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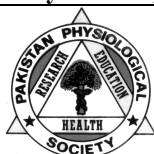
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Email: tyar@iau.edu.sa

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Email: drfarmanwazir@hotmail.com

Ghulam Rehmani Lahko
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Hamid Javed Qureshi
Email: hj.qureshi@yahoo.com

Idrees Farooq Butt
Email: idreesfb@yahoo.com

Masood Anwar Qureshi
Email: m.qureshi@duhs.edu.pk

Muhammad Abdul Azeem
Email: azenmu@gmail.com

Muhammad Hamayun Ikram
Email: hamayunikram@gmail.com

Mumtaz Ali Memon
Email: prof_mumtaz@hotmail.co.uk

Saadat Ali Khan
Email: sasaali3y@gmail.com

Shahnaz Javed Khan
Email: shehnazjkan1@gmail.com

Tehseen Iqbal
Email: prof.tehseeniqbal@gmail.com

NATIONAL (EXTRA-ORGANIZATIONAL)

Abdul Khaliq Naveed
Email: khaliqnaveed2001@yahoo.com

Akhtar Sherin
Email: akhtarsherin@yahoo.com

Farooq Rathore
Email: farooqrathore@gmail.com

Jamshaid Akhtar
Email: jamjim88@yahoo.com

Junaid Sarfraz Khan
Email: junaidсарfraz@hotmail.com

Khadija Qamar
Email: colkhadijaqamar@gmail.com

Muhammad Irfan
Email: mirfan78@yahoo.com

Saba Sohail
Email: drsabasohail@hotmail.com

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Email: sairamust@gmail.com

Shaukat Ali Jawaid
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EDITORIAL

COVID-19 VACCINES BOOSTERS: A DOOR WAY OF TREATMENT?

Taimoor Hassan^{*†}, Saleha^{**}, Sana Saeed[†]^{*}School of Pharmacy and School of Medicine, Changzhou University, Jiangsu, China, ^{**}Department of Anthropology, Quaid-i-Azam University, Islamabad, [†]Department of Health Professional Technologies, The University of Lahore, Pakistan

As of September 20, 2021, the entire planet has reported 218.5 million COVID-19 cases, with 4.52 million fatalities. Lockdowns and softening measures have been thrown into turmoil throughout the world since the outbreak. Our social life will only return to normal once an appropriate vaccine is produced and proper authorized preventive techniques are implemented. To tackle this pandemic, governments and health experts all around the globe are experimenting with a range of measures and preventative strategies. Certain countries are considered to be more successful than others in terms of providing safety to their inhabitants and increasing their economic activities. A plethora of vaccinations have been produced, and a research anthology has been published. However, medical personnel are still searching for a viable treatment to limit this pandemic.

Keywords: Coronavirus pandemic, booster vaccines, mass vaccination

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There is a rising worry about the inadequate protection offered by COVID-19 vaccinations against the formation of SARS-CoV-2 viral strains. The United States speaks with Pfizer about delivering equivalent dosages to the most vulnerable people. Israel has begun supplying Pfizer with vaccination booster injections for people at risk. People with COVID-19 vaccinations have reduced the chances of contracting the virus causing the disease, which is less likely to produce the serious signs of sickness. However, the findings suggest that current vaccinations, for example a Delta version, might provide less protection from future variants of SARS-CoV-2.¹ According to Centers for Disease Control and Prevention (CDC) Study Trusted Source, the immune reaction to a delta variation has been decreased compared with prior strains in 95% of patients who have taken both doses of Pfizer-BioNTech or of Oxford-AstraZeneca CVC-19.² According to the Israeli Ministry of Health, the Pfizer-BioNTech vaccine has lowered its efficacy in prevention of symptomatic COVID-19 by 64% six months after inoculation.² However, two Pfizer dose levels have been found to be able to prevent serious sickness in 93% of people. The United States still has to decide whether booster vaccinations should be provided or not. However, it has been pointed out by the CDC and the Food and Drug Administration (FDA), that the authority is ready for booster-dose if and when the study reveals it is required.^{1,2}

Vaccine boosters are supplemental doses of vaccines that must be used to further guard the illness since the benefits of some vaccines are reduced over time. Many viral diseases require booster vaccines such as the yearly flu. However, Diphtheria, Pertussis and Tetanus, once every 10 years are recommended as boosters. Lower dosages are better for some vaccines than a single high dose of immunization. This method permits a long-standing immune response to the

immune system. When the immune system recalls antigen that has already been triggered, the next time it meets them, it may respond more rapidly. Many vaccination boosters have been changed to improve their efficacy in the same way as prior dosages. For example, the influenza vaccine is amended every year to respond better to new changes in influenza viruses.³

For one of two reasons, individuals might choose vaccine boosters. Firstly, our immunity usually decreases with age. If some antigens aren't often exposed to the immune system, they might become less competent to prevent infection or illness. Vaccine boosters assist the immune system to maintain a protective response. The spokesperson on COVID-19 Vaccine Team University of Oxford informed Medical News Today that viral mutations are a further reason we may require booster vaccines. Certain variations have developed in order to bypass certain elements of the human immune response which means that they can infect persons already infected or immunized with the immune response to the virus. On the other hand, the virus cannot avoid all parts of human immune response. Booster vaccinations are beneficial since they can increase portions that the virus shape cannot boost our immune response.^{2,3}

According to CDC the published evidence suggests that the majority of COVID-19 vaccines provide a robust immune response that provides adequate protection against the virus. The duration of protection by COVID-19 vaccinations at their present dose is unclear. Booster doses may assist seniors or those with impaired introductive immune systems since, after the initial vaccinations, their bodies may not have formed a powerful enough immune response.

It is those who are not responding to vaccines where we need be careful.⁴ Many vulnerable people fall into this group, and previous vaccinations have demonstrated that seniors are not necessarily protected

to the same extent as younger people. We should examine carefully if supporting them is appropriate.

According to Oxford Vaccine Team COVID-19, there is nothing contentious scientifically about extra booster vaccines; they function in a two-vaccine schedule in much the same way as the second. The main goal of the vaccinations is to maintain the masses outside of the hospital.⁵

Vaccines have proven that hospitalizations are significantly reduced. According to health pantheons, supply of vaccines will be constrained in the near future. Sir Andrew argues that before providing boosters to others, it is necessary to prioritize those who have not yet had a single injection. Some even ask whether it is morally right to promote vaccine users, when many individuals, especially in poor nations, have not even received a single shot and are thus more at risk for infection. Others are unconvinced that a third vaccination is needed. T and B cells, which are a body immune system component, respond to vaccination by creating a long-lasting viral protection. B cells are the immune system's 'memory cells' which produce virus-binding antibodies. T cells help these B cell antibodies to be synthesized. Some T cells are capable of killing contaminated cells.⁶

One research—not yet reviewed by peers, but accessible on an online pre-print source—shows a long-lasting T-cell response to those recovering from moderate COVID-19. Another study, which was released prior to the preprinting service peer review, found that B cells can develop antibodies which specifically target newer forms of SARS-CoV-2 in people recovering with symptomatic or mild COVID-19.^{4,6}

Vaccination booster dosages researches are currently being conducted including some preliminary inquiries. Although current vaccinations protect against recognized variations, they may diminish as the virus mutates and new forms arise. The antibody responses were decreased when exposed to delta and beta forms of SARS-CoV-2 respectively, discovered in India and South Africa, which were demonstrated by patients who were completely vaccinated with Pfizer-BioNTech and Oxford-AstraZeneca vaccines. Also available online via a preprint site, another research revealed that individuals were immunized from multiple SARS-CoV-2 variations, including the delta form following the single

dosage of the Johnson & Johnson vaccine. The immunological responses to the delta fluctuation seemed lower than in prior SARS-CoV-2 versions.⁷

A statement published on July 8, 2021 by Pfizer and BioNTech stated that the booster dosage of their vaccine 6 months after the second dose induces an immunological response 5–10 times higher than the response after the 2nd dose against diverse virus strains. But they say they are working on an updated version of the vaccination against the delta variant. Several clinical studies were underway to learn more about the protective duration in normal vaccine regimens. How it changes after a 3rd dose, and what happens when the 3rd dose corresponds better to new variants need answers. However, given the recent start of most of the research, findings are not available for a while.^{1,5,7}

It is important to mention that vaccination process is continuing globally. However, with the emergence of novel variants of Coronavirus, it is a need of the hour to administer vaccine boosters as it contracts complications among people. Various studies have proved that it provides a defensive barrier to our body.

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Address for Correspondence:

Taimoor Hassan, School of Pharmacy and School of Medicine, Changzhou University, Jiangsu, People's Republic of China. Cell: +92-321-9400508

Email: Taimoorhassan408.th@gmail.com

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ORIGINAL ARTICLE

FokI VITAMIN D RECEPTOR POLYMORPHISM AS A PREDICTOR OF RESPONSE TO HEPATITIS C ANTIVIRAL TREATMENT

Noora Hassan Hezam Al-Aqmer, Soumble Zulfiqar*, Sibgha Zulfiqar,
Abdul Rauf Shakoori*, Mateen Izhar**

Department of Physiology, Shaikh Zayed Postgraduate Medical Institute, Shaikh Zayed Medical Complex, *School of Biological Sciences, University of the Punjab, **Department of Microbiology, Shaikh Zayed Postgraduate Medical Institute, Shaikh Zayed Medical Complex, Lahore, Pakistan

Background: FokI vitamin D receptor (VDR) genotype ff has been debated as predictor of response to treatment. This study was designed to find out the association of FokI VDR polymorphism with response to chronic hepatitis C daclatasvir- and sofosbuvir-based treatment. **Methods:** This case control study was conducted at Federal Postgraduate Medical Institute, Lahore from Jan 2019 to Apr 2021. It included 66 chronic hepatitis C genotype 3 patients who responded to daclatasvir and sofosbuvir-based treatment (with ribavirin for cirrhotic patients) and attained sustained virologic response (SVR) three months after completion of treatment, and 66 gender and age matched chronic hepatitis C genotype 3 patients who did not respond to the treatment. Demographic data was collected and 3 mL of blood was drawn from each participant. DNA extraction was done followed by PCR-restriction fragment length polymorphism. Samples were run on 12% polyacrylamide gel and visualized under UV light. Data was analysed using SPSS-24. **Results:** Frequencies of FokI VDR genotypes FF, Ff, and ff were 54.5%, 28.8%, and 16.7% in responders, and 60.6%, 36.4%, 3.0% in non-responders. There was a significant association between FokI VDR polymorphism and response to treatment ($p=0.03$). No significant association was found between FokI polymorphism and cirrhosis. Logistic regression showed FokI genotype ff to be a significant predictive factor for a SVR ($p=0.041$). **Conclusion:** FokI VDR polymorphism is associated with response to daclatasvir- and sofosbuvir-based antiviral treatment in chronic hepatitis C genotype 3 patients. FokI genotype ff could be considered as a predictive marker for response to treatment.

Keywords: Vitamin D receptor, VDR Polymorphism, Hepatitis C, Polymorphism, Cirrhosis, Anti-viral

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INTRODUCTION

Hepatitis C is a worldwide health problem. It is estimated that 58 million individuals suffer from chronic hepatitis C infection globally and there are 1.5 million new cases every year.¹ About 20% of chronic hepatitis C patients progress to end stage cirrhotic liver disease or hepatocellular carcinoma.² Pakistan has a high prevalence of 5% nationwide³, and despite the advent of the directly acting antiviral treatment, hepatitis C prevalence is still persistent.⁴

Vitamin D receptor is a nuclear receptor that acts as a transcription factor. The receptor binds to the active vitamin D and mediates its actions.^{5,6} It is encoded by the vitamin D receptor gene on chromosome 12q.⁷ Recent studies have shown association of genetic variations in the VDR gene with susceptibility as well as chronicity of hepatitis C infection.⁸ These genetic variations have also been studied in relation to the response to pegylated interferon and ribavirin treatment.^{9,10}

El-derany *et al*¹¹ found a significant association between FokI VDR polymorphism and the response to pegylated interferon with ribavirin in CHC genotype 4 patients, whereas Wang *et al*¹² found no significant association between FokI genotypes and the response to treatment. Garcia-Martín *et al* reported FokI

VDR genotype ff as a predictor of the response to treatment.¹³

There is a need to study the association of FokI VDR polymorphism with the new directly acting antiviral treatment of hepatitis C virus as pharmacogenetics are different for the different drugs. Considering that, the controversial results of the previous studies, and the proposed importance of FokI VDR polymorphism in predicting the outcome of treatment, this study was designed to find out the association of FokI VDR polymorphism with the response to daclatasvir and sofosbuvir (with ribavirin for cirrhotic patients).

METHODOLOGY

This was a case control study conducted in the department of physiology, Federal Postgraduate Medical Institute from January 2019 to April 2021 after approval from the Institutional Ethical Review Board. Sample size was calculated to be 25 responders to hepatitis C antiviral treatment and 25 non-responders to hepatitis C antiviral treatment at 95% confidence interval and 5% margin of error with expected FokI vitamin D receptor polymorphism frequency of 40% and 90% in responders and non-responders respectively.¹⁴ However, 132 subjects were enrolled (66 responders to hepatitis C antiviral treatment and 66 non-responders to hepatitis C

antiviral treatment) by non-probability convenient sampling from Shaikh Zayed hospital, Lahore. Subjects included males and females aged ≥ 18 years with cirrhotic/non-cirrhotic HCV liver disease who received directly acting antiviral drugs (daclatasvir and sofosbuvir with or without ribavirin), and did the HCV-RNA test 12 weeks after completion of treatment.

Responders were those who maintained sustained virologic response 12 weeks after completion of treatment (Hepatitis C virus-RNA negative). Non-responders were those who did not maintain sustained virologic response 12 weeks after completion of treatment (Hepatitis C virus-RNA positive). Both groups were age and gender matched.

Patients with autoimmune hepatitis, alcoholic liver disease, hepatitis B surface antigen, HIV, hepatocellular carcinoma, decompensated liver cirrhosis, severe renal disorder, uncontrolled diabetes, uncontrolled hypertension, or severe depression were excluded. A written informed consent was taken from each participant and demographic data was recorded. Clinical reports of platelet count, haemoglobin level, prothrombin time (PT), International Normalized Ratio (INR), and liver function tests (LFTs) were recorded.

About 3 mL of blood was drawn from each patient through venipuncture. At School of Biological Sciences (University of the Punjab), DNA extraction from blood was done followed by amplification of the DNA fragment of vitamin D receptor gene containing the FokI restriction site (rs2228570), and then restriction fragment length polymorphism (RFLP) analysis.

DNA extraction was done using the extraction kit (ThermoScientific #K0781) and stored at -20°C . The DNA fragment containing the FokI VDR polymorphism (rs2228570) was amplified using the forward primer 5'-AGCTGGCCCTGGCACTGACTC TGGCTCT-3' and the reverse primer 5'-ATGGAAC ACCTTGCTTCTTCTCCCTC-3'¹⁵ in a 20 μL PCR mixture as follows:

3 μL 2.5 mM dNTPs, 2 μL $10\times$ NH_4SO_4 buffer, 3 μL 25 mM MgCl_2 , 1.5 μL 10 μM forward primer, 1.5 μL 10 μM reverse primer, 0.5 μL 5 U/ μL Taq polymerase, 5 μL DNA, and 3.5 μL water.

The PCR reaction for amplification of the PCR product containing FokI SNP (rs 2228570) underwent 35 cycles consisting of initial denaturation for 5 minutes at 95°C , denaturation for 30 seconds at 95°C , annealing for 45 seconds at 68°C , extension for 45 seconds at 72°C , and then final extension for 10 minutes at 72°C . Genotyping was done by restriction fragment length polymorphism using restriction enzyme FokI (FastDigest Thermo Scientific #FD2144), i.e., 10 μL of PCR (0.2 μg), 2 μL $10\times$ FastDigest Green Buffer, 1 μL FastDigest enzyme, and 17 μL nuclease free water were added and mixed. The

30 μL mixture was incubated at 37°C in a heat block for 5 minutes followed by inactivation for 5 minutes at 65°C and then samples were run on 12% polyacrylamide gel and visualized under UV light.

Data was entered and analysed using IBM SPSS-24. Comparison of age, BMI, platelets count, haemoglobin, total bilirubin, direct bilirubin, Aspartate aminotransferase (AST), Alanine Aminotransferase (ALT), Alkaline phosphatase (ALP), serum albumin, PT, and INR in responders and non-responders was done using *t*-test (for normally distributed data) and Mann-Whitney test (for not-normally distributed data). Frequencies of the FokI VDR polymorphism genotypes were studied in accordance with the Hardy-Weinberg equilibrium. Association of FokI VDR polymorphism with the response to treatment was studied using chi-square test. Association of FokI VDR polymorphism with cirrhosis was studied using chi-square test in responders group and Fisher's exact test in non-responder group respectively. Binary regression was used to assess the association between the different independent variables and the response to treatment, and $p < 0.05$ was considered statistically significant.

RESULTS

There were 40 males and 26 females in each group with Mean age of 50.03 ± 7.53 years in the responders group and 49.02 ± 7.54 years in the non-responders group. No significant differences in gender and age were found between the two groups ($p = 1$ and 0.36 respectively). No significant differences were found in BMI, platelet count, haemoglobin, PT, INR, serum albumin, total bilirubin, direct bilirubin, AST, ALT, and ALP in responders vs. non-responders ($p < 0.05$) (Table-1).

The RFLP analysis showed a single band of 267 base pairs in the wild homozygous FF genotypes (CC), two bands of 208 and 59 base pairs in the mutant homozygous ff genotypes (TT), and three bands of 267, 208, and 59 base pairs were seen in the heterozygous Ff genotypes (CT). The frequency of FokI VDR genotypes, FF, Ff, and ff was 36 (54.5%), 19 (28.8%) and 11 (16.7%) in responders and 40 (60.6%), 24 (36.4%) and 2 (3.0%) in non-responders. Chi-Square test showed a significant association between FokI VDR polymorphism genotypes and response to treatment (Chi-Square=7.023, $p = 0.03$). There was no significant association between FokI VDR polymorphism and cirrhosis in responders and non-responders ($p < 0.05$) (Table-2). Moreover, there was no significant association between FokI VDR polymorphism and gender (Table-3).

Logistic regression showed FokI genotype ff as a significant predictive factor for response to treatment ($p = 0.41$, OR=0.179, 95% CI=0.034–0.929) (Table-4).

Table-1: BMI, platelet count, haemoglobin, prothrombin time (PT), INR, and liver function tests of responders and non-responders

Variables	Responders Mean±SD	Non-Responders Mean±SD	<i>p</i>
BMI (Kg/m ²)	26.97±6.81	28.01±6.46	0.369 ^a
Platelets (×10 ³ /μL)	246.98±70.69	234.55±73.78	0.294 ^b
Haemoglobin (g/dL)	13.15±1.97	12.74±1.96	0.223 ^b
Prothrombin time (Sec)	14.89±0.86	14.68±0.81	0.093 ^b
INR	1.14±0.19	1.14±0.16	0.939 ^b
Serum albumin (g/dL)	3.52±0.58	3.45±0.55	0.578 ^b
Total bilirubin (mg/dL)	0.97±0.57	1.00±0.56	0.571 ^b
Direct Bilirubin (mg/dL)	0.43±0.38	0.44±0.38	0.707 ^b
AST (U/L)	64.41±17.29	65.86±16.78	0.554 ^b
ALT (U/L)	69.42±22.06	70.95±22.21	0.692 ^a
ALP (IU/L)	108.17±21.53	109.82±20.48	0.652 ^a

^aStudent's *t*-test for normally distributed data

^bMann-Whitney test for not normally distributed data

Table-2: Association of FokI VDR polymorphism with cirrhosis

FokI Genotype	Responders		Non-Responders	
	Cirrhotic (n=33)	Non-cirrhotic (n=33)	Cirrhotic (n=33)	Non-cirrhotic (n=33)
FF	17	19	21	19
ff	5	6	0	2
Ff	11	8	12	12
Chi-Square/Fisher's exact test	0.676 ^a		1.751 ^b	
<i>p</i>	0.713		0.592	

^aChi-square test, ^bFisher's exact test

Table-3: FokI VDR polymorphism genotypes in males and females [n (%)]

VDR Polymorphism Genotype	Responders		Non-Responders	
	Male	Female	Male	Female
FF	24 (60)	12 (46.2)	25 (62.5)	15 (57.7)
ff	4 (10)	7 (26.9)	2 (5.0)	0 (0.0)
Ff	12 (30)	7 (26.9)	13 (32.5)	11 (42.3)
Total	40 (100)	26 (100)	40 (100)	26 (100)
Chi-Square/Fisher's exact test	3.313 ^a		1.422 ^b	
<i>p</i>	0.191		0.512	

^aChi-square test was used, ^bFisher's exact test was used

Table-4: Logistic regression analysing potential predictors of SVR in chronic hepatitis C patients

Variables	B	<i>p</i>	OR (95% CI)
FF	Referent		
ff	-1.721	0.041*	0.179 (0.034-0.929)
Ff	-0.033	0.939	0.968 (0.418-2.239)
Age	-0.022	0.406	0.979 (0.930-1.030)
Gender	0.219	0.606	1.245 (0.542-2.857)
Smoking	-0.516	0.320	0.597 (0.216-1.652)
BMI	0.019	0.542	1.019 (0.959-1.082)
Platelets	-0.003	0.339	0.997 (0.992-1.003)
Haemoglobin	-0.143	0.163	0.867 (0.709-1.060)
Cirrhosis	-0.106	0.860	0.900 (0.278-2.908)
Total bilirubin	0.241	0.798	1.273 (0.201-8.055)
Direct bilirubin	-0.291	0.853	0.747 (0.034-16.203)
AST	-0.002	0.907	0.998 (0.970-1.027)
ALT	0.006	0.534	1.006 (0.986-1.027)
ALP	0.002	0.867	1.002 (0.982-1.022)
Serum albumin	-0.191	0.646	0.826 (0.366-1.864)
Prothrombin time	-0.301	0.292	0.740 (0.423-1.295)
INR	0.028	0.986	1.029 (0.041-25.749)

*Significant

DISCUSSION

Our study found a significant association between FokI VDR polymorphism and the response to DAAs (Daclatasvir and sofosbuvir with/without ribavirin) in HCV genotype 3 patients with a protective role (in favour of response to treatment) of ff genotype. These results were in line with the results of El-Derany *et al*¹¹ whose study showed a significant association of FokI VDR polymorphism genotypes with the response to pegylated interferon with ribavirin and showed the homozygous FokI ff genotype to predict SVR in hepatitis C patients. In their regression analysis, the mutant 'f' allele was significantly associated with responding to the treatment and achieving SVR.¹¹

On the contrary, Arai *et al*¹⁶ found no significant association between FokI VDR polymorphism and the response to hepatitis C treatment. Their study was on genotype 1 chronic hepatitis C patients and the hepatitis C genotype could itself influence the response to treatment, thereby influencing the impact that a VDR polymorphism might have.

Though, Cusato *et al* and Abdelsalam *et al* found an association between FokI polymorphism and response to pegylated interferon and ribavirin, ff genotype was associated with relapse rather than response to treatment.^{17,18} Similar to our results, García-Martín *et al* also reported the mutant 'f' allele of FokI VDR polymorphism to be significantly associated with achieving SVR and related inversely with failure of therapy in chronic HCV patients.¹³

The mechanism by which FokI VDR polymorphism influence the response to treatment is not known. It has been suggested that the two alleles 'F' and 'f' result in the production of two VDR variant proteins which function as transcription factors and their interaction with other co-transcription factors might cause an organ-specific/cell-specific expression that is different for the different VDR variant proteins.¹⁹ FokI VDR polymorphism resulted in different effects on immunity and that was seen at the transcriptional activity level as well as at the level of cytokine synthesis and proliferation by immunity cells.²⁰

The above mentioned studies were conducted on patients who had received pegylated interferon and ribavirin, whereas our study is reporting on the association of FokI VDR polymorphism with the response to DAAs (Daclatasvir and sofosbuvir with or without ribavirin) in HCV genotype 3. FokI VDR polymorphism could well be considered as a new marker for the prediction of response to treatment in chronic hepatitis C patients.

CONCLUSION

There is a significant association between FokI VDR polymorphism and the response to the daclatasvir and sofosbuvir-based antiviral treatment in chronic hepatitis C genotype 3 patients. FokI homozygous mutant ff genotype could be used to predict the response to treatment.

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Addresses for Correspondence:

Prof. Dr. Mateen Izhar, Department of Microbiology, Shaikh Zayed Hospital, Lahore. Pakistan. **Cell:** +92-300-8447937
Email: mateen.izhar@gmail.com

Dr. Noora Hassan Hezam Al-Aqmer, PhD Scholar, Department of Physiology, Shaikh Zayed Postgraduate Medical Institute, Shaikh Zayed Medical Complex, Lahore, Pakistan. **Cell:** +92-333-4125141

Email: drnooraalqmer@gmail.com

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Contribution of Authors:

NHHA: Study design, data collection, analysis, interpretation, manuscript writing

SZ1: Supervision and review of genetic analysis

SZ2: Supervision of study and critical review of manuscript

ARS: Supervision of study and critical review of manuscript

MI: Supervision of study and critical review of manuscript

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ORIGINAL ARTICLE

COMPARISON OF SERUM HOMOCYSTEINE AND FOLIC ACID LEVELS IN ACUTE MYOCARDIAL INFARCTION AND NORMAL HEALTHY POPULATION

Nimrah Siddique, Muhammad Neaman Siddique*, Ayesha Gohier**, Asima Karim**

Department of Physiology, CMH Institute of Medical Sciences, Bahawalpur, *Hameed Latif Hospital, Lahore,

**Department of Physiology, University of Health Sciences, Lahore, Pakistan

Background: Acute Myocardial Infarction (AMI) is a leading cause of mortality worldwide. An emerging risk factor for AMI is raised levels of Homocysteine (Hcy). Deficient levels of folic acid are associated with Hyperhomocysteinemia (HHcy). Fortification of folic acid has been known to improve endothelial dysfunction. We aimed to determine the levels of Hcy and folic acid in patients with AMI and healthy individuals. **Methods:** This cross-sectional comparative study was conducted on 80 subjects, with 40 subjects in each group. Group A included individuals diagnosed with AMI and group B included healthy individuals. Serum Hcy and folic acid levels were measured by Enzyme Linked Immunosorbent Assay (ELISA). Systolic Blood Pressure (SBP) and Diastolic Blood Pressure (DBP) were measured, and Body Mass Index (BMI) was calculated. **Results:** Increased Hcy and lower serum folic acid levels were found in group A as compared to group B ($p < 0.001$). The correlation between serum Hcy level and folic acid in group A was moderate and negative ($r = -0.48$, $p = 0.001$); for group B it was also moderate and more negative ($r = -0.66$, $p < 0.001$). **Conclusions:** There were high levels of Hcy and low levels of folic acid in diseased group as compared to healthy participants.

Keywords: Acute Myocardial Infarction, AMI, Myocardial Infarction, MI, Coronary Artery Disease, CAD, Folic acid, Homocysteine, Hcy

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INTRODUCTION

The major causes of mortality in both developed and developing nations are Coronary Artery Disease (CAD) and Acute Myocardial Infarction (AMI).¹ Thirty-seven percent of all cases of symptomatic heart failure were ascribed to AMI alone globally and the incidence is anticipated to be doubled in South Asia than in any other region worldwide over the next two centuries.² Persistent and severe post-sternal pain are typical clinical symptoms of AMI.³ Prompt diagnosis of AMI is crucial for the timely application of therapy to preserve cardiac function and limit myocardial injury.⁴ According to WHO criteria, history of chest pain, physical examination, estimation of cardiac troponins levels and electrocardiogram (ECG) are tools for diagnosis of AMI.⁵

Hypertension (HTN) and smoking are the two important risk factors in almost 90 percent of patients with AMI.⁶ One of the risk factors for AMI is raised Hcy levels.⁷ Hcy levels normally vary between 5–15 $\mu\text{mol/l}$.⁸ An unusually higher blood level of Hcy ($>15 \mu\text{mol/l}$) is termed as Hyperhomocysteinemia (HHcy) and is considered mild if values are between 15.1 to 30 $\mu\text{mol/l}$, moderate if between 30.1 to 100 $\mu\text{mol/l}$, and severe if $>100 \mu\text{mol/l}$.⁹ HHcy causes the proliferation and thickening of smooth muscle cell in the intima of the blood vessel and upsurge in the oxidation of low-density lipoproteins which then activates the coagulation system leading to thrombi and thus AMI.¹⁰

Certain vitamin deficiencies like folic acid and vitamin B₁₂ or B₆ also contribute to raised plasma Hcy levels. The real determinant of serum Hcy level is folic acid and there exists an inverse relationship between the levels of these two in the patients suffering from AMI. Folic acid levels between 6 to 17 ng/ml are considered normal.¹¹ In the United States 17,000 deaths from cardiovascular events each year have been prevented by recommending food fortified with folic acid to these patients, thereby reducing their Hcy levels.¹²

The association between Hcy levels and AMI disease is still a matter of controversy and there is a debate going on in literature. One study undervalued the risk of having AMI in patients with high blood Hcy levels.¹³ On the other hand in recent two large studies meta-analysis was done with a total of 6,814 patients, and demonstrated that there was a higher risk prediction of developing AMI when blood Hcy levels were raised.¹⁴ It has been anticipated that metabolic dysfunction occurs in South Asians due to their genetic and lifestyle aspects, and they are susceptible to cardiometabolic ailments; yet it has not been proven.¹⁵ A study from all the provinces of Pakistan found that around 28% of AMI patients were below 45 years of age.¹⁶ In a study at Faisalabad, nearly one-third of patients suffering from AMI were of age <45 years.¹⁷ Definitive data depicting association of raised serum Hcy and lowered folic acid levels with AMI is lacking in Asia in general and Pakistan in particular. The present study was planned to determine and compare the serum

levels of Hcy and folic acid following AMI in the local population to compare them and to determine their importance, if any, as new emerging risk factors.

MATERIAL AND METHODS

In this cross-sectional comparative study, 40 patients (group A) admitted to Cardiac Care Unit of Pakistan Institute of Cardiology (PIC), Lahore with documented AMI showing characteristic ECG signs and rise in troponin I concentration were recruited. From local community 40 healthy volunteers were included as a control group (group B) after considering the inclusion and exclusion criteria. Approval of the study was taken from ethical review board of University of Health Sciences (UHS), Lahore. Convenient sampling technique was used for the collection of samples. Individuals suffering from chronic systemic diseases, pregnant women, smokers, and individuals taking folic acid and/or vitamin B₁₂ were excluded.

All the experimental work was carried out in the Department of Physiology and Cell Biology, UHS, Lahore. Data was collected in October 2018, after obtaining written informed consent from each subject. Information regarding demographic data was recorded. Every individual was assessed clinically by taking history and performing general physical and systemic examination. Systolic and diastolic blood pressure was recorded. Weight in Kg and height in Cm were recorded for calculation of BMI. Under aseptic conditions, 3–5 ml blood was drawn from ante cubital vein. Serum was obtained through centrifugation technique and stored at -70 °C. Serum Hcy and folic acid levels were measured with ELISA kits (Biolab, England). HHcy was considered if Hcy levels were >15 µmol/l. Folic acid levels were taken as deficient if <6 ng/ml.

SPSS-23 was used for data analysis. Mean±SD and median with inter-quartile range was given for quantitative variables. Frequency and percentage were given for gender. Normality of data was checked by Shapiro-Wilk test. For normally distributed data, independent student *t*-test was used and in case of not normally distributed data and for comparison of the mean difference in quantitative variables between groups, Mann Whitney U test was applied. Chi-square-test was applied to determine the gender difference between groups. Pearson correlation coefficients were used for correlation analysis between serum Hcy levels and folic acid levels, and *p*≤0.05 was considered statistically significant.

RESULTS

Sixty-two (77.5%) participants out of 80 were male. In group A of AMI patients, 34 (85.0%) participants were male, whereas in group B (healthy individuals), 28 (70.0%) participants were female. Chi-square test revealed no differences in gender distribution between

groups (*p*=0.11). Mann Whitney U test showed no significant differences between the median age of AMI patients (46.0 years) compared to healthy controls (45.0 years; *p*=0.19). There were no significant differences between the median pulse rate of AMI patients. Group A and B had mean BMI 25.3±3.6 and 23.9±3.4 respectively. No statistically significant differences were noted in mean BMI among both the groups (*p*=0.08). Median SBP and DBP of group A was 110 (110–120) mmHg and 80 (70–90) mmHg respectively and median SBP and DBP of group B was 110 (110–120) mmHg and 80 (70–80) mmHg, respectively. No statistically significant differences were noted in SBP (*p*=0.75) and DBP (*p*=0.98) between the groups (Table-1).

There were significantly raised levels of mean serum Hcy of AMI patients (22.9±6.0 µmol/l) in comparison to healthy controls (12.1±3.1 µmol/l, *p*<0.001). There were significantly lower levels of mean serum folic acid in AMI patients (6.29±1.50 ng/ml) in comparison to healthy controls (9.09±2.14 ng/ml, *p*<0.001) (Table-2).

The correlation between serum Hcy level and folic acid in group A and group B was moderate and negative (*r*= -0.48, and -0.66 respectively; *p*<0.001).

Table-1: Comparison of study parameters between groups A and B (n=40)

Parameters	Group	Mean±SD	Median (IQR)	Min	Max	<i>p</i>
Age	A	46.8±8.8	46 (41–54)	28	66	0.19
	B	43.9±5.2	45 (40–47.5)	28	50	
Pulse rate per minute	A	78.4±10.7	80 (70–90)	60	98	0.05
	B	74.1±8.7	75 (70–80)	60	90	
Body Mass Index	A	25.3±3.6	24.2 (23–28.3)	16.5	31.6	0.08
	B	23.9±3.4	24.1 (21.3–26)	18.5	33.2	
Systolic BP	A	115.9±12.2	110 (110–120)	90	160	0.75
	B	114.1±6.9	110 (110–120)	90	125	
Diastolic BP	A	76.5±8.9	80 (70–90)	60	100	0.98
	B	76.0±7.1	80 (70–80)	60	90	

Table-2: Serum folic acid and Hcy level among groups (Independent Student’s *t*-test) (n=40)

Groups	Mean±SD	Median (IQR)	Min	Max	<i>p</i>
Serum Folic Acid Level					
A	6.29±1.50	6.15 (5.10–7.55)	3.8	9.3	<0.001
B	9.09±2.14	9.15 (7.73–10.88)	4.9	13.5	
Serum Hcy Level					
A	22.9±6.0	22.2 (19.2–27.3)	10.8	37.0	<0.001
B	12.1±3.1	11.4 (9.4–14.5)	7.5	19.1	

DISCUSSION

In the present study it was observed that the ages of AMI patients and the healthy participants were not statistically significantly different. Our results agree with a recent study conducted in Israel¹⁸ and Pakistan¹⁰ which showed no statistically significant difference among different age groups in AMI. However, our results differ from another recent study¹⁹ which showed a significant difference between age groups. Similarly, the gender distribution between AMI patients and healthy participants did not show any statistically

significant difference. These findings are similar with a recent study which also showed no significant difference.²⁰ However, women are comparatively at a higher risk of having AMI and thus fatality due to infarction as compared to men.²¹

Overweight class has been defined as BMI ≥ 25 Kg/m² according to revised criteria for inhabitants of Asia.²² There were no statistically significant differences between both groups which is similar to a study, in which no statistically significant difference was found between both groups.²³ However, these results are in contrast to the results of a study conducted in Pakistan where significant statistical differences were found among both the groups.⁶ Results of our study could be possibly due to limited sample size and unmatched gender between both the groups.

The difference in pulse rate was found to be statistically insignificant between the diseased and healthy participants. These results are comparable to a study in which there was no significant differences in heart rates among AMI patients and healthy individuals ($p=0.26$).²⁴ One possible explanation could be the physiological changes undergoing in the body's vascular system, for example, vasodilation and vasoconstriction of vessels which tries to maintain homeostasis.²⁵ In the current study, no statistically significant differences were observed in mean SBP and DBP between diseased and healthy study participants. However, our results contrast with a previous study in which patients with HTN were at more risk for AMI ($p<0.05$).⁴ That study had a larger sample and it had 251 AMI cases and 464 age-matched and sex-matched non-AMI controls. The insignificant results in our study could be explained for not having a large sample size.

The diseased group in our study had statistically significant higher serum Hcy level as compared to healthier group. Our results are in agreement with the previous work in which Hcy levels were found raised in diseased group compared to healthy group.²⁴ The possible reason for increased Hcy levels in our study could be explained with the increased demands for DNA synthesis when the damaged tissue is undergoing repair which require the methylation of DNA, RNA and proteins. These reactions lead to production of Hcy as the end point in the methylation pathway.

Group A had mean serum folic acid level of (6.29 \pm 1.50) ng/ml and group B (9.09 \pm 2.14) ng/ml. Our results showed a statistically significant difference ($p<0.001$) in average serum folic acid levels between the diseased and healthy participants. The AMI patients had lower serum folic acid level as compared to healthy individuals. One possible explanation could be an unhealthy lifestyle with unbalanced diet especially lacking in fruits and vegetables leading to decreased folic acid and increased Hcy levels in our population.

Our results are similar to a study which showed inverse relationship between the Hcy and folic acid levels in AMI patients.²⁶ These results are in contrast with a study conducted in India.²⁷ The results of our study showed a significant correlation between serum Hcy and folic acid levels, i.e., increased Hcy levels are associated with decreased folic acid levels in both AMI and healthier group, but the correlation was more negative in later. One possible explanation could be that diet of healthier group could have been more fortified with folic acid as compared to diseased group. Another reason could be that healthier participants may be using folic acid supplements. These results are in accordance with a study which showed significant correlation (inverse/negative) between folic acid and Hcy in AMI group.²⁸ Our findings are in contrast with the results obtained in a study which showed no significant correlation between Hcy and folic-acid levels in both groups.²⁹

There were certain limitations of our study. Being a single centre cross-sectional-study, the sample size was small and geographically limited. Further work with better matched sampling and accountability for influencing factors, is required for a better acquaintance of the role of Hcy and folic acid in the pathophysiology of AMI.

CONCLUSION

Hcy levels were higher and folic acid levels were lower in subjects with AMI than the healthy individuals. The results indicate the possibility that decreased folic acid levels due to improper diet as well as lack of supplementation may contribute to the progression of AMI and thus maintaining these levels in normal range may contribute to a better management of AMI.

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Address for Correspondence:

Dr Nimrah Siddique, Senior Demonstrator, Department of Physiology, CMH Institute of Medical Sciences, Bahawalpur, Pakistan. **Cell:** +92-334-4160917
Email: nimrah.siddique@gmail.com

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Contribution of Authors:

NS: Concept, study design, manuscript writing and review

MNS: Data collection, analysis and final review

AG: Statistics, manuscript writing and bibliography

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ORIGINAL ARTICLE

FREQUENCY OF HEPATITIS B AND C VIRUS IN SURGICAL PATIENTS IN A TERTIARY CARE HOSPITAL OF MUZAFFARABAD

Irum Gilani, Ziyad Afzal Kayani*, Sarmud Lateef Awan*, Farzana Sabir*, Naheed Akhter*, Zakir Naqvi*

Department of Public Health & Community Medicine, Health Services Academy, Islamabad,

*Department of Surgery, AJK Medical College, Muzaffarabad, Pakistan

Background: Hepatitis B and C, parenterally transmitted diseases are major health problems all over the world especially in the developing countries. Patients presenting in different hospitals are not routinely screened for hepatitis B and C if they are not symptomatic. Since majority of carriers are asymptomatic, they create a real threat to health staff through self pricks, and other patients who share the same surgical instruments. Objective of this study was to assess the extent of hepatitis B and C in hospitalized surgical patients. **Methods:** This study, a cross-sectional survey, was carried out in Department of Surgery, Abbas Institute of Medical Sciences Muzaffarabad, from June to December 2018. Sample size was calculated using WHO sample size calculator for cross-sectional surveys. Non-probability convenient sampling technique was used. **Results:** Out of the total 100 patients included in this study, there were 54 (54%) males and 46 (46%) females, with ages ranging from 20 to 70 years. Three percent (3%) patients were hepatitis B positive while 12% were hepatitis C positive. Highest frequency of hepatitis C was seen in the age group 61–70 years, while hepatitis B patients were one each in age group of 20–30 years, 41–50 years, and 51–60 years. **Conclusion:** The frequency of hepatitis B surface antigen and hepatitis C virus in patients undergoing surgery is increasing which is a public health concern. There is an urgent need to implement infection control practices and preventive measures.

Keywords: Hepatitis B, Hepatitis C, Screening, Surgical, Healthy, Patients

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INTRODUCTION

Hepatitis B and C, parenterally transmitted diseases, are major health problems all over the world especially in the developing countries.¹ Parenteral transmission is facilitated by contaminated blood and blood products, multiple transfusions, needle sharing, use of contaminated instruments, for example in haemodialysis or surgeries, reuse of contaminated medical devices, tattooing devices, acupuncture needles etc.²

The prevalence of disease among general public due to HBV and HCV infection in Pakistan is 10%³, and in another study it is 4–10%⁴. Operating room personnel including surgeons have the highest risk of exposure.^{5,6} Sharp injuries with needles in the operating room are least likely to be reported⁷ hence putting operating room personnel even at a higher risk. Since majority of carriers are asymptomatic, they create a real threat to health staff through self pricks and other patients who share the same surgical instruments.⁸

Screening for hepatitis B and C should be mandatory as routine pre-operative investigations to assess their prevalence and to plan better preventive strategies against transmission to surgical team, universal precaution by using enhanced personal protective equipment (PPE), post-exposure prophylaxis and patient counselling about disease and further management.⁹

Transmission of hepatitis B and C by percutaneous exposure to contaminated blood is common. The fact that hepatitis virus and the HIV share similar routes of transmission contributes to co-infection. Healthcare personal that have exposure to blood are at risk of infection. As little as 0.01 ml of contaminated blood can transmit infection making it a big problem.¹⁰ The annual incidence of HBV infection in surgeons is estimated to be 50 times greater than that in general population, and more than twice that of physicians.⁹

Exposure rate in operation theatre settings is perceived to be high. Very little is known about the current prevalence of hepatitis B and C among healthy patients booked for surgery. The objective of this study was to assess the extent of hepatitis B and C in hospitalized surgical patients.

PATIENTS AND METHODS

This cross-sectional survey was carried out in the Department of Surgery, Abbas Institute of Medical Sciences (AIMS) —a teaching hospital of AJK Medical College, Muzaffarabad from Jun to Dec 2018. Ethical approval was taken from Ethical Committee of AIMS.

During the preoperative period all the elective patients from 21 to 70 years of age were screened while patients with known hepatitis B and C disease were excluded from the study. Initially patients were screened

by Immunochromatographic device (Rapid testing) and positive samples were confirmed by Enzyme Linked Immunosorbent Assay (ELISA) which has a sensitivity and specificity of 99%.

Sample size calculated using WHO sample size calculator for cross-sectional surveys with 95% CI was 97, and rounded up as 100. Non-probability convenient sampling technique was used.

RESULTS

Out of the total 100 patients included in this study, there were 54 (54%) males and 46 (46%) females, with ages ranging 20–70 years. Table-1 shows age distribution of study participants, maximum number of participants were 20–30 years of age.

Table-2 reveals that total three (3%) patients had hepatitis B infection while 12 (12%) were hepatitis C positive.

Highest Frequency of hepatitis C was in the age group 61–70 years, followed by 51–60 years age group, while hepatitis B patients were one each in age group of 20–30 years, 41–50 years, and 51–60 years.

Table-1: Age Distribution of study participants

Age (Years)	Frequency	Percentage
20–30	38	38
31–40	21	21
41–50	21	21
51–60	12	12
61–70	8	8
Total	100	100

Table-2: Frequency of hepatitis B and C

	Frequency	Percentage
Hepatitis B	3	3
Hepatitis C	12	12
None	85	85
Total	100	100

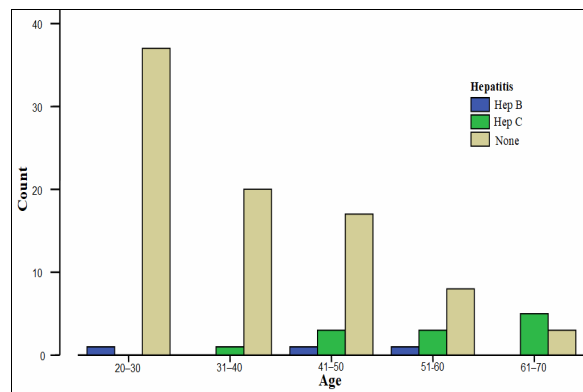


Figure-1: Age-wise distribution of Hepatitis B and C

The frequency of hepatitis in males was higher than females as shown in Figure-2. The percentage of hepatitis B in female patients was 1% while the frequency of hepatitis C was 5%. In male patients the frequency of hepatitis B was 2% while the frequency of hepatitis C was 7%.

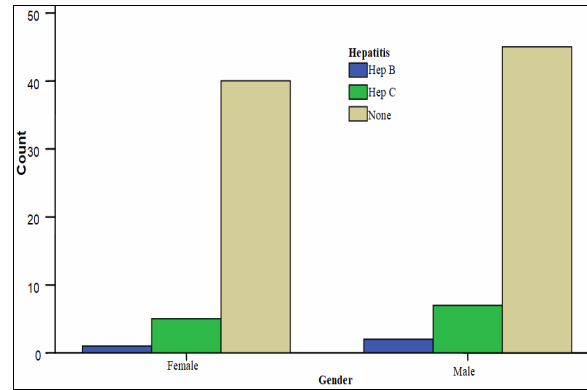


Figure-2: Gender-wise frequency of hepatitis B and C

DISCUSSION

Global prevalence of Hepatitis B varies from high (>8%) in Africa, Asia and Western Pacific, to low (<2%) in Western Europe, North America and Australia.¹¹ The overall prevalence for HBV and HCV was 8.4% and 42.7%, respectively in a study done in Pakistan. Moreover several community-based studies report a higher prevalence of viral hepatitis in Pakistan both in Sindh and Punjab Province.¹²

Studies on understanding the epidemiology of viral hepatitis and related risk factors in Pakistan are limited.¹² Our study documents a low frequency of hepatitis B and increased frequency of hepatitis C amongst admitted patients for surgery. Frequency of HBV was 3% and HCV 12%. Comparing with data HBsAg carrier rate is around 10% in different areas of Pakistan.³ Other studies carried out in different areas of Pakistan for hepatitis B virus frequency reported carrier state as 2.8%¹³, 8.6%¹⁴, and 10%¹⁵ respectively.

Several social factors are responsible for the higher prevalence of HBV and HCV; these include lack of health and safety standards due to insufficient awareness and knowledge of the disease in the general population. Furthermore, the higher prevalence of hepatitis is also linked to the inappropriate disposal of hospital waste in Pakistan.¹⁶

Prevalence of hepatitis C is also seen increasing in another study undertaken at Nawabshah on 523 patients.¹⁷ That study reported 14.3% prevalence of hepatitis C. Such high magnitude of these communicable diseases warrant public health measures to be undertaken and infection control guidelines to be strictly followed for prevention of disease to spread in healthcare workers who are at high risk during surgical procedures.

Positive patients with hepatitis B or C and surgical team require planned management strategies from admission to discharge. Patients with their family should be properly counselled about the disease, treatment, prevention, cost enhancement, and compromised outcome. If there is no emergency,

patients should be properly treated for hepatitis C and vaccinated for HBV. In emergency cases surgical team should use personal protective equipments.⁹

Emphasis should be laid on public health education particularly creating awareness about the risk factors of hepatitis B and hepatitis C, its prevention and control to minimize its transmission. Mass media should be used for this purpose. All patients should be routinely screened for hepatitis B (HBsAg) and hepatitis C (Anti HCV) prior to any surgical procedure.¹⁸

CONCLUSION

The frequency of hepatitis B and C in patients undergoing surgery is going up. This warrants mandatory screening before any surgical procedure, prophylaxis against hepatitis B, and use of personal protective equipment.

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Address for Correspondence:

Dr. Ziyad Afzal Kayani, Professor Department of Surgery, AJK Medical College, Muzaffarabad, Azad Jammu & Kashmir. **Cell:** +92-300-9825279

Email: ziyadkayani@hotmail.com

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Contribution of Authors:

ZAK: Conception, data collection, analysis, drafting

IG: Conception, data analysis, drafting

SLA: Conception, data collection

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ORIGINAL ARTICLE

HAEMATOLOGICAL AND BIOCHEMICAL EFFECTS OF TRANSFUSION OF STORED BLOOD IN TRANSFUSION-DEPENDENT THALASSEMIA PATIENTS

Sidra Humayun, Ghulam Farooq*, Nayab Farid**, Sara Asmat***, Alina Mehwish[†], Mohsin Ali^{††}

Department of Pathology, Muhammad College of Medicine, *Department of Medicine, Hayatabad Medical Complex, **Department of Pathology, Kabir Medical College, ***Department of Community Medicine, Muhammad College of Medicine, [†]Department of Pathology, Rehman Medical College, ^{††}Department of Pharmacology, Muhammad College of Medicine, Peshawar, Pakistan

Background: Thalassemia is a genetic disease in which there is an imbalance in the synthesis of globin polypeptide chains. The current study aimed to determine the haematological and biochemical effects of transfusion of seven days stored blood in transfusion-dependent thalassemia patients. **Methods:** A quasi-experimental study was conducted between January and July 2021 at Muhammad College of Medicine, Peshawar. A total of 20 transfusion-dependent thalassemia patients were selected. The impact of transfusion of 7-days old blood on haemoglobin levels, serum LDH, serum electrolytes, serum-free haemoglobin, serum bilirubin, serum-free iron, serum ferritin, and C-reactive protein were measured. Variations in pre-transfusion and post-transfusion samples were determined using paired-samples *t*-test, and $p < 0.05$ was considered significant. **Results:** There was a non-significant difference in increase of haemoglobin levels ($p = 0.543$) after transfusion of fresh and stored blood. Similarly, RBC counts, MCV, MCH, MCHC showed a slightly lower increase as compared to fresh blood. No differences were seen in platelet count between the two groups. However, the rise in white cells was significantly higher after transfusion of 7-days stored blood as compared to fresh blood ($p = 0.002$). A non-significant increase in post-transfusion LDH ($p = 0.13$), direct bilirubin ($p = 0.76$) and indirect bilirubin ($p = 0.45$) was seen. No differences in creatinine, glucose, and uric acid variations were found. Levels of C-reactive protein showed a significantly higher rise when 7-days stored blood was transfused in comparison with fresh blood ($p = 0.012$). **Conclusion:** At least seven days stored blood can be safely transfused to transfusion-dependent thalassemia patients.

Keyword: Thalassemia, blood transfusion, banked blood, Peshawar, Pakistan

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INTRODUCTION

Red blood cell transfusion is one of the most common medical procedures performed in the world. In the United States of America, an estimated 49 numbers of blood units per 1,000 of the population are transfused in various settings each year.¹ Most blood transfusions are provided in hospital settings to patients undergoing surgery, or to those requiring multiple blood transfusions such as patients with thalassemia or aplastic anaemia.² To establish a continuous supply of safe blood, a comprehensive supply chain mechanism of blood collection, screening, storage, and transfusion is required.³ Blood units are stored in blood banks in refrigerators for up to 42 days before transfusion. During storage, red cells undergo multiple physiologic changes. These generally described as ‘storage lesions’ include haemoglobin oxidation and release of free haemoglobin, RBC membrane structural degradation, and increase cell fragility. Metabolic abnormalities accumulate over time as well which include accumulation of lactate and depletion of ATP and 2,3-diphosphoglycerate. These cells when transfused, are at risk of haemolysis, releasing free iron causing oxidative damage to organs.⁴

Recent clinical and laboratory data suggest that increasing the duration of storage is associated with increased morbidity and mortality. A meta-analysis reported no significant differences in survival comparing transfusion of fresh blood (1–10 days old) with older stored blood (2–3 weeks) in clinical trials. However, an increased risk of death was found with increasing duration of storage in 31 observational studies.⁵ Rapido *et al* showed that autologous transfusion of stored blood after 5 weeks showed marked extra vascular haemolysis, saturated serum transferrin, and circulating free transferrin.⁶ Post-42 days of storage, transfusion of red cells produced extravascular haemolysis and circulating non-transferrin bound iron and was associated with proliferation of *Escherichia coli*.⁷ In another study, up to 21 days stored blood transfusion was not found to be associated with multi-organ dysfunction in patients undergoing cardiac surgery.⁸ However, the majority of these studies are either performed on healthy volunteers or clinically gross outcomes such as morbidity or mortality. Based on this lack of evidence, the American Association of Blood Banks allows transfusion of up to 42 days stored blood. Biochemical evidence of transfusion of stored blood shows that transfusion of

older red cells results in lysis and causes an inflammatory state.⁹ This in an otherwise healthy individual might be of no clinical consequence, however, may be harmful when transfused to patients with compromised hemodynamics.

Thalassaemia major is characterized by severe transfusion-dependent anaemia, ineffective erythropoiesis, and extramedullary haematopoiesis. In addition, there is an enlargement of the spleen with hypersplenism and increased red cell clearance in the sinusoids.¹⁰ Transfusion of older red cells to patients with splenomegaly may probably destroy red cells in the spleen, resulting in the release of haemoglobin, free iron and causing an inflammatory state. There is a need to assess the effects of transfusion of stored blood in such patients. The current study aimed to determine the impact of transfusion of 7-days stored blood on haematological and biochemical parameters in transfusion-dependent thalassaemia.

MATERIAL AND METHODS

This quasi-experimental study was designed to achieve the objectives. Blood samples were collected from patients enrolled at Fatimid Foundation Thalassaemia Care Centre, Peshawar, and the laboratory work was performed at Pathology Department of Muhammad Medical College, Peshawar. The study was performed from January to July, 2021 after ethical approval. The purpose of the study, procedure, risks, and benefits of the study were explained to the parents of the patients and written consent was taken. Patient demographic data such as age, sex, address, phone number, and transfusion demand per month were collected. A personal number was allocated to each patient. A total 20 transfusion-dependent thalassaemia patients with age 5 years and above were included in the study. Blood group of each patient was checked for ruling-out the chance of alloimmunization. Blood donations were collected as per standard practice in blood bags and were screened for Hepatitis B and C, HIV, and Syphilis. Blood bags were then centrifuged in a high-speed blood bank centrifuge (4,000 rpm) for plasma separation. Plasma was transferred in another blood bag and red cell concentrate was stored in the standard blood bank. Blood bags were separated into 'fresh' and were transfused within 2-days of collection, or were labelled as 'stored' and kept in the refrigerator for 7 days before transfusion.

In the first visit, each patient received a transfusion of fresh blood (2-days of storage). Prior to transfusion, a complete general physical examination was performed. Subsequently, blood samples were collected in EDTA tubes and gel tubes containing z-serum clot activator. Samples were transferred to the laboratory for analysis. After that, red cell concentrate was transfused to the patient as standard practice in the

centre. 24-hours after the transfusion, the patient was examined again, and blood samples were collected in EDTA and gel tubes. The patient was informed of their next transfusion schedule after a 2–4 weeks depending upon their haemoglobin level. Upon next visit, the same examination, blood sampling, and transfusion procedure was adopted, except this time, 7-days stored blood was transfused. A 24-hour post-transfusion sample was obtained as before.

In Laboratory, complete blood count (CBC) of blood samples was calculated on Abbott's Cell-Dyn 3200 haematology analyzer. The blood sample was aspirated, and a complete blood count was obtained on the attached computer screen. The machine contains pre-installed reagents for blood count which were supplied by the company. Serum ferritin levels of the samples were measured on Roche's Cobas e411 analyzer. The blood sample was centrifuged at 4000 rpm for 3–5 minutes to obtain blood serum. A 10 µl serum was then aspirated in Roche's Cobas e411 analyzer to measure the serum ferritin level as the machine contain a pre-installed ferritin kit. The machine was pre-calibrated through the company's supplied reagent before the test. Alanine aminotransferase (ALT), bilirubin, uric acid, C-reactive protein (CRP), lactate dehydrogenase (LDH) and glucose levels of blood samples were measured on Roche's Cobas C111 analyzer. The test type was selected from the machine's software and obtained results were recorded.

Participants' demographic and laboratory parameters were entered in Microsoft Excel 365 and statistical analysis was performed through SPSS-23. Differences in each laboratory parameter were calculated. Mean differences and standard deviation were calculated. Paired sample *t*-tests were performed to compare the differences in parameters between fresh blood transfusion and stored blood transfusion, and $p < 0.05$ was considered significant.

RESULTS

The study was conducted on 20 thalassaemia patients including 13 (65%) female and 7 (35%) male patients with a mean age of 17.75 ± 5.03 years. The demographic and clinical data of the patients is shown in Table-1. Transfusion of 'fresh' and 'stored' blood resulted in an increase in blood Hb levels in 24-hour post-transfusion samples. This increase was more when fresh blood was transfused (1.55 mg/dL) compared to old blood (1.35 mg/dL). However, this difference was not statistically significant ($p=0.543$). Similarly, other red cell parameters (RBC counts, MCV, MCH, MCHC) showed a slightly lower increase as compared to fresh blood but these results were not statistically significant. No differences were seen in platelet count between the two groups. Notably, an increase in white cell counts was seen in both groups. The rise in white cells was

significantly higher after transfusion of 7-days stored blood ($1.01 \times 10^9/L \pm 2.03$) as compared to fresh blood ($1.82^9/L \pm 1.1$) (paired sample *t*-test, $p=0.002$) (Table-2).

Table-1: Demographics and clinical features of patients (n=20) [Mean±SD, n (%)]

Variables	Observed Values
Age (Years)	17.55±5.031
Gender Ratio (Male:Female)	1:1.7
Splenomegaly	Yes
	No
Hepatomegaly	4 (20) 16 (80)
Jaundice	9 (45) 11 (55)
Haemoglobin (g/dL)	7.58±1.59
Red Blood Cells ($10^{12}/L$)	2.86±0.64
White Blood Cells ($10^9/L$)	6.25±3.34
Platelets ($10^9/L$)	199.9±147.2
Packed Cell Volume (%)	22.6±5.06
MCV (fL)	79.5±4.85
Mean Corpuscular Haemoglobin (pg)	26.81±2.36
MCHC (g/dL)	33.70±1.75
Bilirubin Direct (mg/dL)	0.53±0.30
Bilirubin Indirect (mg/dL)	1.11±0.99
Glucose (mg/dL)	125.3±76.77
Uric Acid (mg/dL)	4.35±1.10
Lactate Dehydrogenase (U/L)	345.15±173.36
Creatinine (mg/dL)	0.31±0.10
C-reactive protein (mg/dL)	17.30±39.57
Ferritin (ng/mL)	2901.35±1855.63
Iron (µg/dL)	547.19±235.86

Table-2: Difference in haematological parameters fresh blood vs 7-days stored blood (Mean±SD)

Variables	Increase in post-fresh RCC transfusion	Increase in post-stored RCC transfusion	<i>p</i>
Hb	1.55±0.64	1.35±0.46	0.543
RBC	0.52±0.25	0.57±0.24	0.89
WBC	1.01±1.1	1.82±2.03	0.002*
Platelet	19.9±18.17	22.85±26.55	0.65
PCV	4.67±2.51	3.57±1.91	0.91
MCV	1.67±1.87	1.25±0.91	0.17
MCH	0.93±0.64	0.83±0.77	0.46
MCHC	1.33±1.06	0.87±0.84	0.85

*Significant

The older transfused red cells may undergo lysis resulting in increased LDH and bilirubin levels. To assess this, differences in LDH values, pre-and post-transfusion of fresh and 7-days stored blood was calculated and compared using paired samples *t*-test. The mean values of LDH pre-transfusion and post-transfusion of fresh blood in comparison to 7 days banked blood (67.02 ± 101.02 vs 107.30 ± 121.69 U/L) showed a bigger increase in post-transfusion LDH when 7-days stored blood was transfused. However, this was statistically not significant ($p=0.13$). However, there was no significant increase in either direct bilirubin (0.13 ± 0.14 mg/dL vs 0.12 ± 0.15 mg/dL, $p=0.76$) and indirect bilirubin (0.42 ± 0.55 mg/dL vs 0.32 ± 0.32 mg/dL, $p=0.45$). To determine levels of creatinine, glucose, C-reactive protein (CRP), and uric acid were determined to assess the level of body metabolites affected by the storage time of the transfused blood. No difference in creatinine, glucose, and uric acid variations

was noted. Interestingly, levels of CRP which is an inflammatory marker showed a significantly higher raise when 7-days stored blood was transfused in comparison with fresh blood. (1.89 ± 2.38 mg/dL vs 6.43 ± 7.46 mg/dL, $p=0.012$) (Table-3).

Table-3: Differences in biochemical parameters after fresh or 7 days stored blood (Mean±SD)

Variables	Difference after transfusion of fresh blood	Difference after transfusion of 7 days stored blood	<i>p</i>
LDH	67.02±101.02	107.30±121.69	0.13
Bilirubin (Direct)	0.13±0.14	0.12±0.15	0.76
Bilirubin (Indirect)	0.42±0.55	0.32±0.32	0.45
Creatinine	0.10±0.06	0.07±0.06	0.149
Glucose	28.5±46.8	34.40±108.12	0.76
CRP	1.89±2.38	6.43±7.46	0.012
Uric Acid	0.50±0.511	0.42±0.34	0.52

An increase in serum iron levels was noted in transfusion of both fresh blood (155.79 ± 177.77 µg/dL) and 7-days stored blood (91.76 ± 88.87 µg/dL). However, the differences in the serum iron raise were not statistically significant (paired samples *t*-test, $p=0.18$). Transfusion of both fresh and 7-days stored blood resulted in increased iron levels in the post-transfusion sample. In fresh blood transfusion, serum ferritin levels increased by 1072.70 ± 1292.052 ng/mL, and in 7-days stored blood transfusion, serum ferritin levels increased by 826.32 ± 1088.94 ng/mL. These differences were statistically not significant ($p=0.55$) (Table-4).

Table-4: Difference in iron overload between fresh blood and 7 days stored blood

Variables	Difference after transfusion of fresh blood	Difference after transfusion of 7 days stored blood	<i>p</i>
Ferritin	1072.70±1292.052	826.32±1088.94	0.55
Iron	155.79±177.77	91.76±88.87	0.18

DISCUSSION

Our study aimed to investigate the differences in haematological and biochemical markers after transfusion of fresh blood compared to 7-days banked blood. The main purpose of blood transfusion in thalassaemia patients is to raise the red blood cells count by suppressing the ineffective erythropoiesis. As per previous studies performed on different sets of patients to determine transfusion-related differences, no significant difference was found between transfusion of fresh blood and up to 15–20 days banked blood.¹¹ Similarly, we found no significant difference in haematological parameters post-transfusion fresh blood versus 7 days banked blood except non-significant post-transfusion increase in WBCs, platelets, CRP, ferritin, and iron levels. The haemoglobin level was expected to be raised after blood transfusion. According to Linda *et al*, the haemoglobin level raised up to 1g/dL in patients with severe anaemia after transfusion of packed red blood cells.¹² As per our study, an increase in haemoglobin level was observed in both groups

receiving fresh blood and 7 days old blood. No significant increase in MCV, MCH, and MCHC post-transfusion levels were observed in both groups. Although transfusion-dependent thalassaemia patient's red cells have very low MCV, MCH, MCHC, our patients had received multiple transfusions and therefore, their haematological picture was representative of previously transfused blood from healthy donors. Therefore, transfusion of normocytic red cells did not increase MCV, MCH, or MCHC. Similar findings were earlier observed by Spadaro *et al* in anaemic patients post-transfusion blood samples.¹³

In our research study, we also observed a mild but significant post-transfusion increase in WBCs count. The plausible explanation for the increase in WBCs count may be due to the infusion of pro-inflammatory cytokines especially IL-6.^{14,15} A mild leucocytosis is observed in patients who are transfused with leuco-depleted blood.¹⁶ Increased in post-transfusion WBCs count was also found by Hirani *et al*.¹⁷ Another proposed mechanism is the increased concentration of non-transferrin bound iron (NTBI) in the blood leading to the production of pro-inflammatory cytokines including IL-6 and IL-8 which causes leucocytosis in the recipient's blood.¹⁸ More recently, Straat *et al*, concluded that RBCs transfusion bags contain extracellular vesicles (EVs) as supernatant which may increase due to prolong storage of blood. These EVs cause release of TNF, IL-6, IL-8, and IL-10 which results in the production of a strong pro-inflammatory host response, i.e., leucocytosis.¹⁹

Consistent with increased white cell count, we also observed a significant increase in serum C-Reactive Protein (CRP) in post-transfusion samples of patients transfused with old blood. This finding is as per results shown by Kapur *et al*.²⁰ The authors have attributed this increase in CRP to EVs which are found in blood bank stored blood. The EVs have pro-coagulant activity. It may be also due to platelets aggregation because of RBCs membrane damage and thrombocytosis as a consequence of splenectomy. Interestingly, the increased platelets were found more in splenectomized patients than non-splenectomized patients by Trincherio *et al*.²¹

We also tested markers of red cell and tissue destruction (bilirubin and LDH). Although the values of these showed a slight increase in post-transfusion samples from patients transfused with old blood, this was not significant. Blood transfusion-related abnormalities in serum laboratory parameters such as bilirubin and lactate dehydrogenase were demonstrated by Weisen *et al*, in transfusion-dependent patients.²² A non-significant and temporary increase in bilirubin and lactate dehydrogenase levels was observed in patients receiving two packs of RBCs. The increase in bilirubin level is due to the destruction of non-viable RBCs in the first 1–2 hours after transfusion and conversion of

released haemoglobin into bilirubin by the liver. This phenomenon is seen in almost all patients who receive blood. However, the bilirubin level returns to normal after 24 hrs.²³ Hence, reticulocytes count was not performed to detect the significance of increase in bilirubin and LDH levels. Since we tested our post-transfusion samples 24 hours after transfusion, these values might have returned to normal.

Blood transfusion leads to an increase in serum ferritin and iron levels. Increase serum iron concentration causes the transfer of excessive iron to the liver and other organs such as the heart, endocrine glands, and tissues which may lead to severe toxicity and life-threatening medical conditions.²⁴ Cardiac myopathy is one of the most common pathological conditions that results from iron overload and causes most of transfusion-related deaths.²⁵ Production of reactive oxygen species (ROS) is another problem related to iron load. These free radicals cause degradation of cellular lipid contents and cell organelles including DNA leading to the death of cells.²⁶ A patient suffering from thalassaemia or other types of haemoglobinopathies receives about 200–250 mg iron per unit of blood. The body cannot excrete excessive iron and therefore the excess iron is managed through iron chelation therapy.²⁷ Assessment of iron overload is usually performed by measuring serum ferritin level in developing countries. ferritin is an iron storage protein that is used as a marker for analyzing iron load. Serum ferritin, being an acute phase reactant, also increases in inflammatory diseases, liver diseases, renal diseases, and metabolic abnormalities.²⁸ The increase in iron and serum ferritin levels post-transfusion is observed by different studies. Sadoom *et al*, found a significant increase in serum ferritin and iron levels in the Iraqi population.²⁹ An interesting finding in our study was a non-significant increase in blood ferritin and free iron level in post-transfusion blood samples of both groups ($p=0.55$ and 0.18 respectively). Probably, 7-days stored blood caused a more acute increase in serum iron and ferritin in the old-blood transfusion group and the difference was reduced at 24 hours.

CONCLUSION

At least seven days stored blood can be safely transfused to transfusion-dependent thalassaemia major. However, due to limitation of our study budget, large-scale study should be designed in future for generalization of our results.

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Address for Correspondence:

Dr. Mohsin Ali, Senior Lecturer, Department of Pharmacology, Muhammad College of Medicine, Peshawar, Pakistan. **Cell:** +92-321-5275212

Email: mohsin.ibms86@gmail.com

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ORIGINAL ARTICLE

PATTERN OF CHEST INJURIES IN ROAD TRAFFIC ACCIDENTS IN A TERTIARY CARE HOSPITAL

Ziyad Afzal Kayani, Irum Gilani*, Shagufta Manzoor, Sarmud Lateef Awan,
Zakir Naqvi, Shagufta Sabir Qureshi

Department of Surgery, Abbas Institute of Medical Sciences, Muzaffarabad, *Department of Public Health and Community Medicine, Health services Academy, Islamabad, **Department of Anatomy, AJK Medical College, Muzaffarabad, Azad Jammu and Kashmir

Background: Road traffic accident injuries are an important cause of mortality and morbidity. It is the major public health problem in every country across the world and causing approximately 5.8 million deaths per year. The Chest trauma is a disease that has worsened long with growing urbanization and industrialization; due to worldwide increases in violence, constructions and vehicle number. **Methods:** This study was conducted at Abbas Institute of Medical Sciences (AIMS) Muzaffarabad AJ&K from August 2020 to December 2020. The study was approved by Ethical committee of AIMS hospital. Convenient sampling technique was used. Hospital based retrospective descriptive cross-sectional study designs was used to assess patterns and outcomes of chest injuries among patients based on pre designed questionnaire presented to AIMS Muzaffarabad. The collected data were entered to SPSS, version 25 for analyses. **Result:** Most affected age group was young adults (63%) between 18yr-35yr. Nearly three fourth 72% of chest trauma patients were male and 28% were females. Majority of chest trauma patient arrived to health care facilities within 1 to 2 hours of trauma. Rib fracture was the commonest types of chest injury (39%) followed by hemopneumothorax (25%) and pulmonary contusion (7%). With regard to associated body region injured, extremities were the commonest region (47%). **Conclusion:** Chest injuries due to RTA predominantly affect the male and economically productive age group with high morbidity and mortality in this environment.

Keywords: Chest injury, Road traffic accidents, Rib fractures, associated injuries, hemopneumothorax

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INTRODUCTION

Road traffic accident injuries are an important cause of mortality and morbidity. It is the major public health problem in every country across the world causing approximately 5.8 million deaths per year; about 16,000 deaths per day.¹ Injury is affecting and putting a significant health burden on all populations, regardless of age, sex, income, or geographical region. Traffic accidents make up 1.3 million deaths, suicides responsible for 844,000 whereas homicides responsible for 600,000 deaths globally and they are considered as the leading cause of traumatic deaths.² Approximately, 91% of these deaths occur in developing countries including Pakistan.

The Chest trauma is a disease that has worsened along with growing urbanization and industrialization; due to worldwide increases in violence, constructions and vehicle number. The later results in increments of road traffic accidents. It is a major health problem especially for young males. Globally, chest trauma is the third important cause of mortality and morbidity preceded by cancer and cardiovascular diseases being responsible for 10% of all trauma admissions and 25% of trauma-related deaths across the world.³

In Europe and United states, the mortality rate as a result of blunt trauma can be as high as 60% but appropriate and timely diagnosis of chest traumas can

decrease the mortality and morbidity.⁴ Optimal care of severely injured patients needs a multidisciplinary approach starting from the point of injury to a rehabilitation structure in order to return the patient to their maximum potential level of function within a society.⁵

Major thoracic injuries are known as the Deadly Dozen. Six are lethal and six are hidden. The Lethal six are immediate life threatening conditions like Airway obstruction, Tension pneumothorax, open pneumothorax, Flail chest, Massive haemothorax and cardiac tamponade. While the hidden six like tracheobronchial tree injury, pulmonary contusion, blunt cardiac injury, blunt oesophageal rupture, traumatic aortic disruption and traumatic diaphragmatic injury are potentially life threatening conditions.⁶

A Malaysian study reported that trauma was the third cause of admission to hospital and fifth cause of death. Chest trauma was the cause of 10% of all admissions and about a quarter number of deaths were trauma-related.⁷

In Pang study at Klang Valley hospitals, chest injuries accounted for 36.6% of fatalities among the fatally injured motorcyclist.⁸ The aetiology and pattern of chest trauma that vary from one part to the other part of the world have been reported in literature. RTA is the most common cause of chest trauma in majority of studies.

In emergency, patients frequently present with both blunt and penetrating injuries. This includes a spectrum ranging from simple chest wall contusion to severe vital organ injuries.⁹ Most patients admitted with chest trauma are managed conservatively and only a few need a thoracotomy, which is usually performed in a higher proportion of patients who sustained penetrating chest trauma.

Associated extra thoracic organ injury, late presentation beyond 24 h post trauma and severe chest injury with bilateral chest involvement were found to be determinants of mortality in chest trauma.¹⁰

Majority of chest trauma is preventable. A clearer understanding of the aetiology, injury patterns and outcome of these patients is important for establishment of prevention strategies and treatment protocols. A clearer understanding of the aetiology, injury patterns and outcome of these patients is important for establishment of prevention strategies and treatment protocols. The objective of this study was to assess the patterns and outcomes of chest injuries among adult patients admitted with chest trauma in AIMS Hospital Muzaffarabad over 4 months period.

METHODOLOGY

It was a retrospective quantitative descriptive cross sectional study. The study was conducted at surgical department of Abbas Institute of Medical Sciences (AIMS), Muzaffarabad from August 10, to December 10, 2020. The study was approved by Ethical committee of AIMS hospital. The sampling technique used was a convenient sampling. 100 patients between the age of 18–80 years having chest injury during road traffic accident reaching alive in Accident and Emergency of AIMS hospital were included in the study. Between the ages of 18–35 years were labelled as young adults, 35–60 years middle age adults and above 60 years as old patients. Written informed consent was taken from all the patients in the study. Data was based on predesigned questionnaire presented to patients arriving in accident and emergency in AIMS Muzaffarabad. The collected data analysed using SPSS-25.

RESULTS

A total of 100 chest injured patients were enrolled to the study during period of 4 months (August 10, 2020–December 10, 2020). Most affected age group was young adults (63%) between 18–35 years. Nearly three fourth 72% of chest trauma patients were male and 28% were females. Among these patients motorcyclists were the most frequent road users who sustained chest injuries as shown in Table-1.

Majority of chest trauma patient 63% received blunt injuries while 37% received penetrating injuries as shown in Table-2.

Most of the patients (92%) arrived to health care facilities within 1 to 2 hours of trauma. The average duration for hospital stay was 1 to 7 days. Concerning outcome of the patient, 77 were recovered, 15 were referred (with associated injuries) and 8 died in AIMS during treatment (Table-3). Those who died (8%) were the ones with advanced age (over 60 years), had associated injuries (head and neck) and presented late in hospital (after 2 hours).

Rib fracture was the commonest types of chest injury, it accounted for (39%) of chest injuries followed by hemopneumothorax (25%) and pulmonary contusion (7%) as shown in Table-4.

Table-1: Distribution of cases according to types of road users

Valid	Frequency	Percent
Vehicle driver	25	25.0
Passenger	23	23.0
Pedestrian	11	11.0
Motorcyclist	41	41.0
Total	100	100.0

Table-2: Distribution of cases according to nature of chest injury

Valid	Frequency	Percent
Blunt	63	63.0
Penetrating	37	37.0
Total	100	100.0

Table-3: Distribution of cases according to outcomes

Valid	Frequency	Percent
Recovered	77	77.0
Referred	15	15.0
Death	8	8.0
Total	100	100.0

Table-4: Distribution of cases according to pattern of chest injuries

Valid	Frequency	Percent
Rib fracture	39	39.0
Clavicle fracture	10	10.0
Sternal fracture	6	6.0
Pneumothorax	17	17.0
Hemothorax	8	8.0
Cardiac tamponade	5	5.0
Pulmonary contusion	7	7.0
Myocardial contusion	2	2.0
Chest wall contusion	4	4.0
Airway obstruction	2	2.0
Total	100	100.0

DISCUSSION

In this study chest injuries predominantly affected male and economically productive age group. In agreement with the present study, other studies reported similar findings.^{7,11–14}

A detailed literature search is suggestive of RTAs as the main cause of chest injuries worldwide, more so in the developing world.² In the present study also, motor vehicle accidents accounted for almost 62%

of all thoracic injuries. This mechanism of injury is rarely reported in the western literature but is seen more often in developing countries such as our and other Asian and African nations.

Blunt chest injuries were more frequent than penetrating chest injuries in present study. Similarly, the same pattern reported from previous studies in Syria by Al-koudmani, in Tanzania by Lema and in Iran by Mohammadzadeh.^{6,7,13}

Early presentation after a chest injury coupled with prompt and effective management at a trauma centre is the key to a good outcome. However, this study found that most of patients were presented to health care facilities within 1 hours of injury. Our observation is in agreement with a study from Nigeria.¹¹

Moreover, late presentations to hospital (after 2 hours) were highly associated with mortality in the present study. This could be as a due to grossly inadequate ambulance service that usually resulted in a long interval between the accident and the arrival of the victims at the hospital, this needs further exploration in future studies.

Concerning pattern of chest injury majority of the patients sustained rib fracture, i.e., 39% followed by hemopneumothorax and pulmonary contusion i.e., 25% and 7% respectively. In agreement with the present finding study in England reported rib fracture as the most common type of chest injury.¹² Contrasting to the present finding another study in Iran reported hemothorax as the most common type of chest injury.¹⁴

Injury to extremities was the most common extra-thoracic associated body region injured among patients presented with chest injury. This finding was consistent with the study conducted in Syria.⁶ Grave outcome; mortality of 8% were the ones with advanced age (over 60 years), had associated injuries of head and neck was found in this study, this is in agreement with other National and International studies.^{15,16}

CONCLUSION

Road traffic accidents involving motor vehicles are the most common cause of blunt chest injuries predominantly affecting the young male age group. Rib fractures were the commonest pattern of chest injury. Mortality is high in patients with advanced age, associated injuries and late presentation in hospital.

Address for Correspondence:

Prof. Dr. Ziyad Afzal Kayani, Department of Surgery Abbas Institute of Medical Sciences, Muzaffarabad, AJK.

Cell: +92-300-9825279

Email: ziyadkayani@hotmail.com

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Contribution of Authors:

ZAK: Conceptualization, data collection, analysis and drafting

IG: Conceptualization, data analysis, drafting

SM: Data analysis, drafting and proof reading

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ORIGINAL ARTICLE

AMELIORATING EFFECT OF ZINC ON PROTEIN SUPPLEMENT INDUCED DNA AND SPERM DAMAGE IN MALE SPRAGUE DAWLEY RATS

Saira Safdar, Shazia Ali*, Humaira Fayyaz*, Hina Munir**, Sana Majeed*,
Fozia Atif***, Sidra Jabeen[†]

Department of Physiology, Islamabad Medical and Dental College, Islamabad, *Islamic International Medical College, Rawalpindi, **AJK Medical College, Muzaffarabad, ***Fazaia Medical College, Islamabad, [†]HITEC Institute of Medical Sciences, Taxila, Pakistan

Background: Oxidative stress induced DNA damage and impairment in testicular tissue histology are indicative of male infertility. The aim of this study was to evaluate the protective role of Zinc on Protein supplement induced damage to the DNA and testicular tissue histology. **Methods:** The study comprised a total 30 male Sprague Dawley rats divided into Group 1 (n=10) fed on standard laboratory food, Group 2 (n=10) fed on standard laboratory food and dietary supplement powder and Group 3 (n=10) fed on standard laboratory food, dietary supplement powder and Zinc. Serum levels of 8-hydroxy-2'-deoxyguanosine (ng/ml) were estimated by ELISA. Testicular Tissue histology was performed for the detailed examination of Spermatogenic cells, size of seminiferous tubules and lumen of seminiferous tubules. **Results:** Mean±SEM of serum 8-hydroxy-2'-deoxyguanosine (8-OHdG) levels in Group-2 rats was significantly increased ($p<0.05$) as compared to Group 1 rats. While Mean±SEM of serum 8-hydroxy-2'-deoxyguanosine (8-OHdG) levels of Group 3 rats was significantly decreased ($p<0.05$) as compared to Group 2 rats. There was no significant difference in Mean±SEM of serum 8-hydroxy-2'-deoxyguanosine (8-OHdG) levels between Group-1 and Group-3. Evident pathological changes were seen in the testicular tissue of the Group-2 rats, characterized by atrophic seminiferous tubules with reduced number of spermatogenic cells and widening of lumen. Rats of Group 3 received Zinc supplement and showed normal seminiferous tubules with increased number of mature spermatozoa in their lumen. **Conclusion:** Soy protein and silicon dioxide which are the ingredients of protein supplements induce DNA damage and destroys testicular tissue. Zinc has potential to restore the DNA damage and testicular tissue structure.

Keywords: Protein supplements, Soy Protein, Silicon dioxide, Zinc, 8-hydroxy-2'-deoxyguanosine (8-OHdG) and testicular tissue histology

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INTRODUCTION

Dietary supplements are the products containing a dietary ingredient intended to improve the nutritional value of food. About 50% population in United States is consuming these supplements to improve health conditions and to boost energy.¹ There is increasing interest of men in oral intake of whey protein supplements to attain a fit body.² Protein supplements are synthetic form of proteins which are composed of Whey Protein, Soy (Lecithin or Isoflavone), Whey Peptides, Fat-Reduced Cocoa Powder, Flavorings, Sweeteners, Enzyme Complex and preservatives.² These protein supplements contain the ingredients which are negatively affecting the male reproductive health.³ Silicon dioxide and soy are constituents of protein supplements which damage the male reproductive system.⁴⁻⁶

Soy proteins are also called as Phytoestrogens that binds to estrogenic receptors which are located in several hypothalamic nuclei including Hypothalamic preoptic area (centre of sexual behaviour), Pituitary gonadotropes and along lining of Male reproductive tract, Secondary sexual glands, Sertoli cells, Leydig

cells and Spermatids.⁷ It influences pituitary gonadal axis. Foods rich in Soy adversely effects the Male Reproductive system.⁸

Silicon dioxide is used as preservative in protein supplements and is cytotoxic in nature.⁹ One of the association linking Silicon dioxide with damage to male reproductive parameters is the generation of reactive oxygen species as a result of oxidative stress.¹⁰ Silicon dioxide damages mitochondrial cristae in cells leading to decreased ATP generation which causes the oxidative stress leading to the DNA damage.¹¹

Zinc is an important dietary nutrient which has a remarkable effect on male reproductive system as it acts as an antioxidant and protects the epithelial linings of the reproductive system.¹²

Zinc is the component of an important enzyme superoxide dismutase which transforms the superoxide to oxygen and hydrogen peroxide so it have the capability to fight against the oxidative stress.^{13,14} Zinc reduces the amount of free radicals and repairs the DNA.¹⁵

There is scarcity of data about the harmful effects of preservatives used in dietary supplements

and about role of zinc in correcting the male reproductive parameters and DNA damage caused by harmful constituents in protein supplements.

The protein supplements have been reported to cause the problems in male reproductive system and Zinc is well known for improving male fertility. There is no research, which suggests the regular addition of an antioxidant with protein supplements to avoid the damage to male reproductive parameters.

MATERIAL AND METHODS

Total number of 30 male Sprague Dawley rats, 8 weeks old, weighing 250–300 g were included in the study. Male Sprague Dawley rats were distributed into 3 groups as following:

Group 1 (control group) contained 10 male Sprague Dawley rats which were fed on a standard laboratory feed. Group 2 (experimental group) contained 10 male Sprague Dawley rats which were fed on a standard laboratory feed mixed with soy protein (42 mg/week for 10 rats) and silicon dioxide (0.42 mg/week for 10 rats) in powder form. Group 3 (experimental group) contained 10 male Sprague Dawley rats which were fed on standard laboratory feed mixed with soy protein (42 mg/week for 10 rats), silicon dioxide (0.42 mg/week for 10 rats) and zinc (210 mg/week for 10 rats) in powder form.

Male Sprague Dawley rats were acclimatized to the NIH animal house atmosphere at humidity of 50–70% and at a room temperature of 24 ± 2 °C, maintained at 12 hour light and dark cycle. Standard laboratory feed and water was provided *ad libitum*.

Amount of soy protein, silicon dioxide and zinc used in the experiment was calculated by using the formula after incorporating the allowed doses of all three ingredients mentioned in the literature.^{15–17}

As, a biomarker of oxidative stress induced DNA damage serum levels of 8-hydroxy-2'-deoxyguanosine (ng/ml) was measured. The value was assayed by using commercially available ELISA Kits from (Elabsience Biotechnology Co. Ltd., Japan.)

At the beginning of experiment blood collection from male Sprague Dawley was retrieved through Tail Vein method for detecting the DNA damage. Male Sprague Dawley rats were placed in a plastic restraining holder. Tails of the male Sprague Dawley rats were washed with water (20–30 °C) to dilate the blood vessels. The tail was wiped with ethanol and cleaned with the gauze. The 22G butterfly needle was inserted into one of the lateral tail veins at a position approximately 2–3 cm away from the tip of the tail at an angle of approximately 20°. About 1.2 ml of blood was drawn from the tail. Collected blood was added into the labelled gel tubes.

While at the end of the experiment the blood collection from Group 2 and 3 male Sprague Dawley

rats was retrieved through intra-cardiac puncture. Male Sprague Dawley rats of Group 2 and Group 3 were placed in the jar containing cotton, soaked in chloroform. The rats were kept in the jar, until their breathing movements ceased. Male Sprague Dawley rats were sacrificed once their breathing movements were stopped. Sacrificed male Sprague Dawley rats were placed ventrally on their back on dissection board. After palpation of lower rib cage and sternal margin, a 23 G needle was inserted into the heart and 3 mL of blood was drawn by using 3 mL disposable syringe.

Right testis of Group 1, Group 2 and Group 3 of anesthetized male Sprague Dawley rats, were obtained after dissection. Peritoneal cavity was opened through a midline incision along abdominal wall and right testis was dissected out and freed from adherent tissues. Right testis was fixed in formalin saline solution for 24 hours. Ascending grades of ethanol were used to dehydrate the tissue. Tissue was cleared in xylene and embedded in paraffin for 2 hours. Then next day testicular tissue was sectioned by using microtome (thickness of 5 µm). Testicular tissue sections were deparaffinised by xylene, hydrated through an ethanol series of 100%, 90%, 80%, 70%, and 50%. Staining of slides was done using haematoxylin-eosin (HE) stain for the determination of Testicular histology (spermatogenetic cells, size of seminiferous tubules and lumen of seminiferous tubules).

Statistical analysis of the data was done using Statistical package for Social Sciences version 23 (SPSS 23). Results were documented as Mean±SEM. A comparison between the groups was done by using independent sample *t*-test, and $p<0.05$ was regarded as significant.

RESULTS

Comparison of Mean±SEM of serum 8-hydroxy-2'-deoxyguanosine (8-OHdG) levels of Group 1, Group 2 and Group 3 male Sprague Dawley rats is shown in Figure-1. Serum 8-hydroxy-2'-deoxyguanosine (8-OHdG) in Group 2 rats (5.23 ± 2.62 ng/ml) was significantly increased ($p<0.05$) as compared to serum 8-hydroxy-2'-deoxyguanosine (8-OHdG) levels of Group 1 rats (2.19 ± 1.84 ng/ml). There was no significant difference in serum 8-hydroxy-2'-deoxyguanosine (8-OHdG) levels between Group 1 and Group 3. While serum 8-hydroxy-2'-deoxyguanosine (8-OHdG) levels of Group 3 rats (3.42 ± 2.56) was significantly decreased ($p<0.05$) as compared to serum 8-hydroxy-2'-deoxyguanosine (8-OHdG) of Group 2 rats (5.23 ± 2.62 ng/ml).

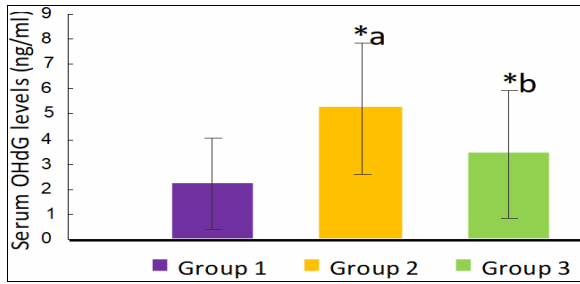


Figure-1: Comparison of Mean±SEM of serum 8-hydroxy-2'-deoxyguanosine (8-OHdG) (ng/mL) levels in Group 1, Group 2 and Group 3.

Under light microscope (40× and 100× Magnification), seminiferous tubules of testicular tissue were observed in all three groups. Group 1 (Control group) Sprague Dawley rats showed normal shaped seminiferous tubules, 7 to 9 layers of

spermatogenic cells were present and lumen of seminiferous tubules was filled with large number of spermatozoa. There was no atrophy of seminiferous tubules and no widening of lumen of seminiferous tubule. While testicular tissue histology of Group 2 male Sprague Dawley rats who were fed with Soy protein and Silicon dioxide mixed with standard laboratory feed showed atrophic seminiferous tubules with 2 to 3 layers of spermatogenic cells and widening of seminiferous tube lumen. However, administration of Zinc along with Soy protein and Silicon dioxide in Group 3 male Sprague Dawley rats showed normal seminiferous tubules with increased number of mature spermatozoa in the lumen and presence of 6 to 8 layers of spermatogenic cells.

The histological changes in testicular segment of all three groups (Group 1, Group 2 and Group 3) are shown in Figure 2, 3, and 4.

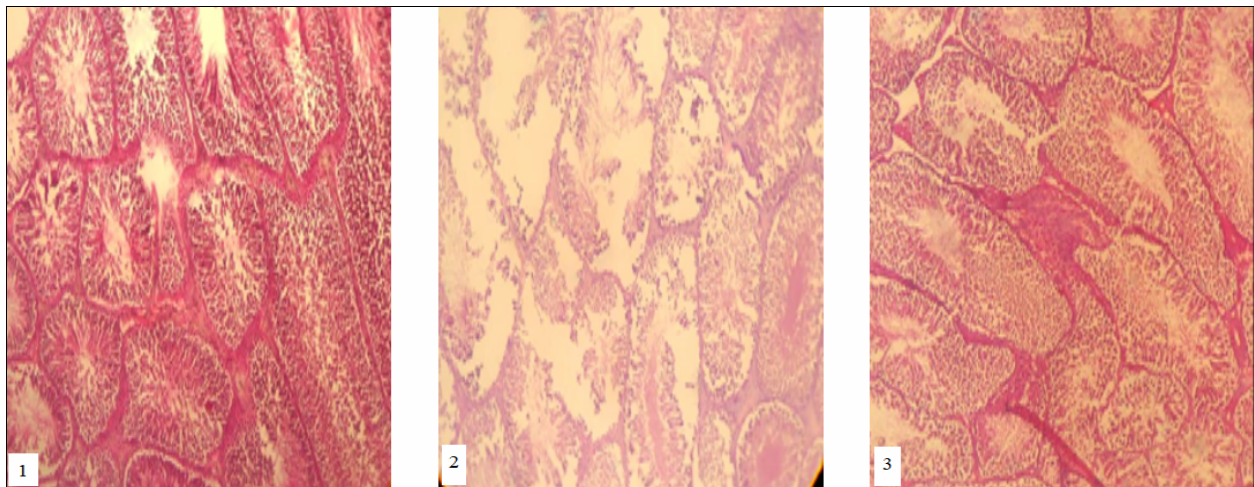


Figure-2: Histological changes in the testicular tissue of Group 1, Group 2, and Group 3. (H&E, ×40)

1. Testicular segment of Group 1 showing normal seminiferous tubules. 2. Testicular section of Group 2 showing atrophic seminiferous tubules. 3. Testicular section of Group 3 showing normal seminiferous tubular.

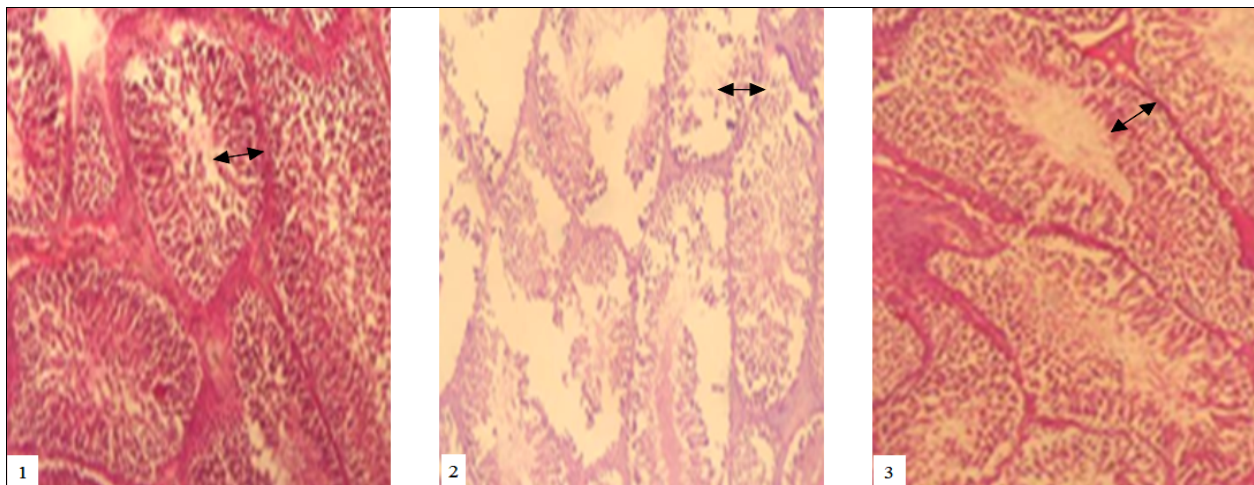


Figure-3: Histological changes in the testicular tissue of Group 1, Group 2, and Group 3. (H&E ×40)

1. Testicular section of Group 1 showing 7 to 9 layers of spermatogenic cells. Testicular section of Group 2 showing 2 to 3 layers of spermatogenic cells. Testicular section of Group 3 showing 5 to 8 layers of spermatogenic cells.

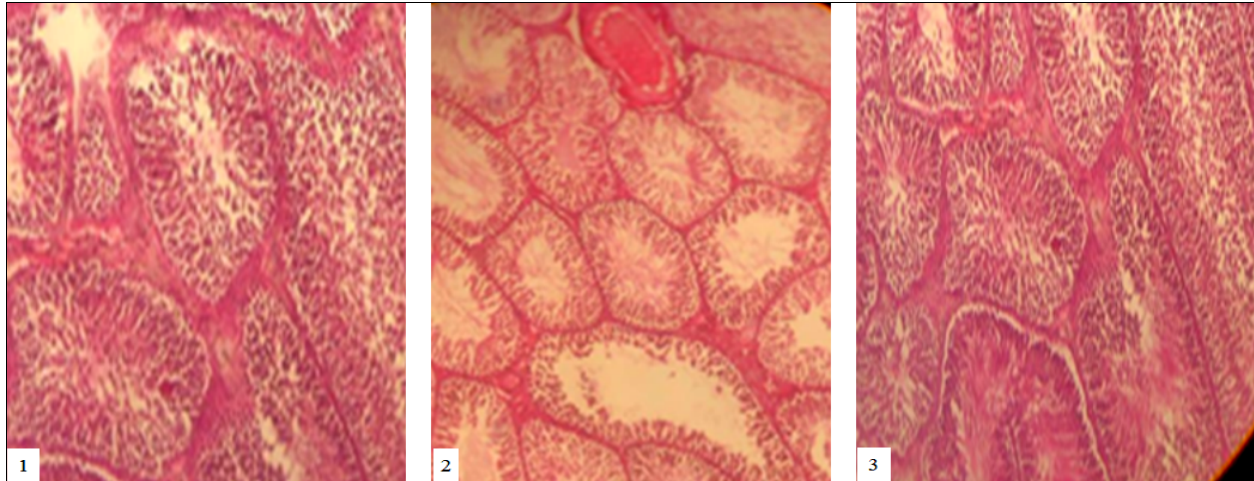


Figure-4: Histological changes in the testicular tissue of Group 1, Group 2, and Group 3. (H&E ×40)

1. Testicular segment of Group 1 showing spermatozoa in the lumen of seminiferous tubules. 2. Testicular section of Group 2 showing decrease number of spermatozoa and widening of lumen of seminiferous tubules. 3. Testicular section of Group 3 showing increase in number of spermatozoa in lumen of seminiferous tubules.

DISCUSSION

Protein is one of the most popular dietary supplements which have gained much popularity amongst athletes and people who do heavy exercise to increase muscle mass and strength. Studies have proved that some important components of protein supplements like Soy protein and Silicon dioxide are detrimental to male reproductive system.^{6,16} The damage induced by these components to the Male Reproductive system may lead to infertility in males. This study explored the harmful effects of Soy protein and Silicon dioxide on male reproductive system and the role of Zinc in restoring fertility.

Current study explored the important biomarker of oxidative stress 8-hydroxy-2'-deoxyguanosine 8-(OHdG). Level of 8-hydroxy-2'-deoxyguanosine (8-OHdG) was raised in male Sprague Dawley rats who were fed with soy protein and silicon dioxide indicating DNA damage. These results are similar to Guo *et al*¹¹, and Pearce *et al*¹⁷, who revealed that oxidative stress increases the level of 8-hydroxy-2'-deoxyguanosine (8-OHdG). Levels of 8-hydroxy-2'-deoxyguanosine (8-OHdG) were remarkably decreased in male Sprague Dawley rats who were fed with Zinc, indicating reversal of DNA damage, these findings are similar to Lee Sr¹³, Jarosz *et al*¹⁸, and Kefer *et al*¹⁹.

Observation of testicular tissue histology after HE staining was included in the present study, which demonstrated that Soy protein and Silicon dioxide consumption resulted in atrophy of seminiferous tubules, decrease in number of spermatogenic cell layers in male Sprague Dawley rats. Similarly, Nurdiana *et al*, showed that administration of soybean extract in male rats resulted in significant damage to the testicular histology.²⁰ These results are different from Khalida *et al*²¹, who concluded that there is no damage to testicular histology of rabbits who consumed body building

proteins containing Soy protein. Our study showed that consumption of Zinc improves the histopathological changes in testicular tissue of male Sprague Dawley rats fed with Soy protein and silicon dioxide, Zinc restores the oxidative damage to testicular tissue caused by the production of reactive oxygen species. These results are similar to Fallah *et al*, research outcomes that supported the fact that Zinc have the antioxidant capacity and have the positive influence on the structure and function of male reproductive system.¹²

CONCLUSION

Outcomes of the present research showed that Zinc has the curative and antioxidant properties in response to soy protein and silicon dioxide-induced testicular tissue damage and oxidative stress. Soy protein and silicon dioxide induce damage in the male reproductive parameters. Zinc has the potential to restore normal functions of reproductive system by reducing levels of 8-hydroxy-2'-deoxyguanosine 8-(OHdG) and by improving Histological features of testes.

RECOMMENDATIONS

Although we demonstrated that Soy protein and silicon dioxide induce detrimental effects on testes and increases serum levels and 8-hydroxy-2'-deoxyguanosine 8-(OHdG). The effects of soy protein on fertilization ability should be checked by mating the male rats in the three groups with normal female rats. Sample size needs to be increased. Additional biochemical and molecular studies are needed to clarify the effects of Protein supplements and antioxidants on the male reproductive system. Furthermore, these therapeutic effects of Zinc on male reproductive system and DNA needs to be clarify via future studies on humans.

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Address for correspondence:

Dr. Saira Safdar, Senior Lecturer, Department of Physiology, Islamabad Medical & Dental College, Islamabad, Pakistan. **Cell:** +92-336-7535388

Email: sairasafdar10@gmail.com

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Contribution of Authors:

SS: Concept, study design, manuscript writing

SA: Final review and approval

HF: Final review and approval

HM: Data collection and analysis

SM: Data collection and analysis

FA: Data collection and analysis

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ORIGINAL ARTICLE

COMBINED EFFECTS OF AGING AND OBESITY ON SERUM TESTOSTERONE LEVELS OF OTHERWISE HEALTHY MALES OF SOUTH PUNJAB

Hamid Hassan, Muhammad Abdullah*, Muhammad Ahmad*, Muhammad Muzammil*, Saadat Ali Khan**, Zunairah Humayun**, Mariyam Khan, Mehreen Zaidi

Department of Physiology, *2nd Year Student, Nishtar Medical University, Multan,

**Department of Physiology, Multan Medical and Dental College, Multan, Pakistan

Background: Aging men experience gradual decline in serum testosterone levels (andropause) which is accentuated if aging is coupled with obesity. This study aimed to note the age and obesity related testosterone decline in men. **Methods:** It was a cross-sectional study comprising of 80 healthy male subjects categorized into younger (20–40 years) and elder (41–60 years) groups which were equally divided into non-obese and obese subgroups. Serum testosterone levels were measured using ELISA. **Results:** Serum testosterone levels (ng/dl) of young non-obese subjects (Group A) were significantly higher [680 (575.0–778.5)] as compared to their elder (Group B) [286.0 (263.5–370.0)] counterparts ($p=0.000$), and so was true for comparison between (Group C) younger [412.5 (338.0–542.5)] and (Group D) elder obese subjects [258.0 (220.0–287.5)] ($p=0.000$). Serum testosterone levels of obese elder subjects (group D), though lower than their age and ethnicity matched non-obese (Group B) counterparts, were not statistically significant ($p=0.114$). Moreover, serum testosterone levels of non-obese (Group A+B) subjects were negatively correlated to Waist Circumference (WC) and Waist Hip Ratio (WHR) [(rho= -0.374, $p=0.018$) and (rho= -0.355, $p=0.025$) respectively] while within obese subjects (Group C+D) serum testosterone levels were negatively correlated to waist circumference only [(rho= -0.643, $p=0.000$)]. **Conclusion:** Circumferential obesity coupled with aging results in a steeper decline in serum testosterone levels which can put obese aging men at high risk of systemic disorders.

Keywords: Obesity, testosterone, aging men, Waist Hip Ratio, WHR, Waist Circumference, WC, Body Mass Index, BMI

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INTRODUCTION

Testosterone, the key androgen, is essential for the endo-metabolic, immunological and psychological well being of men and is of absolute importance for maintenance of their muscle mass and bone composition.¹ Its levels tend to decrease in men, from fourth decade onwards, a phenomenon currently termed as andropause.²

Aging, in men is associated with elevated levels of cytokines, such as Interleuin-1 (IL-1) and Tumour Necrosis Factor- α (TNF α), which cause insulin resistance within hypothalamus and pituitary. This reduces the release of gonadotropin releasing hormone (GnRH) which in turn decreases testosterone levels in aging men.³ Declining testosterone levels result in a reduced fat free mass in elderly men due to reduced stimulation of the cells of myocytic lineage, which leads to high estrogen levels (via conversion of testosterone to estrogen) that further affect gonadotropin secretion and hence testosterone levels negatively.⁴ An enhanced fat mass, due to reduction in viable muscle mass, also increases the levels of adipocytines (such as leptin) which also suppress gonadotropin release and thus further reduce testosterone levels. Moreover, sex hormone binding globulin (SHBG) levels tend to rise

within aging men too which also affects bio-available testosterone levels in a negative fashion.⁵

Obesity is being recognized as the epidemic of 21st century ravaging the societies across globe. It's prevalence amongst various South Asian populations varies. The prevalence of obesity within Pakistani population was around 25% as per statistics of national health survey of Pakistan (1990–94). Certain recent studies are putting its prevalence within Pakistani population to up to 57%.^{6,7} Since men do harbor circumferential obesity specifically, rather than general adiposity, hence WC and/or WHR are better indicators of adiposity/obesity in men as compared to the general parameter of Body Mass Index (BMI). According to World Health Organization (WHO), South Asian men with a BMI of ≥ 25 and/or WHR of ≥ 0.9 are termed as obese.⁸

The dysregulated metabolism within obese people leads to dysregulation of endocrine function (insulin resistance as well as deranged cholesterol/HDL ratio)⁹, immune environment (dysregulation of cytokines¹⁰, and adipocytines like adiponectin and omentin¹¹) along with that of anti-inflammatory substances (Vit D)¹² which create a fertile ground for emergence of metabolic syndromes and multi system failures.

This study on otherwise healthy men of South Punjab was aimed to note the age and obesity related testosterone decline in men so that the clinicians could be provided with scientific data that can help them monitor the systemic environment of aging and obese men in a better way since due to the loss of beneficial effects of testosterone on their internal harmony, they are likely to be at an increased risk of development of a myriad of systemic disorders.

SUBJECTS AND METHODS

It was a cross-sectional comparative study conducted on healthy adult male population of South Punjab. The sample size for each group was calculated with a power ($1-\beta$) of 90% and a significance (α) level of 5% through WHO (Geneva) extended software, 'Sample size determination in health studies: a Practical Manual' version 2.0. Study population consisted of 80 healthy subjects which were equally divided into younger (20–40 years) and elder (41–60 years) groups, each of which was further subdivided into non-obese and obese subgroups. Since testosterone levels tend to decline from fourth decade of life onwards hence the 40th year of life¹³ of study subjects was considered as the cutoff beyond which the subjects were termed as elder. Thus, Group A and Group B consisted of 20 younger non-obese and 20 elder non-obese subjects respectively while Group C and Group D had 20 younger obese and 20 elder obese subjects respectively.

According to WHO (2000 and 2008) guidelines South Asians with a BMI of ≥ 25 and/or a WHR of >0.9 are termed as obese, hence these were the cutoffs adopted for our study. The BMI and WHR of the subjects were measured as per criteria set by WHO. Three ml of venous blood of subjects was drawn in early hours of the morning before which subjects were advised to have an overnight fast of 10 hours. The collected blood samples were immediately centrifuged at a speed of 3,000 rpm for three minutes after which the drawn serum samples were immediately stored at $-20\text{ }^{\circ}\text{C}$ for a later analysis.

Serum testosterone levels were calculated through competitive solid phase enzyme linked radioimmunosorbent assay (ELISA) by using ASTRA BIOTECH Testosterone ELISA Kit Ref: 21-02A (German Make). This assay had an Assay Range of 0.2–50 nmol/L (6–1154 ng/dl), an Assay Sensitivity of 0.2 nmol/L (6 ng/dl), an Assay specificity of 100% for human serum testosterone, an Intra-assay precision of 3.77% and an Inter-assay precision of 7.39%.

Genetically and morbidly obese men (BMI of ≥ 30 as per WHO 2000 guidelines) along with those taking exogenous testosterone were excluded from this study. Subjects were screened, twice in the week preceding sample collection, for deranged fasting blood glucose levels. After screening, subjects who had a

fasting blood glucose level of ≥ 126 mg/dl (WHO 2008 guidelines for hyperglycemia), a systolic blood pressure of ≥ 140 and/or diastolic blood pressure of ≥ 90 (WHO 2013 guidelines for hypertension) on first and/or second screening occasion were excluded from study.

The data were entered on SPSS-22. Data were analysed for normality distribution via Shapiro-Wilk's and Kolmogorov Smirnov's tests and Mean \pm SD of normally distributed, while Median (IQR) of non-normally distributed variables were calculated. Mann-Whitney-U test was applied to compare [Median (IQR)] of serum testosterone levels between various groups. Spearman's rho correlation was applied to determine correlation between various quantitative variables, and $p \leq 0.05$ was considered to be statistically significant.

RESULTS

The Mean \pm SD of normally distributed and Median (IQR) of non-normally distributed parameters of study subjects (of all four groups) have been represented in Table-1. Comparisons in Table-2 show that serum testosterone levels of younger non-obese subjects (Group A) were significantly higher than those of their elder non-obese counterparts (Group B) and so was true for the comparison between obese younger (Group C) and obese elder (Group D) groups indicating that testosterone levels decline significantly with age both with or without obesity. Table-2 also shows that serum testosterone levels of elder obese subjects of study (Group D) were lower as compared to their age matched non-obese counterparts (Group C), though insignificantly on statistical grounds, indicating that when aging in men is coupled with obesity the testosterone decline in men is rather more prominent.

No significant negative correlation was found between testosterone levels, WHR and WC in individual groups. It was, however, found that serum testosterone levels had a negative correlation with waist circumference in both non-obese (Group A+B) and obese (Group C+D) subjects ($p=0.018$ and $p=0.000$ respectively) when combined together regardless of their age. Serum testosterone levels were negatively correlated to WHR in non-obese subjects ($p=0.025$) but a statistically significant result could not be derived for obese subjects in this instance ($p=0.397$). This indicates that serum testosterone levels have a more significantly negative correlation with WC as compared to WHR. These findings have been represented in Table-3.

A correlation of serum testosterone levels, of whole of the study population, with indicators of obesity (such as WC and WHR) has been extended in Figure-1. However, serum testosterone levels (of whole population) had non-significant negative correlation with BMI ($\rho = -0.200$, $p = 0.076$).

Table-1: Median (IQR) and (Mean±SD) of anthropometric parameters of the study population

Parameter	Group A (n=20)	Group B (n=20)	Group C (n=20)	Group D (n=20)
Age (Year)* [Median (IQR)]	25.0 (22.0–25.5)	45.0 (42.0–51.2)	28.5 (25.0–31.0)	49.5 (45.0–50.0)
Height (m)* [Median (IQR)]	1.74 (1.55–1.74)	1.77 (1.74–1.80)	1.70 (1.68–1.74)	1.74 (1.71–1.80)
Waist Circumference (Cm)* [Median (IQR)]	81.28 (76.20–83.82)	83.82 (83.82–86.99)	91.44 (86.36–93.98)	96.52 (93.98–96.52)
Hip Circumference (Cm)* [Median (IQR)]	97.79 (91.44–101.60)	99.06 (96.52–101.60)	99.06 (93.98–101.60)	104.14 (101.6–104.14)
Waist Hip Ratio* [Median (IQR)]	0.83 (0.81–0.85)	0.85 (0.84–0.87)	0.92 (0.92–0.92)	0.92 (0.92–0.93)
Weight (Kg)** (Mean±SD)	66.08±7.56	73.35±3.0	79.91±7.47	84.25±5.25
Body Mass Index** (Mean±SD)	23.24±1.87	23.5±0.93	27.07±1.65	27.46±1.4

*Non normally and **Normally distributed anthropometric parameters of study population

Table-2: Comparison of serum testosterone levels within study groups

Groups in Comparison (n=20)		p
Group A 680 (575.0–778.5)	Group B 286 (263.5–370.0)	0.000*
Group C 412.5 (338.0–542.5)	Group D 258 (220.0–287.5)	0.000*
Group A 680 (575–778.5)	Group C 412.5 (338–542.5)	0.003*
Group B 286 (263.5–370.0)	Group D 258 (220.0–287.5)	0.114

The comparison has been drawn via Mann-Whitney U test.

*Significant

Table-3: Correlations of serum testosterone levels with WC and WHR derived via Spearman's Correlation

Variable	A+B (Non-obese) n=40		C+D (Obese) n=40	
	rho	p	rho	p
WC	-0.374	0.018*	-0.643	0.000*
WHR	-0.355	0.025*	0.138	0.397

*Statistically significant

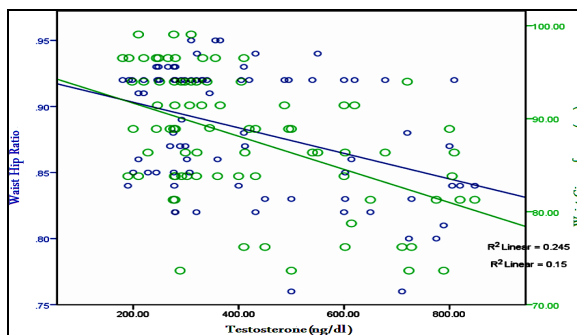


Figure-1: Correlations between testosterone and anthropometric parameters of obesity derived via Spearman's correlation in total study population

DISCUSSION

Testosterone concentrations of the subjects of groups A, B, C and D were compared and it was found that testosterone levels of the young non-obese subjects in group A were the highest, while those of elder obese subjects, in group D were the lowest. Testosterone

levels of younger subjects of group A and C were higher than their elder counterparts in groups B and D regardless of their obesity status indicating an age related decline of testosterone levels in men.

Such findings have also been reported in contemporary literature where it is reported that testosterone levels decline at a rate of up to 2% per year, after third decade of life, because of several changes within the hypothalamo-pituitary axis of aging men. These changes emerge in the background of an age associated testicular impairment which begins after late 30s in men.¹⁴

Testosterone levels of the non-obese subjects of groups A and B were found to be higher than their obese counterparts in groups C and D, regardless of their age, as is projected by other studies too suggesting that testosterone levels decline in circumferentially obese men.¹⁵

It has been proposed that aromatization of testosterone within an enhanced adipose tissue is responsible for testosterone decline in obese men.¹⁶ Obesity related enhancement of adipose tissue is also associated with hyperinsulinemia (as a result of development of insulin resistance) which suppresses the secretion of LH, thus resulting in low testosterone levels.¹⁷ Obesity in men is associated with low levels of SHBG, which also account for low testosterone levels in them.¹⁸

Testosterone levels of non-obese elder subjects of group C, though lower, were not significantly different from their age matched obese counterparts in group D. This is in contrast with the results of other studies which show that testosterone levels decline with obesity.¹⁹

It has been suggested that a single time sampling may reveal inconsistent results regarding testosterone levels as compared to those deduced by serial samplings.²⁰ Since our study was a single time cross-sectional study, this could have led to our results being inconsistent with those being projected by

available literature. This contradiction might have appeared as a result of small sample size too. This supposition is supported by the fact that non-obese subjects of groups A and B combined together, regardless of their age, had higher testosterone levels as compared to their obese counterparts in groups C and D.

Serum testosterone levels of non-obese subjects of groups A and B, regardless of their age showed an inverse correlation with WHR. Testosterone levels in whole of study population were inversely related to WHR. These findings are comparable to studies which suggest that testosterone levels are negatively correlated to WHR.²¹ Increase in WHR is associated with deranged insulin levels and insulin resistance which affects the negative feedback control over hypothalamo-pituitary-adrenal axis²² and results in decreased testosterone levels by disrupting one or more of the control mechanisms involved in its synthesis²³.

Testosterone levels were correlated with WC and had a more significantly negative relationship with WC as compared to WHR (in groups A+B, groups C+D, and in total study population). This finding is in accordance with another study, recently conducted in Pakistan²⁴, which suggests that serum testosterone levels show strong negative correlation with WC as compared to WHR. It is also supported by another research which states that WC is a much better indicator of predicting testosterone decline in men as compared to WHR and BMI.²⁵

Testosterone levels did not have a significant correlation with BMI (neither within groups nor in whole of study population put together). Though a negative correlation of testosterone with BMI has been reported in literature²⁴, it is suggested that only extreme changes in BMI, especially if it crosses the limit of 40, can result in significant decline in testosterone levels. This decline may be attributed to raised leptin levels which suppress testosterone secretion.²⁶ This contradictory result of ours could also be supported by an earlier work where it was suggested that testosterone levels had an inverse relation with BMI in men (if their BMI was ≥ 35) because low LH and SHBG at a BMI of ≥ 35 lead to low testosterone levels.²⁷ Since BMI of our subjects was < 30 hence it's most likely to have led to this finding that stands against popular belief.

CONCLUSION

This study in healthy males of South Punjab revealed that serum testosterone levels decline with enhancing age and adiposity and that this decline is sharpest with the increase of waist circumference in aging men. This extends valuable information to clinicians who, while treating aging men with increased WC, should consider the lack of testosterone's beneficial effects on systemic environment of their patients and consider testosterone replacement therapy for possibly better results.

LIMITATIONS & RECOMMENDATIONS

This was a cross-sectional study with a limited sample size. Further cohort studies with enhanced sample size are recommended.

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Address for Correspondence:

Dr. Hamid Hassan, Associate Professor & Head, Department of Physiology, Nishtar Medical University, Multan, Pakistan. **Cell:** +92-333-6107738

Residence: 311, Shamsabad Colony Multan

Email: ssaaqii@gmail.com

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Contribution of Authors:

HH: Concept, Commencement, Field Work, Statistical Analysis, Referencing

MA: Scripting, Referencing

HBS: Scripting, Referencing

MA: Scripting Referencing

SAK: Final approval

ZH: Fieldwork

MK: Fieldwork

MZ: Fieldwork

ORIGINAL ARTICLE

SELF-REPORTED EXPOSURE TO SECOND-HAND TOBACCO SMOKE AND LUNG FUNCTION IN UNDERGRADUATE MALE MEDICAL STUDENTS IN PAKISTAN

Huma Saeed Khan, Naveen Siddique Sheikh, Maria Muhammad Ayub*, Mehak Tariq, Sidra Zahid, Farida Hafeez

Department of Physiology, CMH Lahore Medical College and Institute of Dentistry,

*Department of Counselling and Psychological Services, Lahore University of Management Sciences, Lahore, Pakistan

Background: Passive smoking, where an individual inhales tobacco smoke, has been associated with many health issues from asthma to cancer and is attributed to affecting pulmonary function tests like the peak expiratory flow rate of individuals. Keeping this in mind, the study was conducted to compare the peak expiratory flow rate of passive smokers with that of non-smokers. **Methods:** A cross-sectional comparative study was conducted from 2017 to 2019 in which 184 male undergraduate medical students enrolled in the study at the time of admission for each successive year after informed consent. Participants were recruited by non probability consecutive sampling technique and divided into two groups based on status of passive smoking, passive smokers (n=97) and non-smokers (n=87). Their height, weight, waist to hip ratio, and peak expiratory flow rate were recorded. The recorded data was analysed on SPSS-26. **Results:** The current study showed that 52.7% of the study participants were passive smokers and 47.3% were non-smokers. A significant difference ($p<0.01$) of peak expiratory flow rate was seen between non-smokers and passive smokers on the Mann-Whitney U Test. As observed by mean ranks, nonsmokers had a higher peak expiratory flow rate (109.86 L/min) than passive smokers (76.93 L/min) ($p=0.01$). Spearman's Rank Correlation Coefficient revealed a positive significant relationship between peak expiratory flow rate and height in passive smokers ($\rho=0.21$, $p=0.04$). **Conclusion:** The peak expiratory flow rate of passive smokers is less than that of non-smokers and there is a positive significant relationship between height and peak expiratory flow rate.

Keywords: environmental tobacco smoke, passive smoking, peak expiratory flow rate, lung function test, second-hand smoke

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INTRODUCTION

Passive smoking has been attributed to 1% mortality occurring worldwide.¹ It is a modifiable risk factor that is associated with a wide variety of preventable diseases.² Passive smoking, i.e., environmental tobacco smoke (ETS) or second-hand smoke (SHS), occurs involuntarily where an individual inhales the mainstream smoke that is expired by the smoker or the sidestream smoke that is diluted in the ambient air, released from burning tobacco.³ In 2015, 4% of deaths resulting from chronic obstructive pulmonary disease (COPD) were attributed to SHS while 3% of deaths due to stroke were linked to SHS.⁴

Passive smoking not only causes adverse effects to an individual's health but also negatively impacts the economy in terms of production losses due to unavailable workforce and healthcare expenditures, these amounted to US\$ 1436 billion in 2012.⁵ In 2018, the economic burden of smoking related illnesses has been attributed to 192 billion rupees (equal to 1.3 billion dollars) in Pakistan.⁶

Second-hand smoke contains around 4,000–4,700 chemicals comprising of hazardous amines, nicotine, hydrocarbons, noxious particles, and metals among others⁷, many of which are reported to

be carcinogenic. These chemicals are irritants that cause stimulation of submucosal irritant receptors which consequently lead to an increment in airway resistance through vagally mediated smooth muscle constriction, impair ciliary movement, thereby decreasing mucociliary clearance, inhibit the function of alveolar macrophages⁸, and cause mucus-secreting glands to undergo hyperplasia and hypertrophy.⁹ They cause proteolytic enzymes to be released from polymorphonuclear leucocytes due to antiprotease inhibition thereby playing a significant role in lung parenchymal destruction and COPD pathogenesis.¹⁰

Passive smoking has even been suggested by a few studies to increase the risk of non-smokers developing heart disease by 25–30%.¹¹ Sudden infant death syndrome in children and infants, certain cancers, respiratory illnesses such as COPD, and cardiovascular diseases such as coronary artery diseases in adults are linked to passive smoking.¹²

Passive smoking induces pulmonary damage slowly, but unfortunately it may not show symptoms until pulmonary functions are compromised. In Pakistan, the prevalence of passive smokers has been estimated to be 69.1% as compared to 63% in Bangladesh.¹³

Peak Expiratory Flow Rate (PEFR) is defined as the maximum flow rate in forced expiration beginning from full inspiration, measured in liters per minute, taking place within the initial 200 ms of expiration.¹⁴ PEFR varies with sex, age, and anthropometric indices such as height, weight, waist and hip circumference, waist/hip ratio, and body mass index (BMI). Race, ethnicity, and the individual's lifestyle such as smoking also influence PEFR.¹⁵

PEFR is useful to assess and monitor respiratory conditions such as restrictive and obstructive lung diseases.¹⁶ It is an easy and non-invasive test that gives a rough estimate of the degree of disease extent. PEFR can be used to monitor lung capacity in the early stages of smoke exposure and hence can be used as a reliable tool to prevent lung damage in smokers.¹⁷

This study was designed with an objective to compare the PEFR in healthy male individuals who were exposed to passive smoke with that of non-smokers.

SUBJECTS AND METHODS

This cross-sectional comparative study was conducted at CMH Lahore Medical College and Institute of Dentistry from 2017 to 2019 to evaluate and compare the PEFR of passive smokers with nonsmokers. The Ethical Review Board's approval was obtained (case# 433/ERC/CMHLMC).

All male students inducted into 1st Year MBBS for three consecutive years (2017, 2018, and 2019) were requested to participate in the study voluntarily at the time of admission in MBBS. A non-probability consecutive sampling technique was used. Sample size of 184 was calculated using the Raosoft sample size calculator, keeping 5% margin of error, 95% confidence interval and 350 as the estimated population size with 50% response distribution.

Participants were interviewed after their informed consent. Boys who had a history of respiratory illness or who had recently suffered a respiratory infection were excluded from the study. Smokers were also excluded from the study. The inducted boys were then divided into two groups: Passive smokers (n=97) and nonsmokers (n=87). Self-reported exposure to passive smoking was defined as being in the same room as a smoker for a minimum of one hour per day for 12 consecutive months or more.¹⁸

Height was recorded to the nearest centimeter (Cm) with the subject standing, and head in the Frankfurt imaginary plane. Weight was recorded in kilograms (Kg) in usual light clothing. Body mass index (BMI) was calculated using the

formula $\text{weight in Kg}/(\text{height in meters})^2$. Waist circumference and hip circumference were measured to the nearest centimeter and waist to hip ratio was calculated. Boys who were obese based upon BMI were excluded from the study.

PEFR was measured using the Wright's peak flow meter by adhering to standard guidelines.¹⁹ Each subject was first explained the technique of performing the lung function test and then was demonstrated as how to perform PEFR before the actual measurement was recorded. Each subject was asked to perform the test thrice in the standing position and the highest value was taken as final.

Data was recorded on a predesigned proforma, and analysed using SPSS-20. Data was explored for normality by using the Shapiro Wilk Test, and accordingly non parametric tests were chosen for inferential statistics. Mann-Whitney U Test and Spearman's Rank Correlation Coefficient were applied to the quantitative parameters of the data, and $p < 0.05$ was considered to be statistically significant.

RESULTS

A total of 184 male students participated in the study. The mean age of the study participants was 18.65 ± 0.88 years. Among the study participants, 52.7% were passive smokers and 47.3% were non-smokers. The median peak expiratory flow rate was 450 (IQR 370–550) L/min. The median height of the participants was 173 (IQR 169–177) Cm and weight was 71 (IQR 62–81) Kg. Median BMI was 23.99 (IQR 20.79–36.83). Participants had a median waist and hip circumference of 86 (IQR 81–91) Cm and 96 (IQR 92–104) Cm respectively, and the median waist to hip ratio of the sample was found to be 0.89 (IQR 0.85–0.91). The results of the anthropometric values and PEFR of all study participants are summarized in Table-1.

When compared by the Mann-Whitney U Test, it was noted that the difference in PEFR between non-smokers and passive smokers is significant ($p < 0.01$). Participants who were nonsmokers had a higher peak expiratory flow rate than passive smokers as suggested by their mean ranks. Non-significant differences were observed on other anthropometric parameters between the two groups (Table-2).

Spearman's Rank Correlation Coefficient was applied to the quantitative parameters of the data. Table-3 shows the result of Spearman's Rank Correlation Coefficient where a positive significant relationship was observed between peak expiratory flow rate and height of the subjects.

Table-1: Anthropometric variables and peak expiratory flow rate (PEFR) reported with their median value, lower quartile (LQ), and upper quartile (UQ) for all study participants (n=184)

Variables	Median	LQ	UQ
PEFR (L/min)	450	370	550
Age (Years)	19	18	19
Height (Cm)	173	169	177
Weight (Kg)	71	62	81
BMI (Kg/Cm ²)	23.99	20.79	36.83
Waist Circumference (Cm)	86	81	91
Hip Circumference (Cm)	96	92	104
Waist to Hip Ratio	0.89	0.85	0.91

Table-2: Mann-Whitney U Test for PEFR with anthropometric parameters between non-smokers and passive smokers (n=184) (Mean Ranks)

Variables	Non Smokers (n=87)	Passive Smokers (n=97)	p
PEFR (L/min)	109.86	76.93	0.01*
Age (Years)	95.65	89.68	0.42
Height (Cm)	84.18	99.96	0.05
Weight (Kg)	88.46	96.12	0.33
BMI (Kg/Cm ²)	90.37	94.41	0.61
Waist Circumference (Cm)	89.16	95.49	0.42
Hip Circumference (Cm)	88.45	96.13	0.33
Waist to Hip Ratio	96.00	89.36	0.40

*Significant

Table-3: Spearman's rank correlation coefficient between PEFR with anthropometric parameters for non smokers and passive smokers

Variables	Age		Height		Weight		WC		HC		WHR		BMI	
	rho	p	rho	p	rho	p	rho	p	rho	p	rho	p	rho	p
Non Smokers	0.09	0.42	0.20	0.06	0.12	0.27	0.00	0.99	0.04	0.72	-0.12	0.27	0.03	0.81
Passive Smokers	0.17	0.10	0.21	0.04*	0.10	0.35	0.19	0.06	0.10	0.31	0.19	0.06	0.05	0.65

*Significant

DISCUSSION

Passive smoking is attributed to cause a decrease in pulmonary functions, as shown by previous researches. In our study, there was a significant difference between the PEFR of passive smokers and non-smokers inducted in the first-year MBBS. The results of our study are in line with the findings reported by an Iraqi researcher whose study demonstrated a statistically significant difference in PEFR between young adult passive smokers and nonsmokers.²⁰ Similar findings have been reported from a study previously done in Lahore which evaluated differences in PEFR between adult passive smokers and nonsmokers to be statistically significant, with nonsmokers having a lower PEFR than their passive smoking counterparts.²¹

The key variables that affect PEFR are the strength of respiratory muscles producing a contraction that results in expiration, lung compliance, airway competency, and resistance offered to airflow.²² Gender, age, height, weight, and body surface area have shown a significant correlation for PEFR in previous studies.²³ The results of our study are also in line with the findings reported by Indonesian researchers, however, their study population comprised of a younger age group between the ages of 10–13 years.²² This shows that the effect of passive smoking is the same on lung function regardless of the age group affected.

Considering gender has been reported to affect PEFR, we conducted the study in only males to rule out gender differences and evaluate the correlates within the same gender for various variables. Our study highlighted a significant positive correlation between height and PEFR, where taller male subjects had a higher PEFR value, a finding which is supported by previously conducted studies.²⁴ With a height

increment, there is an increment in the chest girth, and the thoracic area hence total surface area of the lungs increases. Because taller people have a larger surface area for air exchange than shorter people, a greater amount of air can circulate in and out, resulting in an increase in vital capacity and consequently PEFR in taller people. This is probably because taller subjects have a greater chest volume and with an increment in height the effort by the expiratory muscles and the growth of the airway passages also increases thus increasing PEFR with height as evidenced in previous literature.²⁵ Previous studies have reported that in males, not only does an increase in height causes an increase in PEFR but a weight increment also causes PEFR to increase, this however, does not support our research findings.²⁶ A study done in Nigeria reported a significant correlation between height and PEFR of men subjected to passive smoking, this finding resonates with the findings of our study which showed similar correlation between the two indices, however the study also showed a correlation between PEFR and other anthropometric indices such as weight, which does not support our study findings of nonsignificance between weight and PEFR.²⁷

The current study has a few limitations. The role of other attributes like socioeconomic status, malnutrition, years of exposure to second-hand smoke, and childhood smoke exposure history in non-smokers was not explored. A larger sample size taken at random and evaluating other lung function tests such as FVC and FEV₁ could help improve future researches. The active metabolite of nicotine, cotinine may be measured in passive smokers. Furthermore, evaluating PEFR corrected for forced expiratory pressure could shed more light on the disparities in PEFR results between the two groups.

CONCLUSIONS

First-year MBBS male students who are exposed to passive smoking have lesser PEFR values as compared to their non-smoking non-exposed class fellows. This study highlights the need to create awareness about the detrimental effects of passive smoking on an individual's health and the need for regulation of its control to curb the passive smoking issue.

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Address for Correspondence:

Dr Naveen Siddique Sheikh, Department of Physiology, CMH-Lahore Medical College and Institute of Dentistry, Lahore, Pakistan. **Cell:** +92-321-4153436

Email: naveen.s.sheikh@gmail.com

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Contribution of Authors:

HSK: Conceptualisation, visualization, data curation, methodology design, project administration, reviewing and editing draft, final approval

NSS: Data curation, methodology design, original draft writing, reviewing and editing draft, reference cross-checking

MMA: Methodology design, formal statistical analysis of data on SPSS, result write-up for manuscript

MT: Data curation, methodology design, reference cross-checking

SZ: Data curation, methodology design, reference cross-checking

FH: Methodology design, project administration, Final approval

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ORIGINAL ARTICLE

ENDOSCOPIC SPECTRUM OF OESOPHAGEAL FINDINGS PRESENTING WITH DYSPHAGIA

Shabnam Naveed, Ayesha Nageen*, Priyanka Rani, Syed Masroor Ahmed, Zeeshan Ali

Department of Medicine, Jinnah Postgraduate Medical Centre and Jinnah Sindh Medical University, Karachi,

*United Medical and Dental College, Karachi, Pakistan

Background: The causes of dysphagia may represent chronic benign disorders to life threatening oesophageal neoplasia. The first line investigation in evaluation of dysphagia is upper gastrointestinal endoscopy. The objective of the study was to observe the oesophageal endoscopic findings in patients presenting with dysphagia and their correlation with age and gender. **Methods:** This cross-sectional study was conducted in Jinnah Postgraduate Medical Centre, Karachi, from 2017 to 2019. Adult patients who had their chief complaint as dysphagia underwent endoscopy and their findings were noted on a pre-designed proforma. The findings were analysed on SPSS-22. **Results:** Out of 242, patients, males were 102 (42%) and females were 140 (58%). Among them 19 (8%) were in the age group of 18–20 years, 103 (42.5%) between the ages of 21–40 years, 81 (33.5%) in the ages of 41–60 years and 39 (16%) in the age group of 61 years and above. Pharyngeal growth was seen in 27 (11%) patients. The commonest pathology in oesophagus was oesophageal growth (58, 24%). With respect to age group, oesophagitis was more common between the ages 18–20 years while oesophageal growth was more prevalent in all age groups above 18 years. With respect to gender, oesophageal web/stricture was twice more in females while the oesophageal growth frequency was nearly equal in both genders. **Conclusion:** There is high frequency of oesophageal growth among all age groups and is alarming. In patients with dysphagia, endoscopic evaluation for early detection is crucial.

Keywords: Dysphagia, Upper Gastrointestinal Endoscopy, Oesophageal Mass, Oesophagitis

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INTRODUCTION

Dysphagia is a stressful subjective symptom in which there is a delay or difficulty in deglutition. It could be to solid, liquid or both depending upon the pathology. There are various established factors causing dysphagia. The prevalence of dysphagia is estimated to be around 6–9% in all age groups and 16–22% in patients above 50 years of age.¹ Difficulty in swallowing would lead to a poor diet and malnutrition and as a sequel would cause multiple nutritional deficiencies leading to an immunocompromised state. It impacts the quality of life of a person and adds to the morbidity count of the country. Patients reported psychological impact on their lives with panic and anxiety developing into depression.²

Oesophageal dysphagia is usually due to either structural or inflammatory pathologies such as strictures, rings, webs, malignancy or oesophagitis or motility disorders such as achalasia, ineffective oesophageal motility, oesophageal spasm or oesophago-gastric junction outflow obstruction.³

The dilemma of dysphagia is that in spite of being a critical symptom patients often present late for medical advice. The patients with dysphagia of neoplastic origin frequently present with secondary complications. Some would have the presence of comorbidity which would add to the challenge in their management.

Oesophago-gastro-duodenoscopy is the required diagnostic procedure performed in the evaluation of oesophageal dysphagia since there is a direct visualization of upper gastrointestinal tract and the ability for tissue acquisition for biopsy.⁴ By endoscopic biopsy of any growth malignancies could be ruled out.⁵ An early detection of oesophageal/gastric malignancy will definitely escalate the chance of survival by prompt management.²

In general, symptom of dysphagia can be present in various oesophageal disorders. The endoscopic findings in patients with dysphagia are well known but their frequencies are not very well reported in Pakistani patients. Moreover, there is very insufficient data that associate those endoscopic findings with age and gender. The objective of this study was to determine the pattern and frequencies of different endoscopic findings in patients who present with dysphagia. We also aimed to correlate these endoscopic findings with respect to age and gender.

METHODOLOGY

This cross-sectional observational study was conducted in Jinnah Postgraduate Medical Center, Karachi, from 2017 to 2019. The patients were recruited using consecutive non-probability sampling. Patients included were above the age of 18 years who presented with the principal complaint of dysphagia. After taking consent for being part of study, they underwent upper gastrointestinal endoscopy under standard protocol.

Patients younger than 18 years and with previous history of upper gastrointestinal (GI) endoscopy were excluded from the study. The oesophageal endoscopic findings of patients were noted on pre-designed proforma and standard management plan was carried out. Patients with normal oesophagus on endoscopy were advised any further management, and biopsies were taken where needed. Oesophageal endoscopic findings were categorised according to various abnormalities detected. The prevalence of oesophageal endoscopic findings was ascertained overall and also graded by gender and age groups (18–20, 21–40, 41–60, and ≥61 years). The findings were analysed on SPSS-22. Frequency and percentages were calculated for categorical variables like gender and common upper GI endoscopic findings. Chi-square was taken significant at $p < 0.5$ when variables were cross-tabbed including universal variables of age and gender.

RESULTS

The total patients were 242, males were 102 (42.1%) and females were 140 (58%). There were 19 (8%) patients in the age group of 18–20 years, 103 (42.5%) between the ages of 21–40 years, 81 (33.5%) in the ages of 41–60 years, and 39 (16%) in the age group of 61 years and above.

Growth or mass were present in 27 (11%) patients in the pharynx. Those patients had normal oesophagus on endoscopy.

The endoscopic findings of the oesophagus and the endoscopic impression with respect to gender in the oesophagus are presented in Table-1. The oesophageal findings with respect to age groups are tabulated in Table-2.

Table-1: Oesophageal endoscopic findings and the frequency with respect to gender [n (%)]

Category of findings	Male	Female	Total
Normal oesophagus	27 (26)	45 (74)	72 (29.75)
Oesophageal growth	28 (48)	30 (52)	58 (24.0)
Reflux oesophagitis	16 (49)	17 (51)	33 (13.6)
Oesophageal web/benign strictures	10 (33)	20 (66)	30 (12.4)
Oesophageal ulcers	7 (44)	9 (56)	16 (6.6)
Candidiasis	4 (40)	6 (60)	10 (4.1)
Oesophageal varices	3 (60)	2 (40)	5 (2.07)
Hiatus hernia	4 (100)	0	4 (1.7)
Achalasia	0	3 (100)	3 (1.2)
Stricture plus esophagitis	0	1 (100)	1 (0.4)
Web plus growth	0	1 (100)	1 (0.4)
Stricture plus varices	0	1 (100)	1 (0.4)
Ulcers plus esophagitis	0	1 (100)	1 (0.4)
Candidiasis plus stricture	0	1 (100)	1 (0.4)
Candidiasis plus esophagitis	0	1 (100)	1 (0.4)
Candidiasis plus growth	1(33)	2 (66)	3 (1.2)
Candidiasis plus ulcer	2 (100)	0	2 (0.83)
Total	102	140	242

(Percentages calculated by the frequency of that category found in that gender compared to the overall total in that category)

Table-2: Oesophageal findings with respect to age group [n (%)]

Category of finding	Age (Years)				Total
	18–20	21–40	41–60	61–80	
Normal	7 (36)	32 (31)	23 (28)	10 (25.6)	72
Oesophageal growth	1 (5)	26 (25)	21 (26)	10 (25.6)	58
Reflux oesophagitis	6 (31)	12 (11)	10 (12)	5 (12.8)	33
Oesophageal web/stricture	1 (5)	12 (11)	15 (18.5)	2 (5)	30
Oesophageal ulcers	1 (5)	6 (6)	3 (3.7)	6 (15)	16
Candidiasis	1 (5)	5 (5)	3 (3.7)	1 (2.5)	10
Oesophageal varices	0	1 (0.9)	2 (2.4)	2 (5)	5
Hiatus hernia	1 (5)	2 (1.9)	0	1 (2.5)	4
Achalasia	0	2 (1.9)	0	1 (2.5)	3
Stricture plus oesophagitis	0	1 (0.9)	0	0	1
Web plus growth	0	1 (0.9)	0	0	1
Stricture plus varices	1 (5)	0	0	0	1
Ulcers plus oesophagitis	0	0	1 (1.2)	0	1
Candidiasis plus stricture	0	0	0	1 (2.5)	1
Candidiasis plus oesophagitis	0	1 (0.9)	0	0	1
Candidiasis plus ulcers	0	0	1 (1.2)	1 (2.5)	2
Candidiasis plus growth	0	2 (1.9)	1 (1.2)	0	3
Total	19	103	80	40	242

($p=0.668$, Chi-square test was applied. Percentages shown in brackets are the frequency of that category in that age group to the total in that age group)

DISCUSSION

The results of this study provide an insight into the pathologies prevalent in the community currently. Oesophageal dysphagia is frequently encountered in clinical practice being a distressing symptom and cause should always be diagnosed. Differentiation between benign and malignant lesions is crucial.

In our study the patients that complained most of swallowing problems were in young and middle age group and females outnumbered males. A study conducted by Gupta *et al* in India also showed similar finding that female gender presented in higher number with dysphagia.² However, in terms of age Gupta’s research reported it to be in the middle stage of life.

The endoscopic diagnosis in patients with dysphagia varies in different studies. Etiological analysis of dysphagia is very important as each cause carries different outcomes. In our work the commonest endoscopic lesion was oesophageal growth followed by reflux oesophagitis and then oesophageal web/strictures. Gupta *et al*² also reported the growth as the commonest finding in their study. Krishnamurthy *et al*¹ found that oesophageal stricture was most common pathology. Khan *et al*⁶ suggested that malignant strictures were the commonest problem in dysphagic people. Zameerulla *et al*⁷ in their study found normal

oesophageal findings in higher number with malignant stricture being the next common. A Kenyan study also reported oesophageal cancer as the prevalent diagnosis in patients with dysphagia.⁸

In our statistics presence of growth was seen equally in all age groups with slight preponderance to female gender. However, Several studies reported that oesophageal growth is more common in the old or elderly group and oesophageal squamous cell carcinoma remains the most common histopathology of malignant lesions and hence it should be immediately addressed.^{6,7,9} In our study it was noticed that the presence of growth/stricture more in females compared to males which is in accordance to Khan's study.⁶ An American data also found the probability of oesophageal cancer to be more in white woman.¹⁰ Other studies found malignant strictures nearly equal in both gender.^{3,7}

Our second most common endoscopic finding was of reflux oesophagitis. We found it more common in young age and slightly more frequent in females than males. Kim *et al*¹¹ and Fakhre Yaseri H¹² also revealed similar prevalence rates in terms of gender. However, these studies found reflux oesophagitis to be increasing with age and hence to be common in the elderly. Female gender is a risk factor in gastroesophageal reflux disease. This gender difference could be due to oestrogen or more inclination or consumption of spicy and oily diet by females in our community. This role of oestrogen in reflux esophagitis is also suggested by other researches.^{11,12}

Third common finding was of webs and strictures. The common causes of benign oesophageal strictures are usually oesophageal webs, Schatzki rings, radiation injuries, caustic ingestions, and peptic stenosis.¹³ These disorders are associated with malnutrition, weight loss and increased risk of aspiration. In our study the frequency of strictures was twice common in females. We found these in higher number in patients of young and middle age groups. Our results concur other studies that suggested similar correlation of age and gender with oesophageal web and other benign strictures.^{14,15}

Our study showed that dysphagia is a grave symptom; and it should be investigated promptly. A population based survey of patients with dysphagia in the United States showed that only half of the population actually seeks medical advice for their swallowing difficulties.¹⁶ Most patients just used compensatory ways to handle their issue like by drinking fluids to swallow the food and taking longer time to eat. Increasing awareness in the community for an early medical check-up for dysphagia would be beneficial for such people.

CONCLUSION

Dysphagia is a common clinical problem, and can be associated with serious underlying disorders. The high frequency of oesophageal growth among all age groups is alarming; most of them turned out to be malignant and often already metastasized by the time they are discovered. Clinicians should consider endoscopic evaluation for early diagnosis in patients with dysphagia.

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Address for Correspondence:

Dr. Shabnam Naveed, Associate Professor, Department of Medicine, House# 54/1, 10th Commercial Street, DHA Phase-4, Karachi, Pakistan. **Cell:** +92-300-2298572
Email: shabnamkorejo@gmail.com

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PR: Analysis, agrees to ensure integrity of work

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ORIGINAL ARTICLE

EFFECTIVENESS OF BCG VACCINATION AGAINST SARS-COV-2

Muhammad Talal Khan, Afsheen Mansoor*, Aleena Hussain**,
Muhammad Waqar Hussain***, Farrukh Ahmad[†], Emaan Mansoor^{††}

Department of Dental Biomaterials, Bakhtawar Amin Medical and Dental College, Multan, *Department of Dental Materials, School of Dentistry, Shaheed Zulfiqar Ali Bhutto Medical University, Islamabad, **Student, Islamabad Medical and Dental College, Islamabad, ***Department of Prosthodontics, [†]Department of Oral Medicine, Bakhtawar Amin Medical and Dental College, Multan, ^{††}Islamic International Dental College, Riphah International University, Islamabad, Pakistan

Background: Bacillus Calmette-Guerin (BCG) vaccine is weakened *Mycobacterium bovis* based preparation that provides protective effects and immunotherapy against many ailments and may be tested against SARS-COV-2. Herein, we compare the prevalence of COVID-19 in countries with and without mandatory BCG vaccination program along with a study to examine its incidence in doctors working at quarantine facility with and without history of prior BCG vaccination. **Methods:** For cross-sectional examination, the data was collated from worldometer.info, BCG World Atlas and COVID-19 stats at National Command and Operation Centre (NCOC), Government of Pakistan accessed on Apr 30, 2021. For a real-time analysis, total of one hundred random male and female doctors working at a COVID-19 healthcare facility in Pakistan were voluntarily selected. Chi-square test was used to perform the statistical analysis with the help of SPSS-22. **Results:** The prevalence of COVID-19 in countries with and without a mandatory BCG vaccination policy was found to be 5,512 and 75,592 cases per million, respectively, and mortality rates were 98 and 1,435 per million, respectively. Amongst our randomly selected doctors, only 28% of the BCG vaccinated doctors were affected by COVID-19 contrary to 94% of those who were unvaccinated. **Conclusions:** The incidence of COVID-19 was considerably lower in the doctors with a history of BCG vaccination supported by the evidence that the countries with a universal BCG vaccine policy had a significantly low incidence and lower mortality rate due to COVID-19.

Keywords: Bacillus Calmette-Guerin vaccine (BCG), SARS-COV-2, Tuberculosis, Coronavirus, COVID-19

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INTRODUCTION

The emergence of Corona Virus Disease 2019 (COVID-19) has infected more than 150 million people worldwide with a total of 3,179,925 casualties. Pakistan, alone has reached a total of 820,823 cases with 17,811 mortalities as of April 30, 2021. With the onset of this pandemic, vaccination is considered as the most effective preventive measure for this disease. Bacillus Calmette-Guerin (BCG) vaccine was first introduced in 1921 and has been used since then for the prevention of tuberculosis. BCG vaccines contain a live attenuated strain of *Mycobacterium bovis*, and it protects against various pathogens, some of which may be responsible for acute respiratory tract infections.¹ However, BCG activates the cross-protection that may not be associated to target the disease due to 'Trained Immunity' or innate immune memory.²

In addition to tuberculosis, BCG vaccine has also been utilized for the protection against autoimmune and inflammatory diseases.³ BCG vaccine can speed up the reactivation of the immune system.⁴ Therefore, multiple doses of BCG vaccine have been utilized previously in the treatment of bladder cancer and type-1 diabetes mellitus.⁵ Trained immunity is one of the reason, why many studies

recommend that multiple doses of BCG vaccine should be included in the clinical trials of COVID-19 vaccination.⁶ Some of the recently published studies on COVID-19 suggest that BCG vaccine is potent enough to combat this disease.^{7,8} However, a few studies do not favour the use of multiple doses of intradermal BCG vaccination.⁵

Several epidemiological studies concluded that countries with an approved policy of universal BCG vaccination had a lower incidence of COVID-19 and a lower mortality rate as compared to the countries that did not have a mandatory BCG vaccination policy.⁹⁻¹¹ The current study was carried out to provide a comparative data of selected countries with and without mandatory BCG vaccination policy and to establish the fact that 'BCG vaccination boosts immunity in health care professionals exposed to COVID-19'.

This study was conducted to assess the incidence of COVID-19 in doctors, who were previously administered BCG vaccine in their childhood and those who were not formerly administered BCG vaccine (negative control) and were dealing with COVID-19 patients in a COVID-19 healthcare facility in the city of Islamabad, Pakistan.

METHODOLOGY

The ethical approval of this study was obtained from the institutional review board at Shaheed Zulfiqar Ali Bhutto Medical University Islamabad, Pakistan. The data were compiled from worldometers.info, BCG world atlas and COVID-19 stats at National Command and Operation Centre (NCOC), Government of Pakistan [accessed on April 30, 2021].

For a real-time analysis, a COVID-19 healthcare facility of a tertiary care hospital operating with a compliance to standard operating procedures (SOPs) of World Health Organization (WHO) was selected. Informed consent was obtained from all of the participants of this study and access to this facility was obtained with all implemented ethical considerations and standard biosafety procedures.

A total of one hundred doctors, who were assigned to take care of more than 500±50 COVID-19 positive patients for 6 months at a tertiary care hospital in Pakistan were included in this study. The sample size was similar to that used in previously published studies related to COVID-19.^{12,13} A similar sample size was also calculated according to the sample size calculator to calculate vaccine effectiveness for studies related to COVID-19 where the predicted vaccine effectiveness was 90%, the desired precision width was 30%, and considering that the attack rate of the virus was 30% as suggested by WHO.

The BCG vaccination cards of the healthcare workers were obtained after consent and the evaluation of BCG vaccination scar was performed. Those without the preceding records were confirmed through mantoux test. These doctors were observed for nosocomial infection of SARS Cov-2 despite employment of PPEs with a strict compliance.

Doctors in direct contact with the patients were included in this study while nursing staff, phlebotomists, sanitary workers, infectious waste management personnels, management staff and sample transporters of the hospital were excluded. The individuals with any known health conditions like diabetes, hypertension, pulmonary diseases, rheumatoid arthritis and obesity were also excluded. Only the primary data recorded was analyzed.

All information of the respondents was obtained after proper certification of the Bioethics and Biosafety Committee of the healthcare facility. Chi-square test was used to perform the statistical analysis with the help of SPSS-22. The confidence interval was established at 95% and $p < 0.05\%$ was deemed as statistically significant.

RESULTS

A total of one hundred health care workers, who were assigned to take care of more than 500 COVID-19 positive patients at a tertiary care hospital in Pakistan were included in this study. Out of these 100 individuals, 29 male healthcare workers and 21 female healthcare workers had a prior history of BCG vaccination in their childhood while 30 male and 20 female healthcare workers were never administered BCG vaccine (Table-1).

Doctors were assessed for COVID-19 for a period of six months. Data compiled from 1 to 30 April 2021 revealed that 28% of the doctors who had received a BCG vaccine in their childhood still were affected by COVID-19. Contrary to this, 94% of the workers who were not vaccinated with BCG vaccine in their childhood suffered from SARS COV-2 hospital-acquired infection. The immunized doctors who tested positive for COVID-19 were asymptomatic; however, some alongwith all of the unimmunized individuals, exhibited mild fever, malaise, dry cough, headache and shortness of breath (Table-2).

Countries with and without a universal BCG vaccine policy alongwith their respective prevalence of COVID-19 cases and mortality rate per million are tabulated as Table-3 and 4, and the data has been dichotomized.

Table-1: Total number of immunized and unimmunized doctors included in the study

	Male	Female	Total
Total Participants	59	41	100
Immunization			
Immunized with BCG Vaccine	29	21	50
Unimmunized with BCG Vaccine	30	20	50
Infected			
Immunized infected	8	6	14
Unimmunized infected	28	19	47
Total Infected	36	25	61

Table-2: Association of BCG Vaccine with COVID-19

Vaccine		COVID			p	Unadjusted Odds Ratio	Adjusted Odds Ratio	95% CI for Odds Ratio
		Yes	No	Total				
No	Count [n (%)]	47 (94)	3 (6)	50	<0.001	40.29	44.58	11.46–173.44
	Within COVID-19 (%)	77	7.7	50				
Yes	Count [n (%)]	14 (28)	36 (72)	50				
	Within COVID-19 (%)	23	92.3	50				
Total	Count (n)	61	39	100				
	Within COVID-19 (%)	100	100	100				

Table-3: Countries without a universal BCG vaccine policy

Country	Total Cases	Cases/Million	Deaths/Million
France	5,592,390	85,520	1,594
Finland	86,613	15,612	165
Italy	4,009,208	66,391	1,996
Sweden	967,678	95,326	1,379
Slovenia	239,339	115,112	2,041
Mean	2,179,046	75,592	1,435

Table-4: Countries with a universal BCG vaccine policy

Country	Total Cases	Cases/Million	Deaths/Million
Pakistan	820,823	3,658	79
Bangladesh	756,955	4,559	69
India	18,762,976	13,487	150
Indonesia	1,662,868	6,027	164
Philippines	1,028,738	9,286	155
Afghanistan	59,745	1,507	66
China	90,655	63	3
Mean	3,311,823	5,512	98

DISCUSSION

This study aimed to assess the comparative immunity of doctors against COVID-19. Most of these doctors were previously immunized with a BCG vaccine, while the others had no prior immunization with a BCG vaccine throughout their life. Though, WHO has not yet recommended the use of BCG vaccine for the prevention or treatment of COVID-19 still, it has been thought to enhance immunity.¹⁴⁻¹⁶ Clinical trials are under progress, where doctors in contact with COVID-19 patients are being administered BCG vaccine to evaluate the effects of this vaccine concerning prevention against COVID-19.¹⁷

The results of this study support previously published literature, which suggests that a reduced rate of mortality and morbidity due to COVID-19 disease is observed in the countries where a universal BCG vaccination policy has been implemented.¹⁸⁻²⁰

As of 30 April 2021, a total of 151,240,576 cases of COVID-19 have been reported with 3,182,092 recorded casualties at a rate of 408.2 casualties per million population. Incidence of COVID-19 in BCG vaccinated countries and in countries without a universal BCG vaccine policy was 5,512 cases per million and 75,592 cases per million, respectively. Moreover, the death rates due to COVID-19 in BCG vaccinated countries and in countries without a universal BCG vaccine policy was 98 per million and 1,435 per million, respectively. Pakistan, individually has a total number of 820,823 cases with a gross occurrence of 3,658 cases per million with a mortality rate of 79 deaths per million. According to Global health observatory data repository provided by WHO 91% 1-year-olds in Pakistan are BCG vaccinated in 2020. Another study suggested that 80% of the population was BCG vaccinated.²¹

BCG vaccination is a part of vaccination policy. However, countries like Finland, France, Italy and Sweden require an approval of parents before administration of vaccine and it is not mandatory for all individuals to get vaccinated. If the parents are unwilling to get the vaccine the vaccine is not administered to their children. According to Global health observatory data repository provided by WHO no data for BCG vaccination in 1-year-olds is available for Slovenia, Italy, Finland and France which indicates uncertainty about vaccination. Similarly according to the Global health observatory data repository provided by WHO 26% of 1-year-olds in Sweden are BCG vaccinated.

The dichotomized data of crude case fatality rate (CFR) (deaths/incidence) was 1.33% for BCG and 2.24% for non BCG administering countries. A comparison of total cases from a few randomly selected countries with and without a universal BCG vaccine policy till 30 April 2021, suggests that the average of COVID-19 cases was lower in countries with a universal BCG vaccine policy. Furthermore, the average of the total number of deaths per million was higher in countries without a universal BCG vaccine policy.

This is further strengthened with the statement that BCG vaccines are manufactured under different conditions across the globe generating divergent formulations.^{22,23} A study carried out in Guinea Bissau suggested that BCG vaccinated children have a lower mortality rate of around 50%. Furthermore, it was also noticed that the overall mortality rate was lowered due to the administration of BCG vaccine as it reduced the possibility of respiratory infections and sepsis.²⁴

This study supports the results from previously published studies that the randomly selected countries that do not administer BCG vaccine had a higher number of COVID-19 cases and a higher mortality rate per million individuals. Contrary to this finding, the countries where BCG vaccination policy was in place and the vaccine was administered had a lower mortality rate per million and the total number of cases per million were lower. These findings supported that BCG vaccination might have a potential role in reducing the average of COVID-19 cases and mortality rate in the population under study.

Evidence from previous laboratory and clinical experiments revealed that BCG vaccine might produce nonspecific preventive results against several viral infections in human beings.^{5,25} It has been observed that interferon-gamma (IFN- γ) was presented in a substantial quantity from CD4+ cells that prevented the mice against infection from the virus, hence, it is termed as trained immunity.²⁶ This also

emphasizes that interferon-gamma (IFN- γ) improves immuno-modulatory effects in the body as it enhances presentation and processing of antigens, augments leukocyte trafficking, entices an antiviral situation and promotes antimicrobial features.²⁷ As a consequence of BCG vaccination, pro-inflammatory cytokines like Interleukin-1 β (IL-1 β) cause nonspecific immunity against various viral agents.²⁸ The protection provided by BCG vaccination against COVID-19 is mainly due to its nonspecific effects.^{5,29}

CONCLUSION

The countries with universal BCG vaccination policy have a lower incidence of COVID-19 and a lower death rate in comparison to countries with no BCG vaccine policy. Incidence of COVID-19 was significantly lower in randomly selected doctors who had been administered BCG vaccine in their childhood in comparison to those who had never been administered the vaccine throughout their life. There is a considerable contrast in the incidence and death rate due to COVID-19 in Pakistan as compared to the countries where administration of BCG vaccine is not practiced and this possibly demonstrates enhanced recovery from the disease in Pakistani population.

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Address for Correspondence:

Dr. Afsheen Mansoor, Department of Dental Materials, School of Dentistry, Shaheed Zulfiqar Ali Bhutto Medical University, Islamabad, Pakistan. **Cell:** +92-321-5879166

Email: drafshleenqamar@gmail.com

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Contribution of Authors:

MTK: Substantial/design/data collection an interpretation/script wrining

AM: Substantial/design/data collection an interpretation/script wrining

AH: Conception

MWH: Design and data interpretation

FA: Design and data interpretation

EM: Design and data interpretation

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ORIGINAL ARTICLE

DEMOGRAPHIC AND CLINICAL FEATURES OF CHILDHOOD IDIOPATHIC EPILEPSY AT A TERTIARY CARE HOSPITAL OF PAKISTAN

Syed Sajid Hussain Shah, Bibi Aalia*, Mohammad Ali Raza**, Shahzad Najeeb**, Saima Gillani**, Fauzia Aitazaz***

Department of Paediatric Nephrology, Institute of Kidney Diseases, Peshawar, *KMU Institute of Medical Sciences Kohat, Ayub Medical College Teaching Hospital, Abbottabad, ***Department of Physiology, AJK Medical College, Muzaffarabad, Pakistan

Background: Recent increase in number of children diagnosed with epilepsy raised the need to find out and report the demographic and clinical features of childhood idiopathic epilepsy at a tertiary care hospital. **Methods:** Cross sectional study conducted at Ayub Teaching Hospital from July 2018 to June 2020. Patients aged 1–14 years of either gender diagnosed as idiopathic epilepsy were included from paediatric outpatients, admitted cases and private clinics. Patients with cerebral palsy, dysmorphism and microcephaly were excluded. Demographic data, birth and developmental history details, age of onset of seizures, family history of epilepsy and febrile seizures were documented. Electroencephalograms (EEGs), brain imaging (CT scans and MRIs), where available. Data was analysed at SPSS-20. **Results:** For total 83 (65% males), Mean±SD of age was 6.60±3.64 years. Majority (63.9%) were older than 5 years. Family history of epilepsy and febrile convulsions was positive in 30.1% and 14.5% respectively. Most commonly used drug for seizure control was valproic acid (84.3% patients). Brain CT scan was done in 25.3%, MRI in 16.9% patients, and EEG in 57.8%. Positive family history of febrile seizures was significantly associated with ($p<0.05$) idiopathic epilepsy in males. **Conclusions:** Idiopathic epilepsy affects mostly children above 5 years of age. EEG is the most common investigation performed. When done brain imaging was normal. Valproic acid remains the most commonly used solo antiepileptic in both genders. Male patients are at higher risk of developing idiopathic epilepsy especially if family history of febrile seizures is present.

Keywords: Children, epilepsy, neuroimaging, EEG

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INTRODUCTION

Globally, each year five million people are diagnosed with epilepsy. In developed countries with higher *per capita* income epilepsy is diagnosed in 49 per 100,000 people each year. In poorer countries, the number of diagnosed cases per year can reach as high as 139 per 100,000.¹ Estimated people in United States having epilepsy is 3.4 million with 3 million adults and 470,000 children.¹ According to the latest estimates, about 0.6% of children aged 0–17 years have active epilepsy.^{2,3} This means one out of six children of school going age has epilepsy. Overall prevalence of epilepsy in Pakistan is about 9.99 per 1,000 population. Prevalence is highest in those younger than 30 years of age.⁴

Seizure is a manifestation of epilepsy and characterized by sudden, abnormal, hyper-synchronous discharge of cortical grey matter or neurons with temporary loss of consciousness, abnormal motor activity, autonomic dysfunction and sensory behavior.⁵ Epilepsy and epileptic syndromes were classified as idiopathic depending upon factors such as age at seizure onset, type of seizures, electroencephalographic changes, family history, absence of an anatomic brain lesion. Idiopathic

generalized epilepsy (IGE) is a group of epileptic disorders that are believed to have a strong underlying genetic basis. Patients with an IGE subtype are typically otherwise normal and have no structural brain abnormalities.⁶

In children of developing countries, idiopathic epilepsy is the most common neurological disorder.⁷ As compared to developed countries, the prevalence of epilepsy is more in developing countries due to more complications during deliveries, more chances of intracranial infections going unsuspected and delays in seeking medical help. Rural areas have a higher prevalence in comparison with urban areas, reason of which is not clear.^{8–10} In our hospital we noted recently a surge of children with seizure disorder of various aetiologies, so we decided to conduct this study to highlight the problem in our area. Although studies for epilepsy prevalence and demography have been done elsewhere in Pakistan⁴, through our input we want to contribute in highlighting this important problem in paediatric population of Hazara Division.

METHODOLOGY

This cross sectional study was done in Paediatric B unit of Ayub Teaching Hospital, Abbottabad over two years, i.e., from July 2018 to June 2020 after obtaining

approval from institutional review board. Patients from 1–14 years of age of either gender diagnosed as idiopathic epilepsy on basis of history including antenatal history, examination, normal developmental milestones and investigations including CT scan and MRI and electroencephalogram (EEG) were included in this study. Patients were enrolled from outpatients, admitted cases and patients from private clinic. Patients with cerebral palsy, dysmorphism and microcephaly were excluded from the study.

Sample size was calculated by OpenEpi sample size calculator. The prevalence of epilepsy in children was taken as 5% and estimated sample size was 73 patients. In our study we included 83 patients through non-probability, consecutive sampling after taking written informed consent. Patient’s demographic data comprising age of onset of seizures, family history of epilepsy and febrile seizures, findings on EEG, MRI and CT scan brain (where available) were recorded on the predesigned proforma.

The data was analysed using SPSS-20. Mean±SD was calculated for age of the patients. Frequencies and percentages were calculated for qualitative variables like gender of patients, type of epilepsy, family history of epilepsy and febrile convulsions, type of drugs used for seizure control and investigations done. Chi square test was applied for comparison of nominal data and results considered significant with $p \leq 0.05$.

RESULTS

In this study, total 83 patients were enrolled out of which 54 (65.1%) were males and 29 (37.9%) were females. The mean age of diagnosis of epilepsy was 4.27 ± 3.29 years. Only 5 (6%) patients were less than 2 years of age, while 25 (30.1%) patients were in between 2–5 years of age (Table-1). Majority of patients 53 (63.9%) were more than 5 years of age, whereas 22 (26.5%) patients were less than 2 years, leaving 29 (34.9%) patients with ages between 2–5 years and 32 (38.6%) patients were more than 5 years of age, when they first time presented with history of seizures. Family history of epilepsy was positive in 25 (30.1%) patients while family history of febrile convulsions was present in 12 (14.5%) patients. CT scan brain was done in 21 (25.3%) patients whereas MRI brain was done in 14 (16.9%) patients. All neuroimaging including CT scan and MRI were normal. The EEG was done in 48 (57.8%) patients. Only 28 patients (25%) EEG showed epileptic activity. (Table-1)

Age of patients ranged from 1–14 years with Mean±SD of 6.60 ± 3.64 years, mean for duration of illness was 4.5 years (Table-2).

Though family history of epilepsy was more in male as compare to females yet $p=0.38$ was not significant (Table-3). There was significant relationship

between gender and family history of febrile convulsions with $p=0.037$ as febrile convulsions with family history were more in males (Table-4).

The most common drug used for seizure control was valproic acid, which was used in 70 (84.3%) patients, leviteracetam in 5 (6%) patients, carbamazepine in 7 (8.4%) patients and lamotrigine in one (1.2%) patients. (Tables-1, 5).

Most of the male patients, i.e., up to 89% used valproic acid as sole medication followed by carbamazepine in 5.6%, in females carbamazepine use was slightly higher as up to 13% used it and majority of females, i.e., 76% used valproic acid as only medications, p was insignificant though and only a negligible 1% in both genders used combination treatment of antiepileptic (Table-5).

Table-1: Demographics (n=83)

Variables	Frequency	Percentage	<i>p</i>
Gender			
Male	54	65.1	0.500
Female	29	34.9	
Age Group			
≤1.99 years	5	6.0	0.070
2–4.99 years	25	30.1	
≥5.00 years	53	63.9	
Family history of epilepsy			
Present	25	30.1	0.096
Absent	58	69.9	
Family history of febrile seizures			
Present	12	14.5	0.037
Absent	71	85.5	
Antiepileptic used			
Valproic acid	70	84.3	0.126
Leviteracetam	3	3.6	
Valproic acid and leviteracetam	2	2.4	
Carbamazepine	7	8.4	
Valproic acid and lamotrigine	1	1.2	
CT scan of brain			
Done	21	25.3	0.155
Not done	62	74.7	
MRI of brain			
Done	14	16.9	0.300
Not done	69	83.1	
EEG			
Done	48	57.8	0.142
Not done	35	42.2	
Treatment completed	2	2.4	-

Table-2: Age, weight and duration of illness (n=83)

Variables	Min	Max	Mean	SD	SE
Age (Years)	1	14	6.60	3.63	0.399
Weight (Kg)	5.5	39	18.25	7.60	0.834
Duration of illness (Years)	4	5	4.5	0.707	0.5

Table-3: Correlation between gender and family history of epilepsy (n=83)

Gender	Family history of epilepsy		Total	<i>p</i>
	Yes	No		
Male	18	36	54	0.38
Female	7	22	29	
Total	25	58	83	

Table-4: Correlation between gender and family history of febrile seizures (n=83)

Family history of febrile seizures	Frequency	Percentage	p	
Males				
Present	11	20.4	0.037	
Absent	43	79.6		
Total	54	100.0		
Females				
Present	1	3.4		
Absent	28	96.6		
Total	29	100.0		

Table-5: Correlation and frequencies of gender and antiepileptic used (n=83)

Antiepileptic used	Frequency	Percentage	p	
Males				
Valproic acid	48	88.9	0.126	
Leviteracetam	1	1.9		
Valproic acid and leviteracetam	1	1.9		
Carbamapazine	3	5.6		
Valproic acid and lamotrigine	1	1.9		
Total	54	100.0		
Females				
Valproic acid	22	75.9		
Leviteracetam	2	6.9		
Valproic acid and leviteracetam	1	3.4		
Carbamapazine	4	13.8		
Total	29	100.0		

DISCUSSION

About 50% of epilepsy cases begin in children or in adolescent age and 74% patients with epilepsy are age 18 years.¹¹ Khatri *et al*⁴, reported epilepsy as a common problem in Pakistan with an overall estimated prevalence of 9.99 per 1,000 population. Those younger than 30 years of age and rural population are affected more. Treatment initiation and compliance remains low and compliance is a problem in diagnosed cases. Awareness in people regarding epilepsy and its management is low.

The gender predominance in our study was slightly more as 65% males were affected. Regarding gender predominance this slight difference in generalized seizure were also noted in an Egyptian study¹² and by Carlson C¹³. Both of these studies noted that although difference between genders is only minute with only a slight male predominance in case of generalized seizures, for certain types of seizures significant difference does exist. Beghi E⁵ in one review article gave the incidence and prevalence of epilepsy more in males.

Dragoumi *et al*¹⁴, studied the clinical courses and outcome of idiopathic epilepsy in children along with prognosis. In their study the mean age of seizure onset in children was 6.7 years while in our study mean age of onset of seizures was 4.27±3.29 years. Treatment adherence in our study remained low which is an important problem in long term management of epilepsy and affects the course and outcome of illness. Problem of non-adherence to

AEDs is also reported in other studies. Approximately three fourths of patients with epilepsy shows response to treatment but 12–67% patients relapse after withdrawal of medicine. EEG is diagnostic in about half of patients with epilepsy but literature shows that abnormal EEG done during the withdrawal of antiepileptic drug (AED) is risk factor for recurrence of epilepsy.¹⁵

Modi *et al*¹⁶, reported that early adherence interventions may actually help patients to comply with the treatment and change the course of the disease. Support groups of families can be formed and frequent follow-ups and counselling is important to enhance adherence to the AED used. In comparison to our findings an Iranian study actually reported a high compliance to AED, i.e., 72.4% patients under 18 years of age adhered to the treatment.¹⁷

Children with a positive family history of epilepsy (30.1% patients in our study) are known to be at greater risk of developing epilepsy, as there it is suggested that an underlying genetic cause may be the reason. It is also reported that children with family history of febrile seizures especially complex febrile seizures are at increased risk for IGE. Though in our study the correlation between IGE and family history was not significant but upon splitting find on gender basis males were found to be at higher risk if family history of febrile seizures is positive. Al Habbal A *et al*⁸, did one case control study in Syria regarding the risk factors associated with epilepsy in children. In their there was positive association between family history of epilepsy and febrile seizures.

Kozhanova TV *et al*, did one study regarding diagnosis of epilepsy in children and exome sequencing. In their study, next generation sequencing technique was used and they detected mutations in 57.7% patients.¹⁸ Definitions of epilepsy clearly include that it is associated with recurrent afebrile seizures, but the fact that febrile seizures of childhood also have a genetic predisposition. Although simple febrile seizures is a benign condition but complex febrile seizures in immediate family, siblings or in the past history of patient is a risk factor for developing epilepsy. One study reported that children with febrile seizures have a six times (3%) increased risk of developing future afebrile seizures and epilepsy when compared to controls. Whereas simple febrile seizures without these risk factors have only twofold excess (1%) of developing epilepsy in future. Complex febrile seizure if, repetitive, prolonged and focal place the child at even a higher risk, i.e., 6–8%.^{19–22}

Asghar MA *et al*, did one retrospective cohort study in tertiary care hospitals of southern

Punjab about the prescription patterns of AED.²³ In children it was found that valproic acid was the most common (56%) prescribed drug followed by levetiracetam (27%). In our study valproic acid was the most common prescribed drug in 84.3% patients, carbamazepine in 8.4% and levetiracetam in 6% patients. This shows that higher number of our patients are on valproic acid as compare to above mentioned study while less no. of our patients are on levetiracetam as compare to above study.

In a retrospective study by Crevier-Sorbo G *et al*, it was found that EEG was the most common investigation done in 82% patients and neuroimaging was done in 7% patients to diagnose the epilepsy.²⁴ While in our study, EEG was done in 57.8% of patients and neuroimaging including CT scan and MRI was done in 25.3% and 16.9% patients respectively. Mwipopo EE *et al* studied the profile and clinical characterization of seizures in hospitalized children and concluded that neuroimaging studies were required mostly in children having unprovoked seizures.²⁵ In our study CT scan brain was done in 25.3% and MRI brain was done in 16.9% patients; all studies were normal. Wali A *et al*²⁶, did a study about referral patterns at a neurophysiology centre in Quetta. They found that most of the children were referred by paediatricians for EEG and at the facility, 58% of EEG was done in children less than 18 years of age.

Another important risk factor for childhood IGE is underlying, undiagnosed, covert celiac disease. Canova C *et al*²⁷ concluded that children having celiac disease are at increased risk of epilepsy. In children when no other aetiology is found, screening for celiac disease should be considered.

CONCLUSION

Idiopathic epilepsy is more common in children above 2 years of age; males are affected more than females. EEG was the most common investigation done for epilepsy in our study. Brain imaging of CT scan and MRI brain where available were normal. Valproic acid remained the most commonly used solo antiepileptic in both genders. A positive family history was associated with epilepsy in males.

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Address for Correspondence:

Dr. Saima Gillani, Department of Paediatrics, Ayub Medical College, Abbottabad, Pakistan. **Cell:** +92-3208512073

Email: drsaimagillani@gmail.com

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SSHS: Concept, design and data collection

BA: Critical review

MAR: Literature search

SN: Literature search

SG: Literature search and review

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ORIGINAL ARTICLE

IMPACT OF EDUCATIONAL PEDAGOGY ON REGISTERED NURSES' KNOWLEDGE IN NEUROLOGICAL ASSESSMENT OF TRAUMATIC BRAIN INJURY PATIENTS

Nazia Yousef, Abdullahi Kabir Ozigi, Muhammad Hussain, Kousar Perveen, Muhammad Afzal

Lahore School of Nursing, The University of Lahore, Pakistan

Background: Nurses perform neurological assessment to determine whether patients' neurological condition is intact or there exists some problem. We aimed to assess the effect of educational pedagogy on the knowledge of nurses in neurological assessment of traumatic brain injury patients.

Methods: This quasi-experimental study investigated the effect of educational pedagogy on 70 registered nurses of a tertiary care hospital from April to June 2021. Diploma holder nurses aged 25–50 years working in the morning shift were included. Nurses who had specialization in neurosciences and had planned to go on leave during study period were excluded. A 20-items validated tool was used for assessment. Correct response was marked as '1' and wrong or missed as '0'. Knowledge was categorized as inadequate (1–6 score) <30%, moderate (7–13 scores) 35–65%, and adequate (14–20 scores) as 70–100%. **Results:** Frequency and percentages of demographic and professional variables were checked. Half of the registered nurses had inadequate knowledge (scores 1–6), while rest 50% had moderate knowledge (scores 7–13). No one had adequate knowledge during pre-assessment. The post-test results of the study illustrated that 34 (48.6%) nurses had moderate knowledge. Thirty-two (45.7%) nurses having adequate knowledge (score 14–20). Only 4 (5.7%) nurses had inadequate knowledge. Mean (\bar{x} =13) of the post-test knowledge score was significantly higher than the mean (\bar{x} =7.611) of the pre-test knowledge score. **Conclusion:** Educational intervention was effective in improving knowledge and skills competency of registered nurses in neurological assessment.

Keywords: Knowledge, Traumatic Brain Injury, TBI, Glasgow Coma Scale, GCS, Nursing, Assessment

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INTRODUCTION

Traumatic Brain Injury (TBI) is the disruption of normal brain function due to external injuries caused by blunt head trauma, accident, sudden bumps to the head, violent hitting of the head, or other causes that damage the skull and injure the brain tissues. TBI is a leading cause of death and disability worldwide. Yearly, about 1.5 million people die from TBI and several millions that survive receive emergency treatment.¹ Neurological injuries affect 1.4 million people per year in Pakistan.² Road traffic accidents and sports are two basic factors which lead to traumatic brain injuries. Traumatic brain injuries due to road traffic injuries are reported more as compared to other cases. Majority (60%) of the cases reported are due to the road traffic accidents. In contrast, the hospital-based statistics estimated sports related traumatic brain injuries ranging from 3.5 to 31.5 per 100,000. On the other hand, 170 per 100,000 sports related traumatic brain injuries were reported on community level.³

Usually patients with TBI are received to the emergency department (ED) with altered level of consciousness that needs urgent and efficient assessment. Glasgow coma scale (GCS) is an

assessment tool and was first introduced by Bryan Jennet and neurosurgery professor Graham Teasdale at the University of Glasgow. It provides objective assessment of level of consciousness in all types of medical and surgical patients during neurological assessment. The GCS has 3 main domains of assessment of level of consciousness, namely eye opening (E), verbal response (V), and motor response (M). The highest score of GCS is 15 while lowest scores is 3.⁴ In the extent of critical care, regardless of advancement in technology, neurological assessment plays a crucial part in the diagnosis and management of TBI patients.⁵ Sound knowledge and efficient skill performance of nurses may be helpful to deal with complexities of neurological assessment in such patients.⁶

Nurses and doctors both perform neurological assessment for different purpose. Doctors perform to locate the affected cite of central nervous system, to make a diagnosis for best treatment options. Nurses perform neurological assessment to determine whether patient's neurological condition is intact or there is some problem. They assess the changes in patient's neurological status in response to treatment, and to evaluate life threatening conditions.⁷ Neurological

assessment is performed at the time of admission of patient and then in each shift of duty to assess the effectiveness of treatment and for need of medication. In the extent of critical care, regardless of advancement in technology, neurological assessment plays a crucial part in the diagnosis and management of unconscious patients. Sound knowledge and efficient skill performance of nurses may be helpful to deal with complexities of neurological assessment of unconscious patients with TBI.⁸

Efficient nursing assessment is found to be associated with early recovery of the patients in neuro and general surgery departments. Nurses, working in neurosurgery department have poor knowledge and practices about GCS assessment of unconscious patients. Assessment of the nurses' knowledge and skill about GCS assessment, working with patients in intensive care is needed.⁹ About 40% of all patients admitted in hospitals with traumatic brain injuries, rather to recover, their conditions worsen due to improper assessment and poor management.¹⁰ Moreover, poor knowledge and inefficient skill performance of nurses was found associated with prolong hospital stay of patients in emergency department and intensive care units.¹¹

There is need of changing the learning pedagogies to enhance the knowledge and skill for ultimately best patients' outcome that is the utmost objective of nursing practices. The current study aimed to assess the effectiveness educational pedagogy on knowledge of registered nurses in neurological assessment of patients with TBI.

METHODOLOGY

This study was conducted following guidelines of declaration of Helsinki. Written informed consent was obtained from all participants. Ethical and Research Committee approval (IRB-UOL-FAHS/830-III/2021) was granted by University of Lahore.

In this single group quasi experimental study conducted from April to June 2021, 70 registered nurses were selected from Trauma Centre, General Surgery and Neurosurgery Department of a tertiary care hospital in Lahore Pakistan using purposive sampling technique. Diploma holder nurses aged 25–50 and working in the morning shift were included in this study. Nurses who had specialization in neurosciences or had planned to go on leave during the study period were excluded. The sample size was calculated using mean and standard deviation from previously published studies.¹² After adding up 20% dropout rate and 80% power sample size was calculated as 70.

In pre- assessment knowledge of participants was assessed on a questionnaire at their original job place. This followed a 1-week educational training (PowerPoint lecture cum audio-visual demonstration)

regarding anatomy and physiology of nervous system and neurological assessment of traumatic brain injury (TBI) patients on Glasgow Coma Scale (GCS) was given. This training program was conducted and validated by experts of relevant field. Four weeks were given for implication of knowledge. The participants were reassessed for changes in knowledge using a 20-items multiple choice questionnaire, GCS knowledge assessment too, adopted from published work with permission. Correct response was marked as '1' and wrong or missed as '0'.¹³ Knowledge was categorized as inadequate (1–6 score) <30%, moderate (7–13 scores) 35–65%, and adequate (14–20 scores) as 70–100%.¹⁴ The Cronbach's alpha 0.723 and intra-rater reliability of tool was reported as 0.86.¹⁵

SPSS-20 was used for statistical analysis. Frequency and percentage was checked for demographic and professional variables. Data about knowledge was obtained twice, i.e., before and after educational training. The collected data was in form of whole numbers to check the pre- and post- mean difference applying paired *t*-test, and $p \leq 0.05$ was considered statistically significant.

RESULTS

Total 70 individuals were chosen from Lahore General Hospital. Thirty-six (51.4%) of nurses were single (unmarried), 27 (38.6%) were married, 3 (4.3%) were divorced, and 4 (5.7%) were widows. Twenty-six (37.1%) of the nurses were 25–30 years old, 18 (25.7%) were 31–35 years old, 16 (22.9%) nurses were 36–40 years, and only 10 (14.3%) nurses were above 40 years of age. Most (33, 47.1%) of the nurses had 2–5 years job experience, 25 (35.7%) had 6–10 years, 8 (11.4%) had less than 2 years, and 4 (5.7%) of the nurses had more than 10 years job experience. Regarding work experience in the current department 16 (22.9%) nurses had less than 1 year, 39 (55.7%) of nurses had 1–3 years, 13 (18.6%) had 4–5 years, and only 2 nurse had more than 5 years working experience in the current department. More than half (38, 54.3%) nurses were working in Neuro-Surgery ICU, 20 (28.6%) in Surgical Unit, and 12 (17.1%) nurses were working in Trauma Centre.

Table-1 depicts the frequency and percentage of registered nurses who responded correctly for GCS knowledge questions, before and after educational intervention. Table-2 is showing the comparison of categories of knowledge of nurses (inadequate, moderate and adequate) before and after training session. Paired mean difference for pre- and post-educational effect is presented in Table-3. The mean (\bar{X} =13) of the post-test knowledge score was significantly higher than the mean (\bar{X} =7.611) of the pre-test knowledge score.

Table-1: Pre- and post-educational intervention knowledge about neurological assessment of TBI patients [n=70, n (%)]

Tool Items	Pre intervention correct response rate	Post intervention correct response rate
Pupil reaction is part of the neurological nursing assessment. Which cranial nerve assesses pupil reaction?	42 (60.0)	64 (91.4)
When eye opening is assessed, Which part of the brain is being tested?	22 (31.4)	61 (87.1)
When verbal response is assessed, Which part of the brain is being tested?	15 (21.4)	46 (65.7)
When motor response is tested, Which part of the brain is being assessed?	19 (27.1)	28 (40.0)
Abnormal motor movement would be observed by a positive drift.	20 (28.6)	27 (38.6)
Three indicators of GCS are: Eye opening, best verbal response, and best motor response.	35 (50.0)	33 (47.2)
Which one is the most adequate response when GCS is used? Best response presented by the patient.	22 (31.4)	42 (60.0)
When assessing for eye opening, examiner should start with: Stand next to the patient's bed.	25 (35.7)	47 (67.1)
What is level of consciousness?	22 (34.3)	45 (64.3)
Neurological assessment components for conscious patients, you would include in your assessment, all of the following except: Pulse rate	23 (32.9)	49 (70.0)
A normal neurological finding includes: Oriented to person, place, time, and event	29 (41.4)	46 (65.7)
When testing the best motor response: Record the response in all four limbs	23 (32.9)	40 (57.1)
To test motor response in a tetraplegia patients	18 (25.7)	42 (60.0)
Glasgow Coma Scale lowest score is: 3	22 (34.3)	41 (58.67)
While testing motor response, if patient is unable to comply. You inflict a pain stimulus, and patient pulls his arm away: He has abnormal flexion.	23 (32.9)	44 (62.9)
While asking a patient, 'Where are you now?' patient says he is at his daughter's condominium. He is: Confused.	22 (31.4)	54 (77.1)
Decrease in Glasgow Coma Scale score of '—' is seen as deterioration in conscious level and needs to alert medical team.	41 (58.6)	52 (74.3)
Initially Glasgow Coma Scale was devised to: assess the depth of coma	43 (61.4)	53 (75.7)
Patients are considered comatose, if GCS score is below 8	34 (48.6)	48 (68.6)
In GCS take notes for: Scoring each indicator, total score, and describe when necessary	28 (40.0)	51 (72.9)

Table-2: Comparison of pre- and post-educational intervention knowledge among registered nurses about GCS assessment of TBI patients [n=70, n (%)]

Knowledge category	Knowledge score	Pre knowledge score	Post knowledge score
Inadequate	1-6	35 (50)	4 (5.7)
Moderate	7-13	35 (50)	34 (48.6)
Adequate	14-20	0 (0)	32 (45.7)

Table-3: Paired t-test between pre- and post-knowledge score about GCS assessment (n=70)

	Mean±SD	Mean difference	Paired t-test	p
Pre-test knowledge score	7.611±2.643	5.389	11.146	0.00
Post-test knowledge score	13.000±3.295			

DISCUSSION

Neurological assessment in emergency and intensive care units is a basic skill for nurses. It is recommended that effectiveness of practices could be enhanced through evidence based practices. The findings of present study are contradictory to a previous study¹⁶ that nurses had good knowledge (75%) regarding neurological assessment of traumatic brain injury patients. It was revealed in the present study that 50% nurses had inadequate knowledge and 50% had moderate level of knowledge. The inadequate knowledge in these areas possibly limits their capacity for clinical judgment and decision making in managing unconscious patients.

Current study revealed the same results as previous studies¹⁷ regarding the effectiveness of teaching programs related to GCS for assessment of neurological injuries. This study revealed that the post-

test mean score among staff nurses and knowledge and skills regarding use of GCS was higher than pre-test mean score among staff nurses. This indicates that nurses improved their skill competence after teaching session and also revealed the importance of continuing nursing education.

Nurses working in neurosciences required evidence-based guidelines for assessment of neurological injuries. The findings of this study are supported by previous work conducted by Cook NF.¹⁸

The findings of current study support the need for ongoing education of nurses for neurological assessments to increase skill and confidence in assessment of neurological injuries which ultimately could increase the patient survival rate.¹⁹ This study had similar results as a previous study reporting that there was a significant increase in overall post-survey scores vs pre-survey scores. This study confirms the results of that study²⁰ that neurological assessment is a part of the daily routine for most nurses working in neurology departments, and that nurses with special education and training in Neuroscience nursing have higher competence in consciousness assessment than nurses who only have basic education.

CONCLUSION

Current study disclosed the effectiveness of educational intervention in the enhancement of knowledge and refining the skill competency of registered nurses regarding neurological assessment of TBI patients. A remarkably positive impact of educational pedagogy was demonstrated.

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Address for Correspondence:

Ms. Nazia Yousef, Lahore School of Nursing, The University of Lahore, Lahore, Pakistan. Cell: +92-300-7843065

Email: naziayousef19@gmail.com

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NY: Conceive the idea, design, data collection and manuscript writing

AKO: Overall supervision of the study

MH: Assistance in designing and critical input to manuscript

KP: Data analysis, critical evaluation and redrafting the manuscript

MA: Final draft and made substantial contribution

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ORIGINAL ARTICLE

FREQUENCY OF PSEUDOTHROMBOCYTOPENIA IN OUTDOOR PATIENTS: ROLE OF TRISODIUM CITRATE IN CORRECTING IT

Jamila Farid, Muhammad Idris, Mumtaz Ahmad Khan*, Nasreen Gul,
Shazia Moeen, Shabana Bashir**

Department of Pathology, Ayub Medical College, Abbottabad, *Abbas Institute of Medical Sciences Muzaffarabad,
**AJK Medical College, Muzaffarabad, Azad Jammu and Kashmir

Background: Pseudothrombocytopenia (PTCP) is the name given to spuriously low platelets count given by automated haematology analyser in an otherwise normal and asymptomatic patient having normal platelets count. This may lead to unnecessary investigations and anxiety. The present study was planned to know the frequency of this condition. **Methods:** This cross-sectional, quantitative analytical study was conducted at a tertiary health care facility (Department of Pathology Ayub Medical College Abbottabad) in collaboration with a private sector reference laboratory and research centre for blood diseases (Abbott Clinical Laboratory) over a period of three years. Citrated blood samples of all the participants with low platelet count on CBC (EDTA blood), examination of their Giemsa stained blood film along with a detailed history and complete physical examination. **Results:** As much as 30/15,000 participants had PTCP on initial testing, with a frequency of 0.2%, comprising 14 males and 16 females with male to female ratio 0.875:1. Ten (10) patients had mild, 14 had moderate, and 6 had severe PTCP which was corrected with citrate in 96.66% patients initially. **Conclusion:** Frequency of PTCP was 0.2%, affecting all the age groups with female predominance and the differences between testing on EDTA and citrate was statistically significant ($p < 0.05$), signifying the role of citrate in correcting it.

Keywords: Pseudothrombocytopenia, thrombocytopenia, complete blood counts, platelets, EDTA, Citrate, Platelet aggregation

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INTRODUCTION

Complete blood count (CBC) is one of the common tests performed routinely in patients seeking medical advice and increasing use of haematology analysers have made it much easier than before. It has however given rise to spuriously low platelet count in some patients. This is a benign condition given the name 'pseudothrombocytopenia' (PTCP) sometimes leading to anxiety. Pseudothrombo-cytopenia (PTCP) is spuriously low platelet count when an Ethylenediaminetetraacetic acid (EDTA) blood sample is tested for complete blood count on an automated haematology analyser. It is defined as an inaccurately low estimate of the number of platelets in a sample of blood that is caused by clumping of the platelets in the laboratory sample rather than by a disease that affects platelet production or destruction.¹ It is not something new as it was described for the first time in 1969 by Gowland and co-workers as agglutination of platelets by a serum factor in the presence of Ethylenediaminetetraacetic acid (EDTA), used as an anticoagulant.² Later on it was seen with other anticoagulants including sodium citrate, lithium heparin, disodium oxalate and hirudin.³ Patients with pseudothrombocytopenia are usually asymptomatic. Blood report showing thrombocytopenia is worrisome for the patient and the doctor. It may cause unnecessary treatment delay, unnecessary or

overtreatment, unwanted platelet transfusion, or delay in essential procedures.⁴ Some other aspects of pseudothrombocytopenia have also been reported in the literature. It has been reported in patients with systemic lupus erythematosus and lupus nephritis.^{5,6}

Recently, pseudothrombocytopenia has been reported in COVID-19 patients.⁷ Pseudothrombocytopenia has been reported useful in the diagnosis of scrub typhus.⁸ Platelet aggregates is the hallmark of pseudothrombocytopenia which are not counted by the analyser as platelets but probably as leukocytes. Platelet histogram can provide some clue to this and a well-stained blood film is confirmatory.⁹

Considering the paucity of research on this important clinical entity, the present study was planned to see the frequency of this condition to increase its awareness in health care workers.

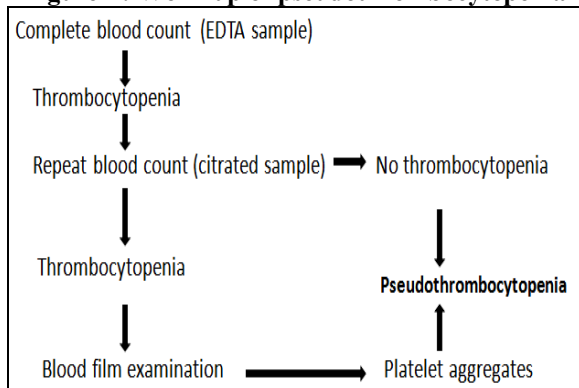
MATERIAL AND METHODS

This was a cross-sectional, quantitative analytical study conducted at a tertiary care health facility (Department of Pathology Ayub Medical College Abbottabad) in collaboration with a private laboratory and research centre for blood diseases (Abbott Clinical Laboratory). The objective of the study was to see the frequency of PTCP in EDTA blood and role of trisodium citrate in correcting it. The study was conducted after taking an informed written consent from each participant. As much as 15,000 patients

were initially recruited in the study by non-random convenience sampling technique over a period of three years (June 2018 to June 2021), referred to laboratory for the work-up of thrombocytopenia noted on complete blood count report (EDTA blood). After history and physical examination, a fresh citrated venous blood sample was taken in the laboratory for repeat blood counts from those patients whose blood counts revealed thrombocytopenia on the initial testing in some other laboratory. Repeat blood count was performed on Mindray BC 500 as the first step. Those with normalization of platelet counts on citrated blood samples were labelled as pseudothrombocytopenia. A well-stained blood film (May Grunewald Giemsa) examination was performed next on samples with low platelets for platelet aggregates by an experienced haematologist as the second step. Blood samples with normalization of platelet count on citrated blood and/or platelet aggregates on blood film, were included in the study. Those without platelet aggregates on blood film, already diagnosed with thrombocytopenia or diseases known to cause thrombocytopenia were excluded from the study. Patients on treatment for thrombocytopenia were also excluded from the study. Work-up of thrombocytopenia is depicted in Figure-1. Classification of pseudo-thrombocytopenia as mild, moderate and severe was done on the analogy of classification of true thrombocytopenia.¹⁰

The patients were divided into three groups depending upon the platelet count by EDTA method. Descriptive statistic was used for independent *t*-test, and $p \leq 0.5$ was considered significant.

Figure-1: Work-up of pseudothrombocytopenia



RESULTS

As much as 30/15,000 (0.2%) patients showed PTCP (Table-1). Three (10%) were <20 years old, 5 (16.7%) >60 years, and the remaining 22 (73.3%) aged 20–60 years. Fourteen participants were male and 16 were female with male to female ratio 0.875:1 (Table-2).

Ten (10) patients had mild, 14 moderate, and 6 patients were with severe PTCP on initial testing (Table-3). Descriptive statistics for platelet count on

EDTA and citrate sample revealed *t*-value as -13.19, and $p=0.00001$ (Table-4). One blood sample (citrate) revealed thrombocytopenia. Platelets aggregates were confirmed on examination of Giemsa stained blood film. The sample was repeated with citrate in a pre-warmed tube kept at 37 °C throughout the testing period and PTCP disappeared (Table-5).

Table-1: Frequency of pseudothrombocytopenia

Total number of participants	15,000
Participants with PTCP	30
% participants with PTCP	0.2%

Table-2: Age and gender-wise distribution of cases (n=30)

Age (Years)	No.	Percent	Male		Female	
			No.	Percent	No.	Percent
<20	3	10.0	2	66.7	1	33.3
20–39	10	33.3	4	40.0	6	60.0
40–60	12	40.0	5	58.3	7	41.7
>60	5	16.7	3	60.0	2	40.0

Male:Female= 0.875:1

Table-3: Severity of pseudothrombocytopenia

Severity	Platelets ×10 ³ /μL	No.	Percent
Mild	100–149	10	33.3
Moderate	50–99	14	46.7
Severe	<49	6	20.0

Table-4: Descriptive statistics

Platelet count ×10 ³ /μl	EDTA sample	Citrate sample
Max	144	155
Min	15	290
R	129	135
Mean±SD	82.9±38.0	206.5±34.37

t-value= -13.19, $p=0.00001$

Table-5: PTCP with EDTA versus Citrate

PTCP (EDTA sample, room temperature)	30
PTCP (Citrate sample, room temperature)	1
Correction of PTCP by citrate	29 (96.66%)

DISCUSSION

The frequency of PTCP was 0.2% in the present study. This is in accordance with the findings of earlier research.¹⁰ A similar study revealed higher incidence of pseudothrombocytopenia (17%) due to delay in the examination of blood and lack of blood film examination facility.¹¹ This is contrary to our findings, as these two factors were excluded in the present study. A study revealed pseudothrombocytopenia in as much as 15.3% patients.¹² Our findings are different from the results of this study. The findings of another study conducted on pseudothrombocytopenia are comparable to our findings with an incidence of 0.27%.¹³ In another study the incidence of pseudothrombocytopenia was 0.15%. Our results are comparable to this study too.¹⁴

Some risk factors have been identified showing higher association with PTCP.¹⁵ In the present study higher association was seen with the increasing age. This is in accordance with the findings

of study under reference. Our findings of higher incidence in male are also different from the results of this study. All the participants of present study were asymptomatic and did not have any recent or previous history of bleeding. This is in accordance with the findings of earlier studies.¹⁶

None of the participants of present study received platelet transfusion on the basis of EDTA based blood report. This is contrary to what has been reported previously.¹⁷ In the present study pseudothrombocytopenia was resolved by trisodium citrate except one patient in which blood sample was kept at 37 °C throughout the testing period. A similar strategy was adopted in a previous study.¹⁸

Prevalence of PTCP depends upon study population. In general population it is up to 0.1%. Higher frequency has been observed in hospitalized and patients already having true thrombocytopenia.^{19–21} Pseudothrombocytopenia has been reported mostly in the form of scattered case reports.^{22–26} A few organized studies or review articles have also highlighted this important clinical condition.¹² Viral infections have been described as a possible cause of pseudothrombocytopenia in a case series.²⁷ The PTCP has been reported in blood donors.²⁸ We did not come across any such case. The present study is one of the pioneer research work in our country on pseudothrombocytopenia.

CONCLUSION

Frequency of PTCP was 0.2%, affecting all the age groups with female predominance. The differences between testing on EDTA and citrate were statistically significant, signifying the role of citrate in correcting it.

LIMITATIONS OF STUDY

Sample size was small compared to the research conducted previously by the others.

RECOMMENDATIONS

Patients presenting with thrombocytopenia should always be advised blood film examination to exclude possibility of pseudothrombocytopenia.

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Address for Correspondence:

Dr. Muhammad Idris, Department of Pathology, Ayub Medical College Abbottabad, Pakistan. **Cell:** +92-333-5037762
Email: midris63@yahoo.com

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JF: Concept and design study, data collection, drafting

MI: Data collection, Statistical analysis, final drafting

MAK: Literature review, data analysis, critical revision

NG: Literature review, proof reading, drafting

SM: Literature review, proof reading, drafting

SB: Data analysis, Drafting, Revision

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ORIGINAL ARTICLE

SOCIO DEMOGRAPHIC FACTORS OF PARENTAL STRESS DURING COVID-19 PANDEMIC RELATED LOCKDOWN IN PAKISTAN

Ahmad Bilal, Zahida Khalid, Sana Fatima

Department of Applied Psychology, The Islamia University of Bahawalpur, Bahawalpur, Pakistan

Background: There had been recent Western studies that reported the parental stress during COVID-19 pandemic. The present study was designed with an aim to find out the socio-demographic factors of parental stress in a sample of parents in Punjab province of Pakistan. **Methods:** Four hundred and sixty-six (466) parents were recruited from different cities of Punjab by administering a Google form based cross-sectional survey over social media platforms including WhatsApp and Facebook. The study was conducted from March to May 2020. The Urdu standardized version of Parental Stress Scale was administered as the main measure. Each participating parent was requested to give explicit consent before enrolling for the study. The data was analysed using SPSS-25. **Results:** There were statistically significant differences in parental stress with respect to marital status, age, gender, and employment status of parents. On the other hand, there were no statistically significant differences in parental stress with respect to the number and age of children, and education of the parents. **Conclusion:** There were 7 socio-demographic factors of parental stress. Some of those factors impact parental stress while some other factors did not impact parental stress. Future studies may be conducted with participants from other provinces too and by including other parenting related variables.

Keywords: COVID-19, Parental Stress, Stress, Punjab, Pakistan

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INTRODUCTION

The world is currently experiencing a new pandemic called Severe Acute Respiratory Syndrome-Coronavirus-2 (SARS-CoV-2) or simply called COVID-19.¹ The WHO imposed Public Health Emergency of International Concern (PHEIC) on 30th January 2020.² As a matter to curtail the spread of COVID-19 pandemic, most countries resorted to lockdown including Pakistan during the 1st wave of the pandemic. The lockdown brought changes in daily routines, adjustment with the remote work style, home-schooling children as schools were shut down during lockdown.³

Living in lockdown is a potentially stressful experience which also affects the parenting practices at home.⁴ The closure of businesses and change in work routine combined with monitoring children at home during the pandemic was the major source of stress for parents.⁵

Recent studies point toward the COVID-19 related parental stress.⁶ A study reported that at least one out of every three parents have experienced the worsening of their mental health during the pandemic.⁷ The new pandemic COVID-19 has brought many stressors, one of them is parental role. Parents experienced stress due to change in parenting practices, the pressure to home school kids, and economic burdens as well.⁸⁻¹⁰ Parental stress may be defined as a kind of psychological reaction that occurs when environmental demands are inconsistent with expectations of self or others or when the parents do not have resources to meet these demands.¹¹ A recent study in Pakistan reported

occurrence of parental stress during COVID-19 pandemic.¹²

The parents who have not directly experienced the pandemic may also get stressed due to COVID-19 pandemic.¹³ The limited interaction and continuous stay at home with a constant monitoring of children and looking after work issues may make parents vulnerable to experience levels of stress.³ The most pronounced stressor experienced by parents during COVID-19 pandemic was change in mood and experience of heightened stress levels.¹⁴ A recent study reported several demographic differences in experience of parental stress during COVID-19 pandemic.¹⁴ The similar study reported that environmental experience of COVID-19 pandemic was the greater source of parental stress.

Despite multitude of work on the psychological aspects of COVID-19 on parents, there is currently lack of empirical research focusing on the demographics of parental stress during COVID-19 pandemic in Pakistan. This lack of research is consistent with previous study as well.¹⁵ The present study was conducted to find out the demographics (gender, age, education, occupation, and marital status of parents; number of children and age of children) of parental stress during COVID-19 pandemic.

METHODOLOGY

The study employed the cross sectional survey research design. The 466 parents, with Mean±SD 34.5±0.71 years of age (fathers=229, mothers=237) from different cities including Rawalpindi, Lahore, Faisalabad, Multan, Dera Ghazi Khan, Bahawalpur, and Rahim Yar

Khan of Punjab Province participated in the online survey administered using Google forms over social media platforms (WhatsApp and Facebook) due to lockdown. The mean age of the parents was around 35 years. The parents living in Punjab province were included in the study while parents living in other provinces or those without internet access were excluded from the study. The study was conducted from March to May 2020.

The data were collected after obtaining the approval from the Research Ethics Committee at the institution of the authors affiliations vide No. REC/B-G4/S20. The participation in the survey implied signing the informed consent as the first part of survey contained informed consent.

The Urdu standardized version of Parental Stress Scale (PSS) was administered to the participants. The original PSS was developed by Berry & Jones in 1995 and had 18 items designed in 5 point likert format. The scores on the PSS range from 18 to 90. The higher scores indicate higher levels of parental stress. The Cronbach Alpha reliability of the scale is 0.83.¹⁶ The locally standardized Urdu version had Cronbach Alpha of 0.81.

The data was analysed on SPSS-25. The analysis was presented by descriptive statistics, independent samples *t*-test, and analysis of variance.

RESULTS

Table-1 shows the socio-demographic characteristics of participants at baseline. There were 466 participants in the study, of which 229 (49%) were fathers and 237 (51%) were mothers. Majority, (106, 22.7%), 128 (27.5%), and 104 (22.3%) of parents belonged to age range of 26–35, 36–45, and 46–55 years respectively. There were only 52 (11.2%) parents of age 56 years or above. The mean age of parents was around 35 years. Most (130, 28%) of them were educated up to Masters and Bachelors (117, 25%). Sixty (13%), and 154 (33%) parents were employed in private and government sectors respectively compared to 30 (6%) unemployed and 139 (30%) stay at home parents. Currently married parents were 430 (92%), 10 (2%) were divorced, and 26 (6%) were widowed; and 91 (20%) parents had 1 child, 174 (37%) had 2–3 children whereas 201 (43%) had 4 children. Majority of children (343, 74%) were under 18 years of age compared to 123 (26%) children of age ≥18 years.

Table-2 describes the result of *t*-tests computed to find out differences in parental stress in parents of younger and older children and between fathers and mothers. The differences were statistically significant for gender ($p < 0.05$) in parental stress of fathers and mothers respectively, although the effect size was small. On the other hand,

there were no statistically significant differences ($p = 0.63$) in parental stress with respect to age of their children.

Table-1: Socio demographic characteristics of participants at baseline (n=466)

Socio Demographic Variables	Baseline Characteristics	n	%
Gender	Fathers	229	49.1
	Mothers	237	50.9
Age Mean±SD		34.5±0.71	
Age of Parents (Years)	18–25	76	16.3
	26–35	106	22.7
	36–45	128	27.5
	46–55	104	22.3
	56+	52	11.2
Education	Matric	103	22.1
	Inter	82	17.6
	Bachelors	117	25.1
	Masters	130	27.9
	MPhil/PhD	34	7.3
Occupations	Stay Home Parents	139	29.8
	Government Jobs	154	33.0
	Business	83	17.8
	Unemployed	30	6.4
	Private Jobs	60	12.9
Parents' Marital Status	Currently Married	430	92.3
	Divorced	10	2.1
	Widowed	26	5.6
No. of Children	1	91	19.5
	2–3	174	37.3
	4	201	43.1
Ages of Children (Years)	1–17 years	343	73.6
	≥18 years	123	26.4

Table-2: Results of several *t*-tests

Variable	Parental Stress						
	Mean±SD	<i>t</i>	Df	<i>p</i>	LL	UL	Cohen's <i>d</i>
Children							
Younger 1–17 Years (n=343)	38.55±8.49	0.48	464	0.63	-1.32	2.18	0.05
Older ≥18 Years (n=123)	38.12±8.45						
Parents							
Fathers (n=229)	37.56±9.13	-2.18	445.96	0.03	-3.25	-0.16	0.20
Mothers (n=237)	39.27±7.71						

LL=Lower limit, UL=Upper limit

Table-3 shows the results of analysis of variance computed to find out the differences in parental stress with respect to marital status and number of children. The results were statistically significant for marital status [$F(2,463) = 8.19, p = 0.000, \eta^2 = 0.03$], although, effect size was small, while non significant for number of children [$F(2,463) = 0.52, p = 0.59, \eta^2 = 0.00$]. The currently married parents had lower parental stress as compared to divorced or widowed parents.

Table-4 presents the results of analysis of variance computed to find out the differences in

parental stress with respect to age, education, and occupation of parents. The results were statistically significant for age of parents [F(4,461)=5.83, $p=0.000$, $\eta^2=0.04$], and occupation status [F(4,461)=7.90, $p=0.000$, $\eta^2=0.06$], although, effect sizes were small while non significant for education of parents [F(4,461)=0.89, $p=0.46$, $\eta^2=0.00$]. The young and unemployed parents had more parental stress as compared to older and employed parents respectively.

Table-3: Analysis of variance for differences in marital status and number of children (n=466)

Variable	Parental Stress			
	Mean±SD	F(2)	p	η^2
Marital Status of Parents				
Married	38.08±8.23	8.19	0.000	0.03
Divorced	48.40±9.58			
Widowed	40.38±9.79			
No. of Children				
1 Child	38.78±8.67	0.52	0.59	0.00
2-3 Children	38.79±9.17			
4 Children	37.97±7.75			

η^2 =Partial Eta Square

Table-4: Analysis of variance for differences in age, education and occupation (n=466)

Variable	Parental Stress			
	Mean±SD	F(4)	p	η^2
Age of Parents				
18-25 y	42.25±9.51	5.83	0.000	0.04
26-35 y	38.46±8.11			
36-45 y	37.17±7.97			
46-55 y	36.81±7.21			
56+ y	39.17±9.59			
Education of parents				
Matric	38.24±8.55	0.89	0.46	0.00
Inter	36.96±7.92			
Bachelor	38.82±8.18			
Master	39.08±8.65			
MPhil/PhD	38.76±9.80			
Occupation of Parents				
Stay at home	39.57±7.87	7.90	0.000	0.06
Govt: Employed	37.80±8.87			
Business	35.31±7.00			
Unemployed	44.46±9.29			
Private Employed	38.73±8.38			

η^2 =Partial Eta Square

support and dual burden of parenting and work activities made the parents experience more stress levels during COVID-19 pandemic.¹⁴

The age of the parent had a significant effect on parental stress. The young parents had experienced more parental stress than parents in middle age group. Likewise, the parents in old age also exhibited greater parental stress. Both the young age and old age of parents was the predisposing factor for experience of parental stress. The parents in young age lacked in parenting experience which made them vulnerable to experience more parental stress. The results are consistent with previous work.¹⁴

The gender of the parents was a significant factor in parental stress. The mothers experienced greater levels of parental stress as compared to fathers. Increased burden of household chores, including baby sitting and managing partner and elder children at home became the source of stress for female parents. Other studies reported similar results.¹⁴

The employment status of the parents was another factor responsible for parental stress. The unemployed parents had shown the highest levels of parental stress as compared to parents who had been employed. The earlier research indicated that economic recession and unemployment was the biggest source of stress among individuals in a society.¹⁸

On the other hand, there had been other socio-demographic factors which had no role in parental stress during COVID-19 pandemic. These were number of children, ages of children, and education of parents. Both number of children and age of children did not significantly impact parental stress. The parents with 1 or more children or having children less than 18 years of age were not different from those parents having 2 or more children or having children of or above 18 years of age. The education of parents also showed no statistically significant differences in parental stress. The education of the parents also did not determine parental stress. It was due to the fact that most parents who participated in the study were educated.

DISCUSSION

The present study was conducted with an aim to find the socio-demographic factors of parental stress in a sample of parents in Punjab province of Pakistan. The present study found that different socio-demographic variables had different effects on parental stress. The parents who had been currently living with their married partners had lower levels of parental stress as compared to divorced or widowed parents indicating that being currently married and living with married partner provides social support. The previous work indicates that the social support in the form of living with the married partner buffers against the stress.¹⁷ Another research indicated that the absence of social

LIMITATIONS AND RECOMMENDATIONS

The present study was limited to participants from Punjab province only. Both members of a parent dyad could not be recruited. The future studies may be conducted with participants from all over Pakistan and by including other variables of interest related to parenting practices.

CONCLUSION

Marital status, age, gender, and employment status of the parents are among the socio-demographic factors

of parental stress during COVID-19 pandemic that have significant impact. Number and ages of children, and educational status of parents do not have statistically significant impact on parental stress during the pandemic.

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Address for Correspondence:

Ahmad Bilal, Assistant Professor, Department of Applied Psychology, The Islamia University of Bahawalpur, Pakistan.
Cell: +92-300-8469524
Email: ahmadbilal4@gmail.com

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Contribution of Authors:

AB: Conceptualization, revising it critically for intellectual content and final approval

ZK: Conceptualization, data acquisition and analysis, writing manuscript and final approval

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ORIGINAL ARTICLE

PROGNOSTIC SIGNIFICANCE OF TUMOUR THROMBUS CONSISTENCY ON CANCER-SPECIFIC SURVIVAL IN RENAL CELL CARCINOMA PATIENTS

Tauheed Farid, Nayab Farid*, Muhammad Izhar**, Sara Asmat***, Sidra Humayun[†], Mohsin Ali^{††}

Department of Urology, Pak International Medical College, *Department of Pathology, Kabir Medical College, **Institute of Kidney Diseases, ***Department of Community Medicine, [†]Pathology, ^{††}Pharmacology, Muhammad College of Medicine, Peshawar, Pakistan

Background: Different studies have focused upon patients suffering from renal cell carcinoma (RCC) with inferior vena cava tumour thrombus. One biotic feature of renal RCC is venous system invasion. Both renal vein and inferior vena cava may be involved. The aim of current study was to evaluate the prognostic significance of the extent of thrombus in renal cell carcinoma patients with involvement of inferior vena cava. **Methods:** A total of 413 individuals were recruited in the study. All collected data were analysed retrospectively. Radical nephrectomy along with tumour thrombectomy was performed in all the participants. The pathological specimens were analysed for morphological feature, i.e., solid vs friable thrombus. To established clinicopathological predictor, Kaplan-Meier estimate and Cox regression analyses were done. **Results:** Friable and solid venous tumour thrombus (VTT) were found in 188 (46%) and 225 (54%) patients, respectively. For solid VTT, the Median Cancer Specific Survival (CSS) was 50 months while for friable VTT, the median CSS was 45 months. Thrombus consistency had no significant association with clinical features such as metastatic spread, pathological stage, perinephric fat invasion, and higher Fuhrman grade. Both survival analysis and Cox regression failed to be considered as a prognostic marker for CSS. **Conclusion:** Thrombus consistency appears not to be independently associated with survival in patients suffering from RCC.

Keywords: Renal cell carcinoma, survival, thrombus consistency, inferior vena cava

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INTRODUCTION

Renal cell carcinoma (RCC) of kidney occurs in both sexes in adult stage of life. RCC comprises 2–3% of all adult malignancies.¹ In the modern world the number of RCC patients is increasing by 2.5% annually. Since 2012, in Asian countries, about 121,000 patients with renal cancer were recorded. In Pakistan there is no authentic and reliable data available for the prevalence of RCC.² Most of the renal cancers are diagnosed incidentally. Amongst renal tumours, RCC constitutes the most frequent diagnosis, i.e., about 90% of total renal malignancies.³ Number of risk factors such as smoking, obesity, and hypertension are responsible for RCC disease.⁴ RCC forms a venous tumour thrombus (VTT) which extends into the inferior vena cava (IVC) as well as into the renal vein.⁵ RCC extension takes place in 4–10% of individuals suffering from it. RCC with VTT is associated with worse characteristics.⁶ Venous migration and VTT formation are distinctive characteristics of RCC and is measured as an adverse prognostic factor for RCC.⁷ In the current study, the aim of the study was to evaluate the prognostic significance of solid versus friable renal tumour thrombus (both renal vein and IVC thrombosis) with mean Cancer Specific Survival (CSS) in RCC patients in Peshawar.

SUBJECTS AND METHOD

A total of 413 patients were enrolled in our study who underwent radical nephrectomy and IVC tumour thrombectomy of solid or friable VTT came to Institute of Kidney Diseases, Peshawar. After surgery, follow-up of the enrolled patients was performed after every four months for the first year, every six months up to the 5th year and annually after that. Those patients with incomplete records of level of tumour thrombus, Fuhrmann grade, TNM (tumour, nodes, metastasis) staging and perinephric fat invasion (PFI) were excluded from the study. The patients were stratified based on their age and gender. By using the Bias software (epsilon, Frankfurt, Germany), all the collected data were analysed. The distribution of inferior vena cava tumour thrombus consistency in definite clinicopathological variables (pathological stage, VTT level, histological subtype, perinephric fat invasion, nodal status, distant metastasis, IVC wall invasion) were measured by means of Chi-square test. Tumour stages were categorised through Fuhrmann grade and was assessed by using Fisher's exact test. Survival analysis was performed by means of Kaplan–Meier analysis and all the variables were compared by means of log-rank test. The clinicopathologic variables were carefully chosen for assessment and their significance on cancer specific survival. Analyses (Univariable and multivariable) were done by means of Cox proportional

hazard regression model to evaluate the influence of variable on survival. All the performed tests were two sided, and $p < 0.05$ was considered significant.

RESULTS

Our results show 54% patients with solid and 46% patients with friable tumour thrombus in the IVC. Of these patients, 215 were pN0/NxM0 while 28 and 170 patients were pN+M0 and pN+M+, respectively. The mean follow-up was 24 months and about 273 (66.1%) enrolled patients died due to RCC during our study period. Eighty-five (20.5%) patients were alive and were in disease-free state while 55 (13.4%) were alive but with distant cancer metastasis. The mean age of the study population was 61.5 years. The frequency of male patients was dominant over female patients and solid IVC VTT was more common than friable IVC VTT in both type of patients. The median CSS of patients with friable and solid thrombus, pathological stage as shown in Table-1. The pathological stage of each renal cell carcinoma sample was determined according to TNM (Tumour, Node, Metastasis) classification. The histological classification of tumour cells was performed as per Fuhrman nuclear grading system. As per Mayo VTT Classification, most of our patients fell into level I and II category (Table-2).

Table-1: Cancer Specific Survival of Patients

Thrombus consistency	No. of patients	Actuarial 5-yr CSS (%)	Median CSS (Months)	p
Friable	188	43.86	45	0.8
Solid	225	49.55	50	

Table-2: Characteristics of RCC patients with IVC TT [n (%)]

Variables	All patients	Friable IVC TT	Solid IVC TT	p
Patients	413	188 (46)	225 (54)	-
Median CSS		45	50	$p=0.8^a$
Pathological State				
pT3 ^b	251 (60.76)	112 (27.12)	139 (33.66)	$p < 0.001^a$
pT3 ^c	130 (31.5)	61 (14.77)	69 (16.7)	
pT4	32 (7.74)	15 (3.63)	17 (4.12)	
Mean Value		188 (45.52)	225 (54.48)	
Fuhrman Nuclear Grade				
G1	9 (2.2)	4 (0.1)	5 (0.12)	$p < 0.001^b$
G2	96 (23.25)	39 (9.4)	57 (13.8)	
G3	194 (47)	83 (20.1)	111 (26.88)	
G4	114 (27.6)	62 (15.01)	52 (12.6)	
Mean Value		44.6%	55.4 %	
VTT Level (Mayo classification)				
Level I	113 (27.4)	48 (42.48)	65 (57.52)	$p=0.03^a$
Level II	136 (33)	75 (55.15)	61 (44.85)	
Level III	85 (20.5)	37 (43.53)	48 (56.47)	
Level IV	79 (19.1)	28 (35.44)	51 (64.56)	
Mean Value		45.5%	54.5%	
Sex				
Male	278 (67.31)	123 (44.24)	155 (55.76)	$p=0.2^a$
Female	135 (32.69)	65 (48.15)	70 (51.85)	
Age				
Mean	61.5	61.0	61.9	$p=0.1^c$

a: Chi-square test, b: Fisher's exact test, c: t-test

Kaplan-Meier curves of cancer specific survival in individuals with friable and solid vena caval tumour thrombus are shown (Figure-1). Log-rank test shown no significant difference in cancer specific survival among individuals suffering from friable or solid IVC tumour thrombus (Table-3). The p of multivariable Cox regression analysis for histological subtype, nodal state and distant metastasis, perinephric fat invasion and IVC wall invasion fall in significant area (Table-4).

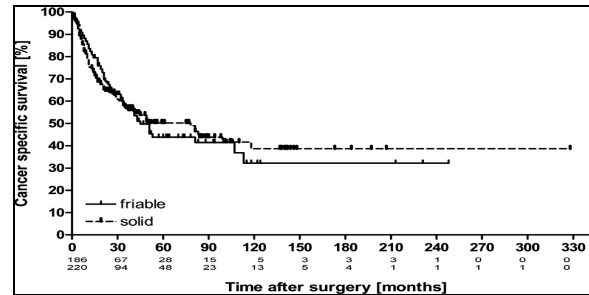


Figure-1: Kaplan-Meier estimates of cancer specific survival probability in patients with RCC and VTT involving the IVC stratified by thrombus consistency

Table-3: Kaplan-Meier estimates and log rank test

Patient at Risk	1-Year	3-Year	5-Year	p
Friable	58	45	15	>0.05
Solid	215	165	76	

Table-4: Multivariate Cox regression analyses predicting cancer specific survival in RCC patients (n=413)

Histological Subtype				
Clear cell RCC	371	169	202	$p < 0.005$
Papillary RCC	29	11	18	
Chromophobe RCC	13	8	5	
Nodal State				
N0/Nx	297	134	163	$p < 0.001$
N+	116	54	62	
Metastasis				
M0/Mx	303	145	158	$p < 0.001$
M1	110	43	67	
Perinephric Fat Invasion				
Yes	266	124	142	$p < 0.001$
No	147	64	83	
IVC Wall Invasion				
Yes	119	60	59	$p < 0.01$
No	294	128	166	

DISCUSSION

Both RCC with VTT are life-threatening conditions and the treatment of RCC with vascular invasion is challenging and controversial. Surgical resection is found to be the best treatment procedure for therapy in non-metastatic disorders.^{8,9}

The thrombus consistency (friable vs solid) in this study was non-significant to CSS. Patients with non-metastatic disease and friable VTT have higher-risk disease than patients with non-metastatic disease and solid VTT. A study by Antonelli¹⁰ supports our results. This suggests that VTT consistency was not an

independent prognostic role in individuals suffering from RCC and no influence of thrombus consistency on CSS had been observed. Both solid and friable VTT showed altered features. In our subjects, poor outcome in individuals suffering from friable thrombus consistency has been found. Novara *et al* and Weiss *et al*, reported that individuals suffering from non-metastatic disorder and friable VTT had higher-risk disease than individuals suffering from non-metastatic disorder and solid VTT.^{11,12} The pathological state (TNM classification) and Fuhrman grading of RCC samples also showed significant association with poor CSS. In individuals suffering from non-metastasized tumour, the Fuhrman grade and thrombus level were identified as independent predictors of poor survival representing the tumour's capability to spread aggressively.¹³

Upon multivariable analysis of overall survival (OS), friable thrombus consistency was found significant in non-metastasized individuals as previously observed by Vergho *et al*.¹⁴ Tumour thrombus grade I and II clinically have shown significantly increased survival in comparison to grades III and IV, and thus proved the histological subtype as independent prognostic marker in non-metastasized individuals. Steffens *et al*¹⁵ also showed that histological parameters of RCC influences the long-term prognosis in patients. Multivariate analysis of thrombus level with perinephric fat and IVC wall invasion showed significant association with poor CSS in RCC patients. These results were found to be similar to previous study by Bertini *et al*.¹⁶

CONCLUSION

Tumour Thrombus Consistency (friable or solid) has no prognostic significance on Cancer Specific Survival in patients with Renal Cell Carcinoma involving Inferior Vena Cava.

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Address for Correspondence:

Dr. Mohsin Ali, Department of Pharmacology, Muhammad College of Medicine, Peshawar, Pakistan. **Cell:** +92-321-5275212

Email: Mohsin.ibms86@gmail.com

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ORIGINAL ARTICLE

RELATIONSHIP OF BODY MASS INDEX AND WAIST HIP RATIO WITH SCORES OF DEPRESSION, ANXIETY AND ACADEMIC PERFORMANCE IN FEMALES

Hamid Hassan, Muhammad Abdullah*, Hafsa Naseer*, Amna Mahmood*, Maria Siddiqui*, Naveera Ayub*

Department of Physiology, *2nd Year Student, Nishtar Medical University, Multan, Pakistan

Background: Enhanced adipose tissue is likely to affect an individual's mental health. This study aimed to link its indices (BMI/WHR) with indices of depression/anxiety in female medical students. **Methods:** One hundred and six female medical students were equally divided into non-obese ($BMI \leq 24.9 + WHR < 0.8$) and obese ($BMI > 24.9 + WHR \geq 0.8$) groups. Depression/anxiety scores were calculated using Aga Khan University Anxiety Depression Scale (AKUADS), Beck's Depression Inventory (BDI) and Beck Anxiety Inventory (BAI) while last exam percentage was considered as an indicator of academic performance. **Results:** AKUADS and BDI scores of obese subjects were higher than those of non-obese subjects ($p=0.018$ and $p=0.030$ respectively) while academic score of obese subjects was lesser than that of non-obese subjects ($p < 0.0001$). Within non-obese and obese subjects, and within total study population AKUADS scores were positively correlated to BMI and WHR ($\rho=0.39$, $p=0.00$), ($\rho=0.38$, $p=0.00$), ($\rho=0.36$, $p=0.00$), ($\rho=0.38$, $p=0.00$) and ($\rho=0.35$, $p=0.00$), ($\rho=0.35$, $p=0.00$) respectively. BDI scores within non-obese group, obese group, and in total population were positively correlated to BMI and WHR too ($\rho=0.40$, $p=0.00$), ($\rho=0.40$, $p=0.00$), ($\rho=0.32$, $p=0.01$), ($\rho=0.34$, $p=0.01$), and ($\rho=0.30$, $p=0.00$), ($\rho=0.31$, $p=0.00$) respectively. AKUADS and BDI scores had a negative correlation with academic score in both non-obese and obese subjects ($\rho=-0.29$, $p=0.03$), ($\rho=-0.30$, $p=0.02$), and ($\rho=-0.44$, $p=0.00$), ($\rho=-0.35$, $p=0.00$) respectively. **Conclusion:** Within obese female medical students enhanced degree of depression/anxiety affects academic performance considerably; this calls for shrewder monitoring of their mental wellbeing.

Keywords: Depression, anxiety, obesity, female medical students, medical education, academic score

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INTRODUCTION

Depression is a common mental disorder characterized by 'a feeling of persistent sadness and a lack of interest or pleasure in activities which used to be rewarding or enjoyable previously'¹ while anxiety is described as 'a feeling of uneasiness and undue concern to a situation that is only subjectively seen as menacing'.² Of the most reliably used inventories to deduce the degree of depression and/or anxiety, within a population, are the Aga Khan University Anxiety and Depression Scale (AKUADS), Beck's Depression Inventory (BDI) and Beck Anxiety Inventory (BAI).

Out of many psychological, social and biological factors governing depression and anxiety, obesity has become a cause of concern in recent times since it has been found that obese individuals are not only at a greater risk of development of aforementioned psychiatric disorders³ but they perform poorly at various fronts of everyday life⁴ (e.g., academic⁵) too.

The World Health Organization (WHO) describes obesity as a medical condition in which excessively accumulated fat in the body may have started to adversely affect health of an individual.⁶ The extent of obesity can be evaluated through a myriad of indices, of

which the two most frequently adopted ones are Body Mass Index (BMI) and Waist Hip Ratio (WHR).⁷

Since, obese women are more likely to harbour stress disorders, contemporary researchers have been trying to correlate anthropometric parameters of obesity (like BMI, Waist Circumference, Hip Circumference and WHR) with scores of depression and anxiety⁸ in their quest to settle the myriad of pathophysiologicals responsible for emergence of stress disorders in them.

Studies have deduced that several endocrine abnormalities tend to exist in obese women with reported changes of BMI and WHR which lead to development of depression and anxiety through several intricate pathways. One of the most probable endocrine parameter likely to be deranged in obese women with shifts in BMI and WHR is their serum oestrogen level.⁹ Researchers suggest that effect of oestrogen on mood is due to its ability to increase the levels of serotonin and beta-endorphins which are the major neurotransmitters associated with the development of mental states that harbour the feeling of generalized wellbeing and content.¹⁰

Certain recent studies have shown that expression of oestrogen receptor α gene is reduced in obese females (as compared to normal weight females)¹¹

which is likely to reduce the positive effect of oestrogen on serotonin levels and hence is likely to result in the development of psycho-psychiatric disorders in obese women¹². Moreover, conditions like Polycystic Ovarian Syndrome (PCOS –low oestrogen state) are also associated with low levels of serotonin and women harbouring them report more symptoms of depression and anxiety.¹³ All these facts put together, do suggest that obese females are likely to be at an increased risk of development of mood disturbances.¹⁴

In this study, we tried to explore if obese female medical students are likely to have low serotonin levels and hence are likely to depict more obvious symptoms of depression and anxiety which in turn is likely to affect their academic performance in a negative fashion.

SUBJECTS AND METHODS

It was a cross-sectional comparative study conducted at various medical institutes of Multan between the months of January and June 2020. The sample size was calculated with a power of 90% and an alpha level of 5% by using the formula:

$$n = \frac{\sigma^2 (Z_{1-\alpha/2} + Z_{1-\beta})^2}{(\mu_0 - \mu_1)^2}$$

from the WHO software ‘Sample size determination in health studies–A practical manual¹⁵ version 2.0.21.

One hundred and six (106) age and ethnicity matched female subjects (aged 18–23 years) were selected through convenience sampling. Subjects were equally divided into two groups. Group A consisted of 53 non-obese female subjects (controls, BMI≤24.9 and WHR<0.8)¹⁶, while Group B had 53 obese female subjects (BMI>24.9 and WHR≥0.81)¹⁶. Students with a BMI>30 and/or WHR>0.94 (morbidly obese females¹⁶) with family history of depression, with previous history of psychiatric illness and/or drug abuse were excluded.

Data was collected after taking written informed consent, from the subjects on a specifically developed proforma. Portable weighing machine was used to measure weight (in Kg), and portable height/length measuring board was used to measure height (m). Waist Circumference (WC) and Hip Circumference (HC) were recorded on WHO procedures of 2008 guidelines. BMI and WHR were derived through standard formulas using these indices. To deduce the degree of depression and/or anxiety AKUDAS (a 25-item, 13 psychological, 12 somatic) questionnaire, available in both English and Urdu was used. The questions were answered numerically (0–4, based on the mood pattern of past 2 weeks)¹⁷. It has a Cronbach’s alpha value of 0.83.¹⁸ BDI-II [a self-reporting questionnaire used worldwide is actually a modified form of its original version (1961) which consists of 21-items, each of which is ranked by subject between 0 and 3 as per severity of features experienced over last 2

weeks. The scores of each question, when added up, determine the intensity of depression (the minimum score being zero and maximum being 63).¹⁹ It has a Cronbach’s alpha value between 0.75 and 0.92²⁰, and BAI based on 4-point Likert scale consists of 21 items for assessment of anxiety as a disorder apart from depressive ailment. Calculation and criteria for BAI is the same as that for BDI, with a range of 0–3 for severity of features.²¹ It has a Cronbach’s alpha value of 0.92²² scales were utilized while percentage of academic score (AS) achieved in last professional exam was considered as an indicator of academia.

The data was entered in IBM SPSS-25 and it was analysed first for normality distribution via Shapiro-Wilk and Kolmogorov Smirnov tests. Most of study parameters were non normally distributed and hence non parametric tests were chosen for inferential statistics. Mann-Whitney U Test was applied to compare [Median (IQR)] scores of anxiety, depression and academic performance (of both groups) while Spearman’s rho correlation was used to derive correlations between psychiatric and anthropometric parameters.

RESULTS

General characteristics of both the study groups have been presented in Table-1. The AKUADS and BDI scores of Group B subjects were higher ($p=0.018$ and $p=0.030$ respectively) than AKUADS and BDI scores of Group A subjects while academic score (AS) of Group B subjects was lesser than academic score of Group A subjects ($p=0.000$). This comparison, drawn through application of Mann-Whitney U Test has been represented in Table-2.

Table-1: Median (IQR) of (non-normally distributed) study parameters

Parameter	Group A (Non-Obese) n=53	Group B (Obese) n=53
Age (Year)	19.0 (19.0–23.0)	22.0 (19.0–23.0)
Height (meter)	1.59 (1.48–1.67)	1.58 (1.47–1.70)
Weight (Kg)	50 (39–64)	65 (55–79)
BMI	19.50 (16.2–24.0)	26.4 (25.0–31.1)
WC (Cm)	72 (52–80)	86 (77–96)
HC (Cm)	97 (80–109)	101 (94–106)
WHR	0.75 (0.64–0.80)	0.84 (0.80–0.92)

Table-2: Comparison of AKUADS, BDI, BAI and academic scores of non-obese and obese subjects [Median (IQR)]

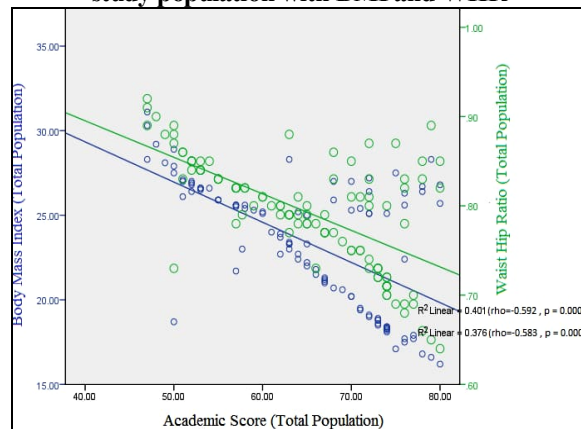
Parameter	Group A (Non Obese)	Group B (Obese)	p
AKUADS	19 (1–60)	29 (1–64)	0.018
BDI	14 (1–48)	26 (1–50)	0.030
AS	71 (50–80)	57 (47–80)	0.000
BAI	15 (1–60)	10 (1–53)	0.137

Within the subjects of Group A, AKUADS and BDI scores were positively correlated to BMI and WHR and inversely to AS and so was true for Group B (Table-3).

Table-3: Correlation of AKUADS, BDI and BAI scores with BMI, WHR and academic score within the groups

Parameter	Group A (Non Obese)						Group B (Obese)					
	BMI		WHR		AS		BMI		WHR		AS	
	<i>rho</i>	<i>p</i>	<i>rho</i>	<i>p</i>	<i>rho</i>	<i>p</i>	<i>rho</i>	<i>p</i>	<i>rho</i>	<i>p</i>	<i>rho</i>	<i>p</i>
AKUADS	0.397	0.003	0.389	0.004	-0.295	0.032	0.366	0.007	0.383	0.005	-0.440	0.001
BDI	0.407	0.002	0.408	0.002	-0.305	0.026	0.328	0.017	0.342	0.012	-0.355	0.009
BAI	-0.208	0.135	-0.191	0.170	0.183	0.190	0.160	0.254	0.189	0.176	-0.254	0.066

Academic scores of the subjects in Group A had a negative correlation with both the indices of obesity in use, i.e., BMI and WHR ($rho = -0.884$, $p = 0.000$) and ($rho = -0.880$, $p = 0.000$) respectively, and so was observed within Group B ($rho = -0.463$, $p = 0.000$) and ($rho = -0.486$, $p = 0.000$) respectively. Also for the whole of study population a negative correlation was seen between academic performance and indices of obesity (Figure-1).

Figure-1: Correlation of academic score of total study population with BMI and WHR

BAI scores were neither significantly different between Group A [15 (1–60)] and Group B [10 (1–53)] subjects ($p = 0.137$), nor showed a significant inverse correlation with academic performance in either of the groups ($rho = 0.183$, $p = 0.190$) and ($rho = -0.254$, $p = 0.066$) respectively. BAI scores neither within Group A nor within Group B had a significant correlation with BMI and/or WHR ($rho = -0.208$, $p = 0.135$), ($rho = -0.191$, $p = 0.170$) and ($rho = 0.160$, $p = 0.254$), ($rho = 0.189$, $p = 0.176$) respectively. Even for the whole of study population put together, a significant correlation of BAI with BMI, WHR and AS could not be derived ($rho = -0.131$, $p = 0.182$), ($rho = -0.119$, $p = 0.223$), and ($rho = -0.026$, $p = 0.792$) respectively.

DISCUSSION

AKUADS and BDI scores of obese individuals of Group B were markedly higher than the scores of non-obese individuals of Group A, a fact which has been highlighted by others²³. The former did not show as good a performance on academic front as the latter indicating that depression caused by obesity-induced

pathophysiological changes affect academic performance in a negative fashion.²⁴

Some studies have been trying to correlate anthropometric parameters of obesity (such as BMI, WC, HC and WHR) with scores of depression and anxiety in females as an enhanced adiposity status adversely affects their psychiatric wellbeing in several ways. One of several probable pathophysiological causes of these is deranged oestrogen level in obese females which lowers the levels of mood related neurotransmitters (like serotonin and beta-endorphins) in them and hence reduces the feeling of generalized wellbeing and content which in turn can result in an enhanced degree of depression and/or anxiety.²⁵

AKUADS and BDI scores (in individual study groups) were positively related to both the indices of obesity, i.e., BMI and WHR. It has been reported that oestrogen within the serotonergic pathways affects the productivity and/or responsiveness of serotonergic receptors within mood controlling centres, e.g., in raphe nucleus. Since obesity is associated with reduced levels and/or resistance to oestrogen, a reduced serotonergic push on mental wellbeing of obese females is evitable, providing a fertile ground for emergence of common mood disorders like depression and anxiety.²⁵

An enhanced adiposity status leads to deranged levels of leptin as well as leptin resistance²⁶ which in turn can reduce the effects of leptin on both NMDA (N-methyl D-aspartate) and α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors within the mood controlling pathways.²⁷ This is most likely to result in the development of depressive disorders within obese females. This postulate, could have been the reason for the results of our study where we found that indices of obesity are positively correlated to indices of psychiatric wellbeing.

Indices of psychiatric wellbeing (AKUADS and BDI) as well as indices of obesity (BMI and WHR) had a negative correlation with academic performance, both within individual study groups as well as within whole of study population. This is in line with results of another study that projects that enhanced WHR and BMI (through intricate pathways already described) lead to an enhanced degree of stress disorders which makes obese individuals to cope with academic stress in a poorer way hence leading to poor academic scores.²⁸

As the fat mass enhances, it affects neuroendocrine²⁹, hypothalamo-pituitary adrenal³⁰ and serotonergic axis³¹ in a negative fashion which becomes

the base over which obese females do develop stress disorders which puts them in a vicious circle of derangements of homeostatic pathways that keep on affecting their mental capacities and hence their academia in the long run.

Though studies have found that BAI scores are positively correlated to indices of obesity³² and are negatively associated with academic performance of students³³, we could not derive such a finding. BAI scores, as per our results, neither had a positive correlation with BMI and WHR nor a negative one with AS. Since we could detect anxiety through AKUADS score and link that negatively to academic performance of study subjects, we conclude that BAI (most likely) is not a sound parameter to judge anxiety, and that AKUADS is a better and accurate indicator of degree of anxiety in the aforementioned population.

CONCLUSION

Obese students harbour significantly higher degree of depression and/or anxiety, as compared to their age and ethnicity matched non-obese counterparts, and they are more likely to collapse under academic and non-academic stress. Parents and educationists should be made aware to keep a vigilant eye on the mental wellbeing of their obese female students so that psychiatric collapses in them could be prevented.

LIMITATIONS & RECOMMENDATIONS

Our study was based on a single time observation, limited to a small sample size comprising of female subjects only. Cohort studies with enhanced samples comprising of both genders for future researchers are recommended.

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Address for Correspondence:

Dr. Hamid Hassan, Department of Physiology, Nishtar Medical University, Multan, Pakistan. **Cell:** +92-333-6107738
Email: ssaaqii@gmail.com

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REVIEW ARTICLE

ABSENTEEISM OF UNDERGRADUATE MEDICAL STUDENTS IN LARGE CLASS FORMAT LECTURES: CAUSES, SOLUTIONS AND THE WAY FORWARD

Rehana Rehman, Tabassum Zehra*, Amber Sultan**, Russell Seth Martins***, Rahila Ali*

Department of Biological and Biomedical Sciences, *Educational Development, **Educational Development and Surgery,

***Medical Student, Aga Khan University Hospital, Karachi, Pakistan

Despite compulsory attendance rule and strict policies, absenteeism from lectures is an ongoing phenomenon which is becoming a point of serious concern because it is associated with inadequate learning and poor academic performance of medical students. Student absenteeism springs from a variety of contributing factors that need to be addressed promptly and collectively. This review gives an overview of some of the factors that account for absenteeism interrelated with students, faculty, curriculum and learning environment. We propose that 'Need Based Standardized Faculty Development Programs', 'Innovative teaching and learning methodologies', 'Good governance' and 'Institutional commitment should aim to replace the traditional passive mode of instruction with active learning and inquiry-based approach to ensure considerable attendance of medical students. We are optimistic that the suggested measures will help to improve the quality and delivery of lectures for better attendance and a shift from traditional way of teacher centred learning, to self-directed learning.

Keywords: absenteeism, medical students, faculty, environment, technology enhanced learning

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INTRODUCTION

Absenteeism, defined as the conscious and deliberate act of being away from the physical space of the classroom, is a pressing concern for institutions of higher learning globally. Medical Colleges are not spared from absenteeism and report it as a common occurrence. Moreover, undergraduate medical students are more likely to be absent than their counterparts in other health science disciplines. This is worrying, since medical colleges are responsible for producing the physicians of the future. This includes inculcating in them key traits such as skill, knowledge, willingness to learn, responsibility, dependability, time-management, and altruism.

Absenteeism from lectures is a point of concern worldwide, as it ties in with inadequate learning and poor academic performance of medical students. Moreover, while many students opt to not attend lectures, others are inattentive during them.¹ The usefulness of lectures in undergraduate medical education has been well documented.² Lectures are one of the important modes of teaching in conventional as well as hybrid medical colleges, despite the adaptation of new curriculum.³ During lectures, committed teachers deliver organized and structured knowledge to students in an interactive way.⁴ This mode of teaching, where the main teaching strategy is the transfer of information by an educator, is considered one of the most economical and productive ways of imparting knowledge.³ Lectures also provide a platform where students can interact with teachers, observe them and identify them as potential role models.

Low attendance hence may affect this process and hinder learning, leading to poor academic performance of medical students and a compromise on the quality of future health care services provided.⁵ Thus, in addition to poorer academic performance, absenteeism also leads to poor motivation to learn, and can have long lasting effects on students' future work ethic, reliability and responsibility as physicians. The unfavourable qualities associated with absenteeism during student life are reflected in the work life of professionals and the quality of health care services provided.

Absenteeism of medical students in lectures adds an unnecessary burden on medical education both nationally in Pakistan and internationally. Since absenteeism is undoubtedly multifactorial, we need to identify a wide range of factors and causes leading to it. This would help the development of solutions aimed to reduce absenteeism, and thus strengthen the learning process and improve the academic performance of undergraduate medical students.

The aim of this review is to identify factors leading to absenteeism and in light of the review suggest solutions to reduce absenteeism.

METHODOLOGY

The authors selected articles from the past five years, and only if a recent article on the area addressed was not available an article more than five-year-old was considered.

Literature search was done extensively to identify different factors leading to absenteeism. After extensive literature search, four important factors were

identified and included in the review article along with possible solutions based on evidence-based medicine.

Search engines for causes and solutions related to students, faculty, curriculum and learning environment were PubMed, ERIC and Google Scholar.

For student related issues, 14 articles were used as a reference and cited, for faculty related issues, 6 were cited, for curriculum related issues, 10 articles were recruited and cited and for learning environment, 5 were cited.

Some of the articles were used more than once as a reference to justify the identified factor related issue along with proposed solutions.

Student-related Issues

Factors intrinsic to the student include lack of interest in the subject matter⁶, poor attitude and low motivation for learning⁷. Poor self-motivation, in particular, is a strong factor contributing towards absenteeism.^{7,8} In addition, bad habits of medical students also contribute to their absenteeism from classes. These include laziness, lack of proper sleep⁹, giving in to peer-pressure^{9,10} and socializing¹⁰. Some other non-academic factors cited by students involve part-time jobs¹¹, family issues^{4,11}, financial constraints⁴ and transport problems¹⁰.

Higher rates of absenteeism have been reported near assessments. This may be due to poor time management during the term⁵ resulting in last minute preparation for examinations.^{6,12}

Other factors include the lack of support by educators^{7,8}, inconvenient lecture timings, poor lecture content and repetition of topics taught, as well as the teaching modality itself. Medical students undervalue lectures, given the numerous textbooks available on every subject to learn theory from.¹³ The majority of students do not attend class due to dissatisfaction with the teaching style in lectures^{6,8,14} and due to their low perception of the quality of lectures. Thus, students also prefer self-study⁶ over, dry and didactic teacher-dominated lectures¹³. To make the matter worse, the lack of any real strictness or consequences of not attending lectures reinforces the triviality that students attribute to lectures.¹³

Solutions to Student-related Issues

Strategies to improve student attendance in medical school should aim to address the issue of dissatisfaction with teaching style by introducing newer teaching and learning initiatives in place of the traditional lectures, that better suit students' preferences. (Table-1).

- Implementation of a student satisfaction-centred monthly teaching evaluation system of all faculty with timely action taken to counteract any areas of deficiency in a particular faculty's teaching method and style. Student evaluation can be obtained through on-the-spot digital evaluation forms accessible easily

through a Quick response (QR) Code displayed on the lecture screen after lecture.¹⁵

- The success of the aforementioned evaluation system will be based upon how well the teaching administration is able to develop their faculty's teaching capability according to the requirements of the student body. This development should be in the form of teacher training programs, where fresher teaching methods (such as small-group activities) and materials (such as animated educational videos) are inculcated into the faculty's teaching style. Student feedback and suggestions should also be considered while implementing these teacher training programs.¹⁶
- Peer tutoring initiatives should be designed and implemented by the administration whereby medical students are chosen through a formal selection process to conduct small group teaching sessions for their peers or juniors. The topics taught in these sessions can be suggested by and decided upon by the student body, and the peer tutors should undergo training programs to equip them for teaching to small groups of students. A similar evaluation system as that suggested for the faculty can also be used to constantly improve the peer tutors.¹⁷

Faculty-related Issues

A recent study showed that several factors contributing to student absenteeism in Pakistan are faculty related.¹³ In this era of modern medical education, the role of teachers to deliver lectures and keep students motivated enough to attend and learn has become a point of concern. 'Boring' lectures delivered by lecturers have been documented as a leading cause of absenteeism in medical students.¹⁸ Therefore, there is a need to strengthen teacher attributes and teaching methodologies to counter student absenteeism.

There are several barriers to the acquisition of the desired changes in medical college faculty in Pakistan. National politics exerts its influence on the selection of medical faculty exclusive of their teaching abilities and communication skills.¹⁹ At the same time, the selection of faculty members is primarily based on their knowledge and clinical skills rather than their teaching abilities.²⁰ Moreover, there are minimal opportunities for faculty development.²¹ Where these opportunities exist, the lack of time and organizational support, faculty disinterest, complexity of the processes involved, and resistance to change have been identified as barriers to professional development. The lack of monitoring, evaluation and improvement of teaching through feedback is hindered by lack of concept of teacher-evaluation by students and peers.²² In addition, an absence of standardized criteria for the professional growth of medical teachers by the country's former regulating authority, the Pakistan Medical & Dental

Council (PMDC), is another factor contributing to the poor faculty development initiatives.

Solutions to Faculty-related Issues

In 2003, the Higher Education Commission of Pakistan realized the importance of introducing Faculty Development Programs (FDP) in equivalence with the international standards of teaching and education.²³ It is emphasized that faculty should be well prepared for their lectures, with full command over PowerPoint, animations, graphic tools and learning activities.¹² (Table-1).

- We recommend that FDPs should be designed to empower faculty to teach a diverse group of students and to learn different teaching methods ('Technology Enhanced/Blended Learning', 'Flipped Classrooms' and 'Effective PowerPoint Presentations') needed to engage millennial students.
- Furthermore, faculty members should be provided with resources, protected time, capacity development grants, appreciation, rewards and recognition, along with opportunities to implement learnt strategies.¹²
- Additionally, there must be a technology-support infrastructure available to help faculty members troubleshoot and solve technology-related problems without delay and disturbance in classroom activities.²⁴
- There should be a system to receive students' feedback, which should be shared with the faculty to take into account for subsequent classes.⁵
- There should be a counselling facility available which helps faculty members understand the basis of students' feedback, so that they can receive this feedback better and improve their performance.²⁴
- Moreover, senior faculty can mentor junior educators by giving them a clear sense of direction and constructive feedback in terms of lesson planning and delivery.²⁵

Curriculum-related Issues

The curriculum encompasses the learning outcomes, content, educational strategies, learning opportunities, assessment and the educational environment.²⁶ It has an important contribution towards students' decision to attend lectures.

Some significant and common factors reported in literature are students' ability to cover curricular content at their own pace, the focus of curriculum on factual material, unengaging learning experiences and assessment based on recall of factual knowledge. Students' decisions on lecture attendance are based on the anticipated learning, lecture content and nature of the material taught.²⁷ Some reported reasons for not attending lectures are poor content, irrelevant curriculum and poor delivery of curriculum.¹² Medical students also cite several other problems with the

medical school curriculum, describing it as too demanding, rigorous^{6,12}, ill-defined¹³ and overloaded⁴.

Moreover, students believe that their primary task is to memorize facts, arguing that the pre-clinical curriculum is mostly fact-based and the exact means by which this information is acquired is insignificant.²⁸

In a traditional lecture-based curriculum, absence from class may not prevent students from achieving the learning objectives, particularly if lectures are available as audio or video recordings.¹ The electronic format of lectures makes it convenient for students cover the lecture content at their own pace without even having to come to class. Additionally, the increasing availability of external educational resources designed to help students prepare for exams undermines the value of attending lectures.²⁸

Solutions to Curriculum-related Issues

The Medical Educator should keep in mind that there is need to revise the curriculum, so that only essentials are covered in lectures, as students prefer independent self-study over attending live lectures. The curriculum should be divided into components that can be covered by self-study or guided group study and those which require hands on practice for optimal understanding. The answer to curricular issues is designing a curriculum which is based predominantly on active learning principles and is grounded on the concept that students will attend classes and learn alongside one another. In conjunction with a curriculum designed on active, integrated and complex learning, there is need to develop assessments that measure higher-order cognitive skills and on which students will perform well only if they have participated fully in classroom activities.¹ Many studies have reported a high degree of student enthusiasm with curriculum promoting deeper learning.¹ Some institutes with strong medical student involvement in research have redesigned and compressed their pre-clinical curriculum, but the impact of these changes is yet to be seen.^{26,28}

In order to solve curriculum-related issues contributing to absenteeism, the curricular content, its placement, delivery and assessment need to be optimized. The following are some suggestions (Table-2).

- For best use of the lectures, medical educators need to identify specific parts of curriculum where lectures may be most effective.²⁷ Curricular content requiring complex learning and integration can be taught via lectures. Lectures should be used to cover difficult concepts, so that even if available digitally, students would prefer attending them in person to grasp core concepts well.²⁷
- The curriculum should be designed with incorporation of active teaching learning strategies which will also promote deeper learning.

- The attendance in lectures can be improved by relay of integrated and complex concepts through interactive teaching and learning methodologies with effective use of audiovisual aids. Educators, however, need to ensure consistency in delivery of curriculum through careful observation of the teaching faculty and class activities.^{1,6}
- To create more meaningful educational experiences for learning essential basic science material, innovative approaches to curriculum design based on student engagement in learning (such as the ‘flipped classroom’) may be more effective than the traditional teacher-centred approach in a larger class format.²⁹ Such approaches also lead to increased levels of student satisfaction and engagement in the curriculum.³⁰
- Informal learning, in which students are free to engage and where what is unrelated directly to curriculum is also taught, is likely to improve attendance and may be a possibility if educators are accepting and forthcoming about implicit curriculum.²⁸

Learning Environment-related Issues

The medical college learning environment encompasses student learning within the structural, social and psychosomatic contexts. It is defined as ‘the sum of the internal and external circumstances and influences surrounding and affecting a person’s learning’.³¹ This includes the curriculum design, infrastructure, accommodation for students, amenities, teaching methods, interactions with faculty and peers, social and academic environment, mentoring system during stress, commitment of the institute with students as well as student motivation.³²

The quality of medical educational programs is related to the educational environment that is being provided to the medical students.³³ A conducive learning environment is nevertheless required for students who come from diverse backgrounds and with different potentials and abilities of self-adaptation with different learning styles.³³ The comforts of one’s home is desirable from the perspective of a lonely and discouraged medical student. Moreover, a student may remain absent because the class environment is not sufficiently conducive for learning. Literature suggests that student attendance and academic performance may be impacted by improved and soundproof infrastructure, proper ventilation and lighting with controlled temperatures. However, there is not much evidence to support this notion.³⁴ Poor infrastructure, including poorly ventilated lecture halls, overcrowding and uncomfortable classroom sitting arrangements are additional important factors for absenteeism.⁵

Solutions to Learning Environment-related Issues

In order to solve learning environment related issues contributing to absenteeism, following are some suggestions. (Table-2).

- The visionary insight of good governance with effective administration should recognize, agree upon and be committed to the processes of educational development.³¹
- A university culture that promotes faculty development in effective teaching methodologies may help to develop a learning environment to engage students and increase student attendance.⁶
- This can be made possible by careful planning of use of existing physical space, learning and human resources for such activities.³¹
- Promotion of interactive learning environments, active teaching with use of information technology and student-faculty interaction with reflection of deep learning experiences are more likely to engage students during lectures.³⁵
- Funds should be judiciously used to build and replenish libraries with latest books and journals. Moreover, free online educational resources should be made available.³¹
- Qualitative studies should be done alone or in addition to the quantitative studies to assess the learning environment.³¹
- In addition, there should be psychosocial support mechanisms to enhance student learning and attendance.

CONCLUSION

Absenteeism is a product that springs from a variety of contributing factors that need to be addressed simultaneously. Innovations in instructional methods focused to engage students can bridge the detachment between ‘digital natives’ and ‘digital immigrants’. The quality of teaching needs an upgrade, through developing the skills of medical teachers with newer technologies. FDPs can play a large role in training faculty to be well versed with new instructional methods, especially those involving technology-enhanced learning. Quality assurance of these initiatives through ongoing monitoring and feedback systems, and accreditation by regulatory bodies is therefore required. Furthermore, positive reinforcement to retain good teachers is required through promotions, bonuses and recognitions. Institutional commitment should be reflected in the form of change in policies and replacement of the traditional passive modes of instruction with active learning and inquiry-based approaches to ensure improved attendance of medical students.

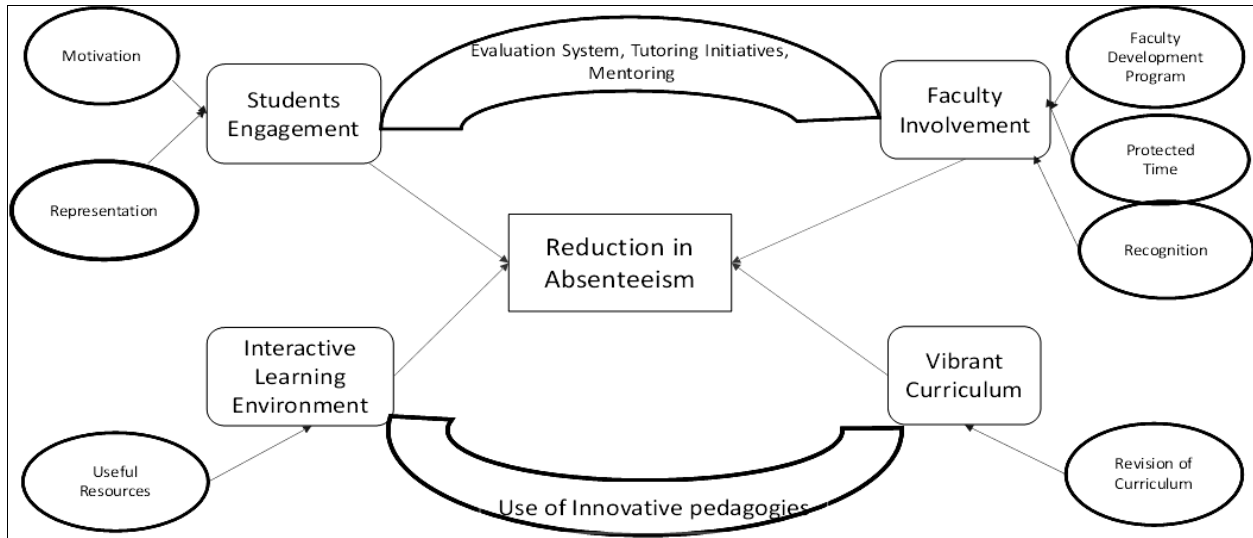


Figure-1: Solutions to reduce absenteeism in Large Class Format

Table-1: Absenteeism reason and recommendation —students and faculty

Author /Year	Title	Objective	Significant Findings	Recommendations
Rao, B., Valleswary, K., Nayak, M., & Rao, N. L. (2016). IOSR Journal of Research & Method in Education (IOSR-JRME), 6, 11-19.	Reasons for Absenteeism among the Undergraduate Medical Students Attending for Theory Classes in Rajiv Gandhi Institute of Medical Sciences (RIMS) Ongole, Prakasam District of Andhra Pradesh: A Self Review.	<ul style="list-style-type: none"> To explore various student, college and external factors for the absenteeism among the medical students To find out the various remedies, suggestions and ideas expressed by the students 	<ul style="list-style-type: none"> Laziness and taking part in extracurricular activities are main student factors for absenteeism. Lengthy classes, lack of clarity among teachers while teaching are some other contributing reasons. Entertainment and sickness of the students were some external factors. 	<ul style="list-style-type: none"> There should be a proper plan to encourage student’s feedback, so that teaching can become more meaningful. Teachers need to adopt creative teaching techniques. Faculty should be well prepared for the classes with use of animation, audio, graphics, to generate interest, curiosity in learning and motivation among students. Faculty skills are enhanced through various training programs to change their orientation from the traditional to interactive and innovative lecture method.
Sharmin, T., Azim, E. Choudhury, S., & Kamrun, S. (2017) Anwer Khan Modern Medical College Journal, 8(1), 60-66.	Reasons of absenteeism among undergraduate medical students: a review	To explore the link between absenteeism and the contributing factors.	<ul style="list-style-type: none"> Poor teaching skills of lecturers leading to boring lectures. Lack of clarity about the topic among teachers while teaching 	Application of strict attendance policy may influence student attendance and medical colleges should reinforce the attendance policy as an effort to improve their student's academic performance.
Rubeena Gul, Hayat Muhammad Khan, Sardar Raheel Alam, Faizan Luqman, Aymen Shahab, Hifsa Sohail J. Med. Sci. 2016, Vol. 24, No. 1: 16-18	Absenteeism among Medical Undergraduate Students	To assess the prevalence and causes of absenteeism among the undergraduate medical students of a Public Sector Medical College in Peshawar	The main reasons reported for missing lectures was lack of free time between classes, dislike of lecturers, teaching style, peer pressure and lack of sleep.	<ul style="list-style-type: none"> There is a need to improve quality of lecture materials and teaching techniques. Regular training for instructors to help them improve their teaching methodologies could be a useful intervention. Peer assisted learning and problem based learning should be introduced in order to make it interactive.
Mukhtar F, Chaudhry AMJ o AMCA 2010;22(3):210-3.	Faculty development in medical institutions: where do we stand in Pakistan?	<ul style="list-style-type: none"> To determine the proportion of medical colleges involved in faculty development activities, to assess the types of faculty development activities, and to identify the factors influencing such activities, along with formulating recommendations for faculty development 	Lack of incentives 20 (54%), lack of faculty interest 15 (40%) and a shortage of trained facilitators 15 (40%) as barriers to faculty development activities.	<ul style="list-style-type: none"> Mandatory training workshops should be conducted by the PMDC, Recognition should be given to faculty undergoing training activities there should be active interaction between institutes to facilitate each other in matters pertaining to medical education

Table-2: Absenteeism reason and recommendation—Curriculum and Learning Environment

Author /Year	Title	Objective	Significant Findings	Recommendations
White C, Bradley E, Martindale J, Roy P, Patel K, Yoon M, Worden MK. Medical education. 2014 Mar;48(3):315-24.	Why are medical students 'checking out' of active learning in a new curriculum?	To understand why (especially with a newly adopted student-centered curriculum) many students were opting to learn on their own outside the curriculum and learning environment.	<ul style="list-style-type: none"> Passive classroom exercises which may not promote active learning and collaborative learning. Students perceive that they are not prepared for adult or active learning 	<ul style="list-style-type: none"> Help students understand the nature of deep learning and their own developmental progress as learners Provide robust faculty development to ensure the consistent deployment of higher-order learning activities linked with higher-order assessments.
Gupta A, Saks NS. Medical Teacher. 2013 Sep 1;35(9):767-71.	Exploring medical student decisions regarding attending live lectures and using recorded lectures	<ul style="list-style-type: none"> To identify factors involved in the decisions first and second year medical students make about attending live lectures, To discover students use recorded lectures, and if their use affects live lecture attendance 	Decisions about lecture placement in the curriculum need to be based on course content and lecturer quality.	Identify parts of curriculum where lectures are effective
Zazulia AR, Goldhoff P. Teaching and learning in medicine. 2014;26(4):327-34.	Faculty and medical student attitudes about preclinical classroom attendance.	<ul style="list-style-type: none"> To investigate differences in medical educator and student attitudes regarding preclinical attendance, To assess the impact of absenteeism on educators and the learning environment, and To explore a possible relationship between attendance and professionalism 	Students view class as a tool to learn factual material and a preclinical students primary task is to learn factual material and the means of mastery is not important	<ul style="list-style-type: none"> Incorporate interactive learning techniques Active educational programs that require attendance and student engagement
T Sharmin, E Azim, S Choudhury, S Kamrun AKMMC J 2017;8(1):60-6	Reasons of Absenteeism among Undergraduate Medical Students: A Review	To explore the reasons of absenteeism in undergraduate students	<ul style="list-style-type: none"> Missing home comforts. Class environment is not conducive enough for learning. Poorly ventilated lecture hall, overcrowding in the classrooms and uncomfortable sitting arrangement in class are important factors for not attending class. Poor infrastructure 	<ul style="list-style-type: none"> Promote conducive Learning environment by ensuring adequate ventilation of the lecture halls. Classroom size should be designed in such a way that all student can be seated comfortably. Infrastructure of the institute should be properly designed according to the educational needs of the students.

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Address for Correspondence:

Dr Rahila Ali, Senior Instructor, Department for Educational Development, Aga Khan University Hospital, Karachi-74800, Pakistan. **Tel:** +92-21-34864502, **Cell:** +92-300-7085702

Email: rahilaali.mazhar@aku.edu

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INFORMATION FOR AUTHORS

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Pak J Physiol receives Original Articles, Review Articles, Case Reports, Short Communication, Letters to Editor, etc. on all physiological topics and medical education. Manuscripts are received for consideration if neither the article nor any of its contents has been or will be published or submitted elsewhere before appearing in the Pakistan Journal of Physiology (PJP). Please submit the article on Open Journal System (OJS) at <http://pjp.pps.org.pk/index.php/PJP> followed by a copy of the complete manuscript with one set of original figures, and a softcopy (MS Word format) on CD. Manuscripts must not be of more than 3,000 words. Use Letter size paper (8.5×11 inch), and double-space throughout for the hard copy only. Address all submissions to **Editor, PJP, C/O JAMC, Ayub Medical College, Abbottabad-22040, Pakistan**. The corresponding author must be identified and address for correspondence with telephone number and email endorsed at the end of the script. An undertaking signed by *all* authors, (certifying originality of work, and that the article has not been submitted, or will not be submitted/published elsewhere before the decision of PJP about it) must accompany the manuscript. The 'Undertaking' is available for download from www.pps.org.pk/PJP/undertaking.pdf. No more than 12 names will be listed under the title; other names will appear in a footnote.

Research and publication ethics: The authors must declare approval of the 'Research Ethics Committee' and clearly mention any 'Conflict of Interest' either in the script or as an attached document.

Title and authors' name: The first page of the manuscript must give the title of article that should be concise and descriptive. Also include on this page the name(s) of the author(s), qualification(s), designation, the name of department and institution from where the work is submitted. Any grant/support that requires acknowledgment should be mentioned on this page.

Abstract: The second page of the manuscript must contain an abstract of not more than 250 words. This abstract should consist of four paragraphs, labelled **Background, Methods, Results, and Conclusions**. They should briefly describe, respectively, the problem being addressed in the study, how was the study performed, the salient results, and what did the author(s) conclude from the results.

Keywords: Three to 10 key words or short phrases should be added to the bottom of the abstract page. Please use terms from the Medical Subject Headings (MeSH) of Index Medicus.

Introduction, Materials & Methods, Results, Discussion, Conclusions, Acknowledgements and References should all start on a separate page from page 3 onwards.

References: The PJP prefers the total number of references in an original article not exceeding 30, while in a review articles not exceeding 60. References must be written single-spaced and numbered as they are cited in the manuscript. The references must be written in Vancouver style. The style for

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Journal article: Badar A. Launching a new journal in the era of publication accountability. *Pak J Physiol* 2005;1(1-2):1.

Book reference: Phillips SJ, Whisnant JP. Hypertension and stroke. In: Laragh JH, Bremner BM, (Editors). *Hypertension: pathophysiology, diagnosis, and management*. 2nd ed. New York: McGraw Hill; 1995. pp.465-78.

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