true.*
Percentages are Row wise. Data are numbers and percentages (%) unless indicated otherwise.

efficacy is checked at 2 different points in time (one being at 3rd week of treatment and other at 6th week). Important being Age (p-value=0.03 at 3rd week and 0.002 at 6th week), co morbidities (p-value=0.001 at 3rd week and 0.001 at 6th week) and duration of diabetes (p-value=0.001 at 3rd week and 0.004 at 6th week), this suggests a strong statistical significance, indicating that these outcomes is unlikely to be due to chance. Also, it can be seen that relation with BMI (p value=0.06) and smoking (p value =0.03) is only significant at week 3. Relation of HbA1c (p value =0.04) is only significant at 6th week.

Discussion

Diabetic peripheral neuropathy presents a formidable challenge for individuals affected by diabetes, significantly impacting their quality of life. The ramifications extend beyond physical discomfort, with potential disruptions to normal sleep patterns and an increased susceptibility to depressive and anxiety disorders.[10] The pharmacological armamentarium for managing this condition includes anticonvulsants, tricyclic antidepressants, opioid-like agents, and serotonin-norepinephrine reuptake inhibitors.[6]

Achieving optimal glycaemic control remains a paramount objective to mitigate the progression of this complication. Emphasizing the importance of compliance, particularly with a focus on single-drug regimens, is underscored by the associated improvements in adherence.[11] Streamlined drug regimens, such as fixed daily dose combinations, have demonstrated enhanced patient adherence in various therapeutic contexts.[12] Notably, randomized clinical trials in healthy adults have revealed superior bioavailability and absorption of fat-soluble thiamine compared to its water-soluble analogue.[13]

The observed efficacy of lipid-soluble vitamin B1 (thiamine) in the current study stands at 76.9%, surpassing the findings of Kew et al, who reported a 47% efficacy in a study involving fat-soluble vitamin B1 analogues over a 6-week period.[14] This disparity may be attributed to the unique genetic makeup of our studied population.

A randomized double-blind pilot study involving 40 diabetic patients with peripheral neuropathy corroborated our results, with a treatment group receiving a fat-soluble vitamin B1 formulation exhibiting significant improvement in neuropathic symptoms. While symptomatic pain relief was evident, vibration sensation scores did not show concurrent improvement.[15] El Hefnawy's comparison of oral vs intramuscular thiamine injection demonstrated a notable decrease in the Diabetic Neuropathic Symptom Score (DNS) across all groups after 14 days of treatment. Similarly, our study documented a substantial decrease in pain on the rating scale, specifically at 68.4%.[16]

Conclusion

In conclusion, the current study, while exhibiting notable strengths in its design and execution, highlights the promising role of fat-soluble thiamine in alleviating diabetic peripheral neuropathy. However, further investigations, incorporating larger cohorts and conducting randomised controlled trials are imperative to ascertain the broader applicability and efficacy of fat-soluble vitamin B1 in managing diverse manifestations of diabetic neuropathy.

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Authors Contribution

AR: Conceptualization of Project
AR, AK: Data Collection
AR, MI: Literature Search
MI, GJ: Statistical Analysis
SK: Drafting, Revision
AR, SK: Writing of Manuscript

Assessment of Patients' Satisfaction Regarding Health Care Services in a Tertiary Care Hospital

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Abstract

Objective: To find out the satisfaction level of the patients regarding the quality of OPD services and its relationship with the sociodemographic factors

Material and Methods: A cross-sectional study was conducted between June to December 2021 in Outpatient Department (OPD) of Fatima Memorial Hospital. After taking approval from Institutional Review Board, 385 patients fulfilling the inclusion criteria were recruited by using non-probability purposive sampling. Following informed consent, a structured pretested questionnaire was used to solicit information from respondents regarding reception area, patient doctor interaction, and overall level of services provided in the OPD. Patient satisfaction was measured on five-point Likert scale. Data was analyzed using SPSS version 27. Simple frequency tables were generated for the qualitative variables. Chi-square test was applied to find out any statistically significant relationship of various sociodemographic factors with patient satisfaction. The p-value of ≤ 0.05 was defined as significant.

Results: The mean age of participants was 36.59±14.59 years. Overall 86.5% patients were satisfied with the OPD services. When satisfaction regarding separate domains of OPD was analyzed, it was found that 76.1% of the respondents were highly satisfied with OPD reception services, and majority (87%) patients were highly satisfied with health care provider services. Around 88.8% patients agreed to come again in order to seek service provision and 87.6% patients supported recommending the hospital to others. No statistically significant relationship between various sociodemographic factors and patient satisfaction.

Conclusion: Majority of patients expressed high satisfaction level with various domains of OPD services and supported recommending the hospital to others based on their experience.

Keywords: patient satisfaction, OPD services, Health care provider services, Health Service readiness, patient doctor interaction

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Introduction

Patient satisfaction is an important and widely used indicator for measuring service quality in health care. It provides valuable insights into patients' perception and experiences with the health care services they receive.[1] Perception of the beneficiaries, which refers to how patients and healthcare service users perceive and experience the healthcare system, is indeed crucial for refining and improving the system.[2] Patient satisfaction has become a crucial component of the present-day health care delivery system. Conducting a patient satisfaction survey to examine the areas impacting satisfaction levels, as well as to find out the causes for discontent, is an established method for improving the quality of care in hospitals.[3] Worldwide several studies have repor-

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ted variable patient satisfaction estimates, ranging from 55 to 99.6%.[4] The evaluation of patient satisfaction takes into account both client- and provider-focused factors. Client-focused aspects are concentrated on how much patients feel their wants and expectations are being met during the provision of health care services, as opposed to provider-focused aspects, which refer to the provision of sound medical facilities.[5] Both provider- and patient-related factors can have an impact on patient satisfaction.[4] It has been found that satisfied patients are generally more compliant with their treatment and follow-up advice.[6] This sequentially translates into better health outcomes, which further reinforces their trust in the health care system and providers, leading to further satisfaction and potentially positive word of mouth recommendations.

Pakistan has a mixed health care system comprising of both governmental and private sectors. Approximately 70% of the population is served by the private sector.[7] According to metrics like overall healthcare quality and patient satisfaction, private hospitals and healthcare facilities routinely perform better than their public equivalents.[8,9] As an important milestone Pakistan established the Punjab healthcare commission in 2010 with the objectives to enhance patient satisfaction levels and complaints that catered to professional accountability.[10]

Outpatient department is an important area of a tertiary hospital where first contact occurs between the clients and the hospital staff. According to research the care offered at the OPD services indicates the overall quality of services of a hospital.[11] This study was conducted to find out the satisfaction level of the patients regarding the quality of OPD services and the relationship of sociodemographic factors with the satisfaction level of the patients. The findings will help health care professionals and administrators to gain insight regarding areas that need further upgrading to enhance patients satisfaction levels. This is of paramount importance as patient satisfaction has been linked to better clinical outcomes and improving patient retention and compliance.

Material and Methods

It was a descriptive cross-sectional study conducted in Outpatient Department (OPD) of Fatima Memorial Hospital. After approval from ethical committee IRB No.FMH-21/03/2024 IRB-1374. The study was conducted between the time period June-December 2021. The calculated sample size was 385 by considering the prevalence of patient satisfaction at 50%. The sampling technique was non-probability purposive sampling. The inclusion criteria for the patients were adults aged 16-80 years, both sexes, ready to give consent and patients who were able to communicate in Urdu. Patients who were not able to participate were excluded. After approval from the Institutional Review Board (IRB), the data was collected from the Outpatient Department of the hospital after taking informed verbal consent from the willing patients on a structured pretested questionnaire. The questionnaire had two sections. First section had questions related to sociodemographic profile and section II had three subsections having questions regarding services provided in the reception area, waiting area, patient doctor interaction, pharmacy services and overall level of services provided in the General OPD. The patient satisfaction was assessed separately for afore mentioned domains. The responses were captured on a likert scale from one to five. For each question, strongly agree & agree were clubbed as satisfied and similarly disagree and strongly disagree were clubbed as non-satisfied. Data was entered and analyzed using SPSS version 27. The quantitative variables were summarized as mean and standard deviation. Whereas the qualitative variables were summarized as frequency (%). Overall satisfaction for reception, waiting area, pharmacy and care provider was graded as poor (0-40%), Satisfactory (41-60%) and High (61-100%). Chi-square test was applied to find out whether there was any statistically significant effect of sociodemographic factors regarding patient satisfaction. The p value of ≤ 0.05 was defined as significant.

Results

Out of 385 patients, 212 (55.1%) were new cases. Most of the respondents 163 (42.3%) were of the age group ranging from 18-30 years and mean age of sample was 36.59±14.59 years. Majority respondents 257 (66.8%) were females. Among 385 patients who visited the OPDs 289(75.1%) were married. Regarding educational status, most of the sampled OPD cases 216 (53%) were in primary -FA/FSc category and only 48 (12.5%) were illiterate. Around 306 respondents (79.5%) had monthly incomes between 20000 to 40000 rupees. Overall satisfaction with the OPD reception services was reported high by 293 (76.1%) respondents, (Fig-1). Out of 385 respondents, 311 (80.8%) were satisfied that the reception area was easy to find, 255(66.2%) agreed that there were adequate signboards, 289(75.1%) were satisfied with guidance provided by the receptionist but only

253(65.7%) were satisfied with the waiting time at the reception. (Table 1) The overall satisfaction for the waiting area was rated as high by 278(72.2%) patients. (Fig-1). It was found that while 264(68.5%) patients agreed that the waiting area was comfortable, only 229(59.4%) patients were satisfied with the waiting time in waiting area and the required time was not acceptable for 21.5% patients. Regarding cleanliness of the toilets, out of 145 patients who availed this facility, 101(69.6%) were satisfied with their functionality but only 66(45.5%) were satisfied with the cleanliness. (Table 1) Around 335 (87%) patients were highly satisfied with Health care provider services and this domain had highest percentage of exceedingly satisfied clients as compared with other three domains. (Fig-1). Most patients (75.6%) were satisfied with behavior of nurses It was reported by 338(87.8%) that health care provider listened to their problems in an attentive manner and majority (91.7 %) patients reported that doctor treated them with respect and courtesy. Similarly 82.6% patients were satisfied with consent taken before examination, however 8% expressed dissatisfaction regarding this. Regarding privacy maintenance during examination (83.1%) patients were satisfied. (Table 2) Regarding OPD Pharmacy services only 169 patients availed them. Among them Overall satisfaction was rated as high by 125 (74%) respondents. (Fig-1). Only 109 (64.5%) were satisfied with the waiting time at the pharmacy and 37 (22%) remained neutral. According to 126 (75.1%) patients the pharmacist was courteous and helpful whereas 7.7% patients disagreed to this. Out of 385 patients surveyed regarding their general satisfaction with the OPD services it was found that satisfaction level was high for 35.6% patients, satisfactory for 50.9% patients and poor for only 13.5% patients. A vast majority 337(87.6%) strongly supported recommending the hospital to others and 88.8% were willing to re visit to seek treatment in future.

Table 1: *Patients' satisfaction regarding reception and waiting area of OPD (n = 385)*

Satisfaction regarding reception of OPDs	Satisfied	Neutral	Dissatisfied
1. Was the reception easy to locate?	311 (80.8%)	38 (9.9%)	36 (9.3%)
2. Were there adequate signboards for guidance?	255 (66.2%)	70 (18.2%)	60 (15.6%)
3. Was the reception well organized and attractive?	282 (73.3%)	67 (17.4%)	36 (9.3%)
4. Was the receptionist courteous and helpful?	294 (76.3%)	60 (15.6%)	31 (8.05%)
5. Is waiting time for getting the OPD ok?	253 (65.7%)	61 (15.8%)	71 (18.4%)
6. Did you get proper guidance at the reception	289 (75.1%)	52 (13.5%)	44 (11.4%)
7. Was there an efficient Queue system available	266 (69.1%)	71 (18.4%)	48 (12.5%)
Satisfaction regarding waiting area of OPDs	**Satisfied**	**Neutral**	**Dissatisfied**
1.Cleanliness of waiting area	284 (73.7%)	66 (17.1%)	35 (9.09%)
2.Waiting area comfortable and well ventilated	264 (68.5%)	64 (16.6%)	57 (14.8%)
3. Waiting time acceptable	229 (59.5%)	73(19.0)	83 (21.5%)
4. Availability of drinking water	185(48.0 %)	104 (27.0%)	96 (25%)

Table 2: *Patient satisfaction related to interaction with health care provider and pharmacy services (n = 385)*

Satisfaction related to health care provider	Satisfied	Neutral	Dissatisfied
1. Nurse was courteous and helpful	291 (75.6%)	50 (13.0%)	44 (11.4%)
2. Doctor's office well organized and comfortable	328 (85.1%)	40 (10.4%)	17 (4.5%)
3. Listened to the your problems	338 (87.8%)	21(5.5%)	26 (6.7%)
4 Treat the patient with respect and courtesy	353 (91.7%)	22(5.7%)	10(2.6%)
5. Doctor spend enough time for consultation	334 (86.8%)	37 (9.6%)	14 (3.6%)
6. Response of physician to queries	335 (87%)	31(8.1%)	19 (4.9%)
7.Proper instructions given by the doctor for medicines	330 (85.7%)	37(9.6%)	18 (4.7%)
8. Consent taken by the physician for physical examination	318 (82.6%)	36(9.4%)	31(8.0%)
9.Maintenance of privacy during examination	320 (83.1%)	35 (9.1%)	30 (7.8%)
10. Advice given for follow up visits	325 (84.4%)	35 (9.1%)	25 (6.5%)
Satisfaction related to pharmacy (n = 169)	**Satisfied**	**Neutral**	**Dissatisfied**
1. Waiting time convenient to you	109 (64.5%)	37 (21.9%)	23(13.6 %)
2. A time-efficient on-site payment system	123 (72.8%)	28 (16.6%)	18 (10.6%)
3. Pharmacist courteous and helpful	127 (75.1%)	29 (17.2%)	13 (7.7%)
4 Medicine required available	126 (74.6%)	19(11.2%)	24(14.2 %)

There was no statistically significant association of age (P=0.69), gender (P= 0.29), education (P=0.28), marital

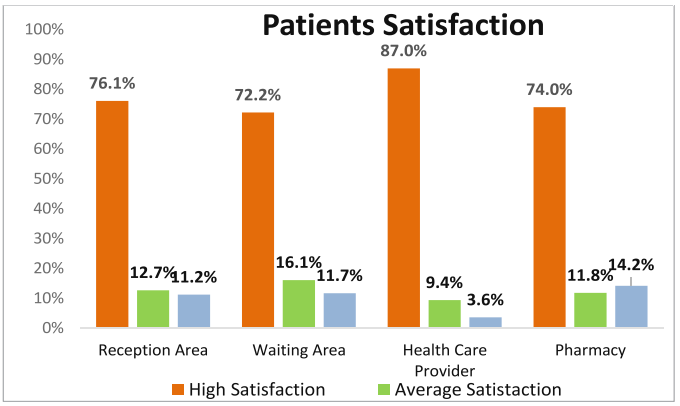

Figure 1: *Overall satisfaction of patients regarding reception area, waiting area, Health care provider and pharmacy.*

status (P=0.77) and monthly income (P=0.24) with the level of satisfaction in current study. (Table 3)

Discussion

Patient satisfaction is at the center of the patient-centered care and is also one of the indicators of the quality of care. The current study was conducted to determine satisfaction level of patients with OPD services of a private sector tertiary care hospital in order to identify the potential areas for improving the health care services and their delivery.

In the current study out of 385 respondents around 86.5% patients were satisfied with the overall OPD services of a private tertiary level hospital. This is in accordance with results of another study where 86.69% patients were satisfied with the overall quality of healthcare they received.[4] However the level is higher than that reported by two studies conducted in India where overall satisfaction level reported by the patients presenting in OPD was72.8% 6 and 84% 12 respectively. Various studies conducted on similar topic in Pakistan have reported much lower patient satisfaction. A study found that only 31% participants reported good satisfaction in a tertiary hospital of Karachi.[13] Contrastingly another

Table 3: *Cross tabulation of various socio demographic variables of patients presenting in OPD of a private tertiary care hospital. (n = 385)*

Variables	Patients' satisfaction level				Chi- square test & P-value
	Poor satisfaction	Average satisfaction	Good satisfaction	Total	
Age in years					
18-30	21 (40.4%)	85 (43.4%)	57 (41.6%)	163 (42.3%)	Chi-square=2.165 P-value=0.705
31-56	23 (44.2%)	88 (44.9%)	68 (49.6%)	179 (46.5%)	
57 and above	8(15.4%)	23 (11.7%)	12(8.8%)	43(11.2%)	
Total	**52 (100%)**	**196 (100%)**	**137 (100%)**	**385 (100%)**	
Gender					
Male	22 (42.3%)	66 (33.7%)	40 (29.2%)	128 (33.2%)	Chi-square=2.952 P-value=0.229
Female	30 (57.7%)	130 (66.3%)	97 (70.8%)	257 (66.8%)	
Total	**52 (100%)**	**196 (100%)**	**137 (100%)**	**385 (100%)**	
Marital status					
Married	37 (71.2%)	148 (75.5%)	104 (75.9%)	289 (75.1%)	Chi-square=0.489 P-value=0.779
Unmarried	15 (28.8%)	48 (24.5%)	33 (24.1%)	96 (24.9%)	
Total	**52 (100%)**	**196 (100%)**	**137 (100%)**	**385 (100%)**	
Education					
Illiterate	6 (11.5%)	26 (13.3%)	16 (11.7%)	48 (12.5%)	Chi-square=2.739 P-value=0.602
Primary – FA/FS.C	30 (57.7%)	115 (58.7%)	71 (51.8%)	216(56.1 %)	
Graduation and above	16 (30.8%)	55 (28.0%)	50(36.5%)	121(31.4%)	
Total	**52 (100%)**	**196 (100%)**	**137 (100%)**	**385 (100%)**	
Family income					
20000 – 60000	46 (88.5%)	157 (80.1%)	103 (75.2%)	306 (79.5%)	Chi-square=4.17 P-value=0.124
61000 and above	6 (11.5%)	39 (19.9%)	34 (24.8%)	79(20.5%)	
Total	**52 (100%)**	**196 (100%)**	**137 (100%)**	**385 (100%)**	

study reported much higher % of satisfied clients with OPD as 97.2%.[14] The difference in results may be due to a variety of factors such as different study setting, type of set up (Govt. vs Private), administrative set up, attitude of healthcare providers, ease and adequacy of service provision and differing sociodemographic characteristics of the patients presenting to the hospitals. All of the aforementioned factors can vary the satisfaction level of patients. However research has shown that apart from these major dimensions relatively minor issues may also influence satisfaction of patients. These may include the length of waiting in the out-patient department, or ease of getting a slip, adequate sitting arrangement and availability of other basic amenities like drinking water and toilets.[15]

The findings regarding satisfaction with reception services are in agreement with results of various studies done in Pakistan and India, where most of the patients were satisfied with registration process and other aspects of the reception area.[6,12,16] However Other studies have reported lower satisfaction including one conducted in Rahim Yaar Khan which showed that 73% of the patients reported over-crowding at the registration counter.[17] According to the second study conducted at Karachi most of the patients left OPD without even getting checkup due to frustration with set up.[18] The lower satisfaction levels in OPD should be controlled by efficient administration to make patient experience hassle free and convenient. The various areas needing focus and where lapses should be prevented include: display of signboards, proper queue system at reception, staff helpfulness and comfort of sitting area.

Regarding the waiting area 73.7% patients were satisfied with its cleanliness. Moreover of those individuals who used toilets only 45.5% were satisfied with their cleanliness and around 25% respondents were not satisfied with the availability of drinking water in waiting area. These results are in accordance with a research conducted in OPD of a tertiary care hospital where 54% were not satisfied with the conditions of the toilets and 30% patients were not satisfied with the availability of drinking water facility.[19] Research shows that having good basic amenities in hospitals is paramount for enhancing the quality of healthcare and improving patient satisfaction and health outcomes. The ease and convenience of patient should be ensured through clean, comfortable waiting areas, a pleasant atmosphere with thoughtfully designed interiors having greenery and natural lighting, easily available safe drinking water and properly maintained hygiene and sanitation facilities.

Another area highlighted by our research was that waiting time was not acceptable for 21.5% patients. This is in contrast to developed countries where most patients are satisfied with the waiting time duration at hospital.[4] The administrative processes should be optimized with efficient appointment scheduling systems and streamlined workflows. Moreover patients should be engaged through informative materials in waiting areas. This will also have additional effect of empowering them with knowledge about their health conditions and treatment options.

A very important component of the OPD services involves interaction of patient with the health care provider. Patients entrust their health and welfare to the doctors, expecting not just technical skill but also empathy and respect. In current study 91.7 % patients reported that the health care provider treated them with respect and courtesy, 86.8% were satisfied with the time given to them during consultation and 85.7% were satisfied with the instructions given to them regarding medication. These levels are bit lower than a study conducted in Peshawar where 90% respondents were satisfied with the time given to them by consultants and 92.5% were content with the conduct of doctors.[4] However the satisfaction levels are higher as compared to another study in Pakistan where high satisfaction with doctors' consultation was reported by 56.4% of respondents only.[13] The difference may be due to different profile of patients shaping their expectations from healthcare providers and different emphasis on this aspect by administrations in various settings.

In this study no relationship was found between various sociodemographic variables and overall satisfaction of patients regarding OPD services. The results are supported by a study in which Patients' satisfaction was reported to be weakly correlated with patient-related characteristics.[12] Similarly other studies reported that there was no statistically significant association between demographic characteristics like age and gender with patient's satisfaction[4,19]. However the findings were in contrast to other studies. Two studies conducted in China and Saudi Arabia reported that satisfaction level of patients was significantly related to age, gender and educational level of the patients.[20,21]

The current study explored the satisfaction of patients with OPD services in detail however more multicenter studies involving a variety of hospitals and catering to patients with diverse background should be conducted

to shed more light on the cardinal predictors of patient satisfaction. It is also recommended that administrators and Healthcare workers should acknowledge patients as partners in their own care journey, respecting their perspectives, and involving them in decision-making processes.

Conclusion

The study revealed that majority of patients expressed high satisfaction level with various domains of OPD services, however waiting time and quality of basic amenities as a part of health services readiness were identified as areas needing further enhancement to ensure maximum patient satisfaction and resultant quality of care in these domains as well. The need for improvement in health services presents a complex task for intellectuals, policymakers, therapeutic experts and hospital administrators to create an environment where patient feels heard, valued and empowered to actively participate in their own health and well-being. This will not only improve satisfaction but also lead to better clinical outcomes in longer run.

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Authors Contribution

SH: Conceptualization of Project
AS, MA: Data Collection
SH, AS, TA: Literature Search
SH, ZP, JI: Statistical Analysis
ZP, JI: Drafting, Revision
SH, ZP: Writing of Manuscript

HPV Infection and its Serotypes in Women with CIN and Cervical Cancer

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Abstract

Objective: To analyze detection of HPV and its subtypes in patients with CIN and Cervical cancer in tertiary care hospitals in Lahore.

Material and Methods: It was descriptive, Cross sectional study conducted in Jinnah, Mian Munshi and INMOL Hospital, Lahore. The study was completed in a duration of 48 months. All patients presenting with symptoms associated with cervical pathology, abnormal cytology on pap smear and confirmed cases of cervical cancer on biopsy were included in this study. Cervical biopsies were taken both for histopathology and HPV testing in patients showing dyskariosis on cytology or visible cervical growth. Confirmed case of carcinoma cervix on biopsy had their sampling done for HPV testing.

Results: There were total 89(81.7%) positive samples for HPV. HPV 16 is the commonest serotype detected (31.3%). HPV 66 was the second commonest variant detected (13.1%). HPV 31, HPV 18, HPV 59 and HPV 45 were the other common serotypes.

Conclusion: Different HPV vaccines available in market provide immunity against 6,11,16,18,31,33,45,52 & 58. However these vaccines are not effective against HPV 66,59,32,61, and 86, which are quite prevalent in our population. So, vaccination and screening should go side by side in Pakistan to cover the disease caused by these non-vaccine type variants.

Keywords: Human papilloma virus, prevalence, distribution of HPV serotypes, invasive cervical cancer, risk factors.

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Introduction

Cervical cancer is the fourth most frequently diagnosed cancer among women worldwide, with an estimated 604,127 new cases and 341,831 deaths in 2020.[1] The highest rates of cervical cancer incidence and mortality are in low- and middle-income countries due to major inequities driven by lack of access to national HPV vaccination, cervical screening and treatment services and social and economic determinants.[2]

Cervical cancer is caused by persistent infection with the human papillomavirus (HPV). Women living with HIV are 6 times more likely to develop cervical cancer compared to women without HIV.[3] Human papillomavirus is a double stranded DNA virus which is sexually transmitted. HPV is now a well-established cause of cervical cancer. HPV types 16 and 18 are responsible for about 70% of all cervical cancer cases worldwide.[4] However, the distribution of HPV subtypes varies in different parts of the world. More than 200 subtypes of HPV are known to exist according to oncogenic potential, HPV can be classified as low risk or high risk. The low risk

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subtypes include 6,11,40,42,43,44,54.61, 70,72,81 and the high risk include 16,18,31,33,35,45,51, 52,58,59,68,73,82.[5] Subtype 58 has been isolated in women with pre-invasive cervical cancer in Thailand, Uganda, Zambia, and Cameroon.[6] Different countries of Europe and America noted that subtypes 31,33,45,52 accounts for most cases of CIN3.[7]

Diagnosing cervical cancer at early stages and thus preventing it via screening measures is very important. During the past 30 to 40 years tremendous efforts have resulted in decrease in the prevalence and resulting deaths due to cervical cancer via introduction of many screening modalities (cervical cytology, HPV DNA detection and VIA) either singly or combined. It has resulted in marked reduction in incidence and mortality in developed countries.[8] In 2019/20 UK NHS has introduced HPV primary screening and cytology side by side with HPV prophylactic vaccination.[9] Non-vaccine types 35,39,51,56,59 are responsible for only 2-11% of CIN2/3, hence a monovalent HPV vaccine can probably prevent the majority of CIN3 irrespective of geographical variations.[10]

In Pakistan 62.8 million woman 15 years and older are susceptible of developing cervical cancer. Cervical cancer ranks as the 5th leading cause of cancer deaths of female cancer deaths in Pakistan. [1]

Annually around 5008 new cases and 3197 deaths occur due to cervical cancer. Prevalence of HPV 16 and 18 in Pakistani women with normal cytology is 0.5%, LSIL is 21.2%, HSIL CIN2 CIN3 CIS is 42.1% and Cervical cancer is 88.1%.[11] In Pakistan, screening of women aged 25-64 years is only 2.3%. The screening program is not very well developed besides vaccines are available to a limited extent.[12]

WHO fact sheet confirms that human papillomavirus subtype 16 is most common type detected in Pakistani women.[13] A study from Karachi reported that 24% of asymptomatic women are HPV 16 and 18 positive, however 88% of women with cervical cancer were screen positive.[14] Another study conducted in twin cities of Punjab (Islamabad and Rawalpindi) showed that HPV 16 is detected in 24.64% among total of 94.81% positive for HPV samples. HPV 18 single is detected in 25.97% and HPV 16 and 18 co-infection found in 40.26%.[15]Our population has different demographic risk factors like being a predominantly Muslim country with regular penile circumcision but poor socio-economic status and poor knowledge of screening results in a very great risk of HPV associated carcinoma and consequent mortality. More than 70% of patients have advanced stages of malignancy in Pakistan on presentation.[16] Other risk factors include unprotected early sex, early marriages, early reproductive cycles and multiparity, smoking and hormonal altered immune system i.e. HIV.[17]

Very few studies have been conducted in Pakistan detecting high risk HPV variants associated with development of Carcinoma cervix and related pathology. No study has been reported about HPV subtype variants in Lahore and adjacent areas. We also need to conduct studies in different areas of Pakistan to know efficacy of vaccines in protecting against infections and cancer development. This study will help us to know the prevalence of HPV viruses in women with moderate and severe dysplasia and carcinoma cervix in our population. By knowing the different subtypes responsible for HSIL, we can use vaccines locally available with confidence or develop an effective one.

Material and Methods

It was descriptive cross-sectional study, conducted in the Department of Gynecology of Jinnah, Mian Munshi, Inmol Hospital Lahore. After the approval from ERB Committee No. 30-01/ERB/30th dated 07-05-2016. Duration of study was 48 months from 1st March 2018 to 28th February 2022 and calculated sample size was 245. Sample technique was non-probability, purposive. Patients presenting with symptoms associated with cervical pathology (post coital bleeding, inter-menstrual bleeding, post-menopausal bleeding, persistent vaginal discharge and pelvic pain), abnormal cytology on pap smear and confirmed cases of cervical cancer on biopsy were included in study. After taking informed consent, detailed history was taken. On speculum examination the cervix was visualized with naked eye and pap smear was taken and recalled after one week. The patient showing dyskariosis on cytology reports had VIA done and biopsies were taken both for histopathology and HPV testing. All biopsy specimens were sent to histopathology in 10% formalin whereas specimen for HPV DNA detection was taken in 0.9% normal saline. However, in cases of visible cervical growth, patients were admitted for EUA and Cervical biopsy and HPV sampling was done. Confirmed case of carcinoma cervix on biopsy

Cytology and histopathology mostly were done by expert pathologist of central lab of Allama Iqbal Medical College/ Jinnah hospital Lahore. HPV DNA detection and PCR were carried out by expert molecular biologist of UHS resource lab and Chugtai Lab Lahore. DNA extraction from samples were done and then case blocks and blank blockers were subjected to real time PCR with modified general primers. 18 different type specific beads for HPV genotyping were used and then sequencing was done. Negative HPV tests were repeated twice taking tissue biopsy as gold standard to avoid any error. So, for 71 tests 100 test kits were used. Bosphore HPV genotyping high risk kit was used, which tested the samples for 18 high risk HPV type 16, 18, 31, 32, 33, 39, 45, 51, 52, 56, 57, 58, 59, 61,66,68,86

Results

A total of 300 women were included in the study. Women who presented with symptoms of irregular bleeding were 224 and 76 had cervical growth on examination. Out of 224, 148 women were reported as Bethesda code 1, had routine follow up. Twenty six of these 224 patients were lost on follow-up. However, 50 women with abnormal cytology on smear were enrolled for biopsy and HPV screening. A total of 109 women with either cervical growth or invasive carcinoma underwent screening for HPV serotypes.

Table 1: *Association of HPV Result with Socio-demographic Variables*

Variables	HPV Result		Total	Chi^2	p-value
	Positive (%)	Negative (%)			
Age (Years)					
≤50	55(78.57%)	15 (21.4%)	70	**0.411**	**0.521**
>50	34 (87.17%)	05(12.8%)	39		
Districts					
Lahore	46 (80.8%)	11(19.29%)	57	**0.023**	**0.879[a]**
others	43 (82.7%)	09 (17.3%)	52		
Total	**89(81.7%)**	**20(18.3%)**	**109**		

[a] *Fisher's Exact test*

Mean age of these women who underwent HPV screening were 49.90±9.39 years with 70(64.23%) were ≤50 years and remaining 39(35.77%) were>50 years; the residence address showed that most of the respondents belong to Lahore 57 (52.29%) followed by Gujranwala 29 (26.6%), Sahiwal 16 (14.6%), Sargodha 08(7.3%), Faisalabad 7 (6.4%) and 01 (0.9%) each in Bahawalpur Division and Multan respectively. The above table showed that among 89 positive cases of HPV there were 61.79% were ≤ 50 years and 38.20% having positive HPV result belonged to >50 years age group. The two attributes were not statistically significant (p=0.521). Whereas, 51.68% with HPV positive results were living in Lahore district and 48.32% with HPV positive result

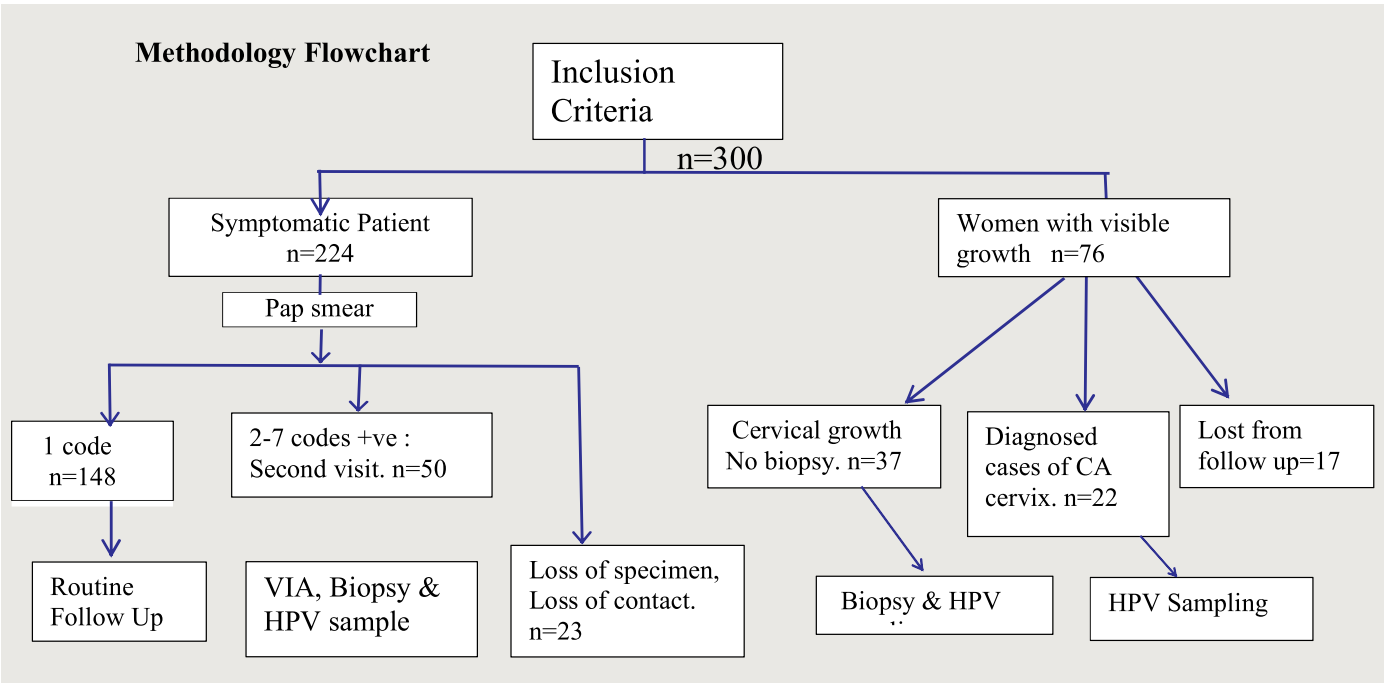

Fig-1: Study methodology.

was dwelling in other districts. The HPV result and districts were insignificant statistically (p=0.879). There were total 89(81.7%) positive samples for HPV

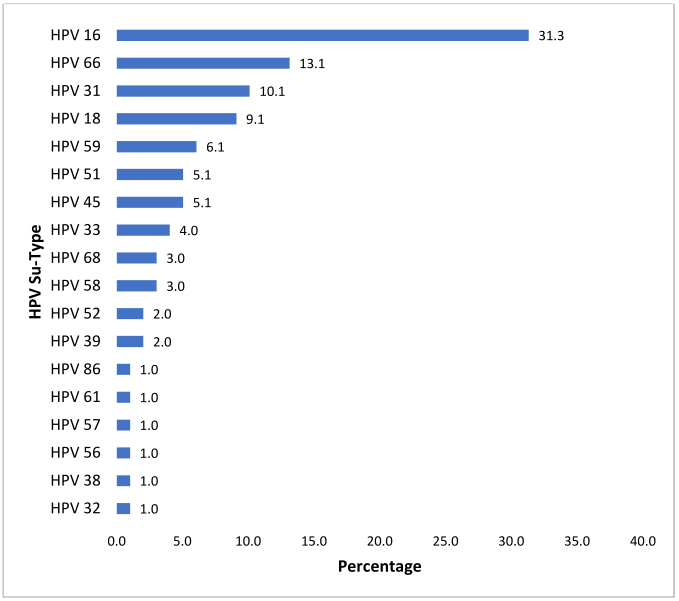

Fig-2: *Graphical Presentation of HPV Type (%) among Female Respondents*

type. HPV 16 is the commonest serotype detected (31.3%). HPV 16 and HPV 66 collectively account for 44.40% of the positive cases. Graphical presentation of percentages of various HPV subtypes found in our samples are as shown below.

Discussion

In 1983 HPV infection was recognized as first tumor virus. Since then over decades of research, it is still thought that HPV is a necessary cause of cervical cancer (99.7% 0f cervical cancer).[18] Regular screening with recall technique and cytology has reduced the disease incidence in developed countries. HPV vaccines solved a major health problem when in 2006 Gardasil a quadrivalent vaccine was first introduced and then in 2008 Cervarix bivalent. A nonvalent vaccine HPV is also available now. In 2009/20 NHS UK has introduced HPV primary screening and cytology side by side with HPV prophylactic immunization program.[19]

In Pakistan, though a preventable disease, the mortality rate is very high as there is no comprehensive national policy for screening and vaccination More than 70% of patients with cervical cancer patients report with very advanced stage of malignancy which results in high mortality rate, increased cost of treatment with poor survival rate.[20]The above-mentioned facts were confirmed in my study also.

In this study HPV 16 being the most common serotype is found in 31.3% of women with cervical pathology. Globally HPV 16 & 18 are found to be responsible for over 70% cases of invasive cancer of the cervix.[21] Similar studies conducted in Punjab and Karachi found HPV 16 to be the most common serotype in cases of carcinoma cervix.[11] In both these studies samples were tested only for HR-HPV 16 and 18 while in my study 17 HR-HPV subtypes have been tested.[22]

Globally HPV 16 is also the most common virus encountered having a proportion of 60.6%. All the vaccines available are effective against HPV 16 (bivalent, quadrivalent, nonvalent).[23]

The second most common HPV type detected in this study is 66 (13.1%). Although HPV 66 is a high-risk virus for cervical cancer but it is not included in first fifteen most common viruses. It may be due to geographical variations. Difference of the risk factors in different populations may account for it as well. No vaccine is yet available against HVP 66.[24]

HPV 31 has a frequency of 10.1% and is the third most common in my samples. Globally it has a frequency of 3.7%.[25] There is greater than average presence of subtype 31 in developing countries (Clifford et al 2005). HPV 31 was the second common (10.9%) in Nordic countries. Only pentavalent vaccines are effective against HPV 31.[26]

HPV 18 having a frequency of 9.1% in my study. However globally it is the second most common type having a frequency of 10.2%.[27] Together HPV 16, 18 and 45 are responsible for almost 90% of cases in most populations. In Pakistan, HPV 18 single had a frequency of 25.97%.[13]All available vaccines are effective against HPV 18. The changes in frequency noted in different areas of even Pakistan demarcates the different risk factors in different areas i.e. use of COCP, smoking, low socio-economic status and poor knowledge of screening.

HPV type specific prevalence varies in different countries and in my study 16, 66, 31, 18, 59, 51 are major five subtypes while other include 45, 33, 68, 58, 52, 39, 86, 61, 57, 56, 38 & 32 in decreasing order as high-risk HPV subtypes for cervical carcinoma and HSIL. HPV 66 has emerged as second common HPV subtype. Another polyvalent vaccine is required to prevent infection from all important subtypes of HPV

in Pakistan.[28]

HPV 61, 32, 86 and 57 found with a variable frequency in my study are not even considered in the first fifteen high risk HPV subtypes all over the world, which is a geographical variation. Further studies should be carried out in all parts of Pakistan before deciding on a national vaccination program.

Screening, knowledge and awareness is very important in developing countries like Pakistan to reduce the burden of late disease similarly vaccines should be developed according to HPV subtypes present in Pakistan.

Conclusion

Different HPV vaccines available in market provide immunity against 6,11,16,18,31,33,45,52 & 58. However available vaccines are not effective against HPV 66,59,32,61, and 86, which are quite prevalent in our population. So, vaccination and screening should go side by side in Pakistan to cover the disease caused by these non-vaccine type variants. Larger studies should be conducted in all parts of Pakistan before forming a vaccination policy.

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Authors Contribution

SY, ZNA, AZ: Conceptualization of Project
ZNA: Data Collection
SY, ZRM: Literature Search
AS, SS: Statistical Analysis
AS, SS: Drafting, Revision
SY, ZRM: Writing of Manuscript

Detection of Hemoglobin Inherited Disorders in Hemoglobin Electrophoresis Sensitivity

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Abstract

Objective: To detect hemoglobinopathies such as β-thalassemia and sickle cell anemia cases using the hemoglobin electrophoresis technique in a population sample from Children's Hospital Lahore.

Material and Methods: This cross-sectional study was carried out from Mar 2023 to Mar 2024 at Thalassemia Department, Fatima Jinnah Medical College, Lahore. Eighty blood samples were drawn from subjects suspected of having hemoglobino-pathies and examined by Hb electrophoresis. The diagnosis of hemoglobinopathies was made based on hemoglobin electrophoresis, sickling tests, and family studies. Individuals with low hemoglobin concentrations accompanied by elevated abnormal hemoglobin percentages were included in this study. Fifty-eight cases of hemoglobinopathies were diagnosed, including 30 (37.5%) with β-thalassemia and 28 (35%) with sickle cell disease carriers.

Results: β-thalassemia and sickle cell anemia carriers were identified in the hematology section of Children's Hospital. Recent hemoglobin protein studies revealed the presence of different common defected hemoglobin types associated with these disorders, distributed as follows: 15 subjects (18.7%) were HbAS (sickle cell minor carriers), 8 (10%) were HbFS (sickle cell minor carriers), 7 (8.8%) were HbS (sickle cell disease), while thalassemic majors were: 9 (11.3%) with HbF and 12 (15%) with HbAF type. Thalassemia minor hemoglobin (HbA2) type represented 7 (8.8%). However, 22 subjects had normal Hb electrophoresis.

Conclusion: The investigations described below demonstrate a rapid and simple method that allows quantitative analysis of the proportions of various hemoglobin forms present. Hemoglobin gel electrophoresis is a simple and convenient technique for studying hereditary hemoglobinopathies at an alkaline pH (4.8 to 6.8). We suggest extending its usage to detect other hemoglobin disorders.

Keywords: Hemoglobin (Hb), Hemoglobinopathies, Sickle cell anemia, hemoglobin disorders, Sickling test.

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Introduction

For the diagnosis of hemoglobinopathies, hemoglobin electrophoresis is the basic technique identifying a diverse array of mutants (genetically different), which are affecting the three-dimensional structures and functionality of hemoglobin. These mutations may cause anemia to fatal diseases. Thalassemia and sickle cell disease represent the most frequent hemoglobinopathies. Hemoglobinopathies is a genetic disorder due to abnormality of hemoglobin and it constitutes several monogenic disorders. Due to these mutations, altered synthesis of hemoglobin causes structural changes in chains, ultimately in 3D folding of the hemoglobin, and leads to severe health conditions like hemolytic anemia, Sickle cell disease, Thalassemia, polycythemia, and erythrocytosis. Thalassemia syndrome is a series of genetic disorders in hemoglobin (Hb) synthesis, characterized by a reduced rate of production of one or more of the globin chains of hemoglobin. About 3%

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of the world population carries genes for β-thalassemia and 3000 mutants of hemoglobin[1]. The adult hemoglobin (HbA) is a tetrameric protein(α2β2) and two types of globin gene clusters are responsible for forming two different genes of a tetramer. β-thalassemias are autosomal recessive inherited blood disorders that result mainly from mutations that decrease (β+) or eliminate (β0) the production of β-globin. This leads to an excess of α-globin, which precipitates in developing erythroblasts, resulting in ineffective erythropoiesis.[2-3] Patients with β-thalassemia are characterized by hypochromic, hemolytic anemia, and dependence on blood transfusions to sustain life.[4]

β-thalassemia is widely distributed throughout the world, with considerable frequencies in the Eastern Mediterranean countries, including Iraq,[5] and the Arabian Gulf region.[6] It has been estimated that 3% of the world's population, or 200 million people, in addition to almost 150,000 affected individuals born annually, carry the β-thalassemia gene. There are several variants of thalassemia due to different structural changes in hemoglobin and each of them has a different severity level. This gene carries a wide spectrum of clinical manifestations, ranging from β-thalassemia intermedia to severe, transfusion-dependent β-thalassemia major. The clinically important feature of β-thalassemia is its interaction with other hemoglobinopathies, like sickle cell disease, in co-inheritance, which improves the hematologic parameters of heterozygous β-thalassemia.[7] β-thalassemia minor is also a type of thalassemia, in which the person is heterozygous for the trait, it inherits one normal and the other one is thalassemia variant gene. Usually, these people do not have any kind of the symptoms but sometimes mild anemia, microcytosis, hypochromia, and increased iron load could be observed. From their diet, the absorption of iron ions increased.

Sickle cell disease (SCD) is a protean disorder caused by elevations of intra-erythrocytes and total blood viscosity. Hypoxia-induced gelation of hemoglobin S (HbS) deforms the erythrocyte and its membrane, causing massive cation loss and increased erythrocyte surface expression of adhesion molecule receptors.[8] This leads to hemolytic anemia and acute vasoocclusion, resulting in organ damage from recurrent erythrocyte sickling, chronic hemolysis, and progressive endothelial vasculopathy.[9]

Like other parts of the country, hemoglobinopathies are an expression of the β-globin gene, leading to changes in the rate of synthesis of β-globin chains of hemoglobin. In classic β-thalassemia major, life expectancy is shortened to 25-30 years on average, due to associated complications such as growth retardation,[10-11] diabetes mellitus, endocrine dysfunction, hypothyroidism, progressive failure, and cardiac complications.[12-20]

Hemoglobinopathies are an important problem in Nassiriyah province (in southern Iraq, with a population of around 1.5 million and over 400 registered patients). The emergence of these disorders is partially due to consanguineous marriages, which are common in this area. This article aims to use hemoglobin gel electrophoresis to detect some hemoglobinopathy forms. With the process of hemoglobin electrophoresis, hemoglobin components can be separated from each other on the basis of their charge and mass value. This approach may contribute to treatment and possibly prevent the transmission of the hemoglobin mutation by identifying carriers, as their offspring are at risk of inheriting the mutation.

Material and Methods

This cross-sectional study was carried out from Mar 2023 to Mar 2024 at Thalassemia Department, Fatima Jinnah Medical College, Lahore. After taking approval from ethical committee Reference No UCP/ORIC/EC/08 Dated 24-08-23. A total of 80 subjects with low hemoglobin levels (under 10mg/dl) suspected of having hemo-globinopathies and voluntary individuals with signs and symptoms of anemia, who attended hospitals, were recruited for this study. All were ethnic Arabs, with ages ranging between 1.2 and 34 years (median age of 7.8 years). They included 38 males and 42 females. 3 ml whole blood samples were collected in EDTA-coated tubes from patients during their hospital visits and used immediately. Hemoglobin level: The level of hemoglobin was checked using the Reflotron Plus (Roche, Germany) Roche diagnostic GmbH machine.

Complete blood count was determined using the Coulter Micro diff II machine according to the manufacturer's instructions, in addition to routine examination of peripheral blood films. A sickling test was performed by adding 50 µl of well-mixed whole blood to 4 ml of phosphate buffer/sodium hydrosulfite solution (one tube for each test and each control). The tube was covered with a cap or parafilm, mixed three or four times, and then incubated in the reading rack for 10 to 20 minutes at room temperature. A positive result was indicated by the presence of turbidity and the absence of visible lines on the reading rack. Electrophoresis of the hemoglobin solutions was carried out in an apparatus (HelloBio), utilizing the alkali denaturation technique as follows: Venous blood was drawn from fasting individuals into EDTA-treated vacuum tubes. About 100-200 µl of whole blood

was added to a tube with 10 ml saline and centri-fuged. 30 µl of the sediment was mixed with 130 µl of hemolyzing solution. Then, 5 µl of each hemolysate was applied across the slits and left for 20 to 30 seconds to allow absorption. The gel was placed in the tank with samples on the cathodic side and run at 200 volts for 20 minutes. The gel was dried completely with hot air (less than 60°C) and stained for 5 minutes with a protein staining solution. The film was destained for 5 minutes in three destaining solution baths and dried again with hot air. The results were analyzed, and the standards were evaluated and analyzed by software using HbAFSA2 and HbAFS protein.

Results

Low hemoglobin concentration is a common manifestation of anemia caused by many environmental factors, such as malnutrition and hemorrhagic conditions, or by hereditary factors, such as hereditary persistence of fetal hemoglobin (HPFH) and hemoglobinopathies (HbP). The normal ranges of Hemoglobin in adults and infants are represented in Table 1. In the case of hemoglobinopathies, the percentage of HbA, HbS, and HBA2 is disturbed due to mutation in the beta chain of hemoglobin. In this study, we found that the age of HbA2, HbAF, and HbAS carriers was older than HbF and HbS hemoglobin type carriers. The 70 unrelated blood samples derived from Iraqi β-thalassemia and sickle cell carriers were analyzed to elicit several forms of sickling and β-thalassemia hemoglobin protein patterns as follows. Table 2 shows the representative characteristics of Beta thalassemia and sickle cell disorders. Thalassemia gene carriers are characterized by the absence of the S form of hemoglobin (HbS), having a high concentration (70-90%) of the fetal form of hemoglobin (HbF), accompanied by an HbA2 hemoglobin pattern as low as normal (less than 1%) as shown in Figure 1, and the highest percentage of hypochromic and reticulocytes obtained by blood film monitoring. In Figure 2, the difference between the normal and variant of hemoglobin polypeptide is also indicated in which Hb-A has normal Glutamate at 6th position, while Hb-S, the variant of polypeptide indicating that valine replaced the glutamate at 6th position, represented in red color.

Table 3: *Comparison of Predictive Values (Bishop Score vs. Cervical Length)*

Hemoglobin	Chains	Normal % Adult	Normal % Neonate
HbA	A_2, β_2	96-98%	27%
HbF	A_2, γ_2	0.5-0.8%	70%
HbA2	A_2, δ_2	1.5-3.2%	0.3%

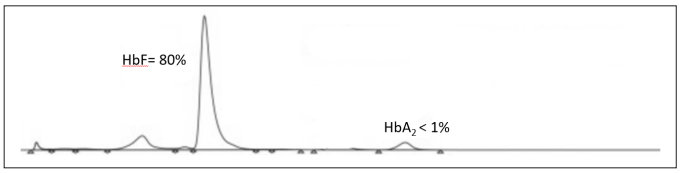

Figure 1: *HPLC of an adult thalassemia disease carrier*

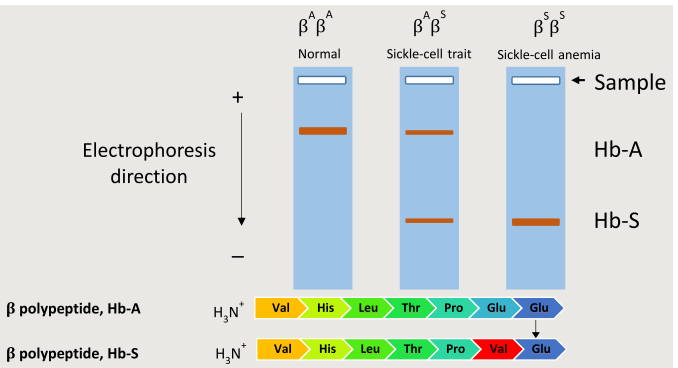

Figure 2: *Electrophoretic hemoglobin pattern of sickling trait*

Table 2: *Representative characteristics of Beta thalassemia and sickle cell disorders*

Hemoglobinopathies	Hb(AS)	Hb(S)	Hb(FS)	Hb(F)	Hb(AF)	Hb(A2)
N	15	7	8	9	12	7
Age (years)	28.3-34	2-28	1.8-5.2	1-2.8	2-6	2.5-7
Hb g/dl	10.04±1.35 8-2	9.3±1.5 7.8-12.2	8.3±1.8 6.2-12	5.3±1.4 3-8	7.4±1.6 5.5-10.8	9±1.3 6.8-10.8
Reticulocytes	2.67±1.06 1.2-4.06	1.83±0.53 1.96-3.2	3.74±1.19 2-5.4	6.1±1.9 4-10	3.3±0.79 2.2-4.8	2.90±0.87 2-3.88
Hb F (%)			48.2±8.19 33.6-60	86.62±5.8 79.8-93.2	40.15±6.77 28.8-48	
Hb A2	1.27±0.45 0.53-2.02	0.92±0.51 0.22-2.0	0.83±0.66 0.1-2.02	0.96±0.47 0.56-26	1.58±0.71 0.8-3.2	9.33±2.49 6.22-12.3
HbS	42.41±6.70 30.8-55	85.04±6.28 78.4-92.06	40.4±6.85 30.8-50.2			

Discussion

Hemoglobinopathies are the most common red blood cell genetic disorder, for its treatment it is necessary to recognize the mutations in hemoglobin chains. Abnormalities of hemoglobin are quite common, mostly it is detected in public by surveys and prevention programs laid for the treatment of hemoglobinopathy. For better confirmation of any abnormality in hemoglobin, at least two methods of detection are used. The more efficient methods of diagnosis are CE-HPLC (Cation exchange high-performance chromatography), CE (Capillary electrophoresis and IEF (isoelectric focusing). CE HPLC is used to diagnose abnormalities in adults as well as in children, it is able to differentiate between SS, AS, SD, S-β+-thal, AC, SC, and C-β+-thal, in blood. Red blood cells become more glycosylated in old age[21]. In the case of isoelectric focusing, variants of hemoglobin having different isoelectric points can be separated from each other at zero pH. In this way, different mutants can be identified so far.

In this study, we found that the age of HbA2, HbAF, and HbAS carriers was older than HbF and HbS hemoglobin type carriers. The 70 unrelated blood samples derived from Iraqi β-thalassemia and sickle cell carriers were analyzed to elicit several forms of sickling and β-thalassemia hemoglobin protein patterns as follows. The presence of mild microcytosis, in the absence of iron deficiency, suggests the presence of β-thalassemia. These carriers or individuals with β-thalassemia trait are essentially normal, although they can usually be detected by screening red cell indices that demonstrate a reduced mean corpuscular volume (MCV) and reduced mean corpuscular hemoglobin value (MCHV). Gel electrophoresis precisely determines the elevation of hemoglobin A2 levels in thalassemia minor. Our result is consistent with other studies previously submitted in literature. This thalassemia intermediate pattern may result from the elevation of fetal hemoglobin (HbF) to around 30-50% as illustrated in thalassemia disease.

Hemoglobin gel electrophoresis may yield a pattern of 65% sickle hemoglobin and 34% F hemoglobin. The presence of normal (A) hemoglobin in combination with more than 90% S hemoglobin has so far only been encountered in sickle cell-thalassemia disease. Blood transfusions are required during the treatment of this disease.

This genetic disorder is accompanied by one normal gene and one sickle cell gene (HbS). It is an autosomal recessive disease, for an infected person it is necessary to be homozygous for the sickle cell genes. A protein with a negative charge, hemoglobin (Hb) is drawn to the anode pole of an electric current and separates from the positive pole as a result. The kind and intensity of the charge, in addition to the hemoglobin type's molecular weight, influence this movement. Currently, the hemoglobin molecule is known to be susceptible to over 300 genetic mutations, some of which have obvious clinical consequences and pose a serious risk to health, particularly in heterozygous conditions.[22]

Certain studies suggest that the fractional HbS content of sickle trait erythrocytes may influence the severity of certain clinical complications.[22] The amount of S hemoglobin in sickle cell trait carriers has been found to vary from 34 to 39%, without any apparent correlations to the severity of their clinical manifestations. We encountered several instances of low hemoglobin values in patients with a positive sickling test, caused by a variety of anemia superimposed on the sickling trait. Electrophoretic analysis of the hemoglobin of some individuals revealed that even in the absence of a positive sickle cell test by blood film test, erythrocytes may still contain small amounts of S hemoglobin, consistent with a study.[23] The reduction of the HbS level in the sickle cell trait associated with α-thalassemia can be explained by a greater affinity of BA than BS chains for α chains in limited supply.

Electrophoresis of hemolysates of patients with sickle cell anemia produced a characteristic pattern, which consisted of a major component of hemoglobin S showing variations from 70 to 91%, and usually with reciprocal values for F hemoglobin (data not shown). Fetal hemoglobin (HbF) is a major contributor to the remarkable phenotypic heterogeneity of sickle cell anemia and influences the levels of disease severity.[24] Since, in some patients with the disease, the minor component was less than 5% or even absent, quantitation of the fetal fraction from gel electrophoresis is necessary.

Our results indicated that SCA patients' blood films are characterized by a low rate of hypochromic and reticulocyte cells by microscopic diagnoses. By indirect investigations, we revealed that some anemia patients may have suffered from transient hemolysis due to pyogenic infection or occurrence of G6PD (glucose-6-phosphate dehydrogenase deficiency), but not due to heritable hemoglobin disorder. In those individuals, hemolysis ceased after appropriate treatment. It may be argued that the high consanguinity rate among homozygous

individuals may have affected the actual frequencies of hemoglobinopathies' emergence. The significant stimulus to carry out such studies was the demonstration that hemoglobin in hemoglobinopathy patients is electrophoretically different from normal adult hemoglobin, and their assessment does not require a complex apparatus and special skills necessary for common detection methods.

Conclusion

Hemoglobinopathies and hemoglobinopathy traits require comprehensive laboratory examination using CBC, blood cell morphology, and qualitative and quantitative electrophoresis. The results indicate the importance of quantifying HbA, HbF, and HbA2 to aid in the differential diagnosis of different types of hemoglobinopathies. This investigation narrates a rapid, efficient, and simple method that can quantitatively analyze the proportions of various hemoglobin variants present in the blood sample. Hemoglobin gel electrophoresis is a simple and convenient technique for studying hereditary hemoglobinopathies at an alkaline pH (4.8 to 6.8). We suggest extending its usage to detect other hemoglobin disorders.

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Conflict of Interest *None*

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Authors Contribution

SI: Conceptualization of Project
AA: Data Collection
SP: Literature Search
SZ: Statistical Analysis
SH: Drafting, Revision
AA: Writing of Manuscript

Understanding Women's Perspectives on Dating Scan: Knowledge, Attitudes, and Behaviors

Nayyer Sultana,[1] Hina Ahmed[2]

Abstract

Objective: To determine the knowledge, attitude and practice among women of reproductive age group regarding benefits of dating scan.

Material and Methods: A cross sectional study was conducted in the Out Patient Department (OPD) of Obstetrics & Gynecology at Central Park Teaching Hospital form November 2021 -April 2022. The married women of reproductive age from 15-45 years were recruited in the study via structured questionnaire. The relationship between the categorical variables was determined by chi-square test by keeping the p value ≤ 0.05.

Results: The average age was 32.09 + 6.96 SD years. Out of all, 103 (25.8%) females were illiterate. Around 35% of the females were matric and below and remainder were above matric to masters. Majority of participants had knowledge about the importance of dating scan in assessment of fetal viability 73.8% (n=295) followed by number of fetuses 70.0% (n=280). While least of study participants (10%) had knowledge of screening of down syndrome, other knowledge parameters were also poorly known by study population. About 23.6% of the females said that they never had dating scan in any pregnancy.

Conclusion: The majority of participants had significantly adequate knowledge regarding importance of dating scan for the detection of fetal viability. Moreover, significantly inadequate knowledge and practice was observed regarding getting it done for calculating accurate dating, congenital abnormalities and screening for Down's syndrome through nuchal translucency.

Keywords: dating scan, knowledge, fetal, attitude, reproductive age, anomalies.

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Introduction

Advancements in medical technology have significantly transformed the landscape of prenatal care, offering a window into the early stages of fetal development that was previously inaccessible. One of the pivotal innovations in this domain is the dating scan, a non-invasive imaging technique that provides crucial insights into the well-being of developing fetus during the earliest stages of pregnancy.[1] The first trimester, spanning from conception to the 14th week of gestation, marks a vital period characterized by rapid embryonic growth and organogenesis.[2] The utilization of dating scan has become a routine component of contemporary prenatal care in many parts of the world. World Health Organization (WHO) aims that every pregnant woman gets comprehensive and quality antenatal care services and recommends at least four contact sessions throughout pregnancy with one in the first trimester.[3] It is done for many indications which include confirmation of intrauterine pregnancy and cardiac activity, to rule out ectopic pregnancy, accurate dating of pregnancy, confirmation of congenital anomalies , number of fetuses determination, evaluation of multiple pregnancy by establishing chorionicity, establishment of causes rela-

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ted with early pregnancy bleeding and evaluation of suspected gestational trophoblastic disease.[4] It is evidence based that first trimester measurement of crown rump length is more reliable than a certain menstrual date in calculating duration of pregnancy and thus unnecessary inductions for presumed prolonged pregnancy may be reduced.[5] Early pregnancy ultrasound can detect up to 63% of congenital anomalies that is helpful for the parents to decide termination of pregnancy at early gestational age.[6]

Despite its clinical advantages, the perception and decision-making processes that underlie the uptake of dating scan among women of reproductive age are influenced by a myriad of factors, including cultural beliefs, socioeconomic status, access to healthcare, and individual preferences.[7] It has been observed that education level is playing a pivotal role in creating awareness among expectant mothers.[8] Globally, there is wide variation of antenatal coverage between and within countries. Factors associated with high antenatal coverage being, urban residence, more education and wealthier status.[9] The rationale of current study is to determine the awareness, attitude and practices among females of reproductive age about the importance of dating scan which will be a step towards behavior change. The results will highlight the importance of early non-invasive screening among the females of reproductive ages.

Material and Methods

A cross sectional study was carried out in the Out-Patient Department of Obstetrics & Gynecology at Central Park Teaching Hospital from November 2021 to April 2022 for assessment of knowledge, attitude and practices regarding dating scan in young females. A total of 400 married women of reproductive age with age range of 15 to 45 years were recruited in this study. Sample size of 400 was computed using WHO sample size calculator by assuming prevalence of first trimester scan at 56 percent with confidence interval of 95% and margin of error at 5%.[10] As per of Helsinki Declaration, ethical letter was obtained from institutional review board of Central Park Medical College & Teaching Hospital Lahore with IRB number as CPMC/IRB-No/1276 dated 28-04-2021 and prior written informed consent was obtained from all study participants. Study participants were recruited by non-random convenient sampling technique. The nulliparous women and the women who were infertile were excluded.

A structured questionnaire was used to collect demographic data and for assessment of knowledge and practice pertaining to dating scan. In this questionnaire, questions about importance and practical appliance of scan because of accurate dating, ectopic pregnancy, number of fetuses, fetal viability, early pregnancy bleeding, fetal anomalies and for down syndrome were asked. Before administration to whole cohort, a pilot was conducted on 20 participants and was validated with alpha Cronbach of 0.9. Once the data collected it was compiled and analyzed by SPSS version 26.0 and was dully compared for errors and omissions. For quantitative variables mean and standard deviation was calculated. The relationship between the categorical variables were determined by chi-square test by keeping the p value ≤ 0.05 with 95% confidence interval and power of study kept at 80%.

Results

The data was collected from 400 females of reproductive age with mean age of 32.09 + 6.96 years. Approximately half of the females had monthly family income of Rs 25000 PKR or more. Out of all 103 (25.8%) of the females were illiterate. More than three-fourth of the females owned the houses. About 59.3% of the females lives in extended family. The most common occupation of the spouse 95(23.8%) was daily wages. Around 324(81.0%) of the females were housewives. **(Table1)**. In our study, majority of participant had knowledge about the importance of dating scan in assessment of fetal viability 73.8% (n=295) followed by number of fetuses 70.0% (n=280) as explained in **(Table2)**. While least of study participants had knowledge of screening of down syndrome through measuring nuchal translucency i.e. 10.2% (n=41), other knowledge parameters were also poorly known by study population as explained in **(table 2).**

It was observed that a large number of females will opt for antenatal visit during first trimester. More than half said that they keep the ultrasound scan done during first trimester safe for reference. Around more than one-third of the females said that dating scan should be done by consultant obstetrician followed by 35.0% of the females who said MBBS doctor. Nearly three-fourth of the females do not get information regarding benefits of dating scan. Among those who get the information, around 14.2% of the females said that the source of information was doctor followed by internet with 3.3%. About 62.8% of the females said that they recommend

Table 1: *Baseline characteristics of the respondents*

Factors	Categories	N	%
Education	Illiterate	103	25.8%
	Primary	48	12.0%
	Middle	40	10.0%
	Matric	52	13.0%
	Intermediate	59	14.8%
	Graduate	55	13.8%
	Postgraduate	39	9.8%
	Hafiz Quran	04	1.0%
Spouse Education	Illiterate	87	21.8%
	Primary	32	8.0%
	Middle	42	10.5%
	Matric	71	17.8%
	Intermediate	47	11.8%
	Graduate	60	15.0%
	Postgraduate	59	14.8%
	Hafiz Quran	02	0.5
Monthly Family Income	Nil	06	1.5%
	Less than 25000 PKR	195	48.8%
	>= 25000 PKR	199	49.8%
House	Rented	85	21.2%
	Owned	315	78.8%
Type of Family	Nuclear	163	40.8%
	Extended	237	59.3%
Occupation	Housewife	324	81.0%
	Daily wages	14	3.5%
	Field worker	03	0.8%
	Bhatta worker	01	0.3%
	Teacher	27	6.8%
	Staff Nurse	05	1.3%
	Medical/ Allied	13	3.3%
	Office worker	06	1.5%
	Entrepreneur	07	1.8%

dating scan (Table 3).

Table 3 indicates the attitudes, practice and purpose of dating scan. Half of the females said that they had ultrasound in all pregnancies. About 23.6% of the females said that they never had dating scan in any pregnancy. The most common purpose of dating scan was fetal viability followed by to know the number of fetuses. The least common purpose was measuring nuchal translucency.

Discussion

Our study represented wide variation in the study population in terms of age, parity, education and socioeconomic status. The current study targeted the women of reproductive age (15-45 years) who were either currently pregnant or had undergone the experience of pregnancy. We examined their knowledge, attitude and practices regarding dating scan.

Regardless of their level of education or socioeconomic standing, approximately three-fourths of the study population knew something about dating scan. More advanced details were related to higher education. Early pregnancy ultrasonography offers crucial details about the gestational age, congenital abnormalities, and the number of fetuses that may influence the antenatal care strategy. In our study, fetal viability was the most well-known of the early pregnancy ultrasound's several components. About 73.8% of women were aware that a first-trimester ultrasound is used to determine the viability of the fetus. In their study of women's knowledge, Mubuuke Aloysius Gonzaga et al. demonstrated that almost all women, regardless of education level, were aware that ultrasound is used to determine fetal viability.[7]

Table 2: *Knowledge of the respondents about dating scan*

Knowledge Parameters	%, (n) n=400		p-value
	Yes	No	
Do you know? Dating scan provides more accurate dating of pregnancy than menstrual date.	39.8%, (159)	60.3%, (241)	0.000*
Do you know? Dating scan can detect ectopic pregnancy early.	49.0%, (196)	51.0%, (204)	0.000*
Do you know? Dating scan can detect number of fetuses.	70.0%, (280)	30.0%, (120)	0.000*
Do you know? In case of multiple pregnancies, ultrasound being done during first trimester may provide valuable information that help in further management plan of pregnancy.	31.8%, (127)	68.3%, (273)	0.000*
Do you know? Dating scan detects fetal viability.	73.8%, (295)	26.3%, (105)	0.000*
Do you know? Dating scan helps detect cause of early pregnancy bleeding like miscarriages and hydatidiform mole.	30.8%, (123)	69.3%, (277)	0.000*
Do you know? Dating scan can detect some fetal congenital anomalies.	34.3%, (137)	65.8%, (263)	0.000*
Do you know? First trimester scan can screen for Down's syndrome.	10.2%, (41)	89.8% (359)	0.002*

Table 3: *Attitude and Practice Trends of the respondents about dating scan*

Attitude	Response	N	%
In your opinion, would you opt for antenatal visit and ultrasound during first trimester?	No	69	17.3%
	Yes	331	82.8%
In your opinion, dating scan should be kept safe for future reference for example dating the pregnancy.	No	171	42.8%
	Yes	229	57.2%
In your opinion, dating scan should be done by	Midwife	41	10.2%
	MBBS doctor	140	35.0%
	Consultant Obstetrician	135	33.8%
	Sonologist	84	21.0%
Do you try to get information regarding benefits of dating scan?	No	305	76.2%
	Yes	95	23.8%
If you try to get information, then the source of information is?	No one	306	76.5%
	Friend	10	2.5%
	Neighborhood	11	2.8%
	LHV	02	0.5%
	Doctor	57	14.2%
	Internet	13	3.3%
	Other	01	0.3%
Do you recommend others to have dating scan?	No	149	37.2%
	Yes	251	62.8%
Practice			
Based on your previous experience, did you have dating scan in?	Never	94	23.6%
	All pregnancies	200	50.0%
	First pregnancy	33	8.3%
	Second pregnancy	18	4.5%
	Current pregnancy	55	13.8%
what was the purpose of getting your dating scan? fetal viability	No	106	26.5%
	Yes	294	73.5%
what was the purpose of getting your dating scan? number of fetuses	No	246	61.5%
	Yes	154	38.5%
what was the purpose of getting your dating scan? early pregnancy complications	No	293	73.3%
	Yes	106	26.5%
what was the purpose of getting your dating scan? fetal anomalies	No	308	77.0%
	Yes	92	23.0%
what was the purpose of getting your dating scan? measure nuchal translucency	No	384	96.0%
	Yes	16	4.0%
what was the purpose of getting your dating scan? see my baby	No	277	69.2%
	Yes	123	30.8%

An important piece of information about the location of the pregnancy can be gleaned from an early ultrasound. 2.7% of maternal deaths resulting from pregnancy are attributed to ectopic pregnancies.[11] Only 49% of women in our study were aware that an ectopic pregnancy could occur and that an ultrasound can pinpoint the pregnancy's location. The incidence of multiple pregnancy is increasing. An early pregnancy ultrasound is crucial for determining the number of fetuses and chronicity in order to plan for antenatal care in the event of monochorionicity and for preventing complications.[12]

In our study, 70% of women knew that early pregnancy ultrasound can detect number of fetuses but only 31.8% of them was aware of the significance of chorionicity and sought out a professional for a comprehensive ultrasound. When determining duration of pregnancy and expected date of delivery, early pregnancy ultrasound is more accurate than menstrual date.[13] Duration of pregnancy calculated by early pregnancy ultrasound as opposed to menstrual date can reduce the incidence of induction of labour for post term pregnancies.[14] Just 39.8% of the ladies in our research were aware of this fact. Only 57% of the women who received early ultrasounds preserved the images for future use. Chan L. et. al demonstrated this knowledge in 50% of women.[15] An early scan performed at 12–13 weeks' gestation by a skilled sonographer can discover approximately 50% of structural anomalies that are prenatally detectable and 100% of those that are anticipated to be detected at this stage. These include neural tube defects, omphalocele and severe skeletal anomalies.[16] In our study, only 34% of women knew that dating scan can detect structural abnormalities and all these women either belonged to health care profession or had higher education. Chen L.et.al described similar findings in his study done on Chinese population, where 35% women knew early pregnancy ultrasound can detect structural anomalies (neural tube defects).[15] In Ethiopia to get an Ultrasound scan done for the purpose of detecting congenital abnormalities was the least common reason identified.[17] A suitable and non-invasive method to check for chromosomal abnormalities is to assess the nuchal translucency during an early prenatal ultrasound between 10 and 13 weeks of pregnancy.[18] Only 10.2% of the women in the current study were aware that Down's syndrome can be detected during an early pregnancy ultrasound, and of those, only 4% asked to have their nuchal translucency measured. In her study on the awareness of prenantal

diagnosis, Ayesha Isani found that just 22% of the study community was aware about Down's syndrome and its screening[19]. Chen L et.al demonstrated this knowledge in 43% of study population.[15] Early detection and treatment of molar pregnancy can lead to improved maternal outcome 20. In our study, only 30 % of the respondents knew the fact that molar pregnancy can be detected on early trimester ultrasound.

For structural anomalies and aneuploidy screening, sonologists need training and a learning curve.[21] Early pregnancy ultrasounds, when carried out by a skilled sonologist, can offer complex information on nuchal translucency and structural anomalies, which aids parents in their decision to terminate the pregnancy at an early gestation. Only 21% of the participants in our study chose a sonologist for their dating scan. The majority of the ladies believed that an MBBS doctor or consultant gynaecologist was qualified enough to do this ultrasonography. 10% of respondents felt a midwife could perform it. These women were all uneducated and older than 40. In our study, 23.6% of women never had dating scan. These were the women who did not receive formal education, their age range was between 40-45 years and their last child birth was in the last 18 years. These were the women according to them this ultrasound was not important to perform that is why they never went for this. Contrary to this, around 70% had their dating scan done and more than 90% acknowledged its importance. Out of these women, 13.8% were currently pregnant and 50% had their last child birth within 5 years. These were the women who received formal education from matriculation to postgraduation. According to the Pakistan Demographic and Health Survey, first trimester prenatal coverage was at 55% in 2017–18 and 15% in 1990–19, respectively.[22] This demonstrates a 27-year increase of 40% in the number of first-trimester visits. This shows how attitudes and behaviours can change throughout time. In Mubuuke et.al study 83% of women had positive attitude towards dating scan[7]. Priyanka Dhiya et al depicted 83% acceptability of first trimester screening.[23] Munim S et.al depicted around 93% women knowing ultrasound is an important investigation but did not exactly knew the exact timing to have one.[24] In a multicenter study done in Ethiopoia, 38% had ultrasound on their own request while 40% had on the request of their health care provider.[25]

Conclusion

The majority of participants had significantly adequate knowledge regarding importance of dating scan for the detection of fetal viability and number of fetuses but the practice was significantly inadequate. Moreover, significantly inadequate knowledge and practice was observed regarding getting it done for calculating accurate dating, detection of hydatidiform mole, congenital abnormalities and screening for Down's syndrome through nuchal translucency. There is need to enhance the knowledge regarding importance of dating scan among the expectant mothers in which the health care professionals, seminars and the social and print media can play a vital role.

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Authors Contribution

NS, HA: Conceptualization of Project
NS: Data Collection
NS: Literature Search
HA, NS: Statistical Analysis
NS, HA: Drafting, Revision
NS: Writing of Manuscript

Association Between Hyperglycemia and Short-term Outcome in Patients with Ischemic Stroke

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Abstract

Objective: To find association between Hyperglycemia and short-term outcome on patients with ischemic stroke.

Material and Methods: A prospective cohort study was conducted in Neurology Department, Sir Ganga Ram Hospital. Study was conducted for 6 months and it included 60 patients. Fasting blood glucose and 2 hours postprandial blood glucose level and history of Diabetes Mellitus (DM) was recorded on 1st day of admission. HbA1C was measured at baseline. All the patients were assessed at 1st admission day as per the modified Rankin Scale (mRS) and were managed as per policy of the department. Patients were followed up on 30th post-stroke day after discharge. On 30th day, they were assessed by mRS, and short-term poor outcome was recorded in both groups.

Results: Relative risk for poor outcome among exposed patients was 4.20 which means that patients in exposed group had 4.20 times more chances of poor outcome as compared to unexposed patients. Patients in the age group 24-35 and 46-55 years had 15.16 and 11 times higher risk for poor outcome in patients with uncontrolled diabetes. The mean age of patients in exposed and unexposed group was 51.30±14.18 and 43.96±13.12 years.

Conclusion: Uncontrolled diabetes was significantly associated with and poses a high risk for short-term poor outcome in ischemic stroke patients.

Keywords: Ischemic stroke, Uncontrolled, Diabetes mellitus, Short term, Outcome

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Introduction

Stroke is a major cause of death in adult population following cardiac diseases and is responsible for about 10-15% of total deaths each year. Also it contributes as a major cause in long-term morbidity among survivors, as about 35% of the sufferers don't get independent in their future life.[1] According to an estimation by World Health Organization, about 15 million people suffer from stroke per year worldwide.[2]The prevalence of stroke in Pakistan is estimated to be around 21.8% which is more than the rest of the world.[3]Diabetes is a well-well-known risk factor for stroke and various consequences.[4] According to Doi Y and colleagues' Hisayama study, diabetics had twice the risk of stroke as non-diabetics in the overall Japanese population.[5] Additionally, diabetics had a worse outcome after a stroke than non-diabetics. Previous studies have found that diabetics have more residual neurological abnormalities and a lower functional result than non-diabetics. As a result, diabetic patients had greater hospital and long-term mortality rates than non-diabetics, albeit further research could not substantiate these results.[6] In one

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study, the poor outcome rate was higher in patients with uncontrolled Diabetes Mellitus (65.9% in uncontrolled diabetes Mellitus vs 5% in control population p-value 0.001).[7]

Only minimal studies have compared the difference in outcome between controlled, uncontrolled and non-diabetics. With the aim of understanding the impact of blood sugar control on short-term prognosis in ischemic stroke, we designed a study to compare outcomes in patients with controlled, uncontrolled, and non-diabetic conditions.

Material and Methods

The study was carried out at Neurology Department, Sir Ganga Ram Hospital, for six months. The study design was prospective cohort study and non-probability consecutive sampling technique was used. After taken approval from ethical committee No102/ERC/IPH Date 20-3 -2024. A sample size of 60(30 in each group) patients was calculated at 5% level of Significance and 80% power of test and taking expected frequency of poor outcome rate in uncontrolled DM (Diabetes Mellitus) is 65.9% and control group is 5%. Approval from hospital ethical review committee was taken. 60 patients (30 exposed and 30 not exposed) fulfilling the inclusion criteria were enrolled in the study. Informed consent was taken from each participant of the study. In all patients fasting blood glucose and 2 hours postprandial blood glucose level were monitored on 1st day of admission and history of DM was acquired. HbA1C was measured at baseline. All the patients were assessed at 1st admission day as per mRS (modified Rankin Scale). They were managed as per policy of the department and after discharge they were followed up at 30th post-stroke day. At 30th day, they were assessed by mRS and short term poor outcome was recorded in both groups. All the collected data was transferred to SPSS version 20 and analyzed accordingly. Frequency and percentages were calculated for qualitative variable like gender. Mean and standard deviation were determined for all quantitative variables like Age and scores in all groups (using mRS scale). The Relative Risk (RR) was determined in all stroke patients in each group. Stratification was done for age and gender using chi square test and p value <0.05 was selected as significant.

Results

Patients were divided into two groups, based on the presence or absence of diabetes. The group of patients with diabetes was labeled as "exposed group" while the group of patients without diabetes was labeled as "unexposed group". Mean age of patients in exposed and unexposed group was 51.30±14.18 and 43.96±13.12 years. In exposed group 15(50%) patients were male and 15(50%) patients were female. While among unexposed group 11(36.7%) patients were male and 19(63.3%) patients were female. Duration of stroke in exposed and unexposed groups was 7.46±2.54 and 7.53±2.68 hours respectively **(Table 1).** At day 0, the mean mRS score of exposed group was 2.57±1.59 while mRS of

Table 1: *Patient demographics*

	Exposed	Unexposed
Patient Age (Years)		
N	30	30
Mean	51.30	43.96
Standard Deviation	14.18	13.12
Minimum	26	24
Maximum	70	64
Patient Gender		
Male	15(50%)	11(36.7%)
Female	15(50%)	19(63.3%)
Duration of Stroke (Hours)		
N	30	30
Mean	7.46	7.53
Standard Deviation	2.54	2.68
Minimum	4	4
Maximum	12	12

Table 2: *Descriptive statistics for mRS Score at Day 0 and Day 30*

	Exposed	Unexposed	Independent samples t-test	P-value
Day 0				
n	30	30	5.600	0.000001 (Significant)
Mean	2.57	0.73		
Standard Deviation	1.59	0.83		
Minimum	0	0		
Maximum	5	3		
Day 30				
N	30	30	5.173	0.000003 (Significant)
Mean	2.77	0.83		
Standard Deviation	1.76	1.05		
Minimum	0	0		
Maximum	5	4		

unexposed group was 0.73±0.83. At day 30, the mean mRS score of exposed group was 2.77±1.76 while mRS of unexposed group was 0.83±01.05 **(Table-2)**. The difference was significant ($p<0.05$). Short term poor outcome was seen in 21(70%) patients in exposed and in 5(16.7%) patients in unexposed group. Relative risk for poor outcome among exposed patients was 4.20 which mean that patients in exposed group had 4.20 times more chances of poor outcome as compared to unexposed patients. Relative risk for poor outcome for patients in the age group 24-35 and 3-45 years was 15.16 and 2.66 and for patients in the age group 46-55 years and >55 years, relative risk for poor outcome was 11.00 and 1.8 hi respectively. For male patients, relative risk for poor outcome was 4.40 and for female patients it was 3.80 which mean that male and female patients in exposed group had 4.40 and 3.80 times more risk for poor outcome as compared to male and female patients in the unexposed group **(Table-3)**.

Table 3: *Short Term Poor Outcome stratified for age and gender*

	Poor Outcome	Exposed	Un-exposed	RR	CI (95%)	p-value
Age						
24-35	Yes	3(60%)	0(0%)	15.16	0.92-249.63	0.015
	No	2(40%)	12(100%)			
36-45	Yes	4(66.7%)	1(25%)	2.66	0.44-15.95	0.524
	No	2(33.3%)	3(75%)			
46-55	Yes	5(83.3%)	0(0%)	11.00	0.74-163.49	0.015
	No	1(16.7%)	6(100%)			
>55	Yes	9(69.2%)	4(50%)	1.38	0.63-3.02	0.646
	No	4(30.8%)	4(50%)			
Gender						
Male	Yes	12(80%)	2(18.2%)	4.40	1.22-15.80	0.002
	No	3(20%)	9(81.8%)			
Female	Yes	9(60%)	3(15.8%)	3.80	1.24-11.61	0.007
	No	6(40%)	16(84.2%)			

Discussion

IIt is widely established that acute and persistent HG (hyperglycemia) is associated with poor outcomes in patients with severe brain damage.[8,9] However, the lowest safe blood glucose level in neurocritical patients has not yet been determined. The brain is extremely sensitive to variations in blood glucose levels, and the severely damaged brain may be even more susceptible.[8] In this study we assessed the association between uncontrolled DM and short term poor outcome in patients with ischemic stroke. Short-term outcome was determined with the help of mRS assessed at 30th day of stroke. As per this criterion relative risk for poor outcome was 4.20 times higher in patients with diabetes as compared to patients without diabetes. Result of this study is in line with the results of an Egyptian study which showed that poor outcome was significantly higher in hyperglycemia patients as compared to controls. i.e. (Patients with Hyperglycemia: 65.9% vs. Control: 5%). In this study relative risk was calculated for poor outcome however in Egyptian study relative risk was not calculated.

Another study stated an interesting finding that raised ABG (Arterial Blood Gas) in patients without DM hospitalized for acute ischemic stroke is related with increased long-term mortality. Elevated ABG, regardless of DM status, was linked to higher in-hospital mortality and LOS[10] .Previous investigations did not reveal a relationship between short-term mortality and hyperglycemia in people with DM. [10,11,12] One study showed that poor pre-stroke glycemic management is an independent predictor of stroke severity, a good predictor of acute and long-term survival, and a reliable marker of neurological functional prognosis. The HR for DM was 6.15 for 30 days' survival following stroke.[13] Contrary to the findings of this study, which showed a substantial risk of poor outcome at the 30th day, researchers found that higher blood glucose levels >6.1 mmol/l (110 mg/dL) in patients with cerebrovascular accident and no history of DM (Diabetes Mellitus) increased the chance of dying within 30 days by thrice.[15]

A recently published study showed that Cumulative incidence of mortality at 30 day was 4.8% in patients with ACS (acute coronary syndrome).[16] The disparity in death outcomes between patients with and without diabetes could be explained by a number of different factors. Patients with diabetes may have higher cutoff levels for stress hyperglycemia than those without[13].Chronically high blood glucose levels and DM therapy may have a neuroprotective effect. Finally, diabetic individuals are more likely to receive therapy for hyperglycemia.[14] Hyperglycemia in acute ischemic stroke patients is recognized to be an independent predictor of increased infarct size, adverse outcome, and a high fatality rate.[17] The severity of stroke symptoms, as well as the extent of

the infarct, cause an increase in cortisol and norepinephrine production, which is linked to stress.[18] Hyperglycemia during the acute stroke phase is a result of relative insulin insufficiency, which is linked to accelerated lipolysis.[17] Patients with these characteristics are more likely to have hyperglycemia during the acute stroke phase, independent of the existence of DM.[19]

Diabetes is a major modifiable risk factor for stroke, particularly ischemic strokes.[20,21,22] Hyperglycemia during the acute stroke phase is linked to poor outcomes in ischemic and hemorrhagic strokes. It needs to be actively remedied, but the appropriate management strategy is unknown. Aggressive glucose control through lifestyle changes or drugs, as well as the alteration of other associated risk variables (such as blood pressure and dyslipidemia), are crucial steps toward effective stroke prevention.[4] Patients must be educated about complications, have regular checkups, and strictly adhere to treatment.

Conclusion

Results of this study showed that uncontrolled diabetes was significantly associated and poses high risk for short term poor outcome in ischemic stroke patients.

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Authors Contribution

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NT: Literature Search

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MJ: Drafting, Revision

KM: Writing of Manuscript

Prolidase Deficiency, A Rare Case Report

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Abstract

Prolidase deficiency is a rare genetic disorder inherited in an autosomal recessive manner. The culprit Peptidase D (PEPD) gene, has been identified through molecular gene testing. Diagnostic methods include assessing reduced prolidase enzyme activity or imidodipeptiduria. This condition is both a metabolic disease and an inborn error of metabolism, characterized by defects in proline-containing protein breakdown, such as collagen. Patients may exhibit dysmorphic facial features, atopy, telangiectasia, skin ulceration, splenomegaly, and recurring infections. Skin ulcer biopsy can rule out vasculitis, and autoimmune activation may lead to an overexpression of the activation marker Human Leucocyte Antigen (HLA DR) on CD4+ and CD8+ T cells, along with high interleukin 18 plasma levels. Multidisciplinary care is essential for proper management.

Keywords: Prolidase deficiency, leg ulcer, autoimmunity, T cell immunity.

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Introduction

Prolidase deficiency is a rare autosomal recessive disorder caused by a gene defect involving prolidase encoding.[1] Diagnosis typically occurs around the ages of eight to eleven years.[1] Prolidase acts as a dipeptidase, cleaving the imide bond present in dipeptides with proline or hydroxyproline at the C terminal position, with its highest activity against glycyl proline.[1] Diagnosis is confirmed through prolidase enzymatic activity assessment.[1] Association with chronic lung disease and systemic lupus erythematosus has been noted.[1] Elevated plasma and urine imidodipeptidases are characteristic Prolidase enzyme is crucial in collagen I biosynthesis and degradation, a key component of the extracellular matrix supporting connective tissues such as skin, bone, cartilage, tendon, and ligament.[2] This deficiency manifests with facial dysmorphism, cognitive impairment, splenomegaly, recurrent infections, and lower limb ulcers.[2] Prenatal testing is recommended for high-risk cases.[2]

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Case Report

A 12-year-old male, born to consanguineous parents with four siblings, presented with a history of multiple admissions due to recurrent furunculosis, recalcitrant leg ulcers, and skin infections for the last 4 years. He has a history of multiple admissions for leg ulcers in different hospitals in the past. He had diffused abdominal pain radiating to the left hypochondrium with complaints of anorexia, abdominal distension, and weight loss of four to five kg over the past six months. He was treated with intravenous antibiotics, painkillers, and dressing. His other siblings are healthy. He exhibited a painful ulcer with purulent discharge surrounding swelling associated with pain in the lower half of his right shin for the last 20 days and abdominal discomfort for 15 days in the dermatology outpatient department at Jinnah Hospital, Allama Iqbal Medical College, Lahore. Differential diagnosis of primary immune deficiency, Sickle cell disease, Chediak-Higashi syndrome, Beta thalassemia, Werner syndrome, and Prolidase deficiency was considered.

Figure 1: *Reveals hypertelorism*

Figure 1: *Prognathism and dysmorphic features*

Figure 2 : *Reveal Bitot spots*

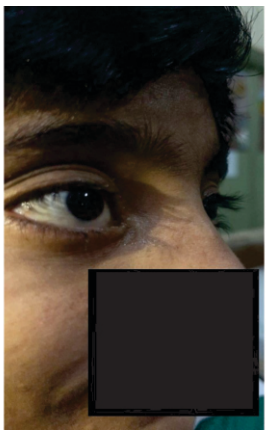

Figure 3:*Reveal Clubbing*

Figure 4: *Leg ulcer with crusting*

Figure 5: *Leg ulcer with surrounding swelling*

Figure 6: *Chest X-ray PA VIEW*

Figure 7: *Teeth of patient*

Figure 8: *High arched palate*

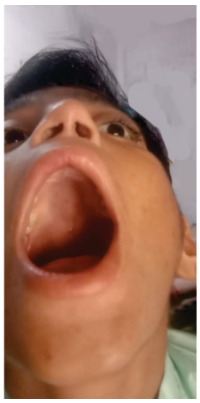

Figure 9: *Cellulitis*

Figure 10: *Cellulitus*

Figure 11: *Leg ulcer with crusting*

Figure 12: *x ray cervical spine lateral view Generalized decrease bone density*

Sclerosis between C1 and C2 vertebrae, Butterfly vertebrae C2 level C6 vertebral body shows decreased height, omovertebral body at C8 level and exaggerated cervical curvature.

Figure 13: *Ultrasound demonstrating splenomegaly of 22.5 cm*

Table 1: *Complete Blood Examination*

	Param		
1	WBC	4.1	10^9/L
2	LYM%	44.1	%
3	MID%	10.4	%
4	GRAN#	45.5	%
5	LYM%	1.8	10^9/L
6	MID%	0.4	10^9/L
7	GRAN#	1.9	10^9/L
8	RBC	4.23	10^9/L
9	HGB	8.6	g/dl
10	HCT	26.9	%
11	MCV	63.6	fL
12	MCH	20.3	pg
13	MCHC	31.9	g/dl
14	RDW_CV	18.3	%
15	RDW_SD	44.9	fl
16	PLT	73	10^9/L
17	MPV	9.7	fl
18	PCT	0.07	%
19	P_LCR	35.3	%
20	P_LCC	25	10^9/L
21	PDW_SD	18.2	fl
22	PDW_CV	19.8	%

Table 2: *Immunoglobulin Levels*

Serum Immunoglobulin E	**19238.9**
Serum Immunoglobulin A	5.23 g/L High
Serum Immunoglobulin G	68.2 g/L High

Table 3: *Gamma-glutamyltransferase Level*

Serum Gamma-glutamyltransferase	89 U/L High

Table 4: *Complement C3 Level*

	Serum Complement-3 (C3)	**120**
1	Female 1 - 14 years:	82 - 173 mg/dL
2	Female > 15 years:	83 - 193 mg/dL
3	Male 1 - 14 years:	80 - 170 mg/dL
4	Male > 15 Years:	82 - 185 mg/dL

Table 5: *Complement C4 Level*

	Serum Complement-4 (C4)	**26**
1	Female 1 - 14 years:	13 - 46 mg/dL
2	Female > 15 years:	15 - 57 mg/dL
3	Male 1 - 14 years:	14 - 44 mg/dL
4	Male > 15 Years:	15 - 53 mg/dL

General Physical Examination:

The patient appeared pale and afebrile, with vital signs within normal limits with a pulse rate of 82 per minute, respiratory rate of 16 per minute, and blood pressure of 120/80. Physical examination revealed pallor, high-arched palate, clubbing, hypertelorism, low-set ears, a depressed nasal bridge, hyperflexible joints, dry skin, trichomegaly, and Bitot spots in the eyes.

Systemic Examination:

Abdominal examination revealed a palpable spleen. Cognitive impairment and the psychiatric evaluation revealed conversion disorder.

Cutaneous Examination:

A 4cm x 6cm leg ulcer with an overlying crust was observed.

The patient was managed with oral antibiotics and daily dressing with pyodine along with oral Vitamin A and Vitamin D, nutritional support was advised by a nutritionist, and psychological counseling of the patient and her family was done. The patient was advised to follow up.

Surveillance:

Skin ulcer follow-up for malignant transformation, eye evaluation, dental examination, Counselling for the avoidance of rigorous sports, timely diagnosis of chest infections, and addressing educational needs of the patient.

Discussion

Prolidase deficiency is an inborn error of amino acid metabolism and the first case was reported by Goodmen et al. in 1968.[2] Ninghty three cases of prolidase deficiency have been reported in the literature so far.[2] The literature about the Pakistani study shows two cases of prolidase deficiency in Pakistan.[2] Its incidence is estimated at 12 per 1,000,000 births.[2] Multidisciplinary management is essential for prolidase deficiency and genetic counseling is recommended.[3] Insulin therapy has been associated with improved wound healing in ulcers due to prolidase deficiency,[4] with topical insulin therapy emerging as a novel treatment modality to minimize systemic side effects like hypoglycemia and hypokalemia. Topical insulin application promotes macrophage infiltration and phagocytosis.[4] Interleukin 10 levels play a role in angiogenesis and granulation tissue formation, facilitating keratinocyte migration and elevating keratinocytes, fibroblasts, and endothelial cells at the wound site. Associated conditions include Crohn's disease, arthritis, uveitis, erythema nodosum, and pyoderma gangrenosum.[5] Colonoscopy may reveal pseudopolyps, serpiginous ulcers, and pancolitis, necessitating immune and rheumatologic evaluations in patients with prolidase deficiency and Crohn's disease.[5] Associations with systemic lupus erythematosus, juvenile idiopathic arthritis, psoriatic arthritis, and elevated ANA levels have been identified.[6] The gene responsible for prolidase deficiency is located on the long arm of chromosome 19 Immunological impairment may manifest as elevated Immunoglobulin A, Immunoglobulin G, and Immunoglobulin M levels, along with decreased complement levels C1q, C3, and C4.[7] Supportive therapy includes a diet supplemented with amino acids, ascorbic acid, and manganese.[7] Alongside topical treatments such as proline, five percent glycerin ointment, and topical tacrolimus.[8] Oral vitamin C and low molecular weight heparin are beneficial for patients at risk of thrombosis.[8]

Conclusion

Prolidase deficiency presents a wide clinical spectrum with multisystem involvement, often involving autoinflammatory processes indicated by elevated interleukin 18 levels. Patients require immunological, rheumatologic, and genetic evaluations for proper management. Oral vitamin A drops can support Bitot spot treatment, while consultation with a nutritionist and psychologist contributes to overall patient well-being.

References

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Authors Contribution

HS: Conceptualization of Project
SR: Data Collection
MJ,US, FK, SL: Literature Search
NAA: Drafting, Revision
HS: Writing of Manuscript