PAKISTAN JOURNAL OF MEDICINE & DENTISTRY

VOLUME: 10, ISSUE: 01

JANUARY 2021 – MARCH 2021

PUBLISHED BY

ZIAUDDIN UNIVERSITY

HEC &
PMDC Recognized

ISSN (Print): 2313-7371
ISSN (Online): 2308-2593

PAKISTAN JOURNAL OF MEDICINE & DENTISTRY

EDITORIAL BOARD

PAKISTAN JOURNAL OF MEDICINE & DENTISTRY

ABOUT PJMD

The Pakistan Journal of Medicine and Dentistry is a quarterly periodic journal that encourages swift publication of original research work on all phases of Medical and Dental Sciences including Anatomy, Biochemistry, Physiology, Pathology, Pharmacology, Community Health Sciences, Family Medicine, Dentistry and other related medical fields. Manuscripts for the journal are selected on the basis of originality and quality of the work, contribution and span of interest to the scientific community.

INDEXED

ISSN: 2308-2593 (online)
ISSN: 2313-7371 (print)
IMEMR
PMDC & HEC RECOGNIZED
CrossRef, Google Scholar, ICMJE

EDITORIAL CORRESPONDENCE & MANUSCRIPT SUBSCRIPTION

All editorial correspondence should be addressed to the Assistant Editor PJMD, Ziauddin University, Clifton Campus, 4/B Shara-e-Ghalib, Block 6, Clifton, Karachi, Pakistan.
Tel: (92 21) 35862937
Ext: 2368
Email: pjmd@zu.edu.pk

WEBSITE

PJMD is available online as an open access peer reviewed journal. It can be accessed through the link http://pjmd.zu.edu.pk

PUBLISHED BY

Ziauddin University, Karachi, Pakistan.

DECLARATION

The editorial board of PJMD has made every effort to retain the authenticity of the material printed in the journal. However, the editors reserve the right to delete or amend the material for the sake of suitability, accuracy or space.

PAKISTAN JOURNAL OF MEDICINE & DENTISTRY

TABLE OF CONTENTS

PAKISTAN JOURNAL OF MEDICINE & DENTISTRY

TABLE OF CONTENTS

EDITORIAL

Molecular Pathology: A Paradigm Shift towards Precision Medicine

Fouzia Shaikh[1], Talat Mirza[2]
[1]Department of Pathology, Ziauddin University [2]Department of Research, Ziauddin University, Karachi, Pakistan.
https://doi.org/10.36283/PJMD10-1/001

Healthcare system is experiencing a paradigm shift to precision medicine. Genotypic–phenotypic affiliation has been found to be a fundamental percept in biology after the completion of Human Genome project. The first era of precision medicine is split into groups and subgroups, making it a meaningful strategy concurrently throughout the clinical phases of drug designing and development. It likewise recommends healthcare reshaping that suggest disease perceptivity or remedial treatment. Thus, translational genomics addresses bench to bedside approach to achieve P4 medicine (*personalized, predictive, preventive, and participatory*), i.e., early disease diagnosis and specifically designed treating plans instead of one size fits all[1].

The ever-increasing importance of targeted therapy in the management of cancerous as well as non-neoplastic diseases calls for novel, advanced techniques of diagnostic pathology. Understanding of tumor development and acquiring information regarding the genetics, transcriptomics, proteomics, metabolomics and epigenetics of pathological lesions, especially cancers, is central to the development of precision medicine. The next generation sequencing (NGS) techniques have proved to be invaluable for gaining insights into molecular profiles of tumors and development of targeted therapies. Even though genomic characterization of tumors has been of immense value, DNA aberrations do not give a clear picture of the related biological pathways. This gap has been very efficiently filled by transcriptomic studies, which have emerged as one of the essential techniques in molecular diagnostics. Moreover, transcriptomics studies have paved the way for the development of bioinformatic tools improving the understanding of differential gene expression concerning biological functions. Targeted breast cancer therapeutics is one of the success stories in the achievements of transcriptomics in cancer management in the near past. Whole genome sequencing has provided substantial knowledge of the genomic profiles of breast cancers including single nucleotide pleomorphism, copy number variations and driver muations[2].

An increasing number of miRNAs are being identified by transcriptomic studies and to date nearly 8600 miRNA genes have been identified. miRNAs play various roles in cell proliferation, fate determination, and differentiation. These appear to contribute to transitions from stem (precursor) cells to differentiated cell types by refining/reinforcing desired gene expression profiles, rather a process of mapping molecular signatures[3]. Further recent field of 'molecular pathological epidemiology' (MPE) is now being recognized for personalized medicine along with public health all through using 'omics' data in population-based studies also. The proposed integration of microbiome (viruses, bacteria, fungi and parasites) into MPE to highlight the reactions and responses of tumor cells, the microbiome, immune cells and other factors of the tumor microenvironment is a step forward[4]. Current advances in tumor microenvironment has accomplished an era of immunotherapies which has proved to be valuable in various tumors e.g. lymphomas. Advancement in understanding complex tissue microenvironment has been accomplished by single-cell analysis to provide useful perceptions on tumor biology and progress.

Liquid biopsy involves the evaluation of circulating tumor DNA (ctDNA), circulating tumor cells (CTCs), and tumor-derived exosomes. Although DNA appears a promising prognostic marker, it cannot supersede biopsy or radiographic assessment. Liquid biopsies can be used as a supplementary standard to follow patients by monitoring the progressive genomic alterations in response to medication[2,3]. Rapid drops in cfDNA concentration and lack of ctDNA in plasma after commencement of therapy indicate treatment response linked with survival[5].

Moreover, digital and telepathology has broadened the scope for diagnostic precision. Infrared microscopy used for the assessment of tumor characteristics has been established as a key technique in the biomedical research promising better patient management. Moreover, Spectral histopathology (SHP) is a new promising tool; though, its use has not been authenticated for clinical practice yet, still the practical implications are expected.

The familiarity with the instructional manual of the master molecule of DNA is now completely sequenced in the form of a chip as an amazing identity card. The present hub of recent advances revolves around molecular signature, microenvironment, liquid biopsy and computational pathology. The mega achievements related to the high-profile diagnostics has amplified in depth understanding of disease processes with advent of targeted therapies. Moreover, the specific approaches for personalized medicine have further revolutionized the strategies and therapeutic outcomes, based on tailored treatment. However, in this era of precision medicine the developed countries are the major beneficiary as compared to resource deficient populations due to financial constraints. The high expenses for molecular diagnostics with lack of supportive infrastructure and expertise is a deprivation of recent advances in technology for the developing countries.

The debate remains that in depth knowledge of molecules altering diseases can lead to mental stress and can have a huge impact on a patient's life. The hidden budget could be potentially lower with the focus on preventive care rather than therapeutic strategies. Precision medicine has the predictive potential in healthcare, but that requires massive infrastructure investments and time to implement. According to a recent analysis, many newly discovered so-called disease-carrying mutations may have limited or no meaningful effect on future illness. Over diagnosis happens when people are diagnosed with a disease that would never actually harm them. Therefore, huge unanswered questions are around to interpret results of genomic testings. Carrying the gene chip is not the total answer to cure through precision medicine, which is, customized approach tailored to the individual patient.

REFERENCES

1. AL-Dewik NI, Qoronfleh MW. Genomics and precision medicine: Molecular diagnostics innovations shaping the future of healthcare in Qatar. Adv Public Health. 2019; 1-11
2. Ma L, Liang Z, Zhou H, Qu L. Applications of RNA indexes for precision oncology in breast cancer. Genom Proteom Bioinform. 2018; 16(2):108-119.
3. Sheu-Gruttadauria J, Xiao Y, Gebert LF, MacRae IJ. Beyond the seed: structural basis for supplementary micro RNA targeting by human Argonaute2. EMBO J. 2019;38(13):1-14.
4. Herrington CS, Poulsom R, Coates PJ. Recent advances in pathology: the 2019 annual review issue of the journal of pathology. J Pathol. 2019;247(5):535-538.
5. Ng CK, Di Costanzo GG, Terracciano LM, Piscuoglio S. Circulating cell-free DNA in hepatocellular carcinoma: current insights and outlook. Front Med. 2018;5:78-87.

ORIGINAL ARTICLE

Evaluation of Naked Eye Single Tube Red Cell Osmotic Fragility Test (NESTROFT) in Screening and Diagnosis of Beta Thalassaemia Trait in Pakistani Population

Rubina Ghani[1], Nazish Jaffar[2], Abdul Rehman[2], Kiran Abbas[2], Sayeda Fatima Ali[1], Ayesha Anum[1]
[1]Pathological and Molecular Laboratories, Musavvir Stem Cell Clinic, Karachi, [2]Department of Pathology, Sindh Medical College, Jinnah Sindh Medical University, Karachi, Pakistan.

ABSTRACT

Background: Beta thalassaemia, an inherited disease that is a cause of continuous burden on the affected families and the society. The objective of the study was to evaluate the efficacy of Naked Eye Single Tube Red Cell Osmotic Fragility Test (NESTROFT) for the detection of beta thalassaemia trait when used for the screening in Pakistani population.

Methods: All participants requesting a complete blood count were included in the study. Informed consent was obtained. Naked Eye Single Tube Red Cell Osmotic Fragility Test was performed with freshly prepared 0.36% buffered saline. Turbidity in the tubes was an indication for individuals to be positive for beta thalassaemia trait. Hemoglobin electrophoresis was performed on all positive cases to confirm the sensitivity and the reliability of the screening test. Chi-Square test was used to test for any significant correlation between the different hematological parameters and other variables.

Results: A total of 812 patients were included in the study from January 2017 to June 2017. The mean age of participants was 35.5 ± 13.6 years. There were 351 (43.2%) male and 461 (56.7%) female participants. A 100% Naked Eye Single Tube Red Cell Osmotic Fragility Test sensitivity was obtained with 65 (8%) positive cases having HbA2 levels of more than 3.2% indicating their true positivity whereas, all 747 (92%) negative cases having HbA2 levels of less than 3.2% indicating their true negativity.

Conclusion: NESTROFT was found a highly sensitive, cost-effective, and a rapid screening test for the identification of carriers of beta thalassaemia trait in our population.

Keywords: Consanguineous Marriage; Population; Thalassaemia; Screening.

Corresponding Author:
Dr. Nazish Jaffar
Pathology Department,
Sindh Medical College,
Jinnah Sindh Medical University, Karachi.
Email: drnazishamin@gmail.com
https://doi.org/10.36283/PJMD10-1/002

INTRODUCTION

Beta Thalassaemia is a group of inherited blood disorders in which the body's ability to produce normal hemoglobin is impaired with reduction in capacity of red blood cells to carry oxygen[1]. Beta thalassaemia is more prevalent in Mediterranean regions, Indian subcontinent, the Middle East, and Southeast Asia[2].

It has been estimated that about 5 to 9 thousand infants are born with beta thalassaemia every year, in Pakistan. However, no central registry is available in Pakistan to ascertain the actual number of cases of the disease per year[3]. Nevertheless, the carrier rate in Pakistan ranges between 5-8%, meaning about 9.8 million people are carriers of beta thalassaemia trait in our population[4]. Following Sickle cell anemia, Beta thalassaemia is considered the second most common haemoglobinopathy, worldwide[5]. Beta thalassaemia major is a severe disease and places a huge economic burden on the health

care setup especially in developing countries like Pakistan and India[6].

The early detection of asymptomatic carriers of thalassaemia (heterozygotes) makes it possible to provide genetic counseling, which may prevent a more severe homozygous condition in the offspring and its fatal outcome. In 2006, Saudi Arabia Health Ministry implemented a mandatory premarital screening of Thalassaemia trait in couples. Ahmad Al Suliman reported the beta thalassaemia trait incidence rate of 3.4% in his population. Parts of the world where blood disorders are highly prevalent, high-risk marriages should be prevented by implying premarital screening[7].

Naked Eye Single Tube Red Cell Osmotic Fragility Test (NESTROFT) is a single tube qualitative osmotic fragility test based on the limit of hypo tonicity, which the red blood cells can withstand. In 2012, Chakrabarti et al. tested the validity of NESTROFT as a screening tool for beta thalassaemia trait. He reported NESTROFT to be 95% sensitive in subjects with at least one family member suffering from beta thalassaemia major while 85.71% sensitive in normal population[6].

In 2007, Mamtani et al. reported her findings that supported the practice of NESTROFT use as a screening tool for beta thalassaemia trait in countries where there is a high prevalence of thalassaemia against minimal resources[8]. Another study from North India in 2013, reported a NESTROFT sensitivity of 100% and a specificity of 85.47% among 150 patients, indicating that NESTROFT is a useful screening tool that is both effective and affordable[9]. The objective of this study was to evaluate the efficiency of Naked Eye Single Tube Red Cell Osmotic Fragility Test (NESTROFT) in determining beta thalassaemia trait in Pakistan population.

METHODS

A prospective, cross-sectional study was carried out at the Pathological and Molecular laboratories, Karachi, Pakistan from 1st January, 2017 to 30th June, 2017. All the patients (812) presenting to the laboratory and requesting a complete blood count (CBC) were enrolled in the study. The study participants belonged to different age groups and had a variable demographic profile. The ethical approval was obtained from the Institutional Review Board of the Molecular and Pathological Laboratories, Karachi, with IRB reference number of MSCC/ER-B/2016/07. Informed consent was taken from all patients prior to sample collection. Both genders that consented were included in the study.

The venous blood of 2.5 ml was collected into a vacuum enabled tube containing Ethylenediaminetetraacetic acid (EDTA) anticoagulant (1.5 ± 0.25 mg/ml). Within two hours of sample collection, NESTROFT and Complete blood counts (CBC) were performed. CBC was done on Automated Hematology Analyzer (BC-3000 Plus-Mindray). NESTROFT was performed using freshly prepared 0.36% buffered saline. In addition, 5 ml sterile test tubes were used each containing 4ml of 0.36% buffered saline. 50µl sample blood was added in each tube. The tubes were left undisturbed at room temperature for at least 30 minutes. The turbidity in the tubes was observed in the presence of a tube light placed behind them. Turbidity in the tubes indicated that the individual was positive for beta thalassaemia trait.

Hemoglobin electrophoresis was performed on all NESTROFT positive cases to confirm the sensitivity and the reliability of the screening test NESTROFT. The formulae used to calculate sensitivity for the NESTROFT is shown below. Sensitivity = True Positive / True Positive + False Negative × 100. Statistical Package for Social Sciences (SPSS v. 25) was used to perform data analysis. Measures of central tendency (mean and median) as well as standard deviation, SD; range: minimum – maximum was calculated.

RESULTS

A total of 812 patients were enrolled in the study between January 1, 2017 and June 30, 2017. The mean age ± SD of participants were 35.5 ± 13.6 years. There were 351 (43.2%) male and 461 (56.7%) female participants. High grade fever and symptoms of anemia were presenting complaints in majority of the patients. The demographic data and presenting complaints of patients are depicted in Table 1. The mean value for red blood indices, white blood cell, and platelet count was observed (Table 1). NESTROFT done in test tubes showed Positive and negative results (Figure 1).

Table 1: Laboratory diagnosis of red blood cell indices, demographic and clinical profile of participants of the study.

Complete Blood Count	(Mean ± SD)	Demographics	Mean ± SD
Hemoglobin (Hb)	12.57 ± 2.10 mg/dl	**Age**	35.5 ± 13.6 years
Red Blood Cell (RBC)	4.86 ± 0.78 X 10^{12} cells per liter	**Gender n (%)**	
Hematocrit (Hct)	42.60 ± 6.59 %	**Male**	351 (43.2)
Mean Corpuscular Volume (MCV)	88.42 ± 11.76 FL	**Female**	461 (56.7)
Mean cell hemoglobin (MCH)	26.06 ± 3.74 pg.	**Signs and Symptoms n (%)**	
Mean cell hemoglobin concentration (MCHC)	29.44 ± 1.98 %	**Fever**	617 (75.9)
White Blood Cell (WBC)	9194.70 ± 6216.74 cells/mm^3	**Lethargy**	523 (64.4)
Platelet count	271,461.82 ± 104194.02 cells/mm^3	**Shortness of breath**	400 (49.2)
Positive cases of anemia n (%)	293 (36.1%)	**Pale skin**	253 (31.2)

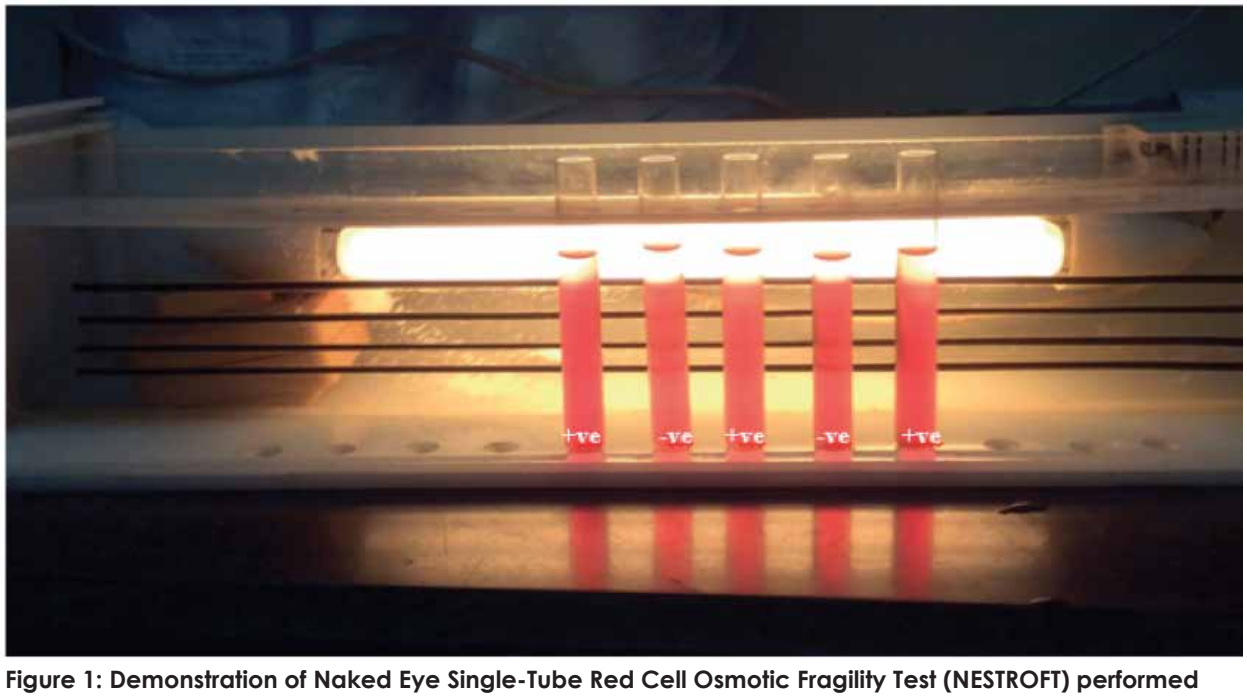


Figure 1: Demonstration of Naked Eye Single-Tube Red Cell Osmotic Fragility Test (NESTROFT) performed during the study.

In the present study, 293 (36.1%) participants were anemic, out of these 205 (69.9%) were female and only 88 (29.1%) were male participants (p=0.000). The mean ± SD hemoglobin level in anemic patients was 10.89±1.92 mg/dl with a range of 4.90 to 13.40 mg/dl. We reported that 65 (8%) participants in our study screened positive with NESTROFT for beta thalassaemia trait.

In our study, Hb electrophoresis for HbA2 levels was measured for all participants to detect the true positive and true negative cases. A 100% NESTROFT

sensitivity was obtained with all 65 (8%) positive cases having HbA2 levels of more than 3.2% indicating their true positivity whereas, all 747 (92%) negative cases having HbA2 levels of less than 3.2% indicating their true negativity (Figure 2).

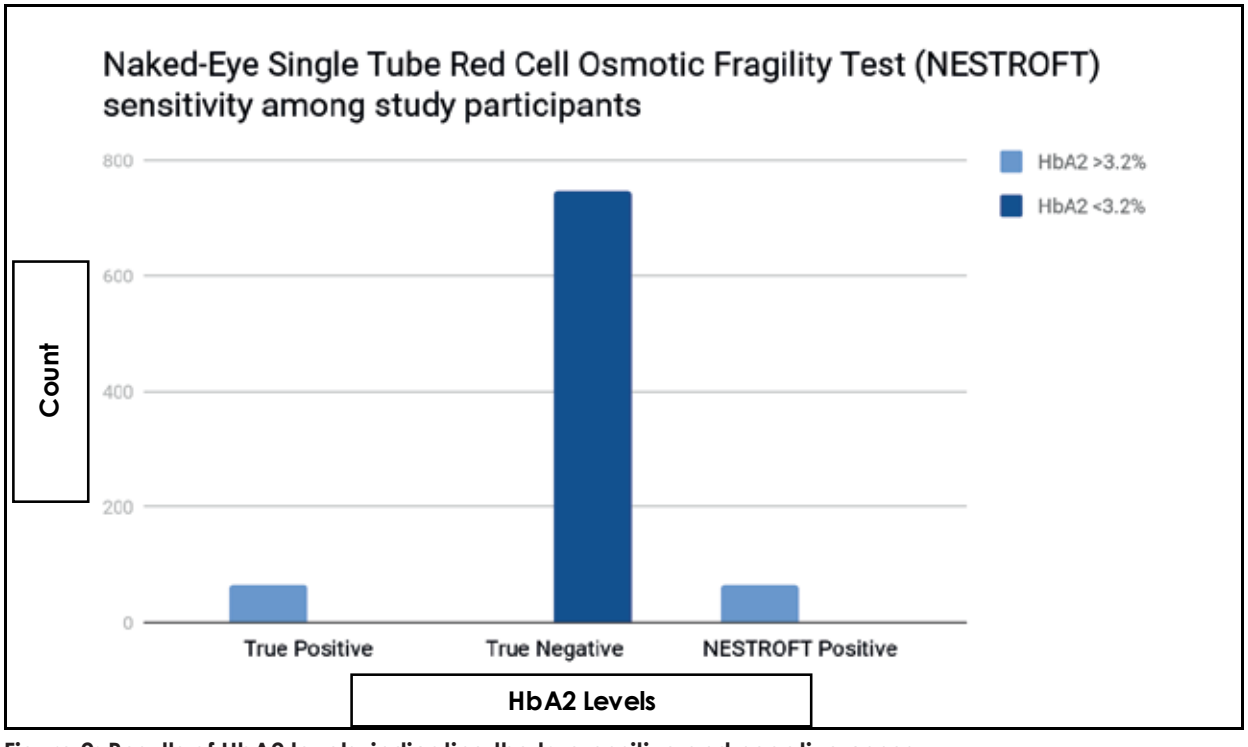


Figure 2: Results of HbA2 levels, indicating the true positive and negative cases.

In the present study, the patients who were screened positive for the beta thalassaemia trait with NESTROFT had reportedly a higher value of red blood cell count (RBC) and lower value of mean corpuscular volume (MCV) and mean cell hemoglobin (MCH) as compared with the subjects who were negative for the trait (Figure 3).

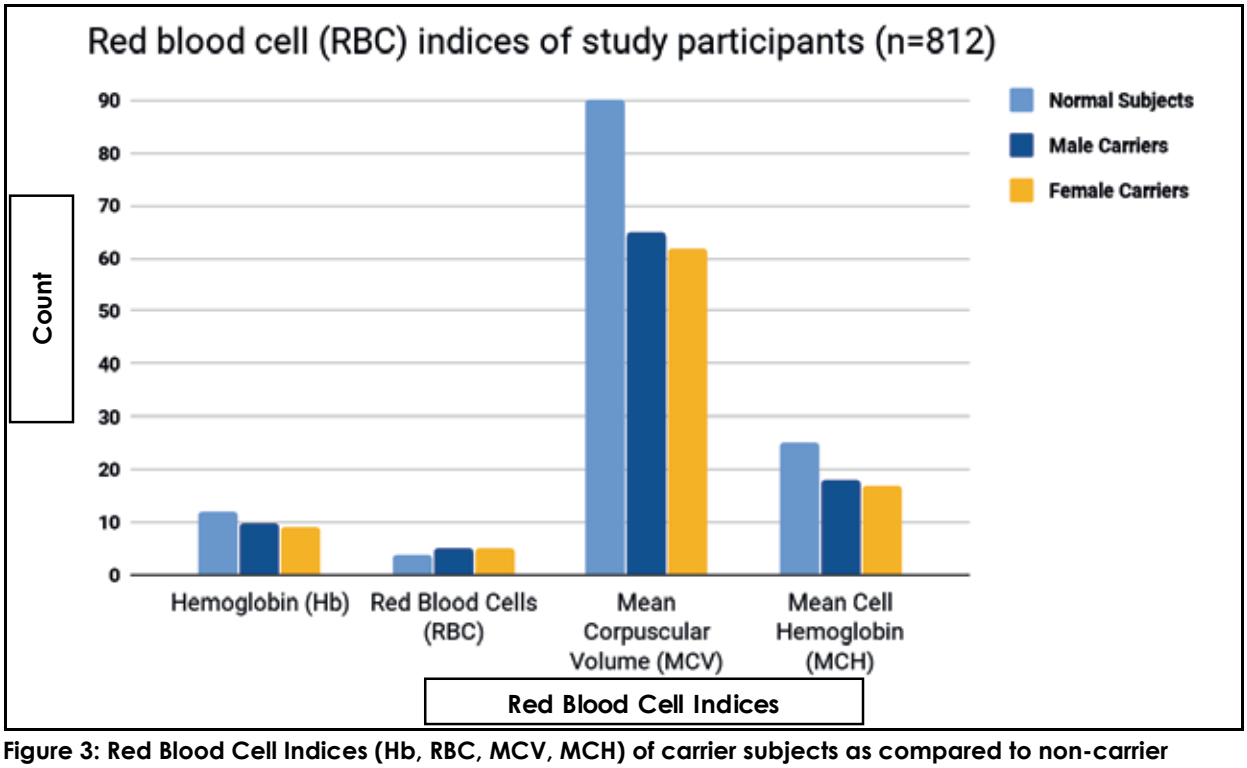


Figure 3: Red Blood Cell Indices (Hb, RBC, MCV, MCH) of carrier subjects as compared to non-carrier subjects in our study population.

DISCUSSION

In the current study, we reported 8% positivity of beta thalassaemia trait in participants using NESTROFT. Furthermore, Hemoglobin electrophoresis confirmed 100% sensitivity of this test observed in all 8% of the samples. Thalassaemia is a hereditary blood disorder that is common in the Mediterranean and South Asian countries including Pakistan. The estimated carrier rate in Pakistan is between 5-7% with over 5,000 new patients being diagnosed with this disease each year[10, 11]. Thalassaemia is not only responsible for huge mortality and morbidity but also causes considerable socioeconomic burden on the affected families and the government.

Many different screening techniques are available for the detection and confirmation of Thalassaemia but most of these tests are expensive hence, not all patients are able to afford them. Besides, Pakistan is a resource-poor region and not every diagnostic laboratory has the advanced equipment to screen and detect even the most prevalent of diseases[12].

Therefore, it is crucial to evaluate the efficacy of various screening methods, which are both accessible and inexpensive. Patients with deranged red blood indices concomitant with symptoms of anemia must be screened thoroughly for beta thalassaemia trait. Presence and relative amounts of abnormal forms of hemoglobin is a preliminary diagnosis of a hemoglobin disorder. Elevated hemoglobin A2 (HbA2) of more than 3.5% as detected on High Performance Liquid Chromatography (HPLC) or cellulose acetate electrophoresis (CAE) is the definitive diagnosis for beta thalassaemia trait[12, 13]. These tests are accurate but costly and labor intensive. Keeping these problems in mind, we sought to evaluate the efficacy of NESTROFT, an inexpensive and a simple technique to screen the carriers of Beta Thalassaemia trait in our population[14].

NESTROFT was introduced in the 1990's and since then many studies have tried to evaluate its efficacy in diagnosing beta thalassaemia trait for screening purpose. NESTROFT is considered to be a very simple and cheap tool to detect carrier state in normal population. However, this test is dependent upon visual interpretation. Gorakshaker et al. determined the inter-observer variability while recording of NESTROFT results by three individuals on blood samples of 380 participants[15]. They observed that there was little variation in recording the negative results while greater variation in positive or doubtful cases. They concluded that NESTROFT should be considered as the investigation of choice for initial screening of Beta thalassaemia trait in Indian population.

In the present study, NESTROFT was reported to be 100% sensitive as confirmed with HbA2 value of more than 3.2 indicating the true positivity of beta thalassaemia trait carriers. Since 1991, many studies were conducted to determine the efficacy of NESTROFT as a screening tool for beta thalassaemia trait among normal population[16-20]. These studies reported that 96-100% of heterozygotes with beta thalassaemia trait could be detected with NESTROFT. Their findings were in accordance with this study. Our study also, supported the claim that NESTROFT is an effortless, economical, and easy to perform technique that could be utilized for screening at a large scale.

A similar study carried out in by Sumera et al. reported NESTROFT to be 93% sensitive and 88% specific. An Indian study showed 100% and 92.9% sensitivity and specificity respectively, claiming that NESTROFT was the most effective, inexpensive, and accurate test for screening for beta thalassaemia trait[16,17].

A study conducted at Jinnah University for women, Karachi evaluated the diagnostic significance of NESTROFT and red blood indices in screening beta thalassaemia minor in subjects. According to the study, compared with the control group, all 100 participants in the carrier group showed a positive NESTROFT test with concomitant low values of MCH and MCV making NESTROFT to be a rapid and a reliable method for the detection of thalassaemia trait among our population[18]. Pakistan is one of the highly prevalent countries suffering from inherited haemoglobinopathies, which poses a huge economic burden on the country[2,19].

A recent study reported that the consanguineous population was significantly associated with an increased prevalence of diseases such as diabetes mellitus, haemoglobinopathy, and mental illnesses[20-22]. It is essential to screen for beta thalassaemia trait carriers among Pakistani population because consanguineous marriages are practiced at an alarmingly high rate of 81% [23]. Therefore, it is important to educate the public about the possible devastating outcomes of a consanguineous marriage and to prevent beta thalassaemia major by genetic counseling and mass screening for beta thalassaemia trait. It was found that with the help of genetic counseling, beta thalassaemia major in infants could be reduced by as much as 90% [12]. In contrast to mass screening by NESTROFT, Baig et al claimed that it is better to do cascade testing of large families with consanguinity practices instead of mass screening in Pakistan, which has limited health resources[23].

A cross sectional study conducted in 2015 in Lahore

where 25 families were screened for beta thalassaemia trait negated our findings. NESTROFT was only 55% sensitive and 64% specific whereas, the Positive Predictive Value was 46% and Negative Predictive Value was 64%. Similarly, about 66.7% detection of beta thalassaemia trait was reported in a study from Myanmar. However, both these studies are insufficient to disregard a seemingly good screening tool, as NESTROFT is highly dependent on the visual interpretation and the concentration of buffered saline[24, 25]. We believe that to consider NESTROFT as a reliable screening tool, studies with larger sample size would be required in future valuable screening tool for identification of carriers of beta thalassemia trait. All NESTROFT positive cases should be advised further workup to confirm their diagnosis. Genetic counseling should be offered to all carriers of beta thalassaemia trait in order to reduce the incidence of beta thalassemia major in Pakistan. We recommend that all families where consanguineous marriages are practiced NESTROFT should be advised to screen for haemoglobinopathy. We recommend studies on larger scale utilizing NESTROFT to establish its reliability as a screening method.

CONCLUSION

NESTROFT is a highly sensitive, cost-effective, and a rapid screening test for the identification of carriers of beta thalassaemia trait in our population. Such selective but extensive screening practice would eventually reduce the burden of thalassaemia major in Pakistan.

ACKNOWLEDGEMENTS

We are thankful to Pathological and Molecular Laboratories, Musavvir Stem Cell Clinic Karachi for providing us the diagnostic facilities.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICS APPROVAL

Ethical approval was provided by Institutional Review Board of Pathological and Molecular Laboratories, Musavvir Stem Cell Clinic, Karachi, Pakistan (MSCC/ERB/2016/07).

PATIENT CONSENT

All the participants in the study gave written informed consent before data collection.

AUTHORS' CONTRIBUTION

RG had conceived the idea and done the laboratory testing; NJ did the manuscript writing. AR did statistics; KA did critical review and editing. SFA did literature review and AA performed the data collection.

REFERENCES

1. Origa R. β-Thalassemia. Genet Med. 2017;19(6):609-619.
2. Ferdousi A, Ahmad M, Sharma JD, Samad R, Ullah AZ. Role of naked eye single tube red cell osmotic fragility test (nestroft) in detecting beta-thalassaemia trait. J Bangladesh Coll Phys Surg. 2018;36(4):145-152.
3. Ansari SH, Shamsi TS, Ashraf M, Bohray M, Farzana T, Khan MT, *et al*. Molecular epidemiology of β-thalassemia in Pakistan: far reaching implications. Int J Mol Epidemiol Genet. 2011;2(4): 403-408.
4. Ali N, Moiz B, Bin Azhar W, Zaidi N, Memon R. Carrier detection for beta-thalassemia trait in general Pakistani population: a way forward. Hematol. 2012;17:4, 237-240.
5. Li CK. New trend in the epidemiology of thalassaemia. Best Pract Res Clin Obstet Gynaecol. 2017;39:16-26.
6. Chakrabarti I, Sinha SK, Ghosh N, Goswami BK. Beta-thalassaemia carrier detection by NESTROFT: An answer in rural scenario? Iran J Pathol. 2012;7(1):19-26.
7. Al-Suliman A. Prevalence of β-thalassemia trait in premarital screening in Al-Hassa, Saudi Arabia. Ann Saudi Med. 2006;26(1):14-16.
8. Mamtani M, Das K, Jawahirani A, Rughwani V, Kulkarni H. Is NESTROFT sufficient for mass screening for β-thalassaemia trait? J Med Screen. 2007;14(4): 169-173.
9. Piplani S, Manan R, Lalit M, Manjari M, Bhasin T, Bawa J. NESTROFT-a valuable, cost effective screening test for beta thalassaemia trait in north Indian Punjabi population. J Clin Diagn Res. 2013;7(12): 2784-2687.
10. Sajjad SF, Ahmad W, Hussain JSS, Asif M, Alam SE. Treatment of chronic hepatitis C in thalassemia major patients. J Pak Med Assoc. 2017;67(6):926-928.
11. Khan MI, Khan HN, Usman M. Beta thalassemia trait; diagnostic importance of haematological indices in detecting beta thalassemia trait patients. Professional Med J. 2018; 25(4):545-550.
12. Hussain Z, Malik N, Chughtai AS. Diagnostic significance of red cell indices in beta-thalassemia trait. Biomedica. 2005;21(2):129-131.
13. Stephens AD, Colah R, Fucharoen S, Hoyer J, Keren D, McFarlane A, International Council for Standardization in Haematology (ICSH), *et al*. ICSH recommendations for assessing automated high-performance liquid chromatography and capillary electrophoresis equipment for the quantitation of HbA2. Int J Lab Hematol. 2015;37(5):577-582.
14. Colah RB. The use of NESTROFT for screening pregnant women for detection of β-thalassemia carriers. J Fetal Med. 2015;2(1):9-10.
15. Gorakshaker AC, Colah R, Nadkarni A, Desai S. Evaluation of the single tube osmotic fragility test in detection of ß-thalassaemia trait. Natl Med J India.

1990;3:171-173.
16. Sumera A, Ahmed S, Ali SA, Khanani R. Evaluation of NESTROFT as a marker of differentiation between β-thalassemia trait and iron deficiency anemia. Int J Collab Res Intern Med Public Health. 2012;4(8):1560-1566.
17. Safia R, Jairajpuri ZS, Khetrapal S, Hassan MJ, Gupta M, Jetley S. An analysis of NESTROFT and Red cell indices in evaluating antenatal mothers for beta thalassaemia trait. Bangladesh J Med Sci. 2018;17(3):411-416.
18. Afshan N, Hussain M. Diagnostic significance of MCV, MCH AND NESTROFT in thalassemia minor individuals. J Biol Res Appl Sci. 2012;3(2):21-24.
19. Khan K, Zahoor S. Pattern of Hemoglobinopathies on HPLC among patients referred to selected centers in Peshawar, Pakistan. Rawal Med J. 2018;43(4):623-626.
20. Bener A, Mohammad RR. Global distribution of consanguinity and their impact on complex diseases: Genetic disorders from an endogamous population. Egypt J Med Hum Genet. 2017;18(4):315-320.
21. Bhinder MA, Sadia H, Mahmood N, Qasim M, Hussain Z, Rashid MM, *et al*. Consanguinity: A blessing or menace at population level? Ann Hum Genet. 2019;83(4):214-219.
22. Romdhane L, Mezzi N, Hamdi Y, El-Kamah G, Barakat A, Abdelhak S. Consanguinity and inbreeding in health and disease in north African populations. Annu Rev Genomics Hum Genet. 2019;20:155-179.
23. Baig SM, Din MA, Hassan H, Azhar A, Baig JM, Aslam M, *et al*. Prevention of β-thalassemia in a large Pakistani family through cascade testing. Public Health Genomics. 2008;11(1):68-70.
24. Akhter MA, Nayyar U, Majeed T, Dar UF. Nestroft validity for screening of extended families of thalassaemia patients. J Fatima Jinnah Med Univ. 2015;9(1):40-43.
25. Aung KM, Lwin AA, Naw H, Mon NC, Soe HO, Oung MT, *et al*. Detection of common beta globin gene mutations on heterozygous beta thalassaemia including hemoglobin E among high school students' population. Int J Comput Eng Res Trends. 2020: 7(1):1-7.

ORIGINAL ARTICLE

Basic Life Support Training as Mandatory Training in Educational Institutions to Deal with Emergencies

Munazza Suharwardy Obaid[1], Saeed Minhas[2], Fouzia Naeem Effendi[3], Shiraz Shaikh[4], Farah Fatima Abbas[5], Ferrukh Zehravi[6], Malik Tajuddin[1], M Bastle Ullah Ansari[7]

[1]Department of Community Medicine, Dow University of Health Sciences and Visiting Academic in Manchester University, [2]Department of Surgery, Jinnah Post Graduate Medical Center,[3]Department of Community Health Sciences, Bahria University Medical and Dental College,[4] Department of Public Health, Jinnah Sindh Medical University, [5]Department of Pathology, Dow University of Health Sciences, [6]Department of Community Medicine, Liaquat National Medical College, [7]Student, Karachi, Pakistan.

ABSTRACT

Background: Injury is a significant issue of public health, a major global burden and first aid training programs incorporated as part of school curriculum will have a considerable impact on public health, and this will in turn reduce out of hospital cardiac arrests. The study aimed to determine the immediate First aid knowledge and long term retention of its knowledge (after three months) of the students (13-18 years), participating in a volunteer program in Karachi.

Methods: This study was conducted in a tertiary care hospital, delivering First aid training to 143 participants by Basic life support (BLS) trained staff in a tertiary care hospital. The design consisted of a pre-test, post-test and follows up assessments, carried out via questionnaire and hands-on assessment. SPSS was used for statistical analysis. ANOVA and Friedman test was applied to evaluate the dependent variable's improvement in knowledge between pre, post and follow up tests.

Results: From 143 participants, 41.5 %, 63.8 % and 61 % satisfactory results were seen in the pre-test, post-test and follow up assessments, respectively. In Pre-test, males scored 18.75 % and the females scored 8.42%, whereas, in the post-test, the scores were 75.7% and 64.58% respectively. Similarly, in follow up evaluations results obtained were 62.1% and 45.8% respectively. Hands-on scores were 88.4% in males and 95.8 % in females.

Conclusion: The level of first aid knowledge was unquestionably less satisfactory in the pre-test evaluations compared to post-test and follows up assessments. Due to its effectiveness, it should be incorporated as part of the school curriculum.

Keywords: Adolescents; Knowledge; Cardiopulmonary Resuscitation.

Corresponding Author:
Dr. Munazza Suharwardy Obaid
Manchester University, Oxford Rd,
Manchester, United Kingdom.
Email: Munazza.obaid@manchester.ac.uk, munazzaobaid@hotmail.com
https://doi.org/10.36283/PJMD10-1/003

INTRODUCTION

When an injury or an illness occurs to a victim, the help and assistance provided by a layperson or trained one, until a definitive medical treatment arrives, is called first aid (FA) [1]. FA includes Basic life support (BLS) which comprises of initial assessment, airway maintenance, and expired air ventilation and chest compressions and is known as cardiopulmonary resuscitation (CPR) [2].

First aid is a huge terminology but some components of first aid skills would include CPR and immediate care of fractures, poisoning, burns of all degrees etc [3]. The eventual goal of first aid is to stop or abolish the possible damage at a given time before reaching a suitable health care centre; consequently, it is important to give fundamental knowledge of first aid to all[4].

Throughout the world, injury is the major killer of children as well as adolescents and accounts for approximately 133,117 deaths in Electronic medical records (EMR) compared to 707,755 globally[5]. First aid provided by the local people can reduce the death toll significantly[6]. FA can be taught from a very young age to school going children[7]. In developed countries, FA is part of the curriculum as students are a good source of transforming information to the community. It has been noticed that high school children are more prone to injuries and have an affinity to be involved in riskier attitudes towards life [2,8]. Prompt response at the right time can save a lot of lives and affect future health and quality of life as well [9].

It has been suggested, training for bystanders has been a means of bridging the gap in localities with inadequate Emergency medical services[10]. General public education campaigns should be encouraged in this field[11]. Research in Pakistan suggested the adolescent age group can be of great help for the family members and can call for help in case of emergency to reduce casualties[12]. Another study from Pakistan quoted that 17.5 % of students have had formal first aid training showing (p-value <0.001) when the comparison was made between trained and non-trained students in answering correctly[13].

In all public settings, first aid measures are an important service that should be provided. In educational services, it is imperative to be able to provide prompt basic first aid[14]. The health problem of a society can be addressed if the students can be appropriately prepared and cultured for healthful living as they have great potential[15]. Hence, this study explored the level of knowledge of the above-mentioned age group along with this it would provide a baseline survey as well as give an update about the improvement brought in because of the intervention. This study would help us evaluate their knowledge about how to resuscitate airway and breathing passage to reduce the morbidity, mortality, and disability due to unintentional injuries. This would also sensitize them with the basic skills and give the confidence to respond in an emergency. This study will develop an evidence-based educational pathway to enable the integration of first aid into the school curriculum by defining the goals to train different age groups. The study aimed to determine the immediate first aid knowledge and long term retention of its knowledge (after three months) of the students 13-18 years old participating in a volunteer program in Karachi.

METHODS

This quasi-experimental study with the intervention of a first aid /Basic Life Support training program was conducted in a tertiary care hospital. The 13-18 years old students were included in the study. Two groups of 25 students were randomly selected from three batches and were trained by the hospital's specially trained Basic life support (BLS) staff. The sample size of 137 was calculated using the software to cater for non-response, incomplete questionnaires, or drop out from the study 10% was added and the final sample size was 150.

The volunteer program took 6 days; the intervention was given in the first two days from 9.30am to 12pm. A pre-test was done on first day of the training and post-test was conducted at the end of the second day after the intervention was completed. Data collection was done with the help of the hospital team as well as the principal investigator. Participants' informed consent was taken from the student's parents. Ethical approval was obtained from the hospital administration to conduct the research and intervention and the IRB reference number was JSMU/IRB/2017/-89.

A questionnaire was used for Assessment of the Victim (6), CPR (11), Breathing (6) and information regarding the infant's assessment (3). Experts and Cronbach's Alpha calculation (0.762) validated the questionnaire. Based on the panel of experts the participant's knowledge was considered satisfactory when the total score was 60% or more and unsatisfactory when it was less than 60 % [16]. SPSS was used for data entry and statistical analysis. In order to compare the three groups repeated ANOVA was used. Friedman test was applied as data were not normally distributed, to evaluate the dependent variable's improvement in knowledge between pre, post and follow up tests and $p < 0.05$ was considered statistically significant.

RESULTS

A total of 150 students were given a questionnaire at three different stages pre-test (before the intervention), post-test 1 (after the intervention), follow up and assessment (after three months). Valid responses collected at the end were 143 making a response rate of 95.3 %. The average age (Table 1) of the participants was (mean±SD) 15.92±1.24 of which 95.9% of the students belonged to matric/O'levels, 35.3% belonged to intermediate/A 'Levels and 10.20% to grade VIII and below. There was a significant change between pretest, post-test and follow up readings (Table 1). The frequency of good

knowledge (≥ 60%) (Table 1) regarding all components of first aid and basic life support program was significantly higher in the post-intervention and follow up phases than the pre-intervention phase. In student's situational knowledge about first aid (Figure 1) throughout the interventions, posttest gives the most elevated results in all the categories.

Table 1: Consolidated pre, post and follow up tests scores of knowledge of first aid.

Sr. No.	Questions Satisfactory Knowledge (60% or above)	Pretest Mean±SD 10.80±2.994 n (%)		χ^2-test (p-value) Pre – Post	Post Test Mean ± SD 16.61± 2.627 n (%)		χ^2-test (p-value) Post – Follow up	Follow Up (After three months) Mean ± SD 15.87 ± 2.915 n (%)		χ^2-test (p-value) Pre – Follow -up
1.	Which of the followings are the Indications for CPR?	95	66.43	2.71 (<0.001)*	127	88.81	0.004 (<0.001)*	106	74.12	0.399
2.	ABC approach in BLS is:	77	53.84	0.005	97	67.83	0.35	88	61.53	0.063
3.	Initial pulse assessment duration is:	43	30.06	1.68 (<0.001)*	137	95.80	0.04 (<0.001)*	119	83.21	1.04 (<0.001)*
4.	The initial BLS steps for adults are:	19	13.28	3.38 (<0.001)*	46	32.16	0.45	40	27.97	0.04
5.	In one man CPR what is the recommended compression to breathing ratio?	24	16.78	2.31 (<0.001)*	137	95.80	0.05	129	90.20	8.24 (<0.001)*
6.	Compression rate	32	22.37	7.76 (<0.001)*	84	58.74	0.05	75	52.44	1.86 (<0.001)*
7.	When re -assessing the Victim's pulse, respiration and chest rise up, we do.	58	40.55	1.00	123	86.01	0.28	126	88.11	0.51
8.	Where can pulse be easily felt (checked)?	51	35.66	1.46 (<0.001)*	125	87.41	0.162	132	92.30	9.00 (<0.001)*
9.	What is the first thing you should do if you find a collapsed person?	50	34.96	9.47 (<0.001)*	50	34.96	0.104	63	44.05	2.16 (<0.001)*
10.	What action would you use to open the Person's airways?	104	72.72	2.09 (<0.001)*	135	94.40	0.49	132	92.30	6.10 (<0.001)*
11.	If the person is not breathing what would you do?	75	52.44	0.027	89	62.23	0.008	65	45.45	0.192
12.	If the person is breathing what would you do?	108	75.52	0.746	123	86.01	0.059	79	55.24	0.091
13.	Where in the chest would you press if you were giving chest compressions?	83	58.04	4.84 (<0.001)*	139	97.20	0.527	137	95.80	1.47 (<0.001)*
14.	When should we stop giving CPR?	53	37.06	2.34 (<0.001)*	79	55.24	0.062		45.45	0.088

**Statistically significant at $p < 0.05$*

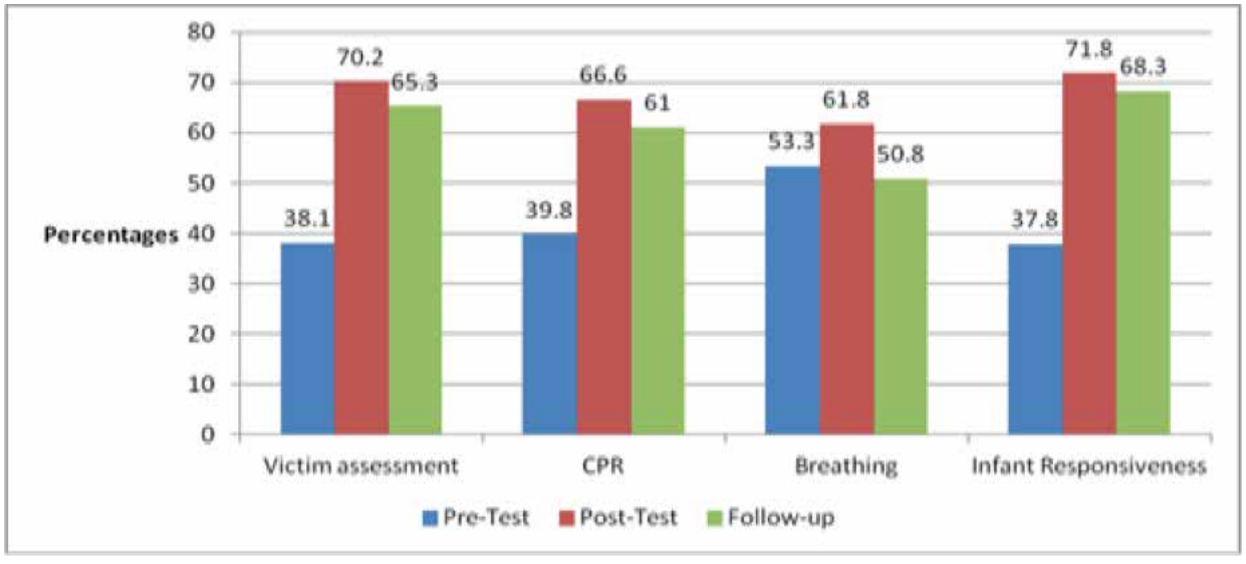


Figure 1: Student's situational knowledge about first aid throughout the interventions.

In pre-test 126 students out of 143 students got less than 60% (Table 2). In post-test only 40 students got less than the required result. None of the students achieved above 90% in pre, post or follow up but in assessment questions, 3 students got more than 90% and only 46 students did not get a satisfactory result. In follow up 45 got less than satisfactory result.

Table 2: Grades of Knowledge achieved by the students in first aid training program.

Test	Grades				
	A* > 91 %	A > 80 – 90.9 %	B > 79.9 -70%	C > 69.9 -60%	F < 60%
Pre - Test	0	0	1	16	126
Post - Test	0	10	27	66	40
Follow - Up Test	0	9	20	53	45
Hands on Assessment	3	10	39	45	46

Males scored better than the females in pretest (18.75%) but in posttest follow up and assessments females scored extremely better, although again in hands on the boys (93.3 %) fared well (Table 3).

Table 3: Consolidate levels of test among gender, education, and different age groups.

Parameters	Pre - Test	Post - Test	Follow Up	Hands on Assessment
Gender				
Female (95)	8.42 %	75.7 %	62.1%	88.4%
Male (48)	18.75 %	64.58 %	45.8%	95.8%
Levels of Education				
Secondary (14)	0 %	13.9 %	10.1%	13.9%
Matric (78)	7.5 %	67.08 %	51.8%	72.1%
Intermediate (51)	22.4%	79.5 %	65.3%	59.1%
Age Groups				
13 - 15 years (53)	7.5 %	64.1%	54.7%	86.7%
16 - 18 years (90)	14.4 %	76.6 %	57.7%	93.3%

It is seen that in pre-test students acquired above satisfactory results at intermediate and matric levels respectively whereas none of the students from secondary level (15) got satisfactory result.

In posttest intermediate level, students got highest (79.5%) whereas, matric students got just above satisfactory results (67.08%) and secondary levels getting the lowest (13.9%). A slight decline of percentages was observed at follow up from Intermediate to secondary levels whereas the hands on practice got maximum 72.1% from matric students (Table 3). In all the levels of intervention higher, the ages better the results.

DISCUSSION

Pakistan trenchantly expends minimal per unit of the population on health and contains one of the high-rise death rates because of contagious diseases[17]. A review of 10 studies revealed that laypersons range from 10.7% to 65% attempt giving emergency assistance considering that 83.7% give incorrect first aid [18]. The intention was to observe that training of this age group will drive to advancement in their present knowledge. Volunteers from a tertiary care hospital aged 13-18 years with a mean age of 15.29±1.24 years were chosen as this age cohort has remarkable keenness and zests to inculcate and obtain knowledge and to brighten their skills by hands-on rehearse[19]. In a study of Zagazig University, the prepa-ratory school children aged 11-16 years got signifi-cant results in pre-post and pre-follow up (p<0.001) in basic life support and choking[16].

In another study, it was seen that concept of CPR training can be conveyed very effectively to schoolchildren[20]. A study in primary and secondary schools bystanders CPR (BCPR) achieved positive results before and after the training[21]. Results of pre, post and follow-up combined scores conclude significant changes. The standard knowledge of BLS had a mean value of 10.8±2.99. Whereas an analogous study observed very low, BLS and choking baseline result in school students[22]. Many different Medical school's studies also showed a low level of first aid knowledge[23].

In post-test, the acquired results were above 60 % where as they dropped in the follow up (3 months after the intervention). These results were consistent with another study where the intervention was given to school health advisors in Qassim province KSA (Kingdom of Saudi Arabia) in which it showed significantly higher results in post-intervention and follow up phases[19]. Masih et al. showed low pre-test, post-test knowledge mean scores as 27.32 ±5.73 and 34.76 ± 4.35 respectively[23]. In the current study it was observed that in pretest males fared better than females but the other two times namely post-test and follow up girls got better results. While in hands on practice boys scored more than the girls. An Indian study in undergraduate students showed contrasting results that 11.5% of males and 15.4% of females had a satisfactory level of knowledge on first aid measures[24].

This study included different levels of education specifically secondary,matric and intermediate but none of the students got a satisfactory result from the secondary level in Pre-test, whereas intermediate was better than matric students. The Post-test and follow up results of intermediate students were better than the matric and secondary students. Hands on practice had better results from matric students, the second was intermediate students, and secondary student's scores were the least. The results were similar to the Student's knowledge as documented by another study[23].

A comparison was done between 13-15, 16-18 years old in this study and it was seen that at all the levels i.e., pre, post-test, follow up, and assessment higher the age better the results were achieved. This is consistent with the study on health advisors that showed with increasing age and education level that the trainees achieve better scores[21]. The strength of the study was that it is an interventional study and since the participants were chosen from an already running volunteer program, the budget was immensely reduced. Limitations were time and budget constraints due to which randomized control trial could not be executed which would have been an ideal study design otherwise. The participants selected from schools would have given results that are more generalizable but selection bias was there, as permissions were not allowed, all the aspects of first aid could not be explored due to lack of time and budget.

Knowledge and skills about FA may be integrated and CPR skills may be focused and improved. Evidence based studies should be conducted in schools at large to assess knowledge and attitudes towards FA/CPR in society. Incidences and consequences of school-age child injuries may be assessed by further researches. The finding of the research strongly recommends that these training may be made part of the school curriculum.

CONCLUSION

First aid training delivered to the volunteers was very effective and more training may be conducted at larger scales in schools. Adding first aid training in the curriculum may act as fundamental for present and future national emergencies as well as normal control.

ACKNOWLEDGEMENTS

The authors would like to acknowledge their teachers, families, unit heads and students for being an inspiration.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICS APPROVAL

Ethical Approval was obtained from the hospital administration to conduct the research and intervention. The proposal was submitted to Institutional Review Board of APPNA Institute of Public Health with the IRB reference number: JSMU/IRB/2017/-89.

PATIENT CONSENT

Informed consent was obtained from parents of all participating students' (as the participants are less than 18 years) and confidentiality was maintained.

AUTHORS' CONTRIBUTION

MO developed the concept, wrote the introduction and also contributed in methodology development and helped in data collection. SM helped in development of the concept and writing of methodology and results. FE did data analysis and wrote the results. SS helped in data analysis as well. FA wrote results and discussion while FZ helped in discussion and abstract writing. MT helped in introduction writing too. BA did data analysis, results formulation and results writing.

REFERENCES

1. De Buck E, Laermans J, Vanhove AC, Dockx K, Vandekerckhove P, Geduld H. An educational pathway and teaching materials for first aid training of children in sub-Saharan Africa based on the best available evidence. BMC Public Health. 2020; 20:1-6.
2. Panchal AR, Berg KM, Hirsch KG, Kudenchuk PJ, Del Rios M, Cabañas JG, *et al*. 2019 American Heart Association focused update on advanced cardiovascular life support: use of advanced airways, vasopressors, and extracorporeal cardiopulmonary resuscitation during cardiac arrest: an update to the American Heart Association guidelines for cardiopulmonary resuscitation and emergency cardiovascular care. Circulation. 2019;140(24):e881-e894.
3. Al-Musa HM, Bharti RK, Alsamghan AS, Asiri M, Alqahtani MS, Al-qahtani D, *et al*. Knowledge of first aid skills among medical students in King Khalid University, Abha, Saudi Arabia. People's J Sci Res. 2017;10(1):1-6.
4. Ganfure G, Ameya G, Tamirat A, Lencha B, Bikila D. First aid knowledge, attitude, practice, and associated factors among kindergarten teachers of Lideta sub-city Addis Ababa, Ethiopia. PloS one. 2018;13(3):e0194263:1-15.
5. Al-Hajj S, El Bcheraoui C, Daoud F, Khalil I, Moradi-Lakeh M, Abu-Raddad LJ, *et al*. Child and adolescent injury burden in the eastern mediterranean region: Findings from the Global Burden of Disease 1990-2017. BMC Public Health. 2020;20:1-10.
6. Chokotho L, Mulwafu W, Singini I, Njalale Y, Maliwichi-Senganimalunje L, Jacobsen KH. First responders and prehospital care for road traffic injuries in Malawi. Prehosp Disaster Med. 2017;32(1):14-19.
7. Başer M, Çoban S, Taşci S, Sungur G, Bayat M. Evaluating first-aid knowledge and attitudes of a sample of Turkish primary school teachers. J Emerg Nurs. 2007;33(5):428-432.
8. Afifi RM, Zaytou SS, El Raggal AA, Qulali A, Ayoub HA. Involvement of male youth into accidents in upper Egypt: Pattern and risk analysis. Health. 2015;7(08):965-974.
9. Peterson TD, Noland S, Russell DW, Paradise NF. Bystander trauma care training in Iowa. Prehosp Emerg Care. 1999;3(3):225-230.
10. Olumide AO, Asuzu MC, Kale OO. Effect of first aid education on first aid knowledge and skills of commercial drivers in South West Nigeria. Prehosp Disaster Med. 2015;30(6):579-585.
11. Wilks J, Kanasa H, Pendergast D, Clark K. Emergency response readiness for primary school children. Aust Health Rev. 2016;40(4):357-363.
12. Asim M, Ahmad B, Salam M. Household's willingness to pay for solid waste management, parks maintenance and horticulture services (selected areas of Karachi-case in point). Glob Manag J Acad Corp Stud. 2016;6(2): 116-128.
13. Khan A, Shaikh S, Shuaib F, Sattar A, Samani SA, Shabbir Q, *et al*. Knowledge attitude and practices of undergraduate students regarding first aid measures. J Pak Med Assoc. 2010;60(1):68-72.
14. Mohammed AA. The influence of training program on knowledge and practices of preparatory schools' children related to the selected first aid. Am J Nurs. 2018;6(4):158-163.
15. Deekshitha P, Dhivya K, Pravallika S, Lavanya D, Kesini M. Evaluation of knowledge, attitude and practice on first aid measures among students. Indian J Public Health Res Develop. 2018;9(10):50-56.
16. Wafik W, Tork H. Effectiveness of a first-aid intervention program applied by undergraduate nursing students to preparatory school children. Nurs Health Sci. 2014;16(1):112-118.
17. World Health Organization. World health statistics 2010 [Internet]. World Health Organization, 2010 [cited 2020 Sep 16]. Available from: https://ww-

w.who.int/whosis/whostat/2010/en/
18. Tannvik TD, Bakke HK, Wisborg T. A systematic literature review on first aid provided by laypeople to trauma victims. Acta Anaesthesiol Scand. 2012; 56(10):1222-1227.
19. Campbell S. Supporting mandatory first aid training in primary schools. Nurs Stand. 2012;27(6): 35-39.
20. Jones I, Whitfield R, Colquhoun M, Chamberlain D, Vetter N, Newcombe R. At what age can schoolchildren provide effective chest compressions? An observational study from the Heartstart UK schools training programme. BMJ. 2007;334(7605):1201-1203.
21. Behairy AS, Al-Batanony MA. Effectiveness of first-aid and basic life support intervention program on school health advisors. J Health Med Nurs. 2015; 24:136-144.
22. Sharma S. Knowledge of first aid among undergraduate students of KMC, Mangalore. New Indian J Surg. 2011;2:p.401.
23. Masih S, Sharma RK, Kumar A. Knowledge and practice of primary school teachers about first aid management of selected minor injuries among children. International Journal of Medicine and Public Health. 2014;4(4): 458-462.
24. Kanstad BK, Nilsen SA, Fredriksen K. CPR knowledge and attitude to performing bystander CPR among secondary school students in Norway. Resusc. 2011;82(8):1053-1059.

ORIGINAL ARTICLE

Frequency and Trends of Prostatic Diseases in a Subset of Karachi Population: A Retrospective Study

Asma Shabbir[1], Umaima Tariq Khan[2], Diya Nisar[2], Parversh Kumar Rath[2], Cheena Kumari[3], Talat Zehra[1], Syed Mehmood Hasan[1]

[1]Department of Pathology, Sindh Medical College, Jinnah Sindh Medical University, [2]Student, Sindh Medical College, Jinnah Sindh Medical University, [3]House Officer, Sindh Medical College, Jinnah Sindh Medical University, Karachi, Pakistan.

ABSTRACT

Background: Prostatic diseases are a leading cause of frequent urinary complaints in elderly males. The most common diseases include prostatitis, benign prostatic hyperplasia, and prostate cancer. This study aimed to determine the frequency of the prostatic diseases, their trend over the years, and the association of age and diagnosis.

Methods: A retrospective descriptive study was conducted in Basic Medical Sciences Institute (BMSI) at Jinnah Postgraduate Medical Centre, Karachi. Data was collected from the histopathological files and all the cases diagnosed from 2014 to 2018 were included. Statistical analysis was done using SPSS with 95% confidence interval and *p*-value of ≤ 0.05 was considered significant. The Chi-square test was applied to find the association with age and year of diagnosis.

Results: Total 163 prostate cases were documented from 2014 to 2018, out of which 98 (60.1%) were of benign prostatic hyperplasia, which was most commonly diagnosed in men aged, 61-70. Prostatic adenocarcinomas having Gleason scores 8-10 were frequent and commonly seen in men aged 51-60 years. An increasing trend (*p*-value=0.053) was observed in the diagnosis of these prostatic diseases within these five years.

Conclusion: Benign prostatic hyperplasia is the most frequently (*p*-value=0.140) diagnosed prostatic disease in men, followed by adenocarcinoma and prostatitis. Advanced stage prostatic adenocarcinoma was diagnosed more frequently 17(10.4%) and at an earlier age. There is an increase in the number (*p*-value=0.053) of cases of prostatic diseases diagnosed each year. National-level studies and health policies are needed to facilitate early diagnosis and treatment.

Keywords: Prostate; Prevalence; Benign Prostatic Hyperplasia; Prostatic Cancer; Gleason Score; Prostatitis.

Corresponding Author:
Dr. Asma Shabbir
Department of Pathology,
Sindh Medical College,
Jinnah Sindh Medical University,
Karachi, Pakistan.
Email: drasma52@gmail.com
https://doi.org/10.36283/PJMD10-1/004

INTRODUCTION

The prostate is an important exocrine gland that is anatomically located underneath the urinary bladder and is a part of the male reproductive system. It synthesizes and secretes prostatic fluid, a component of semen, which helps in the protection and nourishment of sperms. The prostate gland also assists in the ejaculation of semen into the urethra by contraction of its muscles. As the age advances, this gland becomes susceptible to many diseases such as inflammation, infections, benign enlarge-

ment, and neoplasia. These disorders are an important cause of frequent urinary complaints such as poor flow, nocturia, frequency, and urgency; commonly referred to as lower urinary tract symptoms (LUTS) in the ageing male population[1]. Lower urinary tract symptoms negatively influence the patient's quality of life and these patients reported depressive symptoms and diminished sexual activity more frequently than those without lower urinary tract symptoms diminish. Moreover, patients with LUTS also reported poor overall health and decreased productivity at work due to their symptoms[2].

Prostatitis is defined as a surge in the inflammatory cells present in the prostate following an infectious or inflammatory cause which may present as acute bacterial prostatitis, chronic bacterial prostatitis, a chronic pelvic pain syndrome, or asymptomatic prostatitis[3]. It is the third most commonly diagnosed urinary tract disease in men, following benign prostatic hyperplasia and prostate cancer[4]. Previous studies suggested that prostatitis could be associated with or lead to the development of other diseases of the prostate gland like benign prostatic hyperplasia and prostate cancer[5,6].

Benign prostatic hyperplasia (BPH) is a very common histological diagnosis of prostatic diseases in ageing men. It can be described as the benign overgrowth of prostatic tissue, which results in glandular enlargement, leading to constriction of the urethra and ultimately causing lower urinary tract symptoms and urinary retention[7]. According to a global study, the prevalence of benign prostatic hyperplasia was found to be 14.8% at 40 years of age and 36.8% in ages 80 and above[8]. This suggests that the prevalence of BPH increases with increasing age. Another study conducted in North America reported that about 50% to 75% of men over the age of 50 years and about 80% of men over the age of 70 years suffered from benign prostatic hyperplasia and associated LUTS[9].

Prostate cancer is the second most common malignancy diagnosed in males after lung cancer and is the fourth leading cause of mortality in males worldwide (6.7% of total male mortality by cancer)[10]. The incidence rates are highest in developed countries like Australia/New Zealand (86.4%), Northern Europe (85.7%), Western Europe (75.8%), and North America (73.7%) [11]. Studies have reported that there is a direct correlation between the Human Development Index (HDI) of a country and the incidence of prostate cancer. Therefore, the incidence rate of prostate cancer, which used to be relatively lower in the developing Asian countries, is seen to be increasing steadily over the past decade and this may be due to the adoption of western lifestyles and excessive fatty diet[12,13]. Asian countries with high HDI such as Israel, Turkey, Lebanon, Singapore, Japan, and South Korea were seen to have a higher incidence of prostate cancer and this incidence is predicted to increase significantly in the future[14]. It has become the sixth most frequent cancer among men in Asia, the average mortality rate being 3.8 per 100,000[15].

The number of cases of prostate cancer documented in Pakistan has increased in the past decade. According to the Karachi Cancer Registry (KCR), there has been a 60% rise in the prostate cancer cases observed from 1995-2002[16]. A recent study shows that the overall prevalence of prostate cancer in Pakistan is 5%[17]. Cancer registry of Shaukat Khanum Memorial Cancer Hospital for the year 2018 reported prostate cancer to be the most common malignancy diagnosed in males[18]. Punjab Cancer Registry report of 2018 documented that prostate cancer is the second most frequently diagnosed cancer of males[19]. Unfortunately, despite the high prevalence of prostatic diseases, no recent studies have been conducted related to all prostatic pathologies in Karachi. Therefore, this study aimed to identify the frequency of prostatitis, benign prostatic hyperplasia, and prostate cancer at a tertiary care hospital in Karachi. Furthermore, the trends of these diseases over the study period from 2014-2018, and the association of each condition with the age of the patient had also analysed. This will help in better prevention, earlier diagnosis, and treatment of those affected by these diseases and serve as a starting point for future research.

METHODS

A descriptive cross-sectional study was conducted to evaluate the frequency of different prostatic diseases in Karachi (prostatitis, benign prostatic hyperplasia, and prostatic cancer) and to assess their possible association with age. After getting approval from the Institutional Review Board (IRB number: JSMU/IRB/2019/-192), data was collected from the histopathological records present at the Basic Medical Sciences Institute (BMSI) at Jinnah Postgraduate Medical Centre (JPMC). This data included the records of patients from diverse areas of the country as the location of the institution being a major tertiary care hospital. The authors noted down all histologically diagnosed patients with prostatitis, benign prostatic hyperplasia, and prostatic cancer from the year 2014 to 2018.

Cases of benign prostatic hyperplasia that were associated with prostatitis and prostate cancer were also noted. The biopsy specimens were transferred to 10% neutral buffer formalin and gross features of samples were examined. Paraffin blocks were prepared for subsequent staining and microscopy. Tissue sections (3-4 µm) were cut from paraffin blocks and treated with haematoxylin and eosin (H and E) stain. Then examine under the microscope. The registration number of each patient, year of

diagnosis, age, histological diagnosis, and Gleason scores (for grading of prostate cancer) was also recorded. Furthermore, Gleason scores were stratified into four groups as follows: 2-4 (very low grade), 5-6 (low grade), 7 (medium grade), and 8-10 (high grade) [20]. Cases with deficient data were excluded from the study.

After collection, the data was entered and statistically analysed using Statistical Package for the Social Sciences (SPSS) version 23.0. The frequency of each prostatic disease and mean age of the patients was calculated. The Chi-square test was applied to assess both the association of prostatic diseases with the age of the patients as well as to see the pattern of prostatic diseases over the 5 years being studied (2014-2018). Furthermore, an analysis was done to find the frequency of Gleason scores seen in different ages of prostatic cancer cases[20]. The authors set a 95% confidence interval and a *p*-value of ≤ 0.05 was considered significant.

RESULTS

The age of patients in this study ranged from 41 to 90 years in which most of the patients were between 61-70 years of age. From the data collected, 145 (88.9%) specimens were of benign prostatic hyperplasia, 17 (10.4%) were of prostate cancer and 1 (0.6%) was of prostatitis. Out of the 145 specimens (Table 1) of benign prostatic hyperplasia, 98 (60.1%) were of isolated BPH, 43 (26.4%) were associated with prostatitis, and 4 (2.5%) with metaplasia.

Table 1: Data from prostatic diseases according to age at diagnosis.

Age in Years	Prostatitis n(%)	Benign Prostatic Hyperplasia (BPH) n(%)	BPH with Prostatitisn (%)	BPH with Metaplasian (%)	Prostatic Adenocarcinoman (%)	*p*-Value
41-50	0	5 (3.1)	3 (1.8)	0	0	0.140
51-60	0	30 (18.4)	19 (11.7)	1 (0.6)	7 (4.3)	
61-70	1 (0.6)	52 (31.9)	10 (6.1)	3 (1.8)	5 (3.1)	
71-80	0	11 (6.7)	8 (4.9)	0	4 (2.5)	
81-90	0	0	3 (1.8)	0	1 (0.6)	
Total	1 (0.6)	98 (60.1)	43 (26.4)	4 (2.5)	17 (10.4)	

Chi-square test applied.

An increasing trend (Table 2) was observed in the number of prostatic diseases being diagnosed each year (*p* value= 0.053). There were 14.7% cases in 2014, 15.3% in 2015, 19.6% in 2016, 24.5% in 2017, and 25.8% in 2018. This increasing trend was particularly apparent in cases of isolated BPH and BPH with prostatitis. Out of the total cases of isolated BPH, 9.2% of cases were diagnosed in 2014, and this number grew to 14.1% in 2018. Similarly, cases of benign prostatic hyperplasia (BPH) with prostatitis grew from 3.1% in 2014 to 9.2% in 2018. No significant association was seen between the prostatic diseases and age at diagnosis (*p*-value= 0.140).

Table 2: Information related to different prostatic diseases over 5 years.

Diagnostic Parameters	Year n (%)					*p*-Value
	2014	2015	2016	2017	2018	
Prostatitis	0	0	0	0	1 (0.6)	0.053
Benign prostatic hyperplasia	15 (9.2)	20 (12.3)	18 (11.0)	22 (13.5)	23 (14.1)	
Benign prostatic hyperplasia with prostatitis	5 (3.1)	2 (1.2)	7 (4.3)	14 (8.6)	15 (9.2)	
Benign prostatic hyperplasia with metaplasia	2 (1.2)	2 (1.2)	0	0	0	
Prostatic adenocarcinoma	2 (1.2)	1 (0.6)	7 (4.3)	4 (2.5)	3 (1.8)	
Total	24 (14.7)	25 (15.3)	32 (19.6)	40 (24.5)	42 (25.8)	163 (100)

Chi-square test applied.

Benign prostatic hyperplasia (Figure 1a, b) was most diagnosed in men aged between 61-70 years.

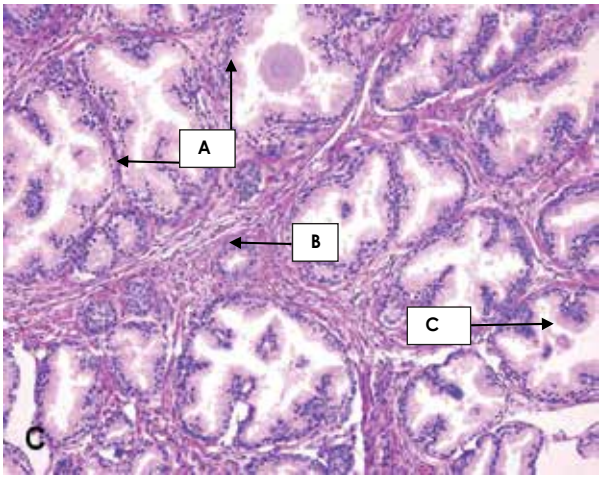


Figure 2a: Benign prostatic hyperplasia (H and E, 40x). Arrow A: Proliferating glands, Arrow B: Fibromuscular stroma arrow C: Corpora amylacea.

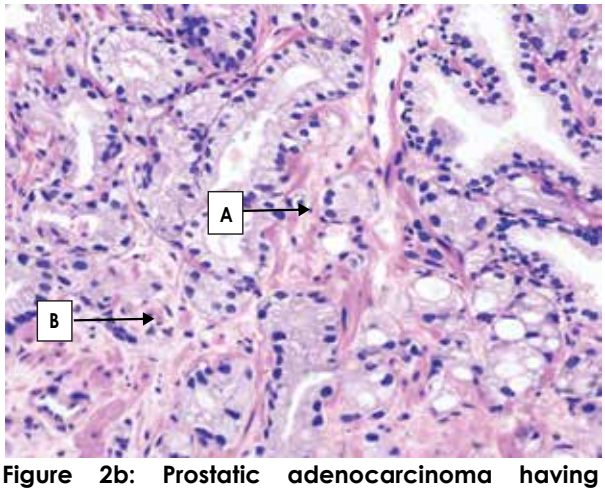


Figure 2b: Prostatic adenocarcinoma having Gleason score-7 (H and E, 40x). Arrow A: Small acinar arrangement, a few with well-formed lumina and back-to-back arranged glands, Arrow B: Less intervening stroma with malignant cells arranged in cords.

Poorly differentiated cases of adenocarcinoma (Table 3) having Gleason scores 8-10 were seen to be more common in our study and were most commonly seen in men aged between 51-60 years (*p*-value=0.050).

Table 3: Gleason scores seen in different age groups.

Age (years)	Gleason Score 2-4 n (%)	Gleason Score 5-6 n (%)	Gleason Score 7 n (%)	Gleason Score 8-10 n (%)	*p*-Value
41-50	0	0	0	0	
51-60	0	0	0	7 (41.2)	
61-70	0	1 (5.9)	0	4 (23.5)	0.05 0
71-80	0	0	1 (5.9)	3 (17.6)	
81-90	0	1 (5.9)	0	0	

DISCUSSION

Prostatic diseases remain the most common cause of lower urinary tract symptoms in the ageing male population worldwide. Prostatic diseases occurring in a patient interfere substantially with their quality of life, and if diagnosed late, may lead to poor prognosis and early death of the patient, in case of carcinoma.

The results of this study infer that benign prostatic hyperplasia was the most commonly diagnosed lesion of the prostate gland (88.9%), was isolated in most of the cases, and was most frequently seen in 61-70 years of age. Similar results have been found in a study conducted in India, a neighbouring country of Pakistan[21]. A study conducted at Dow University of Health Sciences Karachi in 2013 reported the frequency of benign prostatic hyperplasia to be 87.5% [22]. Another study, conducted in King Edward Medical University Lahore, reported their frequency to be 77.0% [23]. The age group affected most frequently in both of these studies is 61-70 years, which is similar to this study. The similarity in these results can be explained by multiple studies conducted in the west, which show that the development of benign prostatic hyperplasia is strongly associated with age and it is often considered a normal part of the ageing process[9,24]. A literature review carried out in France using data from 1990 to 2018 also observed that the demographic trends and increase in expected lifespan has led to a steady rise in the prevalence of Benign prostatic hyperplasia (BPH) in many countries worldwide, making it a major health concern[25].

Moreover, in this study, BPH was found to be associated with inflammation in some cases (26.4%), and very rarely with metaplasia (2.5%). The association of BPH with metaplasia is poorly understood but it

has been suggested that hyperplasia may increase the risk of developing prostatic metaplasia especially in Asian populations[15]. This study also found an increasing trend in the number of cases of prostatic diseases over the study period. This rise is notably evident in the cases of benign prostatic hyperplasia. A mere 9.2% of the cases of isolated BPH were diagnosed in 2014, whereas, in 2018, this percentage raised to 14.1%. Such rise was also evident in the cases of BPH associated with inflammation (prostatitis), the lowest number of cases being reported in 2015 (1.2%), and the highest in 2018 (9.2%). The increase in the incidence of BPH may be associated with certain changes in the lifestyle and an increased life expectancy due to advancements in health care. Hence, screening processes should be regularly performed in elderly men and in individuals with a higher risk to promote early diagnosis and a better prognosis of the disease.

Prostate cancer was found in 10.4% of all the cases in the current study. The number was similar to the previous single-centre studies conducted in Karachi, Lahore, and Faisalabad, which reported their frequencies to be 12.5%, 13%, and 13.5% respectively[22,26,27]. The incidence of prostate cancer is said to be high in developed countries because of their lifestyle, but in recent years, it has been reported that this incidence is rising in the developing countries as well due to the rapid urbanisation and westernisation and modification of lifestyle[10,12]. In Pakistan, Shaukat Khanum Memorial Cancer Hospital and Punjab Cancer Registry have reported an increase in the number of cases diagnosed with prostate cancer in recent years[18]. According to the Punjab Cancer Registry, the incidence of prostate cancer has increased in the last three years, being 7.2% in 2016 and 8.3% in 2018[26]. As presented in the Fourth Asian Prostate Cancer study meeting, various countries reported an increase in the number of cases being diagnosed. In China, the incidence of prostate cancer is rising more rapidly than any other cancer, it is also reported to be the third most common cancer of men in Singapore and its incidence is rising steadily in many other countries like Japan, Korea, Malaysia, and Vietnam[27]. However, such an increase in the cases of prostate cancer was not noted during this study.

Since cancer registry centers are not functional in Sindh, the exact frequency at which prostate cancer is being diagnosed in the province cannot be calculated. Hence, there is a dire need to establish regional as well as national cancer registry centers so that the overall frequency of prostate cancer can be calculated and compared with the statistics of other countries[12].

In this study, it was also observed that almost half of the cases of prostate cancer documented in the last five years were diagnosed at an earlier age (<60 years). This finding may be associated with western studies where the incidence of prostate cancer was observed to be increasing in younger ages[28]. On the contrary, a study conducted in Peshawar reported their mean ages to be 65 years or older. Unfortunately, sufficient data is not available regarding the genetic variants in this society. Therefore, genetic screening for prostate cancer remains a clinical entity that may be beneficial in the early diagnosis of prostate cancer and requires more consideration[28].

The Gleason score also has an important association with age at diagnosis. In our study, the scores of 8-10 were most common and were found throughout the fifth, sixth, and seventh decades of life. Additionally, the scores of 8-10 were mostly seen in men aged between 51-60 years. Studies conducted in the west have suggested that the probability of being diagnosed with a high Gleason score increases with increasing age[29]. However, such an age-related association was not observed in this research. Furthermore, the results of previous research carried out in Aga Khan University Hospital found Gleason score 7 to be the most common score in their study at the time of diagnosis[28]. However, there is no recent data available in the study population to compare and evaluate the possible causes of finding higher scores at the time of diagnosis. Possible reasons for this could be the rapid urbanization, changes in lifestyle, or exposure to carcinogenic substances, which lead to the disease occurring at an earlier age, and hence by the time of diagnosis, the disease has progressed to its later stages. Early pre-prostate-specific antigen (PSA) screening i.e., screening at or before 50 years of age, has been proven to be very helpful in predicting the diagnosis of advanced-stage prostate cancer later in life, therefore, it may be suggested that the screening processes for prostate cancer should be started earlier than that practiced now[25].

One limitation of this study was that since this was a single-centre study, the data was not representative of the entire region. Furthermore, there is a lack of awareness about prostatic diseases in the general population, as proposed by a recent study conducted in Karachi, which reports that as much as 64% of men aged 45 and above were unaware of prostatic diseases and approximately 85% of them were not screened[30]. Therefore, patients may not be aware of these prevention practices and fail to consult a physician when the disease is at its initial stage. In this way, a large number of cases of prostatic diseases may either remain undiscovered or only become apparent when the disease has progressed into its late stage. This not only leads to poor prognosis, but it also means that many subclinical cases would be missed in this study sample. There is a dire need for awareness among the

public regarding prostate health as well as the need for early screening practices so that diagnosis can be made before the disease progresses to an advanced stage. Additionally, more multicentre studies in Karachi and at the national level are needed to find out the frequency of prostatic diseases in our population[30].

CONCLUSION

In this study, a higher prevalence of benign prostatic hyperplasia (88.9%) was observed as compared to the other prostatic diseases and it was seen to be most frequently diagnosed between the ages 61-70 years. Adenocarcinoma of the prostate, although less common overall (10.4%), was frequently of higher grade (Gleason score 8-10) and diagnosed in the age group of 51-60 years. An increasing trend in the number of prostatic diseases diagnosed each year was observed, most notably in cases of benign prostatic hyperplasia.

ACKNOWLEDGEMENTS

We would like to thank Prof. Shehnaz Kher and Dr. Naushaba Rahat for allowing us to collect data from the Basic Medical Sciences Institute at Jinnah Postgraduate Medical Centre (JPMC).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICS APPROVAL

This research was initiated after obtaining approval from the Institutional Review Board of Jinnah Sindh Medical University, Karachi (IRB number: JSMU/IRB/2019/-192).

PATIENT CONSENT

Not applicable since data was collected from the histopathological files and the study has been exempted from full IRB review.

AUTHORS' CONTRIBUTION

AS contributed in conception and design of the study, acquisition, and analysis of data, and critical review of the manuscript. UT drafted the manuscript, and critically reviewed it. DN took part in critical analysis, data interpretation and manuscript writing. CK and PK also performed the data acquisition and critical review. TZ also collected the data, did the critical analysis and SMH preformed the critical review.

REFERENCES

1. Oyelekan A, Ogunsemi O, Afe T, Ayoade B, Nwokoro C, Oluyemi O, *et al*. Sleep quality and lower urinary tract symptoms among patients with prostatic diseases. Ann Afr Surg. 2020;17(2):69-71.
2. Kim TH, Han DH, Ryu DS, Lee KS. The impact of lower urinary tract symptoms on quality of life, work productivity, depressive symptoms, and sexuality in Korean men aged 40 years and older: a population-based survey. Int Neurourol J. 2015;19(2): 120-129.
3. Smieško GM. Acute and chronic prostatitis. SANAMED. 2020;15(1):71-76.
4. Khan FU, Ihsan AU, Khan HU, Jana R, Wazir J, Khongorzul P, *et al*. Comprehensive overview of prostatitis. Biomed Pharmacother. 2017;94:1064- 1076.
5. Repetto E, Sosa A, Colla R, Revol M, Metrebian E, Metrebian S. Relationship of prostatitis in the appearance of prostate cancer and benign prostatic hyperplasia. Revista Cubana de Urología. 2019;8(1):22-33.
6. Zhang L, Wang Y, Qin Z, Gao X, Xing Q, Li R, *et al*. Correlation between Prostatitis, benign prostatic hyperplasia and prostate cancer: A systematic review and meta-analysis. J Cancer. 2020;11(1): 177-189.
7. María Molero J, Miñana B, Palacios-Moreno JM, Téllez Martínez-Fornes M, Lorite Mingot D, Agra Rolán A, *et al*. Real-world assessment and characteristics of men with benign prostatic hyperplasia (BPH) in primary care and urology clinics in Spain. Int J Clin Pract. 2020;74(11):e13602:1-12.
8. Lee SWH, Chan EMC, Lai YK. The global burden of lower urinary tract symptoms suggestive of benign prostatic hyperplasia: A systematic review and meta-analysis. Sci Rep. 2017;7(1):7984:1-10.
9. Egan KB. The epidemiology of benign prostatic hyperplasia associated with lower urinary tract symptoms: prevalence and incident rates. Urol Clin North Am. 2016;43(3):289-297.
10. Ferlay J, Colombet M, Soerjomataram I, Mathers C, Parkin D, Piñeros M, *et al*. Estimating the global cancer incidence and mortality in 2018: GLOBOCAN sources and methods. Int J Cancer. 2019;144 (8):1941-1953.
11. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68(6):394-424.
12. Taitt HE. Global trends and prostate cancer: a review of incidence, detection, and mortality as influenced by race, ethnicity, and geographic location. Am J Mens Health. 2018;12(6):1807-1823.
13. Hassanipour-Azgomi S, Mohammadian- Hafshejani A, Ghoncheh M, Towhidi F, Jamehshorani S, Salehiniya H. Incidence and mortality of prostate cancer and their relationship with the Human Development Index worldwide. Prostate Int. 2016;4(3):118-124.
14. Pakzad R, Mohammadian-Hafshejani A, Ghoncheh M, Pakzad I, Salehiniya H. The incidence and mortality of prostate cancer and its relationship with development in Asia. Prostate Int. 2015;3(4): 135-140.

15. Chen R, Ren S, Yiu MK, Fai NC, Cheng WS, Ian LH, *et al*. Prostate cancer in Asia: a collaborative report. Asian J Urol. 2014;1(1):15-29.
16. Bhurgri Y, Kayani N, Pervez S, Ahmed R, Tahir I, Afif M, *et al*. Incidence and trends of prostate cancer in Karachi South. Asian Pac J Cancer Prev. 2009;10(1):45-48.
17. Idrees R, Fatima S, Abdul-Ghafar J, Raheem A, Ahmad Z. Cancer prevalence in Pakistan: meta-analysis of various published studies to determine variation in cancer figures resulting from marked population heterogeneity in different parts of the country. World J Surg Oncol. 2018;16(1): 129-139.
18. Collective Cancer Registry Report From December 1994 Till December 2018 [Internet]. Shaukat Khanum Memorial Cancer Hospital and Research Center, Pakistan; 2018 [cited 11 March 2020]. Available from: http://shaukatkhanum.org.pk/
19. Punjab Cancer Registry Report [Internet]. 2018 [cited 1 February 2020]. Available from: http://punjabcancerregistry.org.pk/reports/PCR_2018.pdf
20. Gordetsky J, Epstein J. Grading of prostatic adenocarcinoma: current state and prognostic implications. Diagn Pathol. 2016;11(1):25-32.
21. Rajani R, Mehta N, Goswami H. Histopathological study of prostatic lesions at tertiary care centre. Int J Clin Diagn Pathol. 2020;3(2):172-176.
22. Aslam HM, Shahid N, Shaikh NA, Shaikh HA, Saleem S, Mughal A. Spectrum of prostatic lesions. Int Arch Med. 2013;6(1):36-40.
23. Sarwar A, Rathore Z, Rathore S, Butt R. Prostatic diseases common cause of morbidity in adult males. Pak J Med Health Sci. 2016;10(2):526-529.
24. Vuichoud C, Loughlin KR. Benign prostatic hyperplasia: epidemiology, economics and evaluation. Can J Urol. 2015;22(Suppl 1):1-6.
25. Dai X, Fang X, Ma Y, Xianyu J. Benign prostatic hyperplasia and the risk of prostate cancer and bladder cancer: a meta-analysis of observational studies. Med (Baltimore). 2016;95(18):3493:1-8.
26. Punjab Cancer Registry Report [Internet]. 2016 [cited 3 March 2020]. Available from: http://punjabcancerregistry.org.pk/reports/PCR_2016.pdf
27. Youl Lee J, Taniguchi T, Zhang K, Ng CF, Hakim L, Umbas R, *et al*. Report of the forth Asian Prostate Cancer (A-CaP) study meeting. Jpn J Clin Oncol. 2019;49(6):581-586.
28. Rasul G, Khan I, Jan MA, Ahmad T, Khattak IU, Aslam M. Frequency of incidental prostate cancer in patients presenting with palpable enlarged prostate gland. J Postgrad Med Inst. (Peshawar-Pakistan). 2019;33(3):210-215.
29. Muralidhar V, Ziehr DR, Mahal BA, Chen YW, Nezolosky MD, Viswanathan VB, *et al*. Association between older age and increasing Gleason score. Clin Genitourin Cancer. 2015;13(6):525-530.
30. Tanveer M, Tahir F, Majid Z, Hussain MM, Asghar SA, Qadar LT, *et al*. Awareness and self care practices of elderly men regarding prostate diseases in Karachi, pakistan. Cureus. 2019;11(5):4617.

ORIGINAL ARTICLE

In Vitro, D-Ribose and Formaldehyde Glycating Effects on Hen Egg White Lysozyme

Faizan-ul-Hassan Naqvi[1], Umaira Zakir[1, 2], Rizma Khan[2]
[1]Department of Biochemistry, University of Karachi, Karachi, [2]Department of Molecular Genetics, Dr. Ziauddin Hospital, Karachi, Pakistan.

ABSTRACT

Background: Glycation causes severe damage to the protein structure, instigating different diseases like cataracts, nephropathy, vasculopathy, retinopathy, atherosclerosis, neurodegenerative disease, diabetes, and age-dependent complications. Formaldehyde, a pollutant present in human habitation, is produced endogenously or exogenously during cooking or incinerating wood, paints, furniture, chipboards, fabric etc. Its higher concentrations can cause cell damage that promotes the formation of DNA/Protein cross-links. The present study aimed to evaluate the glycating effects of formaldehyde on hen egg white lysozyme in comparison with known glycating agent D-ribose.

Methods: In this, *in-vitro* study, hen egg white lysozyme (HEWL) glycation with different concentrations of formaldehyde (0.25mM, 0.5mM, 1mM and 2mM) and D-ribose (0.01mM, 0.05mM, 0.1mM and 0.5mM) was examined using two different experimental conditions: concentration and time duration. Further cross-linking of protein was also analysed using SDS-PAGE technique.

Results: Glycation of HEWL treated with formaldehyde increased with increasing concentrations (0.25mM, 0.5mM, 1mM and 2mM) and time duration (1, 3, 7 and 15 days). Cross linking of HEWL showed visible glycation at 2mM concentration. Cross-linked HEWL products gave dimer at 0.25mM and 0.5mM and trimers at 1mM and 2mMat 3, 7 and 15days. However, compared to formaldehyde, D-ribose glycation at different concentrations (0.01mM, 0.05mM, 0.1mM and 0.5mM) did not show the prominent cross linking of protein.

Conclusion: Formaldehyde was found to be a more potent glycating agent compared to D-ribose. Compared to D-ribose, formaldehyde can produce protein misfolding and can be used in clinical research to establish the role of formaldehyde in patients with diseases.

Keywords: Formaldehyde; D-Ribose; Lysozyme; SDS PAGE.

Corresponding Author:
Dr. Rizma Khan
Department of Molecular Genetics,
Dr. Ziauddin Hospital, Karachi, Pakistan.
Email: rizma.khan@zu.edu.pk
https://doi.org/10.36283/PJMD10-1/005

INTRODUCTION

Glycation is a general term that covers the progression of complex and unconstrained responses between sugars and amino protein groups producing Schiff bases[1], which are being rearranged and form Amadori products. This product is stable and degraded to highly reactive dicarbonyl compounds, which can interact with sulfhydryl, amino and guanidine groups of protein to generate cross-linked product called advanced glycation end products (AGEs)[2], linked to pathophysiological complications, such as cataracts, nephropathy, vasculopathy, retinopathy, atherosclerosis and neurodegenerative disease[3].

Among the protein glycation, hen egg white lysozyme (HEWL) has attracted the greatest concern and interest because HEWL is structurally like human lysozyme, and in vitro fibrils derived via

HEWL are comparable to fibrils produced in patients[4]. The ability of HEWL fibrils to cause apoptosis in neuroblastoma cells enhances the HEWL a reasonable model to examine the amyloid development in vitro[5]. Possible glycation sites of HEWL are the a-amino group at N-terminus, also the lysine or hydroxylysine residue group[6]. Some research has shown the inclusion of specific proteins in the development of AGEs and correlated with amyloid formation[7]. In view of the above data and its role in the regular immune system, we choose HEWL to inspect the glycation effects.

D-ribose, a glycating agent, and reducing monosaccharide, is predominantly active in protein glycation, leading to the production of AGEs[8], which leads to cell dysfunction and death[9]. It is present in every living cell and is a key component of many biomolecules that play an important role in metabolism[10]. Research shows that glycation with D-ribose produces AGEs faster than glucose glycation, which takes a longer time[11].

Formaldehyde (FA), a product of cell metabolism, is naturally present in human habitation along with its endogenous production, we are also exposed to FA exogenously during cooking, incinerating wood, paints, furniture, chipboards, fabric etc. FA can cause tau protein misfolding and globular aggregation that is harmful to hippocampal neurons[12]. It may also be exposed to certain neurodegenerative diseases[13]. FA at 1mM, increases apoptotic activity and reduces mitosis in tumour cells and endothelial cultured cells, while its high concentration of approximately 10mM causes cell damage and necrotic cell deaths[14] and leads to the formation of cross-links between DNA and Protein, which cause damage to DNA. It is also considered a cross linking agent and can respond with thiol and amino groups of various proteins, which can lead to protein polymerization[15].

HEWL along with sugars has the capacity to generate cross-linking oligomers. Our examination compared the characteristics of lysozyme ribosylation with those of FA in vitro glycation, to determine the potential for aggregate and polymer formation. This study can be beneficial to understand the impacts of FA glycation on the structure of HEWL, its cross linking of protein and its effect on human health. This study aimed to evaluate the glycating effects of formaldehyde at 37°C on HEWL at pH 7.4 in comparison with known glycating agent D-ribose.

METHODS

In this in-vitro study Hen egg white lysozyme (HEWL) (10mg/ml) was added in 0.1M sodium phosphate buffer of pH 7.4 at 37°C for 1, 3, 7, and 15 days in the presence and absence of 0.01mM, 0.05mM, 0.1mM and 0.5mM D-ribose. To prevent bacterial growth, sodium azide (1mM) has been used and incubated under sterile laboratory conditions. At certain times, aliquots were withdrawn and stored at -20°C for further analysis. In this research, native HEWL alone incubated at 37°C in the absence of D-ribose is used as a control.

Also, incubation of HEWL (10mg/ml) was carried out in 0.1M sodium phosphate buffer (containing 1mM sodium azide to prevent bacterial growth) in the absence and presence of formaldehyde (FA) of concentratons: 0.25mM, 0.5mM, 1mM and 2mM, the reaction mixture was incubated at 37°C at different time intervals (1, 3, 7, and 15 days). After incubation Eppendorf tubes were removed and reacted solutions were immediately placed at -20°C for further evaluation. The corresponding HEWL without FA was used as the control.

Glycation of HEWL with D-ribose and FA were analysed using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). Aliquots collected at different time intervals were combined in the ratio of 1:1 with sample-diluting buffer (SDB, 2X) containing 10% SDS (4ml), bromophenol blue (few crystals), β-mercaptoethanol (1ml), glycerol (2ml), and tris-HCL (1M, 1.25ml). The Eppendorf tube containing SDB sample was capsized capped and boiled for 2 min in a water bath at 100°C. After heat treatment, 10µl of the solution was loaded into a well of prepared SDS PAGE gel (10%) and electrophoresis was carried out with the help of mini-protean 3 system, Bio-Rad, Hercules, CA according to standard Laemmli method[16]. For staining, Coomassie Brilliant Blue G-250 (CBBG) was used and destaining was accomplished with aqueous acetic acid/methanol solution (100ml of acetic acid and 200 ml of methanol per litre). The gel was scanned using a gel documentation system (Uvitec, UK).

RESULTS

At first day incubation, the expression of HEWL did not change when the concentration of FA increased compared to the control on SDS-PAGE (Figure 1a). When HEWL was incubated with FA for 3, 7 and 15 days and compared with the control, increased expression of HEWL products were observed (Figure 1b-d), and more prominent glycation was seen at 15 days of incubation (Figure 1b-d), proving the glycating effect of FA increases with the increase in days.

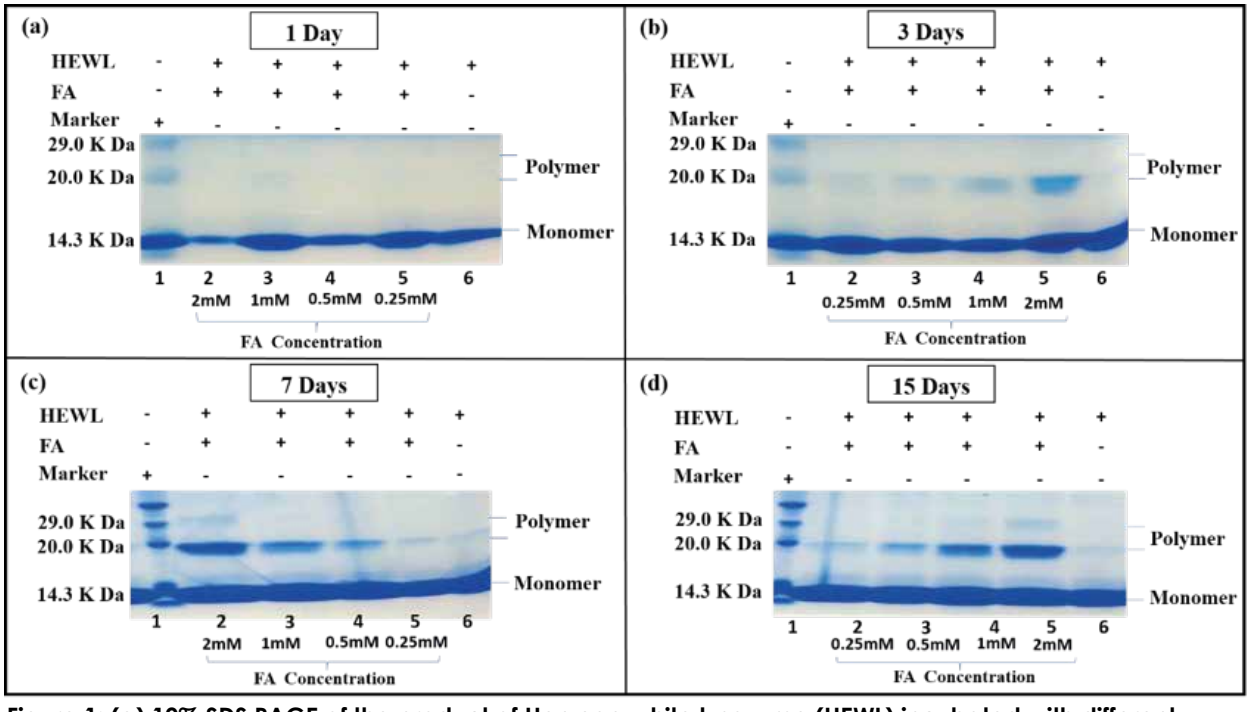


Figure 1: (a) 10% SDS PAGE of the product of Hen egg white lysozyme (HEWL) incubated with different concentration of formaldehyde(FA) (0.25mM, 0.5mM, 1mM and 2mM) at 37°C for 1 day (b) 3 days (c) 7 days. (d) 15 days.

Moreover, cross linking of HEWL increased with the increase of concentration as well as incubation days compared to control (Figure 2a-d), a more visible glycation was noted at 2mM concentration. Cross-linked HEWL products represented dimer and trimer of HEWL compared to the size of un-glycated HEWL (~14 K Da) and these products were observed at the high molecular weight. The presence of cross linking products increased from 0.25mM - 2mM over time (Figure 2a-d). The only dimer was observed at 0.25mM and 0.5mM (Figure 2a-b), but when the concentration increases to 1mM and 2mM, trimers were also seen at 3, 7 and 15 days (Figure 2c-d).

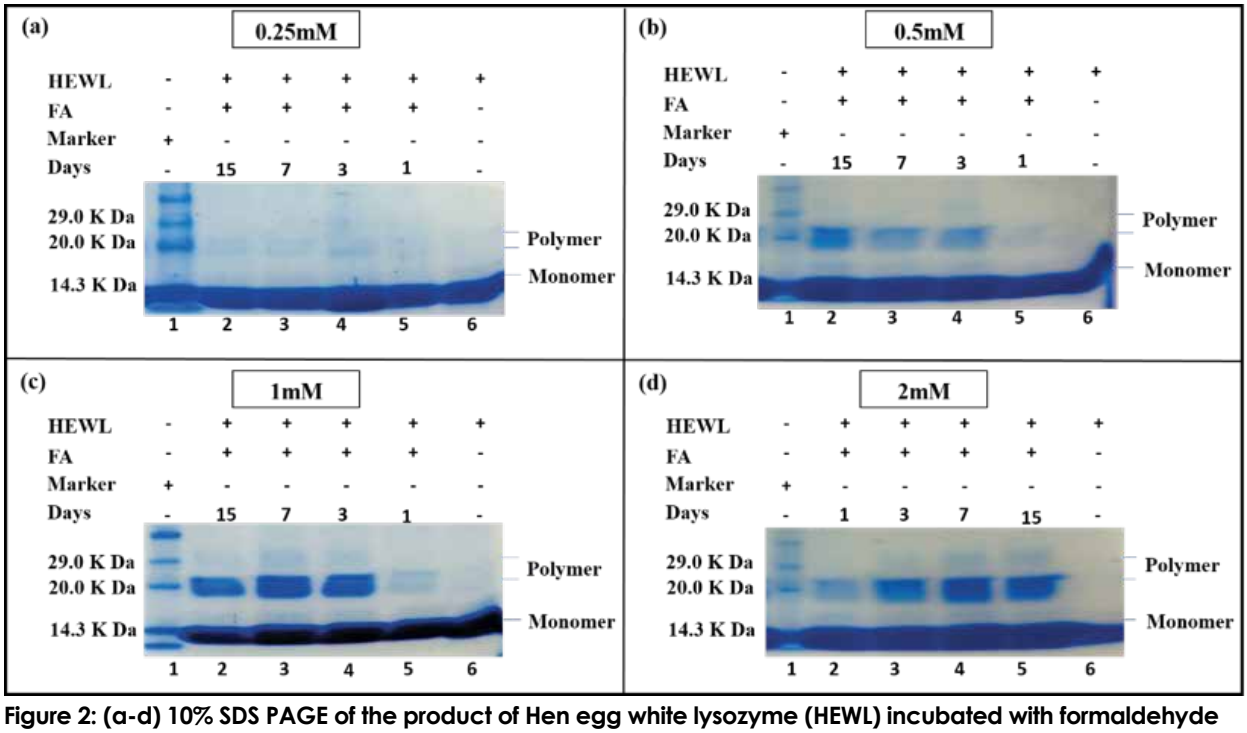


Figure 2: (a-d) 10% SDS PAGE of the product of Hen egg white lysozyme (HEWL) incubated with formaldehyde (FA) 0.25mM, 0.5mM, 1mM, 2mM for 1, 3, 7, and 15 days at 37°C.

HEWL was treated at 37°C during 1, 3, 7 and 15 days at different concentrations of D-ribose. The results show that the expression of HEWL does not change on first day when the concentration of D-ribose increases compared to the control (Figure 3a). With 3 days of incubation, protein glycation increases with the increase of concentration, but no significant change occurs (Figure 3b). At 7 days of incubation, more prominent glycation was seen only at 0.5mM D-ribose concentration (Figure 3c). Moreover, the cross-linking in HEWL increases with the increase in the concentration of D-ribose compared to the control at 15 days of incubation (Figure 3d), proving that glycation of HEWL with D-ribose increases with the increase of days.

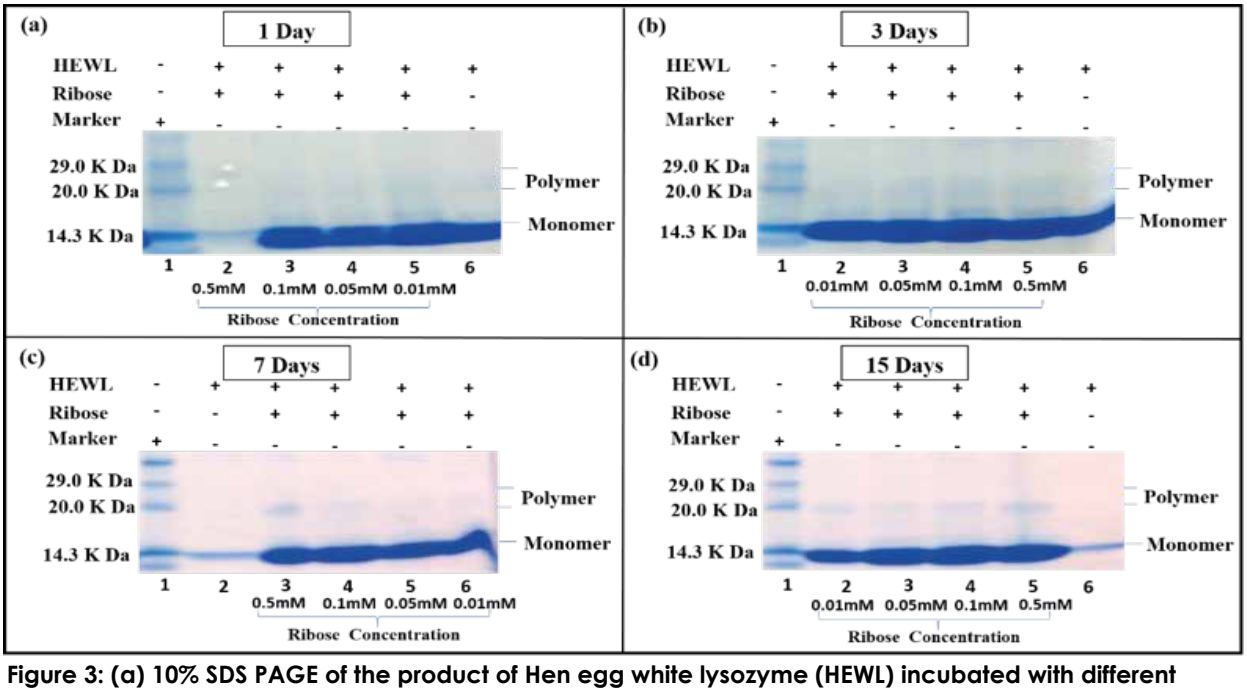


Figure 3: (a) 10% SDS PAGE of the product of Hen egg white lysozyme (HEWL) incubated with different concentration of D-Ribose (0.01mM, 0.05mM, 0.1mM and 0.5mM) at 37°C for 1 day. (b) 3 days. (c) 7 days. (d) 15 days.

The increase of concentration (0.05-0.5mM) of D-ribose, cross linking of HEWL increases while at 0.01mM concentration no significant change has been observed (Figure 4a-d).

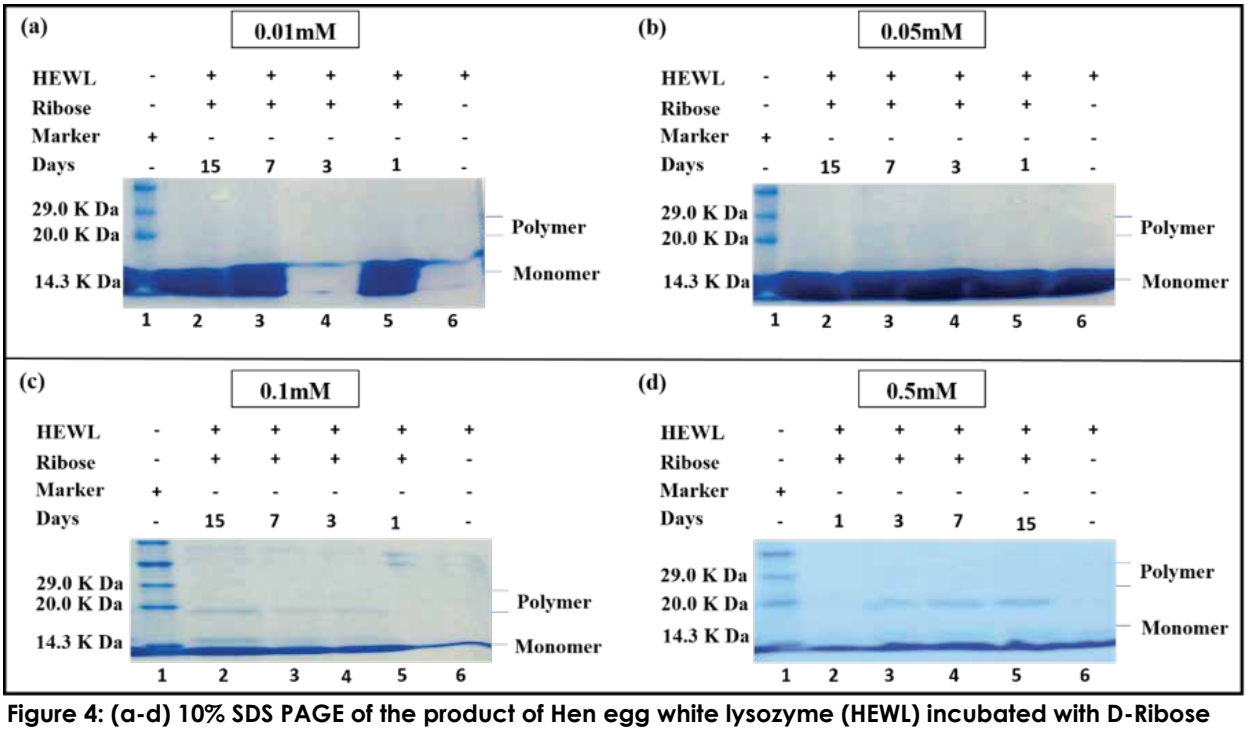


Figure 4: (a-d) 10% SDS PAGE of the product of Hen egg white lysozyme (HEWL) incubated with D-Ribose at 0.01mM, 0.05mM, 0.1mM, 0.5mM for 1, 3, 7, and 15 days at 37°C.

DISCUSSION

Disordered proteins, have previously been reported, and are involved in complicated disorders like cancer, cardiovascular disease[17], neurodegenerative diseases[18], or diabetes[19]. Previously research shows that formaldehyde (FA) of different concentrations (FA ≥ 0.1mM) when reacted with BSA protein, results in the neuronal cell lines (SH-SY5Y) being cytotoxic[20]. Another study revealed that BSA glycation with ribose sugar leads to the production of advanced glycation end products (AGEs), which also results in the neuroblast cells cytotoxicity[21].

To examine either glycation was faster with FA or D-ribose, we separately incubated HEWL with different concentrations of FA and D-ribose and analysed the results using SDS-PAGE. Formaldehyde is a known pollutant present in human habitation, exogenous exposure of FA is motor vehicle exhaust, power plants, petroleum refineries, cooking operations, incinerating wood burning, paints, furniture, chipboard, fabric, smoke etc. so it proven that humans are continuously exposed to FA. Along with its exogenous exposure, it is also endogenously produced as a metabolic by-product. The safe concentration of formaldehyde exposure is 0.1 mg/m^3 [22], but a survey showed that its concentration in home and workplaces exceed the above guideline value[23], that are responsible to increase the risk of various diseases. Under the highlights of the previous study, we designed our goal to find out the glycating effects of FA and D-ribose at the different concentrations on lysozyme and their cross-linked product. In the first part of our, study HEWL was incubated at 37°C for 1, 3, 7 and 15 days with different FA concentrations. With the passage of days (1-15days incubation), high molecular HEWL products were formed, showed that the glycating effect of FA increases with the increase of days. In Addition, at 37°C, HEWL was incubated at different FA concentrations for different intervals. It was observed that with the increase of FA concentration (0.25-2mM), cross-linking of HEWL increased and more prominent glycation was observed at 2mM concentration.

However, in the second part, HEWL was incubated at 37°C for 1, 3, 7 and 15 days at various concentrations of D-ribose. The study results illustrated that glycation of HEWL with D-ribose increases with concentration and time dependent manner. D-ribose is a monosaccharide that is naturally present in the mitochondria of cells that is responsible for energy production[24]. In the healthy individual, the concentration of D-ribose in cerebrospinal fluid and blood are 0.01-0.1mM[25]. To the best of our knowledge, we treated HEWL with different concentrations of D-ribose for various intervals. Out of these, three concentrations of D-ribose are normal in a range that is present in healthy individuals, but the 0.5mM is increased concentration than normal and we had compared the glycating effect of all these concentrations. We also found that at the concentration of 0.01mM D-ribose, the structure of the HEWL does not change compared to the control. While the cross-linking of HEWL at 0.05mM, 0.1mM and 0.5mM increase with the time of glycation compared to the control. Our result indicated that cross linking of HEWL was more prominent at 0.5mM concentration of D-ribose sugar, which further proved that an increase of concentration of D-ribose directly increased the glycating effects on lysozyme and its dimers and trimers[25].

In the comparison of the glycating effects of both FA and D-ribose at its physiological concentrations, it has beenproved that polymerization of HEWL increases with the concentration and time dependent manner and FA was more reactive glycating agent than D-ribose. FA at its physiological concentrations of 0.5mM and 1mM also showed glycation, which was more prominent at 2mM. During glycation, 2-fold increase expression of HEWL was observed at 2mM FA concentration. However, HEWL bands incubated with D-ribose was not as prominently observed as HEWL incubated with FA under similar conditions. In this study, we found that compared to FA, D-ribose did not show the prominent polymerization of protein. These findings concluded that HEWL is more prone to FA glycation compared with D-ribose. In addition to the above results, this research will be beneficial to understand the impacts of FA glycation on the structure of HEWL as well as its results in cross linking of protein. Moreover, this study will be beneficial for future studies, which will help in pre-clinical and clinical research to establish the role of FA in patients with diabetes and neurodegenerative diseases.

CONCLUSION

The glycation promoted the cross linking of HEWL. Compared to D-ribose, FA is a potent glycating agent, can produce protein misfolding and cross-link HEWL product, as observed by SDS-PAGE. FA showed more glycation of HEWL with the increasing concentration and days of incubation, compared to D-ribose.

ACKNOWLEDGEMENTS

We graciously thank Dr. Sajjad Haider Naqvi, Department of Biochemistry, University of Karachi, for providing facility to conduct this research work.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHORS' CONTRIBUTION

FN and UZ performed all experimental work and acknowledged valuable material inside these articles. RK and UZ contributed equally in writing the manuscript. All authors read and approved the final version of the manuscript.

REFERENCES

1. Ali A, Devrukhkar J. *In vitro* study on glycation of plasma proteins with artificial sweeteners. Acta Biol Szeged. 2016; 60:65-70.
2. Allaman I, Bélanger M, Magistretti PJ. Methylglyoxal, the dark side of glycolysis. Front Neurosci. 2015;9:1-23.
3. Bhattacherjee A, Dhara K, Chakraborti AS. Bimolecular interaction of argpyrimidine (a Maillard reaction product) in in vitro non-enzymatic protein glycation model and its potential role as an antiglycating agent. Int J Biol Macromol. 2017; 102:1274- 1285.
4. Walker AR, Baddam N, Cisneros GA. Unfolding pathways of Hen Egg-white lysozyme in ethanol. J Phys Chem. 2019; 123:3267-3271.
5. Bystrenova E, Bednarikova Z, Barbalinardo M, Albonetti C, Valle F, Gazova Z. Amyloid fragments and their toxicity on neural cells. Regen Biomater. 2019; 6:121-127.
6. Suarez HS, Piñales RA, Santos JG, Barreto GE, Navarrera LP, Moreno JA, *et al*. Prediction of glycation sites: new insights from protein structural analysis. Turk J Biol. 2016;40(1):12-25.
7. Sadowska-Bartosz I, Stefaniuk I, Galiniak S, Bartosz G. Glycation of bovine serum albumin by ascorbate in vitro: Possible contribution of the ascorbyl radical? Redox Biol. 2015; 6:93-99.
8. Akhter F, Khan MS, Alatar AA, Faisal M, Ahmad S. Antigenic role of the adaptive immune response to d-ribose glycated LDL in diabetes, atherosclerosis, and diabetes atherosclerotic patients. Life Sci. 2016; 151:139-146.
9. Gao Y, Wake H, Morioka Y, Liu K, Teshigawara K, Shibuya M, *et al*. Phagocytosis of advanced glycation end products (AGEs) in macrophages induces cell apoptosis. Oxid Med Cell Longev. 2017;2017: 1-11.
10. Wei Y, Han CS, Zhou J, Liu Y, Chen L, He RQ. D-ribose in glycation and protein aggregation. Biochim Biophys Acta. 2012; 1820:488-494.
11. Chen Y, Yu L, Wang Y, Wei Y, Xu Y, He T, *et al*. d-Ribose contributes to the glycation of serum protein. Biochim Biophys Acta Mol Basis Dis. 2019; 1865(9):2285-2292.
12. He R. Effects of formaldehyde on protein (Tau) aggregation and cytotoxicity. Formaldehyde Cogn. 2017;121-142.
13. Spence PS. Formaldehyde, DNA damage, ALS and related neurodegenerative diseases. J Neurol Sci. 2018; 391:141-142.
14. Szende B, Tyihak E. Effect of formaldehyde on cell proliferation and death. Cell Biol Int. 2013; 34:1273-1282.
15. Hoffman EA, Frey BL, Smith LM, Auble DT. Formaldehyde crosslinking: a tool for the study of chromatin complexes. J Biol Chem. 2015; 290:26404-26411.
16. Laemmli, UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nat.1970; 227: 680-685.
17. Uversky VN. Wrecked regulation of intrinsically disordered proteins in diseases: pathogenicity of deregulated regulators. Front Mol Biosci. 2014; 1:1-6.
18. Martinelli AHS, Lopes FC, John EBO, Carlini CR, Ligabue-Braun R. Modulation of disordered proteins with a focus on neurodegenerative diseases and other pathologies. Int J Mol Sci. 2019; 20:E1322:1-34.
19. Du Z, Uversky VN. A comprehensive survey of the roles of highly disordered proteins in type 2 diabetes. Int J Mol Sci. 2017; 18:10:1-44.
20. Naqvi SH, Naqvi SFH, Yang L, Naqvi IH, Farhan M, He R. Treatment of bovine serum albumin with formaldehyde would result in cytotoxicity to SH-SY5Y cells. Pak J Biochem Mol Biol. 2014; 47:111-117.
21. Wei Y, Chen L, Chen J, Ge L, He RQ. Rapid glycation with D-ribose induces globular amyloid-like aggregations of BSA with high cytotoxicity to SH-SY5Y cells. BMC Cell Biol. 2009; 10:1-15.
22. World Health Organization (WHO). WHO guidelines for indoor air quality. Selected pollutants2010 [Internet]. WHO Regional Office for Europe, Copenhagen, 2010 [cited 2019 Sep 16]. Available from: https://apps.who.int/iris/bitstream/handle/10665/260127/9789289002134-eng.pdf?sequence=1&isAllowed=y
23. Nielsen GD, Larsen ST, Wolkoff P. Re-evaluation of the WHO (2010) formaldehyde indoor air quality guideline for cancer risk assessment. Arch Toxicol. 2017; 91:35-61.
24. Mahoney DE, Hiebert JB, Thimmesch A, Pierce JT, Vacek JL, Clancy RL, *et al*. Understanding D-ribose and mitochondrial function. Adv Biosci Clin Med. 2018; 6:1-5.
25. Cai Y, Liu J, Shi Y, Liang L Mou S. Determination of several sugars in serum by high-performance anion-exchange chromatography with pulsed amperometric detection. J Chromatogr A. 2005; 1085:98-103.

ORIGINAL ARTICLE

The Operative versus Conservative Approaches in the Management and Treatment of Lumbar Spinal Stenosis

Faiza Sharif, Ashfaq Ahmad, Syed Amir Gilani
New Campus, the University of Lahore, Lahore, Pakistan.

ABSTRACT

Background: Lumbar Spinal stenosis is the narrowing of the spinal canal at any lumber level. Lumbar spinal stenosis has multiple severities and both conservative and operative treatment options. The objective of the current study was to compare the results of operative and conservative approaches in spinal stenosis treatment.

Methods: This cross-sectional study was conducted from January 2019 to June 2019. The data was collected from different hospitals in Lahore (Ittefaq, General, Jinnah, and Hameed Latif). Participants (n=121) of both genders, pre-diagnosed with Lumbar spinal stenosis and symptoms history of 10 weeks (confirmed on imaging) were included. Interventions were decompressive surgery and conventional conservative management. The outcome measures were body pain, functional activities, and the Oswestry Disability Index. An independent sample t-test was used to compare the results between the two groups. A $p \leq 0.05$ was considered statistically significant.

Results: The patients undergoing surgery had statistically significant (p=0.00) advantages of surgery compared to the non-surgical group at 3 months. The 25(36%) of patients remained the same after conservative treatment with 51(72%) having pain radiation and 47(66%) with neurological deficit. The effect of treatment for body pain was 7.8 (95%CI, 8.6, 6.9), physical function −1.3 (95%CI, −0.6, −2.2), and Oswestry Disability Index was −3.4 (95%CI, −2.7, −4.1).

Conclusion: Patients who had surgery of spinal stenosis showed marked improvements in body pains, functional activities, and Oswestry disability index compared to conservatively treated. Patients, health care providers, and other stakeholders may get benefit from the findings of this study.

Keywords: Spinal Stenosis; Laminectomy; Physical Therapy.

Corresponding Author:
Dr. Faiza Sharif
University Institute of Physical Therapy,
New Campus, the University of Lahore,
Lahore, Pakistan.
Email: faiza.sharif@uipt.uol.edu.pk
https://doi.org/10.36283/PJMD10-1/006

NTRODUCTION

Lumbar Spinal stenosis is the narrowing of the spinal canal at any lumber level. This narrowing causes unrestricted pressure on peripheral nerves and spinal cord[1] which in turn results in excruciating pain, numbness, and lower limb weakness[2]. Patients with lumbar spinal stenosis most often have lower back pain radiating into the leg, and these symptoms appear while walking. This pathology is degenerative and severely compromises normal functional activities, the ability to walk normally, and ultimately compromises the quality of life[3].

Lumbar spinal stenosis has a wide range of treatment options because the disease has many levels of severities[4]. A comprehensive physical examination, physical manipulations, radiographic studies

and imaging are of utmost importance in making clinical diagnosis[5]. At present, conservative management i.e. an important initial step in managing lumbar spinal stenosis, can give temporary relief for a short period of time[6]. Physical therapy management can help patients for up to six months or a year, despite its increasing use in recent years, the use of non-steroid anti-inflammatory medicines, pain killers and corticosteroid injections prove to be little beneficial[7,8]. Lumbar spinal stenosis has been labeled as are current indication for spine surgery in patients older than 60 years of age. Indications for spinal surgery seem to differ widely depending on the severity of symptoms. Patients having spinal stenosis usually are asymptomatic on radiographs, therefore careful screening of patients is an especially important and clinical correlation between radiographic findings and symptoms plays a key role in management[9,10].

At present, patients with spinal stenosis have both surgical and non-surgical treatments available. The choice of surgical treatment depends on the patient's quality of life and severity of stenosis[6,11], whereas non-surgical conservative treatment is usually done to relieve major clinical symptoms of instability caused by degeneration, although it provides temporary relief to the patients. It can be complicated to decide which treatment is better than other[12,13]. Spinal surgery to treat spinal stenosis has been used extensively for the past few decades, therefore there are multiple complex surgical procedures[14]. The procedure of laminectomy to decompress neural structures has been increased in addition to lumbar fusion. This has reduced the risk of instability and deformity later in life. Lumbar fusion surgery is another surgical procedure, which is widely used now a day[15].

However, it is very difficult to compare and conclude between these treatments because the diagnosis and treatment options are very intricate and these are based on decisions relying on the patient's signs and symptoms, radiological findings, and comorbidities in every patient. The clinical guidelines of North American Spine Society (NASS) in 2008[16] described that no intervention for lumbar spinal stenosis is beneficial in improving a patient's condition due to the natural disease history and also decompression surgery is more effective than other interventions in patients having moderate to severe symptoms of lumbar spinal stenosis[17].

Surgery for lumbar spinal stenosis has been more effective as compared to conservative treatment, but up to the researcher's knowledge, no such comparative studies have been done in Pakistan for the management of spinal stenosis. Therefore, this study aimed to compare the results of different operative and conservative approaches in the management and treatment of lumbar spinal stenosis.

METHODS

This cross-sectional study was conducted from January 2019 to June 2019. The data was collected from different hospitals in Lahore (Ittefaq, General, Jinnah, and Hameed Latif) and compiled at the University of Lahore. The ethical review board of faculty of allied health sciences, University of Lahore approved the study. The study used a non-probability convenient sampling technique and a total of 121 participants of both genders, with neurogenic claudication of radiating pain in legs for at least 10 weeks were included in the study. Each patient signed informed consent. The radiographic findings also confirmed the diagnosis of spinal stenosis at one or multiple levels. The total sample was determined using the following equation, which kept the error margin equivalent to 5% and the significance level equal to 95%. Sample size calculation in the World Health Organization (WHO) edition 2.0.21 of the health studies by using 95% significance level, 1.76% population proportion and 5% expected margin of error[14]. Patients with ankylosing spondylitis, spinal tumors, cauda equina and lumbar instability symptoms were excluded. The conservative management options were physical therapy, chiropractic, Nonsteroidal anti-inflammatory drugs (NSAIDs), epidural injections and analgesics.

The surgical procedure was posterior decompressive laminectomy and non-surgical procedure was "conventional care", which included physical therapy, patient education, and a home exercise plan with the administration of NSAIDs if tolerated by the patients. The outcome measures were Short form survey (SF-36) body pain, functional activities and the Oswestry disability index measured at 3 months. The difference in the mean values changes from baseline between operative and conservative treatment groups. The SF-36 scores have a range of 0-100, with lower scores demonstrate more severe symptoms; the Oswestry Disability Index ranges from 0-100, with higher scores indicates higher severe symptoms. Treatment comparisons were made at the designated follow uptime. Data was collected through standard questionnaires 3 months after treatment of surgical and conservative treatments and SPSS (Software Statistical Package for Social Science) version 24.0 was used as a statistical tool. An independent sample t-test was used to compare the treatment results between surgical and conservative groups. A $p \leq 0.05$ was considered statistically significant.

RESULTS

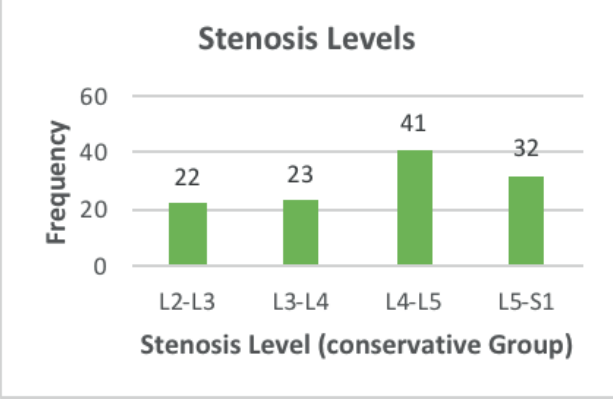
A total of 121 participants were divided into two groups, surgical group (50) and conservative non-surgical group (71). Most of the patients in both groups were males 50(70%). The mean age (SD) of surgical group was 64.7 years and the conservative

group was 67.6 years with a mean body mass index (BMI) of 29.6 and 28.4 respectively. In both groups, patients had comorbid diseases like hypertension, diabetes, and osteoarthritis. Patients were unable to perform straight leg raise and pain radiating towards leg with 42(84%) positive Straight Leg Raise (SLR) and 47(94%) pseudoclaudication experienced surgery later. Since, n=61(86%) of the patients in the operative group had severe symptoms of lumbar spinal stenosis. The most common site for spinal stenosis was L4-L5. Interventions were posterior decompressive surgery and conventional conservative management. The outcome measures (Table 1) were body pain, functional activities and the Oswestry disability index measured 3 months after either treatment.

Table 1: Characteristics of patients describes the demographic details and comorbidities of the patients at baseline.

Treatment Received		
Characteristics	**Operative (n=50)%**	**Conservative (n=71)%**
Mean age	64.7	67.6
Males	35(70)	50(70)
Marital status (married)	44(88)	66(92)
Work status		
Full/part time	8(16)	7(10)
Retired	38(76)	55(78)
Disabled	3(6)	6(8)
Others	1(2)	3(4)
Body mass index (BMI)kg/m^2	29.6	28.4
Comorbidities		
Hypertension	33 (66)	61(86)
Diabetes	11(22)	9(13)
Osteoporosis	6(12)	1(4)
Time since most recent pain>10 weeks	36(72)	44(62)
Satisfaction with symptoms - very satisfied	39(78)	48(68)
Outcome		
Getting improved	43(86)	41(57)
Staying about the same	5(10)	25(36)
Getting worse	2(4)	5(7)
Pseudoclaudication if any	47(94)	38(54)
SLR or Femoral Tension	42(84)	33(47)
Pain radiation – any	45(90)	51(72)
Any Neurological Deficit	43(86)	47(66)

Both groups had almost the same age group i.e., 64.7 and 67.6 respectively (Table 1) with a greater number of male patients in both groups. The patients receiving surgical treatments had severe pain, poor function and disability compared to the conservative group. Patients who had surgery were more satisfied with the improvement of their signs and symptoms when compared with the non-operative group. Level of stenosis among operative and conservative groups is shown in Figure 1.



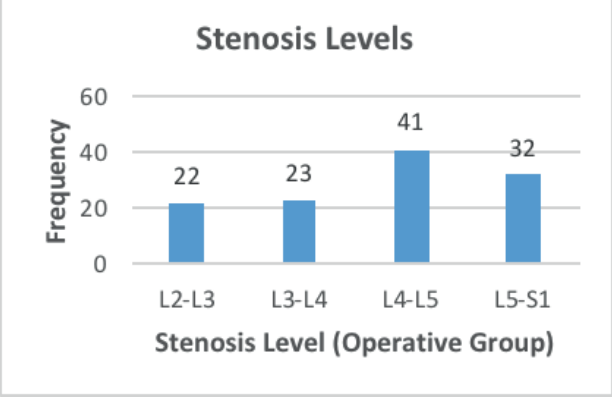


Figure 1: Level of stenosis among operative treatment group and conservative treatment group.

The mean time for lumbar surgery was 110 minutes. No significant difference was seen in the intraoperative complications. The most common postoperative complication was dural tear 5(10%). Intra-operative complications of aspiration, nerve root injury, vascular injury or surgical procedure at the wrong site were not reported. For 8 weeks post-operatively; (Table 2) none of the bone graft complications, leakage of CSF, nerve root injury, cauda equina injury were reported.

Table 2: Operative procedure and complications.

Procedure	n=50
Posterior Decompression laminectomy	44(88%)
Surgery time	110 mins
Laminectomy level	
L2-L3	1(2%)
L3-L4	7(14%)
L4-L5	41(82%)
L5-S1	1(2%)
Post-operative mortality (death within 3 months of the surgery)	0(0%)
Intra-operative complications	
Dural tear/leakage of spinal fluid	5(10%)
Other	1(2%)
None	44(88%)
Post-operative complications	
Wound hematoma	1(2%)
Wound infection	1(2%)
Others	4(8%)

Surgical patients had statistically significant effects in treatment than the conservative group for all outcome measures (Bodily Pain, Physical Function, and Oswestry Disability Index) (Table 3). Patients' after 3 months postoperatively indicate major improvement in operative group as compared to the conservative group (*p*=<0.001).

Table 3: Analysis of outcome measures after 3 months of treatments received.

Outcome Measures	Operative (n=50)	Conservative (n=71)	Effect of Treatment (95%CI)	*p*-Value
SF-36 Bodily Pain (BP) (0-100)	23.2 ± 2.3	15.4 ± 2.2	7.8(8.6, 6.9)	0.00
SF-36 Physical Function (PF) (0-100)	16.2± 2.3	17.6± 2.2	−1.3(−0.6, −2.2)	0.001
Oswestry Disability Index (ODI) (0-100)	−16.1 ± 1.9	−12.7 ± 1.8	−3.4(−2.7, −4.1)	0.00

DISCUSSION

The results of the present study showed that surgery is more effective than conservative management for spinal stenosis. These results are similar to a study carried out by A Delitto et al. which concluded surgery as a more effective treatment in terms of improvement in pain and physical function 4 years post-operatively[19]. Another randomized controlled trial was done to find the long-term results of lumbar spinal surgery which showed better results in pain reduction and function improvement postoperatively[20].

However, a systematic review conducted by Zaina et al. concluded that it is not clear which treatment is better than the other in spinal stenosis management and surgical treatment, more over surgery has many side effects than conservative treatment. The above study results are in contrast to the current study[21]. Similarly, Masakazu and Minetama et al. conducted a study to find out the long-term comparative effects of surgical and conservative treatments for spinal stenosis and found out that there is no significant difference between both groups in all outcome measures except physical function [22,23].

Contrary to our study, Patel et al. found a significant role of conservative managements currently used to treat lumbar spinal stenosis. The conservative therapies having positive results include minimal invasive decompression and spinal cord stimulation. Drugs used to treat lumbar spinal stenosis such as systemic prostaglandin analogs and epidural drugs such as calcitonin showed early results but need further evaluation for clinical use[24]. Another contrasting study of this study was done by Oka et al. which compared the effectiveness of different conservative treatments (pharmacology, exercise, and acupuncture. They concluded that acupuncture is more effective than physical exercise and pharmacological treatment for lumbar spinal stenosis[23].
However Jung et al. concluded in a prospective study that patients of lumbar spinal stenosis without any instability respond to conservative treatment and reported less pain and more functional improvement through 1 year as compared to surgical treatment[25].

In lumbar spinal stenosis patients without instability, non-surgical treatment resulted in less pain improvement and functional recovery through 1 year. The limitations of this study were the small sample size and the follow-up time was also short. It is recommended to conduct more studies on a larger scale with a large sample size and follow-up time up to 4 years postoperatively to see the long terms advantages of surgical and conservative treatments for spinal stenosis.

Studies with detailed protocols and descriptions of non-surgical treatments are lacking in the literature. Research into treatment for lumbar spinal stenosis may be much improved by the development of standard diagnostic criteria and clinical outcomes. Most likely, the highest cost-effectiveness would be achieved, if the patient could be correctly selected for one or the other treatment option, which warrants further research on the identification of predictive factors for treatment success.

CONCLUSION

Surgical approaches have marked effects compared to conservative approaches in the treatment and management of Lumbar spinal stenosis. Patients who had surgery showed significant improvements in body pains, functional activities and the Oswestry disability index than patients who were treated conservatively. Patients, health care providers and other stakeholders would benefit from the findings of this study.

ACKNOWLEDGEMENTS

We are especially thankful to Ittefaq, General, Jinnah, Hameed Latif Hospitals for their assistance and facilitation in collecting the data.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICS APPROVAL

The study was approved from the Faculty of Allied Health Sciences, The University of Lahore Ethical Review Committee (IRB-UOL-FAHS/725/2019).

PATIENT CONSENT

Both verbal and written Informed consent was taken from all patients.

AUTHORS' CONTRIBUTION

The study was conceived and designed by FS. She was also responsible for data collection, analysis, interpretation, manuscript writing, and data management. AA helped in writing this manuscript and monitored the accuracy and integrity of this article. SAG supervised and provided intellectual support for the manuscript conception and study design. He also critically analyzed the article.

REFERENCES

1. Siccoli A, Marlies P, Schröder ML, Staartjes VE. Machine learning–based preoperative predictive analytics for lumbar spinal stenosis. Neurosurg Focus. 2019; 46(5): E5:1-9.
2. Lønne G, Fritzell P, Hägg O, Nordvall D, Gerdhem P, Lagerbäck T, *et al*. Lumbar spinal stenosis: comparison of surgical practice variation and clinical outcome in three national spine registries. Spine J. 2019;19(1):41-49.
3. Lafian AM, Torralba KD. Lumbar spinal stenosis in older adults. Rheum Dis Clin. 2018; 44(3): 501-512.
4. Lurie J, Tomkins-Lane C. Management of lumbar spinal stenosis. BMJ. 2016; 352: h6234:1-13.
5. Tobert DG, Harris MB. Degenerative lumbar spinal stenosis and spondylolisthesis. InPrinciples of orthopedic practice for primary care providers 2018 (pp. 47-59). Springer, Cham.
6. Zweig T, Enke J, Mannion AF, Sobottke R, Melloh M, Freeman BJ, *et al*. Is the duration of pre-operative conservative treatment associated with the clinical outcome following surgical decompression for lumbar spinal stenosis? A study based on the Spine Tango Registry. Eur Spine J. 2017;26(2): 488-500.
7. Hammerich A, Whitman J, Mintken P, Denninger T, Akuthota V, Sawyer EE, *et al*. Effectiveness of physical therapy combined with epidural steroid injection for individuals with lumbar spinal stenosis: a randomized parallel-group trial. Arch Phys Med Rehabil. 2019;100(5):797-810.
8. Minetama M, Kawakami M, Ishimoto Y, Nagata K, Teraguchi M, Kitagawa T, *et al*. Two-year follow-up of physical therapy versus surgical therapy for patients with lumbar spinal stenosis: GP077. Spine J Meet Abstr. 2017: p. 219.
9. Försth P, Ólafsson G, Carlsson T, Frost A, Borgström F, Fritzell P, *et al*. A randomized, controlled trial of fusion surgery for lumbar spinal stenosis. N Eng J Med. 2016;374(15):1413-1423.
10. Aichmair A, Burgstaller JM, Schwenkglenks M, Steurer J, Porchet F, Brunner F, *et al*. Cost-effectiveness of conservative versus surgical treatment strategies of lumbar spinal stenosis in the Swiss setting: analysis of the prospective multicenter Lumbar Stenosis Outcome Study (LSOS). Eur Spine J. 2017;26(2):501-509.
11. Dobkin BH. Provocative walking test of strength for diagnosis, management, and outcome assessment of symptomatic lumbar spinal stenosis. Neurorehabil Neural Repair. 2019; 33(12):1003-1007.
12. Pazarlis K, Punga A, Schizas N, Sandén B, Michaëlsson K, Försth P. Study protocol for a randomised controlled trial with clinical, neurophysiological, laboratory and radiological outcome for surgical versus non-surgical treatment for lumbar spinal stenosis: the Uppsala Spinal Stenosis Trial (UppSten). BMJ Open. 2019; 9(8): e030578:1-6.
13. Weber C, Lønne G, Rao V, Jakola AS, Solheim O, Nerland U, *et al*. Surgical management of lumbar spinal stenosis: a survey among Norwegian spine surgeons. Acta Neurochir. 2017;159(1):191-197.
14. Machado GC, Maher CG, Ferreira PH, Harris IA, Deyo RA, McKay D, *et al*. Trends, complications, and costs for hospital admission and surgery for lumbar spinal stenosis. Spine. 2017;42(22):1737-1743.
15. Machado GC, Ferreira PH, Yoo RI, Harris IA, Pinheiro MB, Koes BW, *et al*. Surgical options for lumbar spinal stenosis. Cochrane Database Syst Rev. 2016;11: CD012421.
16. Watters III WC, Baisden J, Gilbert TJ, Kreiner S, Resnick DK, Bono CM, *et al*. Degenerative lumbar spinal stenosis: an evidence-based clinical guideline for the diagnosis and treatment of degenerative lumbar spinal stenosis. Spine J. 2008;8(2):305-310.
17. Inoue G, Miyagi M, Takaso M. Surgical and nonsurgical treatments for lumbar spinal stenosis. Eur J Orthop Surg Traumatol. 2016; 26(7): 695-704.
18. Amir Y, Halfens R, Lohrmann C, Schols J. Pressure ulcer prevalence and quality of care in stroke patients in an Indonesian hospital. J Wound Care. 2013; 22(5): 254-260.
19. Delitto A, Piva SR, Moore CG, Fritz JM, Wisniewski SR, Josbeno DA, *et al*. Surgery versus nonsurgical treatment of lumbar spinal stenosis: a randomized trial. Ann Intern Med. 2015;162(7):465-473.
20. Brøgger HA, Maribo T, Christensen R, Schiøttz-Christensen B. Comparative effectiveness and prognostic factors for outcome of surgical and non-surgical management of lumbar spinal stenosis in an elderly population: protocol for an observational study. BMJ Open. 2018; 8(12): e024949:1-9.
21. Zaina F, Tomkins-Lane C, Carragee E, Negrini S. Surgical versus non-surgical treatment for lumbar

spinal stenosis. Cochrane Database Syst Rev. 2016; (1):1-31.
22. Minetama M, Kawakami M, Nakagawa M, Ishimoto Y, Nagata K, Fukui D, *et al*. A comparative study of 2-year follow-up outcomes in lumbar spinal stenosis patients treated with physical therapy alone and those with surgical intervention after less successful physical therapy. J Orthop Sci. 2018;23 (3):470-476.
23. Oka H, Matsudaira K, Takano Y, Kasuya D, Niiya M, Tonosu J, *et al*. A comparative study of three conservative treatments in patients with lumbar spinal stenosis: lumbar spinal stenosis with acupuncture and physical therapy study (LAP study). BMC Complement Altern Med. 2018;18(1):1-7.
24. Patel J, Osburn I, Wanaselja A, Nobles R. Optimal treatment for lumbar spinal stenosis: an update. Curr Opin Anaesthesiol. 2017; 30(5): 598-603.
25. Jung JM, Hyun SJ, Kim KJ, Kim CH, Chung CK, Kim KH, *et al*. A prospective study of non-surgical versus surgical treatment for lumbar spinal stenosis without instability. J Clin Neurosci. 2020;80:100-107.

ORIGINAL ARTICLE

Mean Adiponectin Levels and Frequency of Hypoadiponectinemia in Gestational Diabetes Mellitus

Saba Raza[1], Usman Ali[1], Adnan Mustafa Zubairi[1], Erum Salim[2]
[1]Department of Chemical Pathology, Dr. Ziauddin University Hospital, North Nazimabad, Karachi,
[2]Clinical Laboratory, Dr. Ziauddin University Hospital, Clifton, Karachi, Pakistan.

ABSTRACT

Background: Insulin resistance, a major pathogenic factor in Gestational Diabetes Mellitus (GDM), has an inverse correlation with adiponectin; therefore, the role of adiponectin in the pathogenesis of GDM has been suggested. The study aimed to determine mean adiponectin levels and the frequency of hypoadiponectinemia in patients with GDM.

Methods: The study was carried out at Dr. Ziauddin University Hospital, Karachi from June 2016 to December 2017. A total of 99 women of age group 15-45 years diagnosed with GDM on Oral Glucose Tolerance Test (OGTT) at 24-28 weeks of gestations were included in the study. Patient's data was recorded on proposed proforma, regarding age, gestational age, parity, ethnicity, Body Mass Index (BMI). Serum samples for quantitative estimation of Adiponectin were collected while performing OGTT and were analyzed on Immunoassay in conjunction with control materials. Mean±Std. was calculated for quantitative variables. Percentages and frequencies were calculated for categorical variables. For significant differences between categories, an independent t-test and Chi-square test were used.

Results: High frequency of hypoadiponectinemia was observed in GDM women with mean adiponectin levels of 6.90±2.86 µg/ml (95%CI: 6.33 to 7.47). Out of 99 GDM women, 91(91.92%) had hypoadiponectinemia while 8(8.08%) had normal Adiponectin levels. A significant (p=0.05) association was found between BMI and hypoadiponectinemia.

Conclusion: In the majority of GDM females, hypoadiponectinemia was observed. The high frequency of hypoadiponectinemia suggests further large-scale studies, are warranted to ascertain the diagnostic accuracy of adiponectin as a potential biomarker for GDM.

Keywords: Gestational Diabetes; Adiponectin; Pregnancy; Glucose Intolerance.

Corresponding Author:
Dr. Saba Raza
Department of Chemical Pathology,
Dr. Ziauddin University Hospital,
North Nazimabad, Karachi, Pakistan.
Email: saba_raza@hotmail.com
https://doi.org/10.36283/PJMD10-1/007

INTRODUCTION

Pregnancy is a physiological state, which often leads to the development of certain metabolic complications. Gestational Diabetes Mellitus (GDM) is at present the most commonly diagnosed metabolic disorder in pregnant women. GDM is defined as "any degree of glucose intolerance with onset or first recognition during pregnancy" [1]. The frequency of GDM is increasing worldwide with a higher frequency among the Asian population[2]. A study done in Pakistan reported the frequency of patients diagnosed as GDM on the Glucose Tolerance Test as 26.3% [3]. According to a study, mean adiponectin levels of 5.43±2.28 were found in patients diagnosed with GDM and was proved lower when compared to patients without GDM[1].

Gestational Diabetes Mellitus usually develops in the second trimester of pregnancy due to increased

insulin resistance and insufficient β-cell compensation. Different diabetogenic hormones secreted from the placenta such as placental lactogen, growth hormone, corticotrophin-releasing hormone and progesterone lead to insulin resistance and thus women whose pancreatic function is not ample to produce sufficient quantities of supplementary insulin to overcome this insulin resistance produced during pregnancy by alterations in diabetogenic hormones develop Gestational Diabetes. Existing data suggest that β-cell insufficiency and pregnancy-induced insulin resistance in GDM can result from various factors including obesity, inflammation and autoimmune diseases[4].

Advanced maternal age, obesity, high parity, family history of GDM, or type 2 Diabetes Mellitus are different risk factors associated with GDM.[2,3] Oral Glucose Tolerance Test (OGTT) is the gold standard for diagnosis of GDM. Several criteria have been proposed to date for the diagnosis of GDM and guidelines vary among different countries[5]. Early screening and diagnosis of GDM is important to avoid neonatal complications as neonatal hypoglycemia, macrosomia, etc. Preeclampsia, increased chances of cesarean section and type 2 diabetes mellitus are among the maternal complications[6].

Nutrition counseling and diet therapy are helpful in the management of GDM. If target glucose levels are not achieved with diet therapy alone, pharmacotherapy is indicated[7]. Adipose tissue is an important endocrine and metabolically active organ. It secretes various types of adipokines including leptin, adiponectin, resistin, visfatin, and omentin, etc. Adiponectin is a protein hormone and has many functions in the body like enhancement of insulin sensitivity, inflammation resolution and vasculature protection[8]. According to a study, adiponectin levels differ among different ethnicities[9].

Adiponectin has a role in process of metabolism of glucose in pregnancy, its levels decrease in pregnancy and are correlated with increased insulin resistance. Although the association of hypoadiponectinemia with GDM is well established as reported in a study, which showed adiponectin levels were significantly decreased in GDM[10], yet it was required to study, its significance in our population because of genetic variations exists among different ethnicities. If such a relationship is also found in our patients with GDM, this could be used as a potential biomarker to predict GDM in early pregnancy and hence, appropriate therapeutic interventions and lifestyle modifications can be used to prevent the GDM and its complications. This study aimed to determine mean adiponectin levels and the frequency of hypoadiponectinemia in patients with Gestational Diabetes Mellitus (GDM).

METHODS

This study was a cross-sectional study conducted from June 2016 to December 2017 in the department of Chemical Pathology at Dr. Ziauddin Hospital, North Nazimabad, Karachi, Pakistan. This study included ninety-nine pregnant females aged 15-45 years diagnosed as GDM by Oral Glucose Tolerance Test (OGTT) between 24 to 28 weeks of gestation. Non-probability consecutive sampling was done. The ethical approval was taken from the Ethical Review Committee (1541019SRPAT) of the Hospital. All women were given information about the purpose of the study and consent was taken. Participant socio-demographic information including age, parity, and ethnicity were collected through interviews. The gestational week was estimated according to the Last Menstrual Period (LMP). The weight of the participant was measured on a digital weighing scale in kilograms. Standing body height was taken by height scale, Body Mass Index (BMI) was calculated (weight in kg/height in m^2), and the questionnaire was filled. The diagnosis of GDM was made on OGTT when any of plasma glucose levels (measured Fasting≥ 92mg/dL, 1-hour ≥ 180mg/dL, 2-hour≥ 153mg/dL after 75gram oral glucose load) are met or exceeded as per American Diabetes Association (ADA) guidelines[11]. Hypoadiponectinemia was labeled with Adiponectin levels below12.4 µg/ml[12].

Women with hypertension, GDM history in a previous pregnancy, known case of Diabetes Mellitus (type 1 and 2), family history of Diabetes Mellitus, and evidence of twin pregnancy on ultrasound, evidence of Polycystic Ovary Syndrome on ultrasound, smoker, and history of use of corticosteroids during last 6 months were excluded from the study. Blood samples for laboratory measurement of Adiponectin were drawn in a serum separating tube during the OGTT. The blood samples labeled were sent to the Chemical Pathology department and centrifuged and the sample was transferred to aliquot and frozen at -70˚C until testing of adiponectin was analyzed on Immunoassay. Internal quality control materials were used for quality control of the process.

Data was organized and entered on SPSS version 22. Results of quantitative variables as age, gestational age, BMI and serum adiponectin levels were shown as mean and standard deviation. Results of categorical data like parity, ethnicity, and hypoadiponectinemia were expressed as a percentage (%). The rest of the potential effect modifiers as gestational age, age, BMI, ethnicity, parity were controlled through stratification. The student's t-test and ANNOVA was applied post-stratification for the quantitative outcome (i.e. serum adiponectin levels) and the Chi-square test was applied for the qualitative outcome (i.e.

hypoadiponectinemia). The result was considered statistically significant with a *p*-value ≤0.05.

RESULTS

The descriptive statistics of patients are shown in Table 1. Mean Adiponectin levels in patients with GDM were 6.90±2.86 µg/ml (95%CI: 6.33 to 7.47).

Table 1: Clinical statistics of the selected patients for the study.

Variables (Mean± SD)	Groups	n (%)	Mean Adiponectin Levels µg/ml	*p*-Value*
All Patients	-	99(100)	6.90±2.86	-
Age (Years) (30.0±4.9)	15-25 26-35 36-45	16(16.2) 74(74.7) 9(9.1)	6.80±3.28 6.79±2.67 7.95±3.71	0.518
Gestational Age (Weeks) (25.40±0.82)	24-25 >25	38(38.4) 61(61.6)	7.00±2.95 6.83±2.84	0.779
BMI (Kg/m²) (28.97±5.78	Normal (18.50-24.9) Overweight (25-29.9) Obese (>30)	24(24.2) 38(38.4) 37(37.4)	7.59±3.63 7.37±2.88 5.98±2.02	0.043
Parity	Nulliparous Primiparous Multiparous	23(23.2) 34(34.3) 42(42.4)	6.57±2.71 6.67±2.99 7.26±2.88	0.556
Ethnicity	Balochi Muhajir Punjabi Pashtun Sariki Sindhi	3(3.0) 30(30.3) 30(30.3) 11(11.1) 3(3.0) 22(22.2)	4.20±1.96 7.51±2.94 7.15±3.35 5.90±1.75 6.26±2.15 6.68±2.51	0.322

** p < 0.05 considered significant*

The frequency of hypoadiponectinemia in patients with GDM was 91.92% (91/99) as shown in Figure 1.

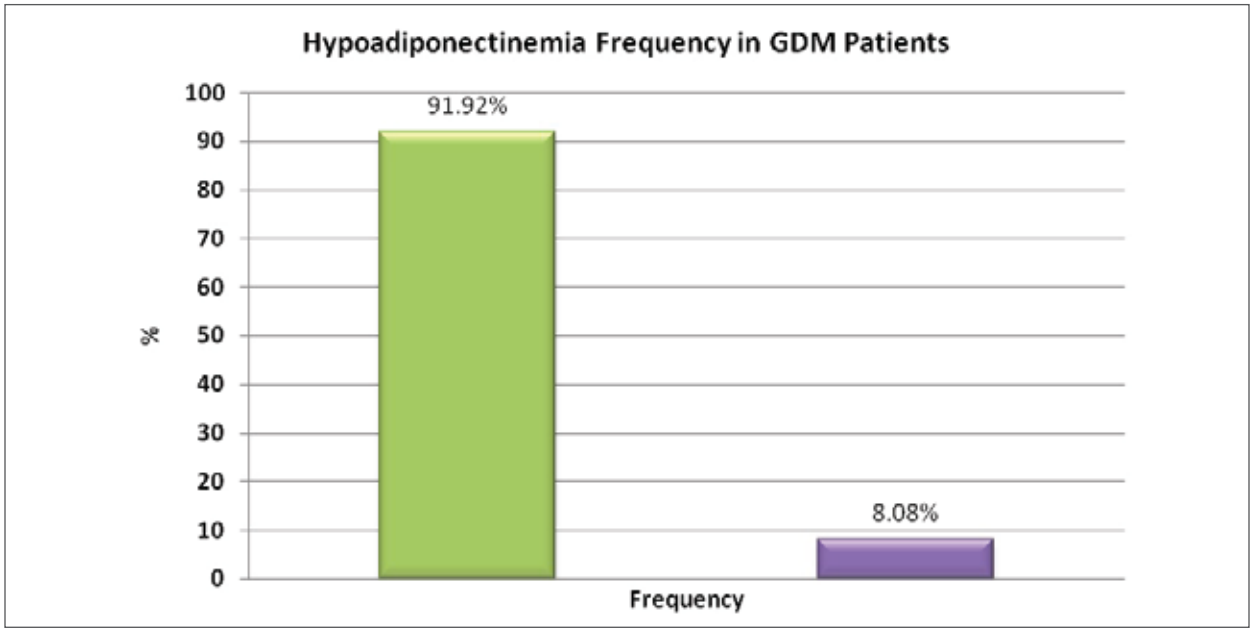


Figure 1: Frequency of hypoadiponectinemia in patients with gestational diabetes mellitus.

The majority (74) of women belonged to the age group of 26-35 years. Twenty-four (24.2%) women had normal BMI, thirty-eight (38.3%) were overweight and thirty-seven (37.3%) were obese. Patients mainly belonged from Muhajir (30.3%) and Punjab (30.3%) ethnicity (Table 1).

Stratification analysis was performed and one way ANNOVA and t-test was applied to compare mean Adiponectin levels, which were not significant among all age groups, gestational age groups, ethnicity, and parity groups though mean Adiponectin levels (Table1) were low in obese women as compared to normal and overweight women.

No significant difference was found in the frequency of hypoadiponectinemia using CHI-square test regarding age groups, gestational age, parity and ethnicity groups (Table 2) while the frequency of hypoadiponectinemia was high in obese cases (p=0.05) (Figure 2).

Table 2: Frequency of hypoadiponectinemia in patients with gestational diabetes mellitus by age, gestational age, parity and ethnicity.

Variables	Groups	n (%)	Hypoadiponectinemia		*p*-Value*	Chi-Square
			Positive	Negative		
Age (Years)	15-25 26-35 36-45	16(16.2) 74(74.7) 9(9.1)	15(93.8%) 69(93.2%) 7(77.8%)	1(6.3%) 5(6.8%) 2(22.2%)	0.263	2.67
Gestational Age (Weeks)	24-25 >25	38(38.4) 61(61.6)	35(92.1%) 56(91.8%)	3(7.9%) 5(8.2%)	0.957	0.003
Parity	Nulliparous Primiparous Multiparous	23(23.2) 34(34.3) 42(42.4)	22(95.7%) 32(94.1%) 37(88.1%)	1(4.3%) 2(5.9%) 5(11.9%)	0.477	1.48
Ethnicity	Balochi Muhajir Punjabi Pashtun Sariki Sindhi	3(3.0) 30(30.3) 30(30.3) 11(11.1) 3(3.0) 22(22.2)	3(100%) 27(90%) 26(86.4%) 11(100%) 3(100%) 21(95.5%)	0(0%) 3(10%) 4(13.3%) 0(0%) 0(0%) 1(4.5%)	0.680	3.15

** p < 0.05 considered significant*

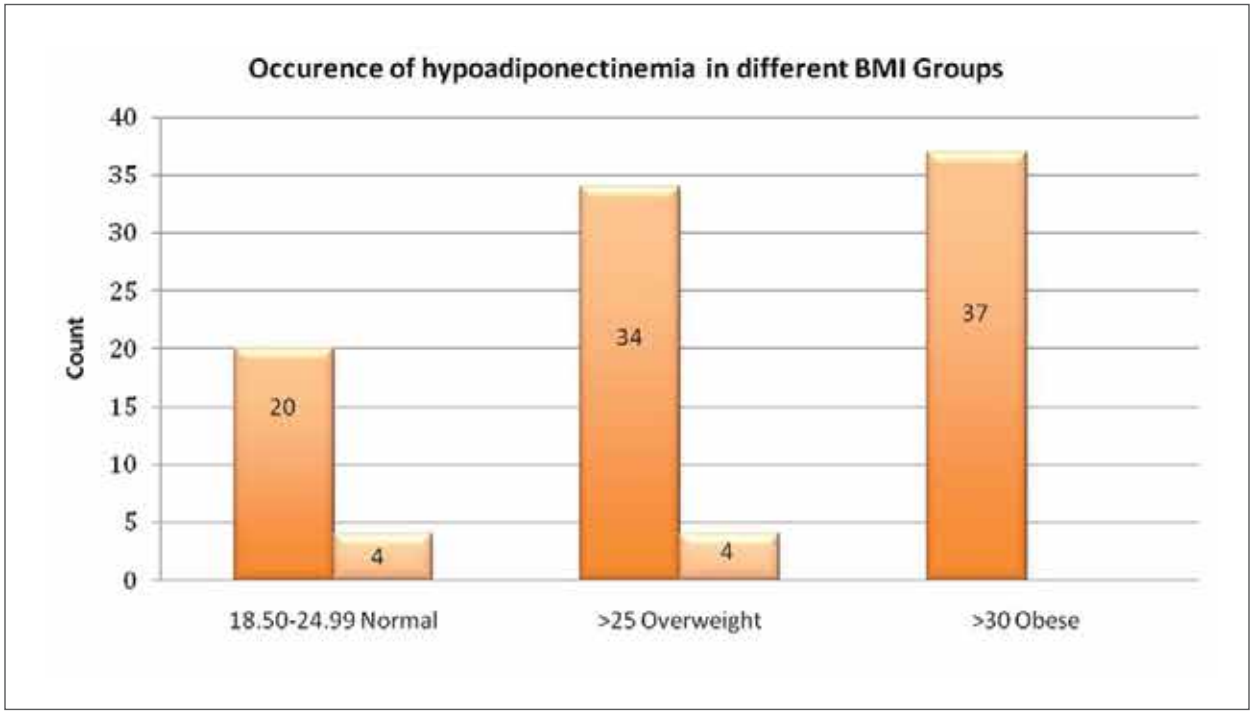


Figure 2: Frequency of hypoadiponectinemia in various groups of BMI.

DISCUSSION

Among all pregnant females diagnosed with GDM 91.92% (91/99) had decreased Adiponectin levels labeled as hypoadiponectinemia (when using a cutoff value of <12.4µg/ml) compared to study conducted by Chen et al. in 2012 which stated the frequency of hypoadiponectinemia as 50.7% in GDM patients (using a cutoff of <9 µg/ml). At the cutoff of <9µg/ml, frequency of GDM in our study was 80.80%, which is high in comparison with the study done by Chen et al[13]. Another study found decreased concentrations of Adiponectin in GDM[14]. In our study, mean adiponectin levels in patients with GDM was 6.90±2.86µg/ml compared to mean adiponectin levels of 7.06±3.69µg/ml. This can be due to ethnic variability, and genetic control of the production, secretion and degradation of adiponectin[15].

The mean age of pregnant females in our study, was 30±4.81 years whereas, a study done in India in 2016 showed the mean age of pregnant females with GDM 28.9± 3.64 years[16]. The mean gestational age (weeks) was 25.40±0.82 compared to a study mean gestational age was 27.1±0.6[15]. This study showed a significant difference (*p* value=0.043) in mean adiponectin levels among BMI groups with lower mean adiponectin levels in obese women which was supported by a study also showing low levels in obese women with GDM [17].

Years of research on adiponectin has emerged as a sizeable factor in the pathogenesis of GDM that may link insulin resistance and beta-cell dysfunction[18,19]. Some studies found that low levels of Adiponectin were linked with an increased risk of developing GDM and may be utilized as a biomarker in the first trimester of pregnancy to predict the risk of developing GDM in pregnant females[20-23].

Adiponectin levels vary among different ethnicities[24,25]. In our region, not much attention has been paid to the assess frequency of hypoadiponectinemia in GDM. When compared among different ethnicities in our population, serum adiponectin levels varied and were comparatively lower in Balochi subjects with mean adiponectin levels of 4.20±1.96µg/ml. In our study, which included GDM patients from 24-28th weeks of gestation, we found a high frequency of hypoadiponectinemia in GDM, making it a potentially good marker for screening of GDM. On the other hand, adiponectin levels have been shown to exhibit variation through different trimesters of pregnancy[14].

The limitations of my study were lack of multicenter data collection, lack of information about pre-pregnancy adiponectin concentrations, in the first trimester of pregnancy and after delivery to determine whether adiponectin concentrations have returned to normal. Our study was a cross-sectional study, therefore further large scale diagnostic accuracy studies are required to be performed in our population, to establish the sensitivity, specificity, Receiver Operating Characteristic (ROC) curve so that a reliable cutoff can be obtained for screening and diagnosing Gestational Diabetes Mellitus.

We are quite optimistic that advanced research about the processes involved in the development of insulin resistance during pregnancy and different phenomena causing a decrease in adiponectin levels in GDM will lead to proactive approaches to improve the health of mothers and neonates in the times to come.

CONCLUSION

The mean Adiponectin levels in women with GDM was 6.90±2.86 µg/ml and 91.92% cases were having hypoadiponectinemia. The findings suggest that a decline in Adiponectin levels may play a part in the development of insulin resistance in patients with GDM.

ACKNOWLEDGEMENTS

The authors would like to acknowledge Ms. Sadia Ghani for assistance in sample analysis.

CONFLICT OF INTEREST

There is no conflict of interest to be declared.

ETHICS APPROVAL

The study approval was obtained from the Ethics Review Committee of the Ziauddin University Hospital (1541019SRPAT).

PATIENT CONSENT

Verbal and written informed consent was obtained from all patients.

AUTHORS' CONTRIBUTION

SR, ES and UA designed the project, SR gathered the writing material and done the data collection activity. UA performed the statistical analysis. AMZ and ES reviewed the manuscript.

REFERENCES

1. Saini V, Kataria M, Yadav A, Jain A. Role of leptin and adiponectin in gestational diabetes mellitus: a study in a North Indian tertiary care hospital. Internet J Med Update. 2015;10(1):11-4.
2. Lee KW, Ching SM, Ramachandran V, Yee A, Hoo FK, Chia YC, *et al*. Prevalence and risk factors of gestational diabetes mellitus in Asia: a systematic review and meta-analysis. BMC Pregnancy Childbirth. 2018;18(1):494-513.
3. Fatima S, Rehman R, Alam F, Madhani S, Chaudhry B, Khan TA. Gestational diabetes mellitus and the predisposing factors. J Pak Med Assoc. 2017;67(2):261-265.
4. Sharafat J, Ahmed U, Anwar MK, Saad K. Association of adiponectin with insulin resistance in gestational diabetes mellitus. Pak J Physiol. 2019;15(2):25-27.
5. Bhavadharini B, Uma R, Saravanan P, Mohan V. Screening and diagnosis of gestational diabetes mellitus – relevance to low and middle income countries. Clin Diabetes Endocrinol. 2016;2(13)1-8.
6. Makwana M, Bhimwal RK, Ram C, Mathur SL, Lal K, Mourya H. Gestational diabetes mellitus with its maternal and foetal outcome: a clinical study. Int J Adv Med. 2017;4(4):919-925.
7. Walker JD. NICE guidance on diabetes in pregnancy: management of diabetes and its complications from preconception to the postnatal period. NICE clinical guideline 63. London, March 2008. Diabetic Med. 2008;25(9):1025-1027.
8. Wang Z, Scherer P. Adiponectin, the past two decades. J Mol Cell Biol. 2016;8(2):93-100.
9. Parvaresh Rizi E, Teo Y, Leow MK, Khoo EY, Yeo CR, Chan E, *et al*. Ethnic differences in the role of adipocytokines linking abdominal adiposity and insulin sensitivity among Asians. J Clin Endocrinol Metab. 2015;100(11):4249-4256.
10. Mohammadi T, Paknahad Z. Adiponectin concentration in gestational diabetic women: a case-control study. Clin Nutr Res. 2017;6(4):267-276.
11. American Diabetes Association. 2. Classification and diagnosis of diabetes: standards of medical care in diabetes—2019. Diabetes care. 2019 Jan 1;42(Supplement 1):S13-S28.
12. Retnakaran R, Hanley A, Raif N, Hirning C, Connelly P, Sermer M, *et al*. Adiponectin and beta cell dysfunction in gestational diabetes: pathophysiological implications. Diabetologia. 2005;48(5):993-1001.
13. Chen X, Scholl TO, Stein TP. Hypoadiponectinemia: Association with risk of varying degrees of gestational hyperglycemia and with maternal ethnicity. Int J Diabetes Mellitus. 2012;02(02): 196-202.
14. Bozkurt L, Göbl CS, Baumgartner-Parzer S, Luger A, Pacini G, Kautzky-Willer A. Adiponectin and leptin at early pregnancy: association to actual glucose disposal and risk for GDM—A prospective cohort study. Int J Endocrinol. 2018;2018:546 3762:1-9.
15. Naghshineh E, Faryadras N, Rouholamin S. Is there any relation between adiponectin levels in the first trimester of pregnancy and gestational diabetes? Womens Health Gynecol. 2016;2(4):32-35.
16. Bhograj A, Suryanarayana KM, Nayak A, Murthy SN, Dharmalingam M, Kalra P. Serum adiponectin levels in gestational diabetes mellitus. Indian J Endocrinol Metab. 2016;20(6):752-755.
17. Ramirez VI, Miller E, Meireles CL, Gelfond J, Krummel DA, Powell TL. Adiponectin and IGFBP-1 in the development of gestational diabetes in obese mothers. BMJ Open Diabetes Res Care. 2014;2(1): 1-8.
18. Plows J, Stanley J, Baker P, Reynolds C, Vickers M. The pathophysiology of gestational diabetes mellitus. Int J Mol Sci. 2018;19(11):3342: 1-21.
19. Barbe A, Bongrani A, Mellouk N, Estienne A, Kurowska P, Grandhaye J, *et al*. Mechanisms of adiponectin action in fertility: An overview from gametogenesis to gestation in humans and animal models in normal and pathological conditions. Int J

Mol Sci. 2019;20(7):1526.
20. Madhu S V, Bhardwaj S, Jhamb R, Srivastava H, Sharma S, Raizada N. Prediction of gestational diabetes from first trimester serum adiponectin levels in Indian Women. Indian J Endocr Metab. 2019;23:536-539.
21. Thagaard IN, Krebs L, Holm JC, Lange T, Larsen T, Christiansen M. Adiponectin and leptin as first trimester markers for gestational diabetes mellitus: a cohort study. Clin Chem Lab Med. 2017;55(11):1805-1812
22. Iliodromiti S, Sassarini J, Kelsey TW, Lindsay RS, Sattar N, Nelson SM. Accuracy of circulating adiponectin for predicting gestational diabetes: a systematic review and meta-analysis. Diabetologia. 2016;59(4):692-699.
23. Lorenzo-Almorós A, Hang T, Peiró C, Soriano-Guillén L, Egido J, Tuñón J, *et al*. Predictive and diagnostic biomarkers for gestational diabetes and its associated metabolic and cardiovascular diseases. Cardiovasc Diabetol. 2019;18(1):1-16.
24. Chen X, Scholl T. Ethnic differences in maternal adipokines during normal pregnancy. Int J Environ Res Public Health. 2015;13(1):8:1-13.
25. Jara A, Dreher M, Porter K, Christian L. The association of maternal obesity and race with serum adipokines in pregnancy and postpartum: Implications for gestational weight gain and infant birth weight. Brain Behav Immun. 2020;3:100053:1-8.

ORIGINAL ARTICLE

Prevalence of Types, Frequency and Risk Factors for Complications after Exodontia

Naseer Ahmed[1], Abhishek Lal[2], Maha Shakeel[2], Danish Cyrus[2], Fatima Tuz Zehra[2], Ahsan Ayub[2]
[1]Department of Community Dentistry, Altamash Institute of Dental Medicine, Karachi, [2]Student, Altamash Institute of Dental Medicine, Karachi, Pakistan.

ABSTRACT

Background: Exodontia is one of the most frequently carried out procedures by a dental surgeon, mostly on an outpatient department basis. The reasons for performing exodontia include non-restorable teeth, periodontal disease, dental trauma, impacted tooth, orthodontic treatment and toothache. Complications in dental extraction are a commonly encountered problem in dental clinics. This knowledge can help dental surgeons make extractions less invasive, traumatic and complicated, enabling quicker recovery of the socket. The aim of this study was to recognize types, frequency and risk factors for complications after exodontia.

Methods: This cross-sectional study enrolled patients who had exodontia done from July- September 2019, visiting OPD of the Oral surgery department of Altamash Institute of Dental Medicine, Karachi. Risk factors included demographic data, general health, past medical and dental history. Spearman's correlation test was used to establish any relationship of variables with complications.

Results: The study patients (126) included 72 females (mean age 39.1±13.39) and 54 males (mean age 41.1±14.93). The overall complications rate was found to be 7.1%, mainly arising from maxillary and mandibular third molars. The most common complications encountered were Hemorrhage, Pain, and Trismus. Increasing age and specific teeth extracted were associated with an increased risk for complications. However, post-operative complications which were encountered most of them were minor and handled on an outpatient department basis.

Conclusion: Frequency and risk factors for complications after exodontia were found low3 (37.5%). While age and teeth extracted cannot be directly altered, these factors maybe indirectly modified, resulting in a potential decrease of postoperative complications.

Keywords: Oral Surgery; Exodontia; Complications.

Corresponding Author:
Dr. Naseer Ahmed
Department of Community Dentistry,
Altamash Institute of Dental Medicine,
Karachi, Pakistan.
Email: rnaseerahmed.t@gmail.com
https://doi.org/10.36283/PJMD10-1/008

INTRODUCTION

Exodontia, also known as dental extraction is a procedure whereby one or more than one tooth is extracted out of their sockets. There are many reasons for the dental surgeon to perform exodontia which includes severe dental pain, non-restorable tooth, periodontal disease, carious teeth, dental trauma, retained deciduous teeth, increase mobility, a patient requiring dentures, dental abscess, orthodontic treatment, impacted tooth and sometimes teeth associated with tumors[1,2].

Dental extraction can be performed using two different approaches. First is called closed extraction, where only dental forceps and elevators are used to extract the teeth out of their sockets with no elevation of the flap[3]. The second approach

is called open extraction or surgical extraction where the flap is raised surgically using a scalpel and adjunctly bone is drilled using a hand piece with forceps and elevators also being used[4]. Indications for both closed and open extractions are different from the initial approach usually being closed. Certain indications make open extractions the first choice, which includes impacted teeth, unusual root morphology, and dilacerations of root tips along with ankylosed tooth, hypercementosis, and teeth lying close to vital structures[4].

Exodontia, one of the commonest procedures performed in dental practice, can be associated with postoperative complications with significant biological and social impact. Complications are unfortunate events that tend to increase morbidity, more than what would be expected from a specific operative procedure in normal circumstances[2]. Although they are uncommon, their happening leads to a prolonged period of treatment, which is uncomfortable for the patient as well as the dental surgeon. Thus, careful attention to detail is required which includes a detailed case history, routine investigations; for example, radiographs and blood tests are a core part of exodontia[5].

Complications are part of almost every procedure that a dental surgeon can perform but some procedures are more prone to complications than others are, exodontia is one of them. Experienced dental surgeons do anticipate and many times are prepared for such circumstances which if taken care of before the procedure usually leads to an uneventful procedure, no complications postoperatively, and no additional follow-ups for the patients[4].

So, preoperative assessment of unchangeable risk factors includes age[5,6], gender, medical history of patients such as conditions associated with wound healing problems (that is chronic hepatitis), diabetes, hypertension, blood dyscrasia (hemophilia, thalassemia) or drug-induced reactions were also considered risk factors leading to complications after exodontia[7,8]. Moreover, factors considered modifiable are the use of tobacco, oral hygiene, and oral contraceptives.

Anticipated (clinically and radiologically) potentials intra-operative risk factors of exodontia are, dental surgeon's experience, the time duration of surgery, the technique of anesthesia administration, intrasocket medications, and topical anesthesia[9,10]. Finally, post-operative, early and late, complications commonly encountered include pain, swelling, trismus, hemorrhage, dysesthesia, severe infection, fracture, dry socket, damage to adjacent teeth, and displaced teeth. Most of these complications are temporary but some are permanent which may lead to a functional deficit. The main objectives of this investigation were to identify risk factors associated with post-operative complications as well as the types and frequency of complications after extractions.

METHODS

The study design was cross-sectional using random sampling to collect data. The study subjects were recruited from patients at Altamash Institute of Dental Medicine, Karachi, Pakistan OPD between July 2019 to September 2019. The patients who had one or more dental extractions with evidence of post-operative follow up were included to access outcomes. Patients presenting to the OPD with co-morbidities such as hypertension, diabetes, hepatitis, blood dyscrasias were also included. Patients without evidence of post-operative follow-up were excluded from the sample. The Ethics Review Committee of Altamash Institute of Dental Medicine approved this study (AIDM/EC/06/2019/10).

The predictor variables, that is, risk factors or exposure were grouped into the following settings of variables: demographic, medical and dental history, anatomic, and operative. Before performing surgery, the evaluation included a medical and dental history of the patients, personal information including name, gender, age, education, occupation, residence, and marital status.

Patients with a positive medical history that might affect immunity or contraindicate surgery e.g., cardiovascular diseases, diabetes mellitus, bleeding disorders, liver or kidney dysfunction, respiratory ailment, hepatitis B or C were part of this study. A positive dental history was recorded in the following circumstances: teeth sensitivity, bleeding gums, orthodontic treatment, and difficulty in moving jaws, mobile teeth, and bruxism. The consumption of tobacco, betel nut, and naswar (powdered tobacco) was also taken into account. The presence or absence of postoperative complications after performing exodontia was the primary outcome variable with risk factors associated with postoperative complications and their frequencies being the secondary outcomes of the study.

After extractions were carried out occurrence of the following complications were observed: pain, swelling, trismus, hemorrhage, damage to adjacent teeth, displaced teeth, infection, ulceration, dry socket, and fracture. For statistical analysis, SPSS was used. For correlation between variables, Bivariate analysis was used to compare variables which include age, gender, medical history, dental history, habits, location of extraction and teeth extracted were compared with the occurrence of complications after exodontia was performed. Spearman's statistical test was used to perform this analysis and a p-value of ≤ 0.05 was considered statistically significant.

RESULTS

The results indicated that between the months from July to September 2019, a total of 126 patients, consisting of 72 (57.1%) females and 54 (42.9%) males who visited the Oral Surgery Department, who had one or more teeth extracted by a Dental Surgeon were finalized. The sample's mean average age was 39.1±13.39for females and 41.1±14.93-for males. Descriptive Statistics are shown in Table 1.

Table 1: Frequency of different teeth extracted in maxilla and mandible.

Kinds of Teeth	Teeth in Females n (%)	Teeth in Males n (%)
Maxillary Anteriors	5(5.1)	7(9)
Maxillary Posteriors	45(46)	24(30.7)
Mandibular Anteriors	5(5.1)	8(10.3)
Mandibular Posteriors	43(43.8)	39(50)

Anteriors: Central incisor to canine, Posteriors: First premolar to third molar.

Among 126 patients, a total of 176 teeth were extracted. Of these 176 extracted teeth, 172(97.8%) closed or non-surgical approach was used to extract the teeth and only 4 (2.2%) teeth required surgical or open extraction approach. 46 (26.1%) extractions were third molars. Patients belonging to the age group of adults and the elderly mainly had their teeth extracted with a smaller number of adolescent and children patients. Patients with a positive medical history were 45 (35.7%). Hypertension and Diabetes were the most common positive factors among recruited patients' medical history. A small number of patients reported having a positive medical history of arthritis, asthma, tuberculosis, and thyroid problems (Table 2).

When these variables of age, gender, medical history, dental history, habits, location of extraction, and teeth extracted were entered in bivariate Spearman's test analysis for correlations, they had an association with the occurrence of complications (Table 2). As the age of the patient increases, the occurrence of complications increases (*p*-value=0.02). A similar relationship was also seen with teeth extracted (*p*-value=0.04). Variables, which had no effect on occurrence of complications, include gender (*p*-value=0.25), medical history (*p*-value=0.16), dental history (*p*-value=0.51), habits (*p*-value=0.92) and location of extraction (*p*-value=0.41) as shown in Table 2.

Table 2: Significance of variables in relation to complications.

Parameters	Correlations with Exodontia	*p*-Value
Age	-0.40	0.00
Gender	0.08	0.37
Medical History	0.18	0.04
Dental History	-0.09	0.33
Habits	-0.23	0.01
Location of teeth	-0.09	0.30
Complications	-0.18	0.04

Considering the habits of the patients, smoking, paan/gutka, and betel nuts consumption was frequently encountered with a small number of naswarusages. In our study, the major cause of performing dental extractions was pain. A small number of patients with caries, mobility swelling gums, missing tooth, sensitivity, trauma, retained deciduous teeth, patient wish, loose denture, extraction needed, and prostheses needed also required dental extraction to be performed (Figure 1).

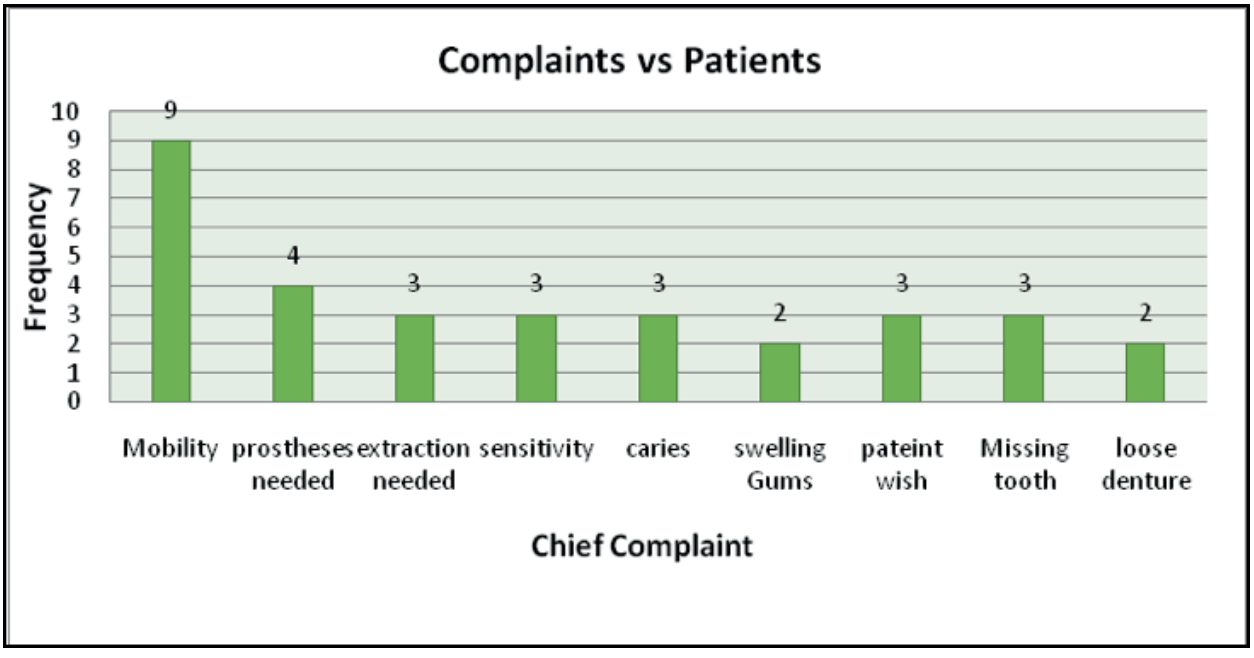


Figure 1: Assessment of chief complaints of the patients.

Teeth most frequently extracted were in the posterior segments of both maxilla and mandible i.e., the molars and they were generally extracted under local anesthesia. In two exceptional cases, general anesthesia was required, although no complications were reported among them. The number of teeth extracted in the anterior segment of both maxilla and mandible was less. There was no use of preoperative antibiotics. In our study, no complication of adjacent tooth damage, ulceration, a displaced tooth, infection, dry socket, and fracture was reported. All of the reported complications were found in teeth undergoing closed extraction, with no complications arising from open extractions. In types and frequencies of postoperative complications after exodontia, most frequently encountered complications after extractions were pain, trismus, and hemorrhage. There was no case of damage to neither lingual nerve nor the inferior alveolar nerve. Post-operative complications that were encountered most of them were minor and handled on an outpatient department basis.

Of the 8 patients in whom complications postoperatively were found, 3 (37.5%) of them had a positive medical history of diabetes, asthma, thyroid problems, and hypertension. The majority of these patients were females belonging to the age group of adults. None of these patients consumed tobacco, alcohol, betel nut, or naswar. Considering gender, complications mainly arose in females. Of a total of 98 teeth extracted in females, complications were found in the extraction of 12 (12.2%) teeth mainly molars. On the other hand, in males of the total of 78 teeth extracted, the complication was found in 1 (1.3%) tooth which was a maxillary third molar. Except for two cases, all of the above-mentioned dental extractions were performed under local anesthesia. No case was reported of using preoperative antibiotics. Patients who did not come for follow-up were excluded from this study.

DISCUSSION

The overall complication rate in the study was 7.1%. Besides, the majority of the complications were related to molars, particularly third molars[11,12]. The investigation outcomes concluded trismus and pain as the most frequently encountered complications. Teeth that are most commonly extracted includes mainly third molars, with anterior dentition both in maxilla and mandible being less frequently extracted[13,14].

Extractions were performed on the patients mainly because the tooth had an unfavorable prognosis for restorative treatment with extraction then being the most likely outcome. Patients present to the dental surgeon quite late when saving the tooth becomes less likely as compared to patients frequently visiting the doctor at least once or twice a year normally. The complication rate in the study is within the range reported in the referenced literature. Studies similarly concluded results corresponding to this study, although there was no presentation of dry socket (Alveolar Osteitis) during the determined duration of this study in literature, the dry socket has been documented[15,16].

Almost all of the post-op extraction complications were present in females with the adult age group[17]. Males had significantly a smaller number of compli-

cations[18]. Consistent with the previous studies, the most common complications after exodontia was associated with third molars[19,20]. Post-operative extraction complications these days tend to be less frequently encountered in OPD mainly because of better techniques used for extractions, the doctor's understanding of the complications, how they can be avoided, better treatment of the complications if encountered, and how to prevent them. In our study, we encountered pain, trismus, and hemorrhage as the most frequently occurring complications[19,21]. whereas, in the literature, the most frequently occurring complications include post-operative pain, hemorrhage, dry socket and infection[7,22].

The second objective of our study was to identify any risk factors associated with post-operative extraction complications. We concluded from our data, age and teeth extracted were contributing factors that might lead to complications. Although at times, there is insufficient data to make a judgment regarding these mentioned risk factors[23].

Hypertension and Diabetes were found to be the most frequently encountered medical condition of patients visiting the OPD and the complications were also found in these patients after exodontia making both of these conditions potential risk factors for complication post extractions[24]. Although no significant relationship can be concluded from our data as complications of trismus and pain were found in one patient who was diabetic. Patients frequently have been positive for dental history mainly gingivitis and periodontitis, but its presence does not predispose patients to postoperative complications as we found in this study.

In the current study, age correlates and acts as a risk factor for post-operative extraction complications[25]. Although different results regarding age is mentioned in the literature[16]. This positive correlation could be due to an increase in bone density as a person ages and this leads to difficulty in extraction, which in turn increases the chances of complications particularly in third molars. A medical history may also be a contributing factor in complications mainly because of patients not keeping in check their health like maintaining blood pressure and blood sugar levels. This study did not find any positive correlation of patients with a positive social history of e.g. smoking, pan, betel nut and naswar with post-op complications, although tobacco is often a very well documented factor in literature[23,25]. Gender and age are often considered as positive factors for complications.

Pain and stress are documented to positive risk factors associated with post-operative exodontia complications[23]. Therefore, varying from patient to patient; anxiety should be taken care of as this will decrease procedure duration, extraction will be less traumatic, and exceeding cartridges of local anesthesia will not be required. All these factors are known to be of hindrance during the procedures. Several methods are able including intravenous sedation and administration of various drugs available usually a few hours before the procedure or the night before[25]. In this study, complications that occur after exodontia have been reported. One of the limitations is that the study included a smaller sample size. Another is that it would have been better if other relatable risk factors regarding complications after exodontia were also included in this study.

CONCLUSION

The frequency and risk factors for complications after exodontia were found low. While age and teeth extracted cannot be directly altered, these factors maybe indirectly modified. It is of vital importance that a dental surgeon recognizes the complications that occur or might occur so that it can be handled accordingly. Dental surgeons must recognize the impending complications and manage them accordingly

ACKNOWLEDGMENTS

The authors would like to acknowledge all the participants that were part of this study.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICS APPROVAL

The Ethics Review Committee of Altamash Institute of Dental Medicine approved this study (AIDM/EC/06/2019/10).

PATIENT CONSENT

Verbal and written consent were taken from the participants.

AUTHORS' CONTRIBUTION

NA provided his valuable guidance and interpreted the patient's data. AL collected data, literature review, made figures and was a major contributor in writing the manuscript. AA collected data and provided his knowledge in writing the manuscript. DC collected data and formed tables. FTZ collected data and conducted a final revision of the article. MS also collected data and responsible for data management.

REFERENCES

1. Taiwo AO, Ibikunle AA, Braimah RO, Sulaiman OA, Gbotolorun OM. Tooth extraction: Pattern and etiology from extreme Northwestern Nigeria. Eur J Dent. 2017;11(03):335-339.
2. Mamoun J. Use of elevator instruments when luxating and extracting teeth in dentistry: clinical techniques. J Korean Assoc Oral Maxillofac Surg. 2017;43(3):204-211.
3. Jennifer Archibald D. What to know about tooth extraction [Internet]. [cited 2020 Aug 11]. Available from: https://www.medicalnewstoday.com/articles/327170
4. Deliverska EG, Petkova M. Complications after extraction of impacted third molars-literature review. Annu Proc Sci Papers. 2016;22(3):1202-1211.
5. Osunde OD, Saheeb BD. Effect of age, sex and level of surgical difficulty on inflammatory complications after third molar surgery. J Maxillofac Oral Surg. 2015;14(1):7-12.
6. Khosla A, Venkateshwar G, Padhye M, Kakkar S. Complications of exodontia: A retrospective study. Indian J Dent Res. 2011;22(5):633-638.
7. AlHindi M. Dry socket following teeth extraction: effect of excessive socket saline irrigation. J Oral Heal Dent Sci. 2017;1(1):1-5.
8. Azam K, Hussain A, Maqsood A, Farooqui WA. Effects of surgery duration on post-extraction sequelae following impacted third molar surgery by using two different bone cutting methods; a double blind randomized trial. Pak Oral Den J. 2016;36(1):1-1.
9. Azam AN, Ezoddini F, Khalesi M, Gholami L, Momtaz A. Evaluation of the complications after mandibular third molar surgical extraction. Avicenna J Dent Res. 2018;2(1):23-28.
10. Sayed N, Bakathir A, Pasha M, Al-Sudairy S. Complications of Third molar extraction: A retrospective study from a tertiary healthcare centre in Oman. Sultan Qaboos Univ Med J. 2019;19(3):e230-e235.
11. Normando D. Third molars: To extract or not to extract? Dental Press J Orthod. 2015;20(4):17-18.
12. Rakhshan V. Common risk factors of dry socket (alveolitis osteitis) following dental extraction: A brief narrative review. J Stomatol Oral Maxillofac Surg. 2018;119(5):407-411.
13. Mahomed VM and O. Incidence and predisposing factors for dry socket following extraction of permanent teeth at a regional hospital in Kwa-Zulu Natal: Research. South African Dent J. 2016;71(4):166-169.
14. Ahmed A, Mohamed F, Hattab K. Surgical extraction of impacted mandibular third molars: postoperative complications and their risk factors. Jamahiriya Med J. 2009;9(4):272-275.
15. Farshid A, Mohiti A, Ghasemzadeh O. Prevalence and risk factors for complications of mandibular third molar surgery. Am J Oral Maxillofac Surg. 2015;2(1):43-52.
16. Malkawi Z, Al-Omiri MK, Khraisat A. Risk indicators of postoperative complications following surgical extraction of lower third molars. Med Princ Pract. 2011;20(4):321-325.
17. Sigron GR, Pourmand PP, Mache B, Stadlinger B, Locher MC. The most common complications after wisdom-tooth removal: part 1: a retrospective study of 1,199 cases in the mandible. Swiss Dent J. 2014;124(10):1042-1056.
18. Miclotte I, Agbaje JO, Spaey Y, Legrand P, Politis C. Incidence and treatment of complications in patients who had third molars or other teeth extracted. Br J Oral Maxillofac Surg. 2018;56(5):388-393.
19. Kumar MA, Gheena S. Incidence of dry socket after third molar extraction. J Pharm Sci Res. 2015;7(7):451-452.
20. Huang S, Dang H, Huynh W, Sambrook P, Goss A. The healing of dental extraction sockets in patients with Type 2 diabetes on oral hypoglycaemics: a prospective cohort. Aust Dent J. 2013;58(1):89-93.
21. Trybek G, Chruściel-Nogalska M, Machnio M, Smektała T, Malinowski J, Tutak M, *et al*. Surgical extraction of impacted teeth in elderly patients. A retrospective analysis of perioperative complications - the experience of a single institution. Gerodontol. 2016;33(3):410-415.
22. Kapil A, Rohit P, Neha P, Neetu A, VikasB. Complications and risk factor associated with extraction of impacted third molars: A prospective study. Int J Contemp Med Surg Radiol. 2017;2(4):153-155.
23. Bortoluzzi MC, Capella DL, Barbieri T, Marchetti S, Dresch CP, Tirello C. Does smoking increase the incidence of postoperative complications in simple exodontia? Int Dent J. 2012;62(2):106-108.
24. Schwartz-Arad D, Lipovsky A, Pardo M, Adut O, Dolev E. Interpretations of complications following third molar extraction. Quintessence Int. 2018;49(1):33-39.
25. McDowall C, Lydall ES, Moore SC. The potential role of patient stress in rates of dental post-extraction complication. Oral Surg. 2014;7(3):162-167.

ORIGINAL ARTICLE

Prognostic Value of Measuring Handgrip Strength (HGS) for Stroke Patients

Shama Razzaq[1], Tahseen Kazmi[2], Amna Khalid[3], Jamal Abdul Nasir[3], Ahmed Saud[3], Saadia Shahzad[3]

[1]Department of Community Medicine, Jinnah Medical and Dental College, Karachi,
[2]Department of Community Medicine, Central Park Medical College, Lahore,
[3]Department of Community Medicine, Shalamar Medical and Dental College, Lahore, Pakistan.

ABSTRACT

Background: A handgrip dynamometer is a simple, inexpensive and quick method to assess muscle strength. Sequential decrease in handgrip strength has been shown in various studies as a strong predictor of stroke. Therefore, this study aimed to measure muscular strength and determined its association with co-morbidities to predict critical illness in a community based setting of Punjab, Pakistan.

Methods: The study participants (n=152), were recruited through convenient sampling, during a community-based survey. Muscle strength was assessed with the handgrip dynamometer and quantified according to high, average and low percentile. Chi-square test was done to assess the distribution and multinomial logistic regression analysis to identify the factors associated with them.

Results: Out of 152 participants, mean age 44.5±15.3 years, 95(62.5%) were females and 57(35.5%) males. Handgrip strength measurement showed that 38(25%) of participants had high muscle strength, 80(52.6%) average and 34(22.4%) had low muscle strength. Low muscle strength was significantly higher among females (OR: 7.9, 95% CI: 2.4-27.1) as compared to the males. In general participants having diabetes had low muscle strength (p<0.011), but in hypertensive the association was not significant (p<0.21).

Conclusion: Overall patients at risk of stroke such as diabetics had significant low muscle strength but in hypertensive the association was not significant (p<0.21). More studies with bigger sample size are required to make it a predictive marker for stroke and cardiovascular diseases. It is easy to measure and is a low-cost technique for risk scoring and risk prediction in a community-based setting at an early stage.

Keywords: Muscles; Risk; Adult; Community, Dynamometer.

Corresponding Author:
Dr. Shama Razzaq
Department of Community Medicine,
Jinnah Medical and Dental College, Karachi,
Shaheed-e-Millat Road, Karachi, Pakistan.
Email: shama.razzak@gmail.com
https://doi.org/10.36283/PJMD10-1/009

INTRODUCTION

The importance of muscle mass, strength, and metabolic function in the performance of exercise is a well recognized fact. Loss of muscle strength or changes in skeletal muscles may cause physical weakness and metabolic variations such as insulin resistance, hyperlipidemia and increase in blood pressure[1,2].

Lower muscular fitness is emerging as a recognized determinant, in addition to previous evidence-based contributing common risk factors for non-communicable diseases (NCDs) such as low physical activity, obesity, poor diet, tobacco use, increased blood pressure, high blood sugar and increased blood cholesterol level[3,4]. Epidemiological studies suggest that declining muscle strength is well linked as one of the potential risk factors for cerebrovascular and cardiovascular diseases including arterial stiffness[4-6].

Various studies have shown that handgrip is a

reliable proxy measure of upper extremity muscle strength and declining handgrip strength has been proved to be a strong predictor of all-cause and cardiovascular mortality in a large multi-country longitudinal study i.e. Prospective Urban-Rural Epidemiology (PURE) study[7-9]. Therefore, handgrip strength is used as an indicator of muscular fitness, nutritional status, and walking performance and muscle mass[10-12].

Moreover, adding handgrip strength (HGS) measurement to traditional office-based risk factors (age, gender, blood pressure, obesity, diabetes mellitus and smoking) in resourced limiting under-served community settings and low and middle-income countries would be beneficial where blood-based measurements are difficult to perform because HGS measurement improves the prediction of office-based risk screeningprocess[9,13]. Whereas, non-communicable diseases (NCDs) have been on the rise and are contributing progressively to the global and local disease burden and need to be identified and prevented early[14].

Keeping in mind the rise of non-communicable diseases among the Pakistani population, the prevalence of coronary artery disease is found to be 29.6%, whereas, males and females are found at equal risk[15]. The situation for stroke in Pakistan is even worse since the risk factors contributing to stroke are massive and projections reported that Pakistan would be the fourth densely populated country concerning diabetic patients and every third person with 45 years of age would be hypertensive by 2020 [16]. Some potential risk factors associated with the declining of handgrip strength includes gender, age, body size, smoking and low physical activity have been reported in neighboring regions[17].

Hence, measuring an HSG which provides a quick, reliable and budgeted procedure for measuring muscle strength may be useful for risk screening of NCDs, and has the potential to apply as a filtering technique in a clinical setting and thus must have a clinical utility[9]. Herein, Pakistan, there is only one study available which was carried out for finding normative data for handgrip strength among Karachi resident[18]. In addition, up to the best of our knowledge, there is no exploration of the factors associated with lower handgrip strength. Therefore, this study aims to assess the handgrip strength and factors associated with lower handgrip strength among adults residing at the urban squatter settlement of Lahore, Punjab.

METHODS

This is a community-based cross-sectional study conducted in two selected Union Councils -120 and 122 of urban Punjab from February to March 2019. During the health camp, the eligibility criteria to recruit study participants included all adults aged 18 years and above of both genders. A total of 152 participants were recruited through a non-probability convenient sampling method.

Informed verbal and written consent was obtained from each participant. The interview was conducted using a structured questionnaire to extract the information about age, gender, present co-morbidities related to diabetes and hypertension, physical activity. That was defined as walk for 30 minutes daily, current medication history in the form of anti-hypertensive, anti-glycemic and lipid-lowering agents, tobacco use defined as current smoking and/or smokeless tobacco use and frequency of consumption of fruits and vegetables per week.

Anthropometric measurements were recorded including height and weight. Measurements of systolic and diastolic blood pressure and random blood sugar were performed according to standardized methods. Institutional Review Board (IRB) of Shalamar Medical and Dental College, Pakistan approved the study (SMDC/IRB/11-12/140).

A hand-held dynamometer could easily be used in clinical settings to measure the trunk muscle strength in patients with stroke because of its accuracy and affordability[19]. Analog handgrip dynamometer was used. Muscle strength was defined and measured with respect to handgrip strength by handgrip dynamometer with a protocol according to a standardized approach where a hand is placed in a vertical position and 90-degree elbow flexion and the whole procedure takes 5 minutes to be completed. The measurement is repeated two times with an interval for a while[20]. Participants with any physical limitations were excluded. Since there was variability in the cutoffs for defining handgrip strength therefore mean standardized score of muscle strength was used in terms of percentiles according to standard method i.e., standardized score was computed by subtracting individual value by mean scores and then divided by the standard deviation. Later, we categorized the sample into high, average and low strength based on the percentile cut-offs i.e. 25th, 50th and 75th respectively[21].

Statistical analysis was carried out using SPSS. Mean± SD for continuous variable and percentage (%) were calculated for categorical variables. Chi-square test was done to assess the sample distribution according to the outcome (handgrip strength) variable and *p*-value less than 0.05 was considered significant. Univariate regression analysis was done and variables with *p*-value <0.05 in univariate analysis were kept further in multinomial regression model for further assessment. Multinomial regression analysis was done since muscle strength

was characterized into three categories i.e. high, average and low muscle strength and therefore to estimate the adjusted odds ratios for associated factors of muscle strength.

RESULTS

A total of 152 participants with mean±SD age of study participants i.e. 44.5 (±15.3), 95 (62.5%) of females and 57(37.5%) males. Overall, 66(43.4%) of them were currently on medications for chronic diseases. Out of the total, 52(34.2%) were consuming fruits for ≥ 3 days per week, while, 101(66.4%) of participants were consuming vegetables for more than three days per week. Participants who walk daily for thirty minutes were 66(43.4%) and those who use any kind of tobacco product currently were 25 (16.4%) (Table 1).

Table 1: Comparison of factors associated with hang grip strength study participant's ≥18 years in urban Punjab, Pakistan.

Characteristics	Total Participants	Muscle Strength			*p*-Value
		High n (%)	Average n (%)	Low n (%)	
	n= 152	38 (25.0)	80 (52.6)	34 (22.4)	
Gender					
Male	57 (37.5)	28 (73.7)	21 (26.3)	08 (23.5)	0.00**
Female	95 (62.5)	10 (26.3)	59 (73.8)	26 (76.5)	
Age					
<40 years	58 (38.2)	13 (34.2)	36 (45.0)	09 (26.5)	0.14
≥ 40 years	94 (61.8)	25 (65.8)	44 (55.0)	25 (73.5)	
Hypertensive	54 (35.5)	9 (23.7)	31 (38.7)	14 (41.2)	0.21
Diabetic	53 (34.9)	13 (34.2)	21 (26.3)	19 (55.9)	0.01**
Physical Activity	66 (43.4)	21 (55.3)	31 (38.8)	14 (41.2)	0.23
Diastolic Blood Pressure					
Raised	37 (24.3)	33 (86.8)	57 (71.3)	25 (73.5)	0.17
Normal	115 (75.7)	05 (13.2)	23 (28.7)	09 (26.5)	
Systolic Blood Pressure					
Raised	39 (25.7)	31 (81.6)	57 (71.3)	25 (73.5)	0.48
Normal	113 (74.3)	07 (18.4)	23 (28.7)	09 (26.5)	
Blood Sugar Level					
Normal	74 (48.7)	17 (44.7)	45 (56.3)	12 (35.3)	0.11
Raised	78 (51.3)	21 (55.3)	35 (43.7)	22 (64.7)	
Taking Medications	66 (43.4)	14 (36.8)	34 (42.5)	18 (52.9)	0.37
Tobacco Use	25 (16.4)	13 (34.2)	10 (12.5)	02 (5.9)	0.00**

Consumption of Fruits per Week					
< 3 days	100 (65.8)	24 (63.2)	51 (63.7)	25 (73.5)	0.56
≥ 3 days	52 (34.2)	14 (36.8)	29 (36.3)	09 (26.5)	
Consumption of Vegetables per Week					
> 3 days	101 (66.4)	28 (73.7)	50 (62.5)	23 (67.6)	0.47
> 3 days	51 (33.6)	10 (26.3)	30 (37.5)	11 (32.4)	

*** p-value < 0.05*

Muscle strength among study participants was found as high 38(25%), average 50(52.6%) and low 34(22.4%) muscle strength while measured according to the percentile (Figure 1).

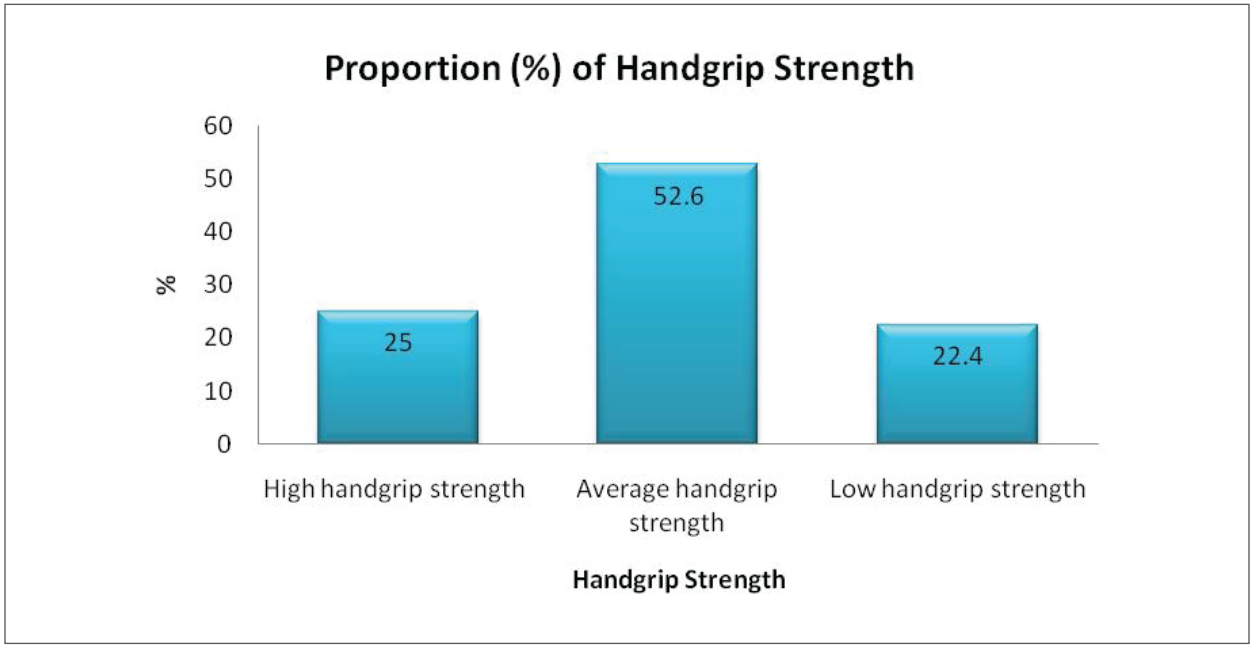


Figure 1: Proportion (%) of with handgrip strength among study participant's ≥18 years in urban Punjab, Pakistan.

Among the total sample, 26(76.5%) of females have low muscle strength compared to only 8(23.5%) of males and had significant results as the *p*-value <0.001). Similarly, 28(73.7%) of males have high muscle strength as compared to 10(26.3%) of females with significant results. The difference between muscle strength of non-diabetic and diabetic participants was statistically non-significant (*p*-value=0.17). Those who are not hypertensive have high muscle strength compared to those who have hypertension (76.3% vs. 23.7%). In this study participants with no tobacco use 25(65.8%) have high muscle strength compared to 13(34.2%) of tobacco users (<0.05) (Table 1).

Multinomial logistic regression analysis (Table 2) for factors associated with average and low muscle strength shows females are 7.9 times more likely to have low muscle strength as compared to males and the result is statistically significant (*p*-value <0.001). With reference to the analogue handgrip dynamometer (Figure 2), two significant aspects were presented which were showing an original position vs. handgrip strength measurement.

Table 2: Multinomial logistic regression analysis for factors associated with hang grip strength among study participant's ≥18 years.

Characteristics	Average muscle strength	*p*-Value	Low muscle strength	*p*-Value
	OR (95% CI)		OR (95% CI)	
Gender				
Male	1		1	
Female	6.4 (2.3-17.2)	0.00	7.9 (2.4-27.1)	0.00 **
Age			Age	
<40 years	1		1	
≥ 40 years	1.3 (0.4-3.8)	0.66	2.4 (0.6-9.7)	0.19
Hypertensive	1.5 (0.5-4.9)	0.38	1.1 (0.3-3.5)	0.91
Non-hypertensive	1		1	
Diabetic	1.1 (0.3-3.7)	0.89	3.1 (0.7-13.2)	0.11
Non-diabetic	1		1	
Physically Active	1		1	
Physically Inactive	1.5 (0.5-3.7)	0.38	1.4 (0.4-4.3)	0.51
Tobacco user	1.5 (0.4-4.9)	0.49	3.2 (0.5-19.1)	0.18
Tobacco Avoidance	1			

*** p-value < 0.05*

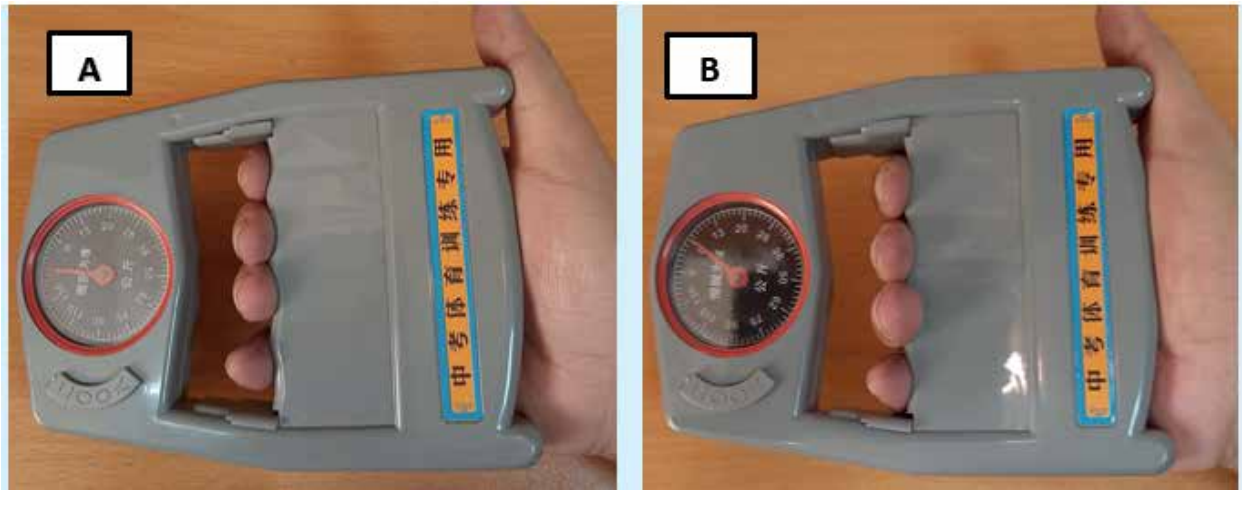


Figure 2: Analogue handgrip dynamometer (Image A: Original position; Image B: Measuring handgrip strength).

DISCUSSION

In this exploratory study, overall 80 adult participants have an average (52.6%) and low (22.4%) muscle strength and findings are comparable to those reported for Malaysian populations[22]. Similar findings were reported for European and American adults where weaker handgrip strength was found overall in the study population[23, 24].

Since previous studies have established a correlation between muscle strength and age where it highlighted that muscle strength increases till the age of 38 years and then decreases steadily after-

wards[22]. In our study, odds ratio of low handgrip strength is high (OR: 2.5) among participants age 40 years and above. However, the association could not be significantly established which might be due to the small sample size. A study showed a pooled data from six cohort studies reporting low handgrip strength as a potential risk factor for all-cause mortality among older age population [25].

Concerning gender, our study is showing significant association and reporting higher odds of low handgrip strength among females compared to males (OR: 6.7). These results are similar to several other previous findings. However, another study has demonstrated a comparative finding among Malaysian adults where male participants had consistently stronger handgrip measurement with an odds ratio of 1.75 times higher compared to females across all age group[22]. Factors underlying the difference in handgrip strength among genders were body mass index and waist circumference, which mediate the effect of handgrip strength. These factors may play role in developing cardiovascular diseases among males and females, which was evaluated in a study conducted among Korean adult population[26].

Abundant evidence suggests that low handgrip strength is significantly linked with a high risk of cardiovascular diseases (CVD) and its related risk factors such as diabetes, hypertension, dyslipidemia and obesity [26,27]. In our study, low strength is higher among those participants who had hypertension (OR: 1.1), diabetes (OR: 2.7) and tobacco users (OR: 3.4). In accordance with our study findings, there are other surveys conducted among Korean and Hispanic population, which is also showing that low handgrip strength is significantly related to cardiovascular risk factors and cardiovascular fitness respectively, among adults [4,26,28]. However, studies have also reported that low handgrip strength is not a predictor of cardiovascular risk[29]. Therefore, the contrary findings among different studies appeal for further large-scale community-based research with a robust methodology.

Physical activity increases muscular functional capacity and cardio-respiratory fitness (CRF) and the latter one is an established predictor for cardiovascular disease morbidity in the future[30]. Similarly, in our study multinomial regression result is showing that odds of low strength is higher (OR: 1.5) among participants with no physical activity. Other robust cohort studies reporting similar findings that low physical fitness is significantly associated with low muscular strength[21,30].

The strength of the study includes the participants were selected from a community, which is challenging in terms of refusals. The measurement of handgrip strength with respect to percentile is representing the meaningful result of the outcome and can be used further for applying certain interventions in a specific community. However, there are some limitations of participants selection in the study were at-risk population; hence, the results cannot be generalized to a healthy population. It is an exploratory study among people who attended the health camp and having co-morbidities so findings cannot be generalized to the whole population and cannot be conclusive. However, the findings can generate only the hypothesis, which can be explored further in future studies. Up to the best of our knowledge, this study is novel for assessing muscle strength in a community-based setting in Punjab

CONCLUSION

The participants are having either average and low muscle strength and regression analysis showed that females are at high risk of having low muscle strength. Therefore, it can be suggested that the handgrip dynamometer, an economic tool, used as a proxy measurement of upper extremity muscle strengthen a community based clinical settings. It is easy to measure and a low-cost technique for risk-scoring and risk-prediction of cardiovascular disease and cerebrovascular disease such as stroke, as evident by literature, and thus to identify high-risk people beforehand.

ACKNOWLEDGMENTS

All authors acknowledge Department of Community Medicine, Department of Marketing and Administrative of Shalamar Medical and Dental College for the smooth rolling of this survey.

CONFLICT OF INTEREST

The authors have no conflicts of interest.

ETHICS APPROVAL

Institution Review Board (IRB) of Shalamar Medical and Dental College, Pakistan approved the study "Prevention of coronary artery diseases in urban slums of Lahore" with reference # SMDC/IRB/11-12/140.

PATIENT CONSENT

Informed verbal and written consent were obtained from participants.

FUNDING

This study was funded through the Shahid Hussain Foundation only for the data collection process and campaign purpose. Funding source did not play any role in the design, reporting and publication of this study.

AUTHORS' CONTRIBUTION

TK conceived the idea and supervised the manuscript writing. SR performed statistical analysis and wrote the initial complete manuscript. AK, AS and JAN did the literature search and contributed in data collection. TK and SS critically reviewed whole manuscript and every author has approved the final verdict of the manuscript.

REFERENCES

1. Jang SK, Kim JH, Lee Y. Effect of relative handgrip strength on cardiovascular disease among Korean adults aged 45 years and older: Results from the Korean Longitudinal Study of Aging (2006–2016). Arch Gerontol Geriatr. 2020;86:103937.
2. Ji C, Zheng L, Zhang R, Wu Q, Zhao Y. Handgrip strength is positively related to blood pressure and hypertension risk: results from the National Health and nutrition examination survey. Lipids Health Dis. 2018;17(1):1-7.
3. World Health Organization. Technical package for cardiovascular disease management in primary health care. Geneva, Switzerland. 2016 [Internet]. World Health Organization, 2016 [cited 2020 Jun 16]. Available from: https://www.who.int/cardiovascular_diseases/hearts/Hearts_package.pdf
4. Artero EG, Lee DC, Lavie CJ, España-Romero V, Sui X, Church TS, *et al* Effects of muscular strength on cardiovascular risk factors and prognosis. J Cardiopulm Rehabil Prev. 2012; 32(6): 351-358.
5. Cooper R, Kuh D, Cooper C, Gale CR, Lawlor DA, Matthews F, *et al*. Objective measures of physical capability and subsequent health: a systematic review. Age Ageing. 2010;40(1):14-23.
6. Fahs CA, Heffernan KS, Ranadive S, Jae SY, Fernhall B. Muscular strength is inversely associated with aortic stiffness in young men. Med Sci Sports Exerc. 2010;42(9):1619-1624.
7. Leong DP, Teo KK, Rangarajan S, Lopez-Jaramillo P, Avezum Jr A, Orlandini A, *et al*. Prognostic value of grip strength: findings from the Prospective Urban Rural Epidemiology (PURE) study. Lancet. 2015;386(9990):266-273.
8. Wu Y, Wang W, Liu T, Zhang D. Association of grip strength with risk of all-cause mortality, cardiovascular diseases, and cancer in community-dwelling populations: a meta-analysis of prospective cohort studies. J Am Med Dir Assoc. 2017;18(6):551.e17-e35.
9. Celis-Morales CA, Welsh P, Lyall DM, Steell L, Petermann F, Anderson J, *et al*. Associations of grip strength with cardiovascular, respiratory, and cancer outcomes and all cause mortality: prospective cohort study of half a million UK Biobank participants. BMJ. 2018 8;361:1-10.
10. Willems SM, Wright DJ, Day FR, Trajanoska K, Joshi PK, Morris JA, *et al*. Large-scale GWAS identifies multiple loci for hand grip strength providing biological insights into muscular fitness. Nat Commun. 2017;8(1):1-2.
11. Springstroh KA, Gal NJ, Ford AL, Whiting SJ, Dahl WJ. Evaluation of handgrip strength and nutritional risk of congregate nutrition program participants in Florida. J Nutr Gerontol Geriatr. 2016;35(3):193-208.
12. Granic A, Jagger C, Davies K, Adamson A, Kirkwood T, Hill TR, *et al*. Effect of dietary patterns on muscle strength and physical performance in the very old: findings from the newcastle 85+ study. PLoS One. 2016;11(3):e0149699.
13. Ueda P, Woodward M, Lu Y, Hajifathalian K, Al-Wotayan R, Aguilar-Salinas CA, *et al*. Laboratory-based and office-based risk scores and charts to predict 10-year risk of cardiovascular disease in 182 countries: a pooled analysis of prospective cohorts and health surveys. Lancet Diabetes Endocrinol. 2017;5(3):196-213.
14. Murray CJ, Vos T, Lozano R, Naghavi M, Flaxman AD, Michaud C, *et al*. Disability-adjusted life years (DALYs) for 291 diseases and injuries in 21 regions, 1990–2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet. 2012;380 (9859):2197-223.
15. Jafar TH, Jafary FH, Jessani S, Chaturvedi N. Heart disease epidemic in Pakistan: women and men at equal risk. Am Heart J. 2005;150(2):221-226.
16. Khealani BA, Hameed B, Mapari UU. Stroke in Pakistan. J Pak Med Assoc. 2008;58(7):400-403.
17. Stenholm S, Tiainen K, Rantanen T, Sainio P, Heliövaara M, Impivaara O, *et al*. Long-term determinants of muscle strength decline: prospective evidence from the 22-year mini-Finland follow-up survey. J Am Geriatr Soc. 2012;60(1):77-85.
18. Jawaria S. Evaluation of pakistani adult handgrip strength reference values through dynamometer. J Pak Orthop Assoc. 2017;29(04):139-144.
19. Karthikbabu S, Chakrapani M. Hand-held dynamometer is a reliable tool to measure trunk muscle strength in chronic stroke. J Clin Diagn Res. 2017;11(9): YC09-YC12.
20. Roberts HC, Denison HJ, Martin HJ, Patel HP, Syddall H, Cooper C, *et al*. A review of the measurement of grip strength in clinical and epidemiological studies: towards a standardised approach. Age Ageing. 2011;40(4):423-429.
21. Timpka S, Petersson IF, Zhou C, Englund M. Muscle strength in adolescent men and risk of cardiovascular disease events and mortality in middle age: a prospective cohort study. BMC Med. 2014;12(1):62-69.
22. Kamarul T, Ahmad TS, Loh W. Hand grip strength in the adult Malaysian population. J Orthop Surg. 2006;14(2):172-177.
23. Petersen P, Petrick M, Connor H, Conklin D. Grip strength and hand dominance: challenging the 10% rule. Am J Occup Ther. 1989;43(7):444-447.
24. Crosby CA, Wehbé MA. Hand strength: normative values. J Hand Surg Am. 1994;19(4):665-670.
25. McLean RR, Shardell MD, Alley DE, Cawthon PM, Fragala MS, Harris TB, *et al*. Criteria for clinically relevant weakness and low lean mass and their longitudinal association with incident mobility impairment

and mortality: the foundation for the National Institutes of Health (FNIH) sarcopenia project. J Gerontol A Biol Sci Med Sci. 2014;69(5):576-583.
26. Lee MR, Jung SM, Kim HS, Kim YB. Association of muscle strength with cardiovascular risk in Korean adults: findings from the Korea National Health and Nutrition Examination Survey (KNHANES) VI to VII (2014–2016). Med. 2018;97(47):1-7.
27. Cheung C-L, Nguyen U-SD, Au E, Tan KC, Kung AW. Association of handgrip strength with chronic diseases and multimorbidity. Age. 2013;35(3):929-941.
28. Xu L, Hao YT. Effect of handgrip on coronary artery disease and myocardial infarction: a Mendelian randomization study. Sci Rep. 2017;7(1):1-5.
29. Kodama S, Saito K, Tanaka S, Maki M, Yachi Y, Asumi M, *et al*. Cardiorespiratory fitness as a quantitative predictor of all-cause mortality and cardiovascular events in healthy men and women: a meta-analysis. JAMA. 2009;301(19):2024-2035.
30. Yates T, Zaccardi F, Dhalwani NN, Davies MJ, Bakrania K, Celis-Morales CA, *et al*. Association of walking pace and handgrip strength with all-cause, cardiovascular, and cancer mortality: a UK Biobank observational study. Eur Heart J. 2017;38(43): 3232-3240.

ORIGINAL ARTICLE

Applicability of Two Non-Radiographic Mixed Dentition Analysis Methods in Orthodontic Patients

Hafsa Mahida, Sarwat Memon, Maham Khan, Farheen Naz
Department of Orthodontics, Ziauddin College of Dentistry, Karachi, Pakistan.

ABSTRACT

Background: Using erupted components of a dental arch to estimate the width of the unerupted dental components are the basis of mixed dentition analysis. Non-radiographic mixed dentition analysis employs a regression equation to assess the width of the unerupted canines and premolars. In this study, we assessed the applicability of two non-radiographic methods of mixed dentition analysis in orthodontic patients.

Methods: This cross-sectional research was carried out from the records of Ziauddin College of Dentistry, Department of Orthodontics, from November 2019 to March 2020. Pre-treatment dental casts of 120 subjects (60 males and 60 females) aged between 12-30 years undergoing orthodontic treatment were selected. The mesiodistal widths from the left first molar to the right first molar were measured using a digital Vernier caliper on pretreatment dental casts of both arches. Bachman's and Tanaka-Johnston methods were applied to estimate the widths of canine and premolars. Gender dimorphism for actual and estimated values was assessed using an independent t-test and a paired t-test was applied for the comparison between the actual and estimated mesiodistal widths of canine and premolar.

Results: The actual and estimated widths of canine and premolars reported 14.3±1.4 years for males and 13.4±1.2 years for females. In addition, the Bachman's and Tanaka-Johnston method overestimated the actual widths of unerupted canine and premolar but the difference was statistically insignificant ($p \geq 0.05$) in both the genders.

Conclusion: The two non-radiographic methods were reliable for mixed dentition analysis with minor overestimation between actual and estimated widths (ICC=0.79). This makes both the methods applicable interchangeably in regular clinical practice.

Keywords: Mixed Dentition; Unerupted Teeth; Dental Models; Radiography.

Corresponding Author:
Dr. Sarwat Memon
Orthodontic Department,
Ziauddin College of Dentistry,
Karachi, Pakistan.
Email: sarwat.memon@zu.edu.pk
https://doi.org/10.36283/PJMD10-1/010

INTRODUCTION

The mixed dentition period initiates, at approximately 6 years of age, once the first permanent molars or incisors erupt. Mixed dentition is a transitory stage demarcated from the time the first permanent tooth erupts and lasts until the last primary tooth is shed[1]. Mixed dentition, from the point of view of the orthodontist, is a crucial stage of occlusal development. Arch length and discrepancy of tooth size are two factors involved in eliciting problems in the mixed dentition phase[1-3]. Dental malocclusion can commonly occur during this phase. To address effectively, this problem, various prediction methods consisting of systems and formulas based on fixed algorithms have been devised. These are termed as mixed dentition analysis. Calculation of the space required and space available is carried

out so that treatment can be accordingly and teeth in the arches can be aligned well. Accurate prediction plays a key role in orthodontic treatment planning. The fundamental principles for mixed dentition analysis are that they should be easy to use, not time-consuming, uncomplicated, have a minimum margin for systemic error, can be carried out in both arches and can be directly carried out in the oral cavity as well[4-6]. These are the methods established to evaluate the mesiodistal widths of the canines and premolars and for this purpose, study casts are used.

The goal of carrying out mixed dentition analysis is to evaluate the amount of space present for the succeeding dentition in each of the dental arches. Once the prediction size of the unerupted permanent teeth is found, the most likely degree of crowding can be established[7]. Early diagnosis and interception of crowding undoubtedly aids in better treatment planning to tackle crowding and thereby the effects produced by it[8-10]. The severity of malocclusion in the future can be markedly decreased using timely interception. There are a variety of accepted methods, broadly divided into 3 categories - regression equations used, radiographs used and a combination of both[11-14].

The most commonly used prediction methods worldwide are Moyer's Prediction Tables and Tanaka-Johnston equations[2-4]. However, it has been established that these are not always accurate when used on populations of varying descent[7,15]. Prediction tables do not give accurate results unless they are made gender and race-specific. There is a dissimilitude in tooth sizes among different racial and ethnic groups, as well as a difference between genders; due to this, there can often be imprecise and faulty results when standardized and non-specific methods are used. It has also been concluded by recent studies, that mandibular incisors alone are not the most accurate predictors. For better accuracy, a sum of incisors and maxillary first molars should be used[16,17]. Moreover, the accuracy and reliability of Bachman's method for mixed dentition analysis was required to be assessed in our population. Therefore, this study aimed to assess the applicability of two non-radiographic methods of mixed dentition analysis in orthodontic patients.

METHODS

This cross-sectional research was done on pre-treatment dental casts of patients seeking treatment at Department of Orthodontics, Ziauddin University, Karachi, Pakistan for a period of 6 months beginning from November 2019 to March 2020. Institutional acceptance was obtained preceding the initiation of the research.

The sample size was calculated n=96, which was augmented to 120 to add 20 percent attrition. The power of the study was kept 80% at a 95% confidence level with a margin of error of 5%. Pretreatment dental casts of patients aged between 12 to 30 years with fully erupted permanent teeth from the second central incisor until the second molar were included in the study. Dental casts of patients with a former history of orthodontic treatment, missing teeth, carious and restored teeth at the measurement landmark (mesiodistally and vestibule-orally), hypoplastic, worn, or with anomalies, were excluded. The principal investigator measured all the study models using a digital Vernier caliper (0-150 mm ME 00183, Dentaurum, Pforzheim, Germany) with an accuracy of ±0.02 mm and repeatability of ±0.01 mm (manufacturer specification). The mesiodistal diameter (MD) of teeth from the right first molar to the left first molar of both maxillary and mandibular arches was measured. The primary investigator randomly picked up thirty dental casts after two weeks and the mesiodistal diameter of teeth was re-measured. Intraclass correlation (ICC) was applied to calculate the intra-examiner reliability for the measurements for mesiodistal widths of canines and premolars.

Tanaka-Johnston and Bachmann's prediction equations (Figures 1 and 2) were used in this research to estimate the mesiodistal widths of the canine and premolars in both arches.

Figure 1: Tanaka and Johnston method for mixed dentition analysis.

Tanaka and Johnston prediction equation:
Maxillary arch = Mesiodistal width of four lower incisors/2 + 11.0mm
Mandibular arch = Mesiodistal width of four lower incisors/2 + 10.5mm

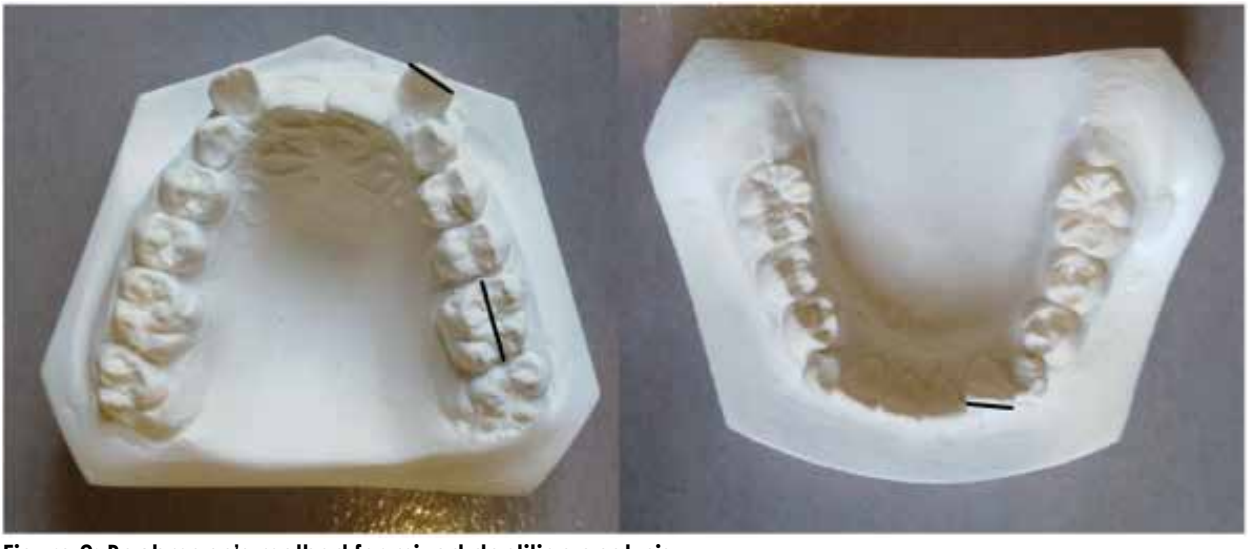


Figure 2: Bachmann's method for mixed dentition analysis.

Bachmann's prediction equation:
Maxillary arch = 0.81 × (22MD) + 0.54 × (26MD) + 0.56 × (32MDD) +6.98
Mandibular arch = 0.71 × (22MD) + 0.39 × (26MD) + 0.86 × (32MDD) + 6.96

The 22 and 26 represent MD (mesiodistal) width of the crowns of the upper and lower lateral incisors, 32 MDD (mesiodistal diameter) of the crown of the left upper first permanent molar. The data was evaluated through SPSS version 21. A paired sample t-test was applied to compare the actual and estimated sum of the mesiodistal widths of canine and premolars for both prediction methods. An independent sample t-test was conducted to compare means of actual and estimated sums of canine and premolars widths in both genders and *p*-value ≤0.05 was considered statistically significant.

RESULTS

The study sample comprised of 120 dental casts of 60 males (14.3±1.4 years) and 60 females 13.4±1.2 years). A gender dimorphism for actual and estimated widths of canine and premolars is shown in Table 1. Although gender variances were visible in the actual and estimated values of canine and premolars in both arches, however, gender variances were statistically insignificant (*p* ≥0.05).

Table 1: Actual and estimated combined widths of canine and premolars in both genders.

Prediction Method (Mean±SD)		Male	Female	*p*-Value
Permanent canine and premolars in maxillary arch		21.80±1.35	22.37±0.96	0.38
Permanent canine and premolars in mandibular arch		20.60±1.51	20.81±1.73	0.82
Tanaka and Johnston	Maxillary arch	21.97 ± 1.29	21.06 ± 1.10	0.50
	Mandibular arch	21.09 ±2.42	20.56 ± 2.25	0.43
Bachmann's Method	Maxillary arch	21.41±0.61	21.2±0.49	0.97
	Mandibular arch	21.05±0.69	21.2 2±0.56	0.63

** p≤ 0.05 as statistically significant; Independent sample t-test.*

The actual and estimated sum of canine and premolars constructed on the methods of Tanaka and Johnston and Bachmann were assessed using paired t-test as depicted in Table 2. Both prediction methods overestimated the actual sum of canine and premolars however the difference was statistically insignificant in both genders. Good intraclass correlation was found between the two sets of measurements (ICC=0.79).

Table 2: Actual and estimated values based on both non-radiographic methods in both genders.

Prediction Method		Gender	Actual Mean±SD	Estimated Mean±SD	Difference Mean±SD	*p*-Value
Tanaka and Johnston	Maxillary arch	Male	21.44±1.54	20.35±1.29	1.09±0.25	0.55
		Female	20.71±0.78	20.96±1.08	-0.25±0.23	0.69
	Mandibular arch	Male	21.09 ± 2.42	20.35±1.29	0.73 ± 2.38	0.43
		Female	20.56 ± 2.25	20.96 ± 5.06	0.39 ± 5.23	0.36
Bachmann's Method	Maxillary arch	Male	21.80±1.35	21.41±0.61	0.39±1.05	0.46
		Female	21.00±1.66	21.42±0.49	-0.42±1.40	0.42
	Mandibular arch	Male	20.60±1.51	21.06±1.05	-0.46±0.89	0.32
		Female	20.18±1.7	21.22±0.56	-0.40±1.42	0.44

*n= 120; * p ≤ 0.05 as statistically significant; Paired sample t- test*

DISCUSSION

Bachmann's Mixed Dentition analysis method employs a regression equation to measure the combined width of the canines and premolars. Kondapaka et al. conducted a study where they compared seven methods of analysis to find the most reliable method. Bachmann's method proved to have an average correlation, which did make it reliable but not the most reliable[16]. Legović et al. compared different mixed dentition analysis methods for predicting the size of unerupted canines and premolars[17]. They found statistically significant differences between mesio-distal and buccolingual measurements justifying the use of both of these dimensions. Bachman Analysis is one such analysis, which makes use of both of these. Amongst the methods that use regression equations, are Bachmann, Gross and Hasund and Tränkmann et al. According to a study by Legović et al, Bachmann's method was the most reliable and significant, therefore in the maxilla for females, and both maxillary and mandibular arches for males observed in this study, had no significance between methods or any statistically significant difference between the genders[17].

In the present study, Bachmann's Method overestimated the sizes from the actual. This may be due to racial and ethnic variation since the method was originally used in children of northwestern European descent. According to Galvão et al. who studied methods of mixed dentition analysis, it is not clinically problematic if a method of analysis overestimates because it leaves some space available; however, it is a problem if the analysis underestimates from the real, as compensating space, in this case, is difficult[18]. In the present study, the difference between the values predicted by Bachman's method and the real values was found to be statistically insignificant. This makes the method applicable to our routine practice.

Tanaka-Johnston Method was created on drawing parallels between size of the teeth and the arch. It is more widely used for people of Northern European descent. The method is based on using simple linear regression equations and the indices used here are the mandibular permanent incisor teeth. The concern shared by most authors who studied the method is that due to its greater use in the North European population, the method's reliability for analysis in other ethnicities could be dubious. In a study by Handayani and Hidayah[19], who assessed the applicability of the method in an Arab population, showed a statistically insignificant difference between the actual values and those predicted by this method. This does not concur with the findings of Lee-Chan et al. in an Asian-American population, who found Tanaka Johnston to overestimate the size of smaller unerupted canines and premolars and underestimated larger canines and premolars. Hence, the method was not found to predict accurately and a reason could be racial and ethnic differences[20].

Another concern has been the method's ability to predict reliable values in all genders. According to Vilella et al. in the Brazilian population though, the method was generally reliable to predict widths in groups of both black and white descent. There was a great disparity in its applicability between the genders where it did not show an acceptable prediction in white women[21].

The present study, which was applied over a Pakistani population and the findings for the Tanaka-Johnston Method do overestimate the sizes from the actual but these are statistically insignificant. There was also a statistically insignificant disparity of the findings between genders. This is not in agreement with the study of Goyal et al. regarding the applicability of Tanaka-Johnston methods. Goyal et al. found that the method significantly overestimates the sizes in a North-Indian population[22], which is to an extent ethnically similar to the Pakistani population. This concurs with a study by Giri et al. where they studied the two methods in a Nepalese mongoloid population[23]. The Nepalese mongoloid ethnicity population exists within the South Asian Association for Regional Cooperation (SAARC) region and is influenced by the Asian race. The findings for this ethnicity concur with the findings for similar Asian ethnicities of other nationalities (such as Asian American) where Tanaka-Johnston method has not proved to be applicable either[20]. The study by Giri et al. is quite important since it discusses a comparison of the general Nepalese population with the Mongoloid ethnicity, which is one-fifth of their population[23].

According to a study in the Nepalese population, the method was found to be applicable in their population[24,25]. Akhtar et al. applied Tanaka-Johnston mixed dentition equation in orthodontic patients presenting to the Armed Forces Institute of Dentistry. They reported that this method overestimated the sized of canine and premolars and the difference was statistically significant in their studied sample[25]. These findings and discussion of the afore-mentioned studies bring to light two areas of potential further research. Firstly, that there could be similarities amongst similar races in different nationalities. Secondly, in multiethnic countries such as Pakistan, India etc., there are strong ethnic variations amongst populations, e.g., Pukhtoons, Balochs, etc. These variations might lead to significant differences in results obtained for the generalized population of the country, which does not take into account ethnic variations[23]. A limitation of this study could be the fact that it was conducted at a single center. Further studies on the applicability of Tanaka-Johnston and Bachman's Method in a Pakistani population, could be multi-centered. Future studies could explore the individual differences in applicability for different ethnicities within the Pakistani population.

CONCLUSION

The Bachman's and the Tanaka and Johnston Method are reliable for analyzing the mixed dentition in a Pakistani population with minor, statistically insignificant differences between actual and predict values, and those between the genders. This makes both the methods applicable to interchangeably in regular clinical practice.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the fellow faculty members and dental assistants at Orthodontic Department, Ziauddin University for their valuable contribution.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICS APPROVAL

The research approval was taken as per departmental protocol before starting the orthodontic patient study.

AUTHORS' CONTRIBUTION

SM conceived the idea, analyzed the patient data and reviewed the manuscript, HM and FN had major contributions in writing of the manuscript and MK collected the data.

REFERENCES

1. Keerthika A, Jeevarathan J, Ponnudurai Arangannal VM, Amudha S, Aarthi J. Mixed dentition analysis procedure: A review. Indian J Public Health Res Dev. 2019;10(12):1131-1136.
2. Thimmegowda U, Divyashree, Niwlikar KB, Khare V, Prabhakar AC. Applicability of Tanaka Jhonston Method and prediction of mesiodistal width of canines and premolars in children. J Clin Diagn Res. 2017;11:16-19.
3. Hashim HA, Al-Hussain HA, Hashim MH. Prediction of the size of unerupted permanent canines and premolars in a Qatari sample. Int J Orthod Rehabil. 2019;10:10-17.
4. Grover N, Saha S, Tripathi AM, Jaiswal JN, Palit M. Applicability of different mixed dentition analysis in Lucknow population. J Indian Soc Pedod Prev Dent. 2017;35:68-74.
5. Baheti K, Babaji P, Ali MJ, Surana A, Mishra S, Srivastava M. Evaluation of Moyer's mixed dentition space analysis in Indian children. J Int Soc Prev Community Dent. 2016;6:453-458.
6. Pintér Z, Rill L, Vitályos G, Kolarovszki B, Frank D. Applicability of the Moyers mixed dentition analysis for the Hungarian population. Orvosi Hetilap. 2019;160(50):1984-1989.

7. Alzubir AA, Abass S, Ali MA. Mixed dentition space analysis in a Sudanese population. J Orthod. 2016; 43:33-38.
8. Sherpa J, Sah G, Rong Z, Wu L. Applicability of the Tanaka-Johnston and Moyers mixed dentition analyses in Northeast Han Chinese. J Orthod. 2015;42:95-102
9. Aljabaa AH, Alqahtani ND, Aldrees AM, Alkofide EA, Al-Sehaibany FS, Albarakati SF. Changes in the mandibular dental arch during the late mixed dentition stage: Experimental study. J Pak Med Assoc. 2019;69:77-81.
10. Al-Dlaigan YH, Alqahtani ND, Almoammar K, Al-Jewair T, Salamah FB, Alswilem M, Albarakati SF. Validity of Moyers mixed dentition analysis for Saudi population. Pak J Med Sci. 2015;31:1399-1404.
11. Kakkar A, Verma KG, Jusuja P, Juneja S, Arora N, Singh S. Applicability of Tanaka-Johnston, Moyers, and Bernabé and Flores-Mir mixed dentition analyses in school-going Children of Sri Ganganagar City, Rajasthan (India): A cross-sectional study. Contemp Clin Dent. 2019;10:410-416.
12. Ravinthar K, Gurunathan D. Applicability of different mixed dentition analyses among children aged 11-13 years in Chennai population. Int J Clin Pediatr Dent. 2020;13:163-166.
13. Bhatnagar A, Chaudhary S, Sinha AA, Manuja N, Kaur H, Chaitra TR. Comparative evaluation and applicability of three different regression equation-based mixed dentition analysis in Northern Uttar Pradesh population. J Indian Soc Pedod Prev Dent. 2018;36:26-32.
14. Cirulli N, Ballini A, Cantore S, Farronato D, Inchingolo F, Dipalma G, *et al*. Mixed dentition space analysis of a southern Italian population: new regression equations for unerupted teeth. J Biol Regul Homeost Agents. 2015;29:515-520.
15. Shobha MB, Ajs S, Manoj K, Srideevi E, Sridhar M, Pratap G. Applicability of two universally accepted mixed dentition analysis on a sample from south-eastern region of Andhra Pradesh, India. Ann Med Health Sci Res. 2016;6:176-180.
16. Kondapaka V, Sesham VM, Neela PK, Mamillapalli PK. A comparison of seven mixed dentition analysis methods and to evaluate the most reliable one in Nalgonda population. J Ind Orthod Soc. 2015;49:3-9.
17. Legović M, Novosel A, Škrinjarić T, Legović A, Mady B, Ivančić N. A comparison of methods for predicting the size of unerupted permanent canines and premolars. The Eur J Orthod. 2006;28:485-490.
18. Galvão MD, Dominguez GC, Tormin ST, Akamine A, Tortamano A, Fantini SM. Applicability of Moyers analysis in mixed dentition: A systematic review. Dental Press J. Orthod. 2013;18:100-105.
19. Handayani FT, Hidayah RA. Applicability of Moyers and Tanaka-Johnston analyses for the Arab population of Pekalongan, Indonesia. Dent J (Majalah Kedokteran Gigi). 2019 30;52:154-158.
20. Lee-Chan S, Jacobson BN, Chwa KH, Jacobson RS. Mixed dentition analysis for Asian-Americans. Am J Orthod Dentofacial Orthop. 1998;113:293-299.
21. Vilella OD, Asuncion PS, Asuncion RL. The Tanaka-Johnston orthodontic analysis for Brazilian individuals. Odonto Sci Mag. 2012; 27(1):16-19.
22. Goyal RK, Sharma VP, Tandon P, Nagar A, Singh GP. Evaluation of mixed dentition analyses in north Indian population: A comparative study. Contemp Clin Dent. 2014;5: 471-477.
23. Giri J, Pokharel PR, Gyawali R, Timsina J, Pokhrel K. New regression equations for mixed dentition space analysis in Nepalese mongoloids. BMC Oral Health. 2018;18(1):214-220.
24. Jaiswal AK, Paudel KR, Shrestha SL, Jaiswal S. Prediction of space available for unerupted permanent canine and premolars in a Nepalese population. J Orthod. 2009;36:253-259.
25. Akhtar F, Tariq S, Jan A, Amin E, Bangash A, Khan M. Comparison of predictive accuracy of Tanaka-Johnston analysis, Melgaco formula and Bherwani's regression equation for patients presenting to armed forces institute of dentistry. Pak Armed Forces Med J. 2020;70:962-966.

ORIGINAL ARTICLE

Isolation and Identification of Microbes on Hands and Mobile Phones Causing Urinary Tract Infections

Anum Liaquat Ali, Umrah Imran, Mehwish Sajjad, Hhira Waseem, Tanzela Khan, Sikandar Ali, Sidra Afzal, Afsheen Khan, Tahira, Bushra Imdad

Department of Clinical Laboratory Sciences (CLS), Dow Institute of medical Technology (DIMT), Dow University of Health Sciences (DUHS), Karachi, Pakistan.

ABSTRACT

Background: Hands and cell phones are the major source of cross-transmission of urinary tract infections. The aim of this study was to isolate, identify and evaluate Gram-negative bacteria from hand and mobile phones.

Methods: This study was conducted in visiting area of Civil Hospital Karachi, Pakistan. Analysis was done by 100 wet sterile cotton tipped swabs, 50 each from mobile phones and hands of their owners. Samples were transported in a Cary Blair transport media, Swabs were streaked on Nutrient agar, Blood agar and MacConkey agar. Organisms were identified by cultural, biochemical, and microscopic characteristics.

Results: Total samples n=100 was collected from hand and mobile phones samples (50 hand and 50 mobile swabs) from the Dow university Hospital and Civil Hospital Karachi were tested. Six species of bacteria were isolated along with their identification during the research study. The isolated bacteria were Serratia, Klebsiella, Pseudomonas, Proteus, Shigella and *Escherichia coli*. The participants' hands showed high bacterial contamination (50%-56%) in comparison to mobile phones. The frequency (%) of bacteria isolated from mobile phone and hand swabs included Serratia, 12 (24%) with the highest quantity and frequently found bacteria. While, the rest of the results reported *Escherichia coli* 10 (20%), Klebsiella 9 (18%), Pseudomonas 5 (10%), Shigella 4 (8%) and Proteus 10 (20%) respectively.

Conclusion: Patient attendants in hospitals and visitors are more susceptible to nosocomial infections through exchange of mobile phones n=12(24%). Therefore, hygienic practice of hands cleaning while mobile using may help to break the transmission cycle of pathogenic bacteria.

Keywords: Urinary Tract Infections; Gram Negative Bacteria; Nosocomial Infections.

Corresponding Author:
Dr. Anum Liaquat Ali
Department of Clinical Laboratory Sciences (CLS),
Dow Institute of medical Technology (DIMT),
Dow University of Health Sciences (DUHS), Karachi, Pakistan.
Email: Anum.liaquat@duhs.edu.pk
https://doi.org/10.36283/PJMD10-1/011

INTRODUCTION

It is an indisputable fact that infectious diseases are increasing day-by-day. In poor countries, peoples of over 25% suffer from nosocomial infections. On the other hand, around 100,000 fatalities occur in U.S. hospitals, as they are responsible for 1.7 million infections. It is certainly true that standard infection control instructions would be useful against one-third of such infections[1,2]. Urinary Tract Infections (UTIs) identification by bacterial test susceptibility, is significant for selecting suitable antimicrobial agent distressing bacterial diseases[3].

The cellular appliance has become one of the most crucial supplements of executive and communal life undoubtedly; it has made a tremendous impact on our lives in this rapidly changing world. It is used

as a fundamental source of communication by affluent societies low-income earners[4]. Mobile phone regularly became new and more complicated in daily life, together with classified and related work capacity With the high rang of mobile phone[5].

Gram-positive bacteria which are related to individuals produced through organisms Gram-negative and generally include urinary frequency, or suprapubic pain, dysuria, urinary urgency[6,7]. The increase use of cephalosporin is responsible for the production highly resistance of Extended-spectrum beta-lactamases (ESBL) microorganisms[8]. These organisms are ubiquitous and find their way into the phones through the skin. This is an inevitability that some bacteria are part of the normal flora of the skin[10]. All microbiologists state that grouping of continuous handling by the phones creates a prime breeding ground for many microorganisms[11].

Hands and mobiles are thought to be a great source of infection that is why they were chosen for the collection of samples. Civil hospital is in the working area of city; the roads around it are always busy with traffic. As people keep their cell phones with them, their cell phones get exposure to the polluted environment. In addition, it is among the largest and economical hospitals of Karachi, for this reason it is preferred by a vast majority of people. Mostly socioeconomic people come here as they are served at a very low cost. However, unfortunately, sanitary rules are not applied here. The lack of availability of cleaning guidelines led to nosocomial infections simply. Hands act as a route of transmission of several infections as UTI. Many people show idleness in washing hands. A very few people are aware of the sanitary practices. Even medical staff show carelessness and treat patients with contaminated hands. As patients are already immuno-compromised, they are more prone to get infection easily[12-14].

The Gram-negative rods isolated from hands and mobile phones, are all responsible for urinary tract infections. Unluckily, in a country like Pakistan, personal hygienic rules are not followed. A common example is hastiness in washing hands after using washroom. The organisms like (*E. coli*, Klebsiella, Serratia, Proteus, Pseudomonas and Shigella) are transmitted from hands to mobiles and vice versa[15-17]. They are responsible for many infectious diseases like UTI (urinary tract infection)[17]. Their presence in samples might be due to the mobile phones are used routinely all day long but not cleaned properly. People may do not wash their hands as often as they should. This study aimed to evaluate the role of mobile phones and hands in the transmission of bacteria.

METHODS

The study was conducted at Dow University of Health Sciences, Karachi. The samples were collected from Dow University of health sciences Karachi and Civil hospital Karachi. Informed consent was taken from the participant included in the study. The research was conducted after approval from the ethics review committee of the Institution of biological, biochemical, and pharmaceutical sciences (IBBPS) (Ref. No. IBBPS/UL/R&DM-003). The samples were collected aseptically with wet sterile cotton swab sticks dipped in Cary Blair transport media.

Total samples 100 were collected from hand and mobile phones, 50 samples collected from the Dow University Hospital (25 hands and 25 mobile) and 50 samples collected from Civil Hospital Karachi (25 hands and 25 mobile). Each swab was streaked on nutrient, blood, and MacConkey agar. The plates were incubated at 37°C for 48 hours and observed for colonial characteristics. Bacteria were identified by their morphological and biochemical characteristics[18]. Gram staining was done for further recognition[19] .

Biochemical reactions were done to verify each bacterial isolate by doing Triple Sugar Iron TSI, Citrate and Oxidase for the identification of Gram-negative bacteria[19]. A total of 25 samples were collected from cell phones, out of which 3 samples were positive. In this research, total samples1oo from hands 50 samples and cell phones 50 samples were collected from persons having age between 28 to 43 all result were analysis SPSS version 6 and standard deviation rang 16.67±9.266.

RESULTS

Total samples 100 were collected from hand and mobile phones, from which six species of bacteria: Serratia, Klebsiella, Pseudomonas, Proteus, Shigella, and *Escherichia coli*. were isolated. The participants' hands showed high bacterial contamination other than mobile phones. Thirty-eight were males and 12 were females. The frequency of bacteria isolated from mobile phone and hand swabs from both hospitals are shown in Table 1.

Table 1: Univariate analysis of factors associated with hand and mobile contamination between Dow University Hospital and Ruth Pfau Civil Hospital, Karachi.

Parameters	Hands		*p*-Value	Mobiles		*p*-Value	Total
	Positive	Negative		Positive	Negative		
Male	15(39%)	5(13%)	**1.000**	15(39%)	3(7.8%)	**1.000**	38
Female	6(50%)	1(8.3%)		4(33.3%)	1(8.3%)		12
DUH	25(50%)	5(10%)	**1.000**	15(30%)	5(10%)	**0.416**	50
CHK	28(56%)	5(10%)		15(30%)	2(4%)		50

Serratia, 12(24%) was the most frequent bacteria isolated (Table 2) followed by *Escherichia coli* 10 (20%), Klebsiella 9(18%), Pseudomonas 5(10%), Shigella 4(8%), Proteus 10(20%).

Table 2: Bacterial species isolated from the hand and mobile phone from Dow University Hospital and Civil Hospital.

Bacteria	Dow University Hospital		Civil Hospital		Frequency Percentage n (%)
	Hand	Mobile	Hand	Mobile	
Serratia	5	1	5	1	12(24)
E. coli	5	1	4	0	10 (20)
Klebsiella	4	1	3	1	9(18)
Pseudomonas	2	1	2	0	5(10)
Proteus	6	1	3	0	4(8)
Shigella	2	0	2	0	10(20)

E. coli was detected in hands among nine individuals and cell phones of one participant having urine infection shown in Figure 1a. Only one had *E. coli* in his cell phone. Serratia was detected in hands among ten and phones of two people had a history of urine infection. Hands of two samples were contaminated with Serratia, however their cellular device showed no bacterial contamination. Shigella was isolated only in hands of 10 participants having diarrhea. Hands of seven participants and cell phones of two respondents with urine infection showed colony of Klebsiella while only cell phone of one participant attendant had a growth of Klebsiella reported in this research study. Hands and cell phones of females with vaginal infection showed growth of Proteus among ten as shown in Figure 1b and Pseudomonas among five participants reported in results.

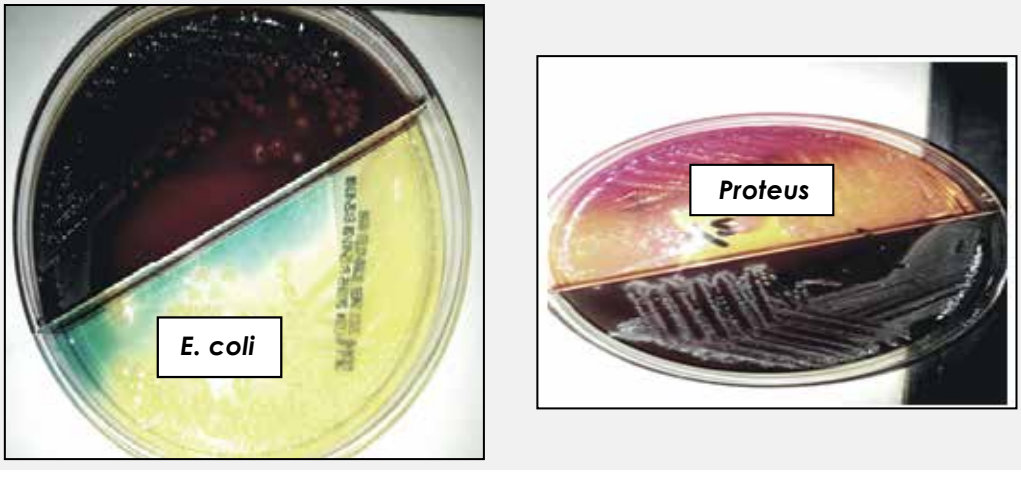


Figure 1a: Isolation of *E. coli* in Agar Plate. 1b: Proteus species isolated in Agar Plate.

DISCUSSION

In this current study total 6 different species of bacteria were isolated which were from the DUH and CHK isolated samples of mobile phones and hands. Therefore, total mobile phone percentage was 4% and hands percentage was about 20%. According to past literature, numerous species of bacteria are considered of pathogens in human, as Serratia, *E. coli*, Klebsiella, Shigella, Proteus, and Pseudomonas. These bacterial species can cause severe diseases including the urinary tract infections. Nosocomial infections continue to pose risks of increased mortality and morbidity rate in patients. The hands of healthcare workers (HCWs) play an important role in transmission of infections[8]. The mobile phones of HCWs have seen many harmful pathogens, which act as a pool for nosocomial infections[20,21]. Thus, the etiological agents of nosocomial infections have found a significant way to spread in our hospitals. Mobile phones appear in direct touch with the hands, face, and act as a prepared site to inhabit various microbes[21].

Klebsiella, a Gram-negative bacteria, member of family Enterobacteriaceae, is a normal flora of human mouth, skin and intestine[22]. Therefore, this study related results also showed this bacterium which was present in hands even more than the mobile phones. Since, people touch their body parts and then use their mobile phones with contaminated hands. For that reason, our study also showed gram negative bacteria. To the best of our knowledge, *E. coli* is another Gram-negative bacterium, which is a part of normal flora of lower intestine. It causes diseases like diarrhea, abdominal cramping, and nausea. It reaches our food through contaminated hand as its route is fecal-oral[23,24]. This research also showed *E. coli* which is more commonly present in hands than on the mobile phones.

Serratia is another Gram-negative rod-shaped bacterium from a family Enterobacteriaceae and the most common specie in this genus is *S. marcescens*. It causes nosocomial infections as it has a propensity to populate the respiratory, urinary tracts, and causes infection of eyes, blood, and wounds. It can transfer by contaminated or infectious people, medical devices, and direct contact to mucous membrane[25,26].

Serratia is also found in plants and animals. As it is very common, here that people pluck flower and leaves, touch domestic animals, and do not wash their hands. Therefore, in this way Serratia causes contamination of hands. Serratia infections are related to hospitalization, likewise, invasive procedure such as intravenous catheterization, respiratory intubation and urinary tract manipulation also contributes in these aspects[27]. This research also goes in the favor of our research study since, Serratia also present more common in hands than on mobiles phones which has confirmed by our research as well.

Pseudomonas causes urinary tract infections and our results also reported the presence of this bacterial infection. It can cause pneumonia, diarrhea, enterocolitis, enteritis, meningitis etc. It contaminates respiratory therapy and anesthesia equipment, intravenous fluids and even distilled water. It also has a capability to survive in disinfectants, as it grows in hexa-chloroform containing soap solution, in antiseptics and in detergents[28]. Our study also supported its presence in hands more as compared to the cell phones. Its presence in wound site causes contamination of hands when a person itches that site. The four Fs, fingers, flies, food and feces by contaminated water and polluted environment normally transmit Shigella[28]. Our study has also revealed the presence of Shigella more in hand than on mobile phones. However, past research reported that Proteus species are the root of hospital-acquired infections, for instance, urinary tract, wounds and burns etc[29]. Within the context of the European region, Proteus species are the second most commonly isolated bacteria after the *E. coli* [29,30].

Hence, we are exposed to these types of organisms, which are very harmful for us. The most critical place for patients is hospital where numerous microbes show their presence. Microbiological standards in hygiene are obligatory for a healthy life, especially people present in hospitals, since visitors are very incautious about their hygiene. They do not wash their hands properly and then use their mobile phones with contaminated hands. Therefore, organisms are transferred from hands to mobiles and infections occur when that contaminated mobile is used. The major visitors should take preventive effort is hand washing, so the bacteria will not transfer from their hands to mobiles. Awareness programs should be started as well so that the people can realize how to care themselves while present in a hospital. Infections can be restricted by taking preventive steps. Thus, we can easily avoid spreading bacterial infection just by using regular cleansing agent and rearranging our environment.

CONCLUSION

Hands and mobile phones are vehicles of transmission of infectious diseases due to the nullifying of sanitary rules. Strict health hygiene should be practiced for prevention. Hands should be properly washed. Developing active preventive strategies like routine decontamination of mobile phones with alcohol containing disinfectant material might be effective in reducing cross infection. Another way to reduce bacterial contamination on mobile phones might be the use of antimicrobial additive materials.

ACKNOWLEDGEMENTS

We are immensely grateful to the patients and their guardians who allowed us to collect samples from their hands and mobile phones.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICS APPROVAL

The research was conducted after receiving approval from IBBPS (Institutional of biological, biochemical, and pharmaceutical sciences) (Ref. No. IBBPS/UL/R&DM-003).

PATIENT CONSENT

The authors obtained both written and verbal consent from the participants involved in this study.

FUNDING

The authors received funding for this research from Dow Institute of Medical Technology (Dow University of Health Sciences).

AUTHORS' CONTRIBUTION

This study was directed and supervised by NHA; ALA had a major role in collection, analysis, and interpretation of data. UI and the rest of the authors assisted in manuscript writing. All authors read and approved the final manuscript.

REFERENCES

1. Mayhall CG. Hospital epidemiology and infection control. Lippincott Williams & Wilkins; 2012.p.1-4.
2. Khan HA, Baig FK, Mehboob R. Nosocomial infections: Epidemiology, prevention, control and surveillance. Asian Pac J Trop Biomed. 2017;7(5):478-482.
3. Salih MK, Alrabadi NI, Thalij KM, Hussien AS. Isolation of path4ogenic gram-negative bacteria from urinary tract infected patients. Open J Med Microbiol. 2016;6(02):59-65.
4. Marchaim D, Kaye K. Infections and antimicrobial resistance in the intensive care unit: epidemiology and prevention 2019 [Internet]. Prieiga per internetą, 2019 [cited 2020 Sep 18]. Available from: https://www. uptodate. com/contents/infection-sand-antimicrobial-resistance-in-the-intensive-care-unit-epidemiology-and-prevention.
5. Mohammed TK, Jwad MA, Kamal O, Abbas AH, Alabbas AS. Isolation of some pathogenic bacteria and fungi from students mobile phones (Part I). Indian J Public Health Res Dev. 2019;10(10):2108-2112.
6. Kline KA, Lewis AL. Gram-positive uropathogens, polymicrobial urinary tract infection, and the emerging microbiota of the urinary tract. Urin Tract Infect Mol Pathogen Clin Manag. 2017:459-502.
7. Zowawi HM, Harris PN, Roberts MJ, Tambyah PA, Schembri MA, Pezzani MD, *et al*. The emerging threat of multidrug-resistant Gram-negative bacteria in urology. Nat Rev Urol. 2015;12(10): 570-584.
8. Sharma BK, Sharma K. Extended spectrum β-lactamase producers in mobile phones and nosocomial infections: Risk factors for infection control practices. Janapriya J Interdiscip Stud. 2019;8:60-74.
9. Acalovschi M, Badea R, Dumitrascu D, Varga C. Prevalence of gallstones in liver cirrhosis: A sonographic survey. Am J Gastroenterol. 1988;83(9): 954-956.
10. Esmaeili Z, Sadeghi J, Razavi S, Oshaghi M, Sayyahfar S, Rahbar M, *et al*. High level of biofilm formation and virulence factors in enterococci species isolated from clinical and normal flora samples. Infect Dis Clin Pract. 2018;26(1):27-30.
11. Kadhem H, Abed Ali A, Hassan O. Isolation and identification of bacteria isolated from different parts of cell phones. World J Exp Biosci. 2016;4(1):29-31.
12. Rathore MH, Jackson MA, Committee on Infectious Diseases. Infection prevention and control in pediatric ambulatory settings. Pediatr. 2017;140(5): 1-25.
13. Johnson CO, Nguyen M, Roth GA, Nichols E, Alam T, Abate D, *et al*. Global, regional, and national burden of stroke, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet Neurol. 2019;18(5):439-458.
14. Jenkins DR. Nosocomial infections and infection control. Med. 2017;45(10):629-633.
15. Aguado J, Silva J, Fernandez-Ruiz M, Cordero E, Fortún J, Gudiol C, *et al*. Management of multidrug resistant Gram-negative bacilli infections in solid organ transplant recipients: SET/GESITRA-SEIMC/REIPI recommendations. Transplant Rev. 2018;32(1):36-57.
16. Presterl E, Diab-El Schahawi M, Lusignani LS, Paula H, Reilly JS. Bacteriology: Selected bacteria and diseases. Basic microbiology and infection control for midwives: Springer; 2019. p.67-84.
17. Alatoom A, Elsayed H, Lawlor K, AbdelWareth L, El-Lababidi R, Cardona L, *et al*. Comparison of antimicrobial activity between ceftolozane–tazobactam and ceftazidime–avibactam against multidrug-resistant isolates of Escherichia coli, Klebsiella pneumoniae, and Pseudomonas aeruginosa. Int J Infect Dis. 2017;62:39-43.
18. Azizah RA. Physical characteristics and antibacterial activities of onion cream ethanol extract (Eleutherine palmifolia) (Against Staphylococcus aureus) [dissertation]. University of Muhammadiyah Malang); 2019. 80.
19. Cheesbrough M. District laboratory practice in tropical countries, part 2. Cambridge university press; 2006. p. 1-15.
20. Miller MA, Hyland M, Ofner-Agostini M, Gourdeau M, Ishak M. Morbidity, mortality, and healthcare burden of nosocomial Clostridium difficile-associated diarrhea in Canadian hospitals. Infect Control Hosp Epidemiol. 2002;23(3):137-140.

21. Mark D, Leonard C, Breen H, Graydon R, O'Gorman C, Kirk S. Mobile phones in clinical practice: reducing the risk of bacterial contamination. Int J Clin Pract. 2014;68(9):1060-1064.
22. Schulz-Stübner S, Kniehl E. Transmission of extended-spectrum β-lactamase Klebsiella oxytoca via the breathing circuit of a transport ventilator: root cause analysis and infection control recommendations. I Infect Control Hosp Epidemiol. 2011;32(8):828-829.
23. Al-Abdalall AH. Isolation and identification of microbes associated with mobile phones in Dammam in eastern Saudi Arabia. J Fam Med Community Med. 2010;17(1):11-14.
24. Nwankwo E, Ekwunife N, Mofolorunsho K. Nosocomial pathogens associated with the mobile phones of healthcare workers in a hospital in Anyigba, Kogi state, Nigeria. J Epidemiol Glob Health. 2014;4(2):135-140.
25. Rabier V, Bataillon S, Jolivet-Gougeon A, Chapplain JM, Beuchée A, Bétrémieux P. Hand washing soap as a source of neonatal Serratia marcescens outbreak. Acta Paediatrica. 2008;97(10):1381-1385.
26. Pitout JD, Sanders CC, Sanders Jr WE. Antimicrobial resistance with focus on β-lactam resistance in gram-negative bacilli. Am J Med. 1997;103(1):51-59.
27. Rusin P, Maxwell S, Gerba C. Comparative surface-to-hand and fingertip-to-mouth transfer efficiency of gram-positive bacteria, gram-negative bacteria, and phage. J Appl Microbiol. 2002; 93(4):585-592.
28. Brooks G, Carroll K, Butel J, Morse S, Mietzner T. Jawetz, Melnick, Adelberg Medical Microbiology, twenty-sixth American edition / first Croatian edition.
29. O'Hara CM, Brenner FW, Miller JM. Classification, identification, and clinical significance of Proteus, Providencia, and Morganella. Clin Microbiol Rev. 2000;13(4):534-546.
30. Liu P, Gur D, Hall LM, Livermore D. Survey of the prevalence of βlactamases amongst 1000 Gram-negative bacilli isolated consecutively at the Royal London Hospital. J Antimicrob Chemother. 1992;30 (4):429-447

REVIEW ARTICLE

Histomorphological Spectrum of Glomerulopathies: A Review

Anam Shaikh, Fouzia Lateef, Talat Mirza
Department of Histopathology, Ziauddin University and Hospital, Karachi, Pakistan.

ABSTRACT

Renal disease and dysfunction is a worldwide public health problem. The underlying pathology in most renal disease is glomerulopathy, largely referred to as glomerulonephritis. It can be primary or secondary to other diseases. A range of morphological patterns was observed in this condition, each with different etiopathogenetic mechanisms, diverse clinical presentation, disease progression and therapeutic responses. We searched the literature using Hinari, PubMed and Google Scholar, for appropriate studies. This review was conducted by employing specified methods and structures using histopathology-confirmed data during the year 2011 to 2020. Thirty-five studies consisting of 13,423 reported renal biopsy cases were covered in this review. The most common indication of the renal biopsy was nephrotic syndrome followed by proteinuria and nephritic syndrome. Focal segmental glomerulosclerosis, minimal change disease, and mesangio-capillary glomerulonephritis among others, were the most frequently reported primary patterns of glomerulopathies. Glomerular diseases remain poorly characterized due to the scarcity of data on histo-morphological patterns of glomerulopathies. The development of registries regarding renal biopsy may offer a chance to characterize the pervasiveness and patterns of glomerulopathies and have a positive impression on chronic/end stage renal disease analysis and treatment since most glomerular diseases are complaisant to treatment.

Keywords: Nephrotic Syndrome; Immunofluorescence; Biopsy; Glomerulonephritis.

Corresponding Author:
Dr. Anam Shaikh
Department of Histopathology,
Ziauddin University and Hospital, Karachi, Pakistan.
Email: anamshaikh67@yahoo.com
https://doi.org/10.36283/PJMD10-1/012

INTRODUCTION

Renal diseases are a global health issue. According to the World Health Statistics 2016 and Sustainable Development Goals (SDG) project, the kidney and urinary tract pathologies, confers to the worldwide burden of diseases, with around 850,000 deaths and 15,010,167 fine-tune conditions every year[1]. They are the 12th cause of death and the 17th cause of disability[2].

Pakistan ranks eighth in renal disease causing 20,000 deaths every year. Additionally, among the 43,000 people dying in the country due to organ failure, more than 45% die of renal failure (both acute and chronic) [3]. The underlying pathology in most cases (33.56%) of renal dysfunction is glomerular dysfunction/disease, largely referred to as glomerulonephritis (GN) [4,5].

In developing countries, glomerulonephritis is a highly reported diagnosis to patients carrying the burden of renal diseases[6,7]. According to the 2012 United States Renal Data System (USRDS) 28.3/million/year is the adjusted renal disease rate due to primary glomerulopathy[8]. Material from the Chinese Renal Data System reveals that the most common cause of renal pathology is glomerular diseases (57.4%) [9]. Data from Africa displays glomerular diseases in 10.2% to 52% of patients with renal disease[10,11].

A variety of morphological pictures are noted in the condition inclusive of minimal change disease, glomerular basement membrane thickening, mesangioproliferative glomerulonephritis, focal and segmental scarring, mesangiocapillary glomerulonephritis, hyalinosis and other rare patterns[12].

Despite the high prevalence and incidence of glomerular disease, the underlying histological variants and morphological patterns are seldom explored, resulting in a lack of understanding of the morphological patterns of the disease.

Recent studies are now showing, morphological patterns of glomerular diseases having different etiological factors, pathogenetic mechanisms and diverse clinical presentation, proteinuric remission, disease progression and therapeutic response[13,14], making it even more important to investigate the matter as this may lead to better prognosis and reduced morbidity and mortality from the disease.

Nephrotic syndrome is a combination of clinical presentation and laboratory outcomes embracing non-selective proteinuria (3.5 g/24 h), low level of albumin in the blood, increased level of cholesterol, and generalized edema[14-16]. Nephrotic syndrome is the most frequent cause of glomerular damage. The glomerular impairment may be unspecified (primary or idiopathic) or because of some known disorders like Systemic lupus erythematosus (SLE) and Henoch- Schonlein purpura (HSP) which is a secondary glomerulonephritis[17].

About 60% of the adult population developed primary GN[18]. There is a very little amount of fluorescence-based documentation in local data particularly, regarding the pathology of adult nephrotic syndrome cases. As per literature, very few past studies were found, purely made diagnosis on light microscopy without the use of immunofluorescence or electron microscope and thus the real picture of glomerular injury was not reflected in nephrotic syndrome[19]. Therefore, these types of studies missed-diagnosed as mesangiocapillary and mesangioproliferative glomerulonephritis, while most excluding other entities like focal segmental glomerulosclerosis[20].

According to current reports, the various region of the world shows the root of nephrotic syndrome is focal segmental glomerulosclerosis[21], followed by membranous glomerulonephritis and minimal change disease with many of other least common patterns. The cases of nephritic syndrome mostly diagnosed as IgA nephropathy[22-24].

In the 1970s, the International Study of Kidney Diseases in Children reported minimal change disease is the most common histopathological pattern in biopsies from individuals with idiopathic nephrotic syndrome[23]. Minimal Change disease reported by other single center studies in 70-90% of cases. Focal segmental glomerulosclerosis was the underlying cause of idiopathic nephrotic syndrome found in only 5-7% of cases[25]. It is believed, however, that children and adults both are on the higher incident of focal segmental glomerulosclerosis[26]. However, there is no comprehensive review of literature reporting the morphological pattern of glomerulonephritis. Consequently, we line-up to review in stock published literature on the histomorphological spectrum of glomerular diseases.

DISCUSSION

This review was conducted according to the guidelines of "Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA)". Studies were collected by using PubMed, Hinari and Google Scholar as a search engine. The search of studies was restricted to three keywords (i.e., Histo-morphology, Glomerulonephritis and Glomerulopathies using the Boolean operator "and") and search results from last decade (2011-2020) were included. Only articles available in English were included. The bibliographies of articles were also inspecting to strengthen the search.

We included, study, which reports only biopsy-proven cases of glomerulopathies, contain a minimum of 50 participants and provided data on morphological types of the reported glomerulopathies. The study whose focus was on the comorbid condition and whose whole unit had a single or specific type of glomerulopathy was excluded. The relevant data were extracted to review the full-text study of selected data. Data collection included the region and country of publication, publication year, the design of the study, number of performed biopsies and their indication, distribution of gender, frequencies of reported histomorphological types and their etiopathogenesis.

Thirty-five complete research papers were reviewed and analyzed for this review study. Out of 35 studies, 18 (51.43%) reported on adults (1st group), 11/35 (31.43%) comprising of adults and pediatrics (2nd group) and 6/35 (20.69%) were limited to pediatric age group (3rd group) (Figure 1). Total, 13,423 documented cases of renal biopsy in the last decade (2011-2020) were evaluated. The study duration stretched from 12 months to up to many years. The prospective study design was found only in eight studies.

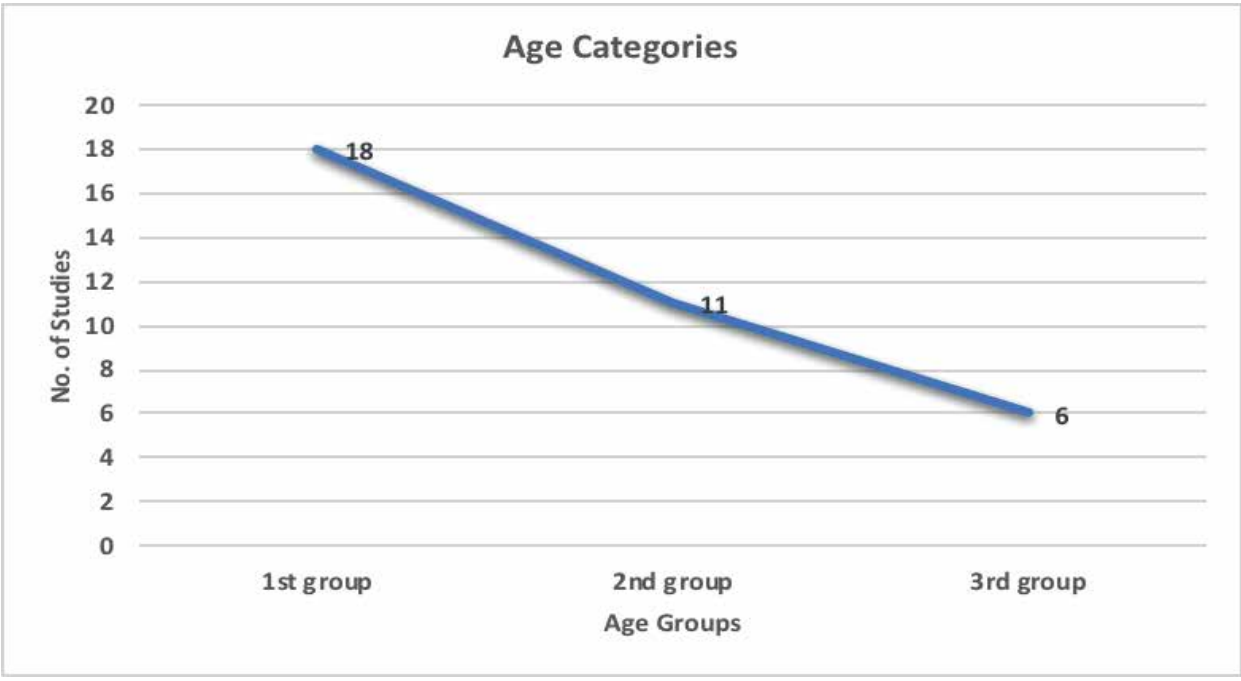


Figure 1: Data regarding the age categories of group individuals.

Male predominance observed in most of the studies: 45.2%-63.7% in 1st group; 47.3%-64.3% in 2nd group and 53.0%-68.8% in 3rd group. In 1st group studies, at the time of biopsy the average age was below 40 years (range 15-70 years), while in 3rd group studies, the average age was below 9.5 years (range 1-14 years). Generally, we found 121 was the median number of cases per the study, however, the vast difference observed in the mean rate of renal biopsy cases reported annually (1st group: 17.1/annum to 134/annum; 2nd group: 7.9/annum to 617/annum and 3rd group: 5.2/annum to 83.1/annum). The most common indication of the renal biopsy was a nephrotic syndrome.

The search comprised of articles from parts of Asia, Europe, and Africa. The highest prevalence among different types of glomerulopathy reported by all 35 studies was minimal change disease 16.5% (95% CI: 11.2-22.6; n=7657). Minimal change disease also reported in 2nd group by the study of Morocco was: 79.2% (68.5-87.6) on the other hand there were two studies from Nigeria which concluded minimal change disease the least common type found[27,28]. Minimal change disease was the highly prevalent type of pattern found in the pediatric group (3rd group) compared to group 1st and 2nd but no accountable difference was found across all the age groups (p=0.496).

The prevalence rate of focal segmental glomerulosclerosis in Africa was 15.9% (11.3-21.1). Among many regions, Focal segmental glomerulosclerosis (FSGS) was the most prevalent type of picture noted in West Africa: 19.1% (5.9-37.4) and it was the least common type in North Africa: 13.2% (9.5-17.5) but there was no significant inter regional difference noted (p=0.889). Other primary patterns of glomerulopathy were observed in 9.2% (95% CI: 6.2-12.7), among them mesangioproliferative glomerulonephritis 11.8% (95% CI: 9.2-14.6), Mesangiocapillary glomerulonephritis 6.6% (95% CI: 4.6-9.0) and membranous glomerulonephritis 2.8% (95% CI: 1.3-4.9). In the African continent, amyloidosis and lupus nephritis were two frequently reported diseases with a mean prevalence of 11.3% and 13.9% respectively.

In Asia, focal segmental glomerulosclerosis and minimal change disease were the commonest morphological variants. Minimal change disease was found in 35.48% of the studies (95% CI: 34.3-41.3). Focal segmental glomerulosclerosis was present among 37.58% of biopsies from Asia (95% CI: 37-38), membranous glomerulonephritis stood at 22.58% (95% CI: 4.1-0.9) and membranoproliferative glomerulonephritis was noted in 16.44% of the biopsies from the region (95% CI: 9.8-24.6). The most prevalent underlying condition in Europe was focal segmental glomerulosclerosis (28%), followed by minimal change disease (19.5%) and then membranous glomerulonephritis (18.5%), diabetic and hypertensive nephropathy, mirroring the high prevalence of diabetes and hypertension in the affluent parts of the world, were the predominant types of secondary renal diseases in Europe.

There is a scarcity of solid data regarding the epidemiology of glomerular diseases in many parts of the world in some measures due to the dearth of renal registries. Due to budget, insufficient skills to perform biopsies, tissue handling and adequate skills to interpret the morphology of renal tissue, especially in Africa and Asia. However, few institutions are efficient enough to perform renal biopsies regularly.

However, from what we gathered in this review, we have found that minimal change disease and focal segmental glomerulosclerosis are the frequently reported glomerular pattern in Asia,[14] Europe and Africa. This may be because of nephrotic syndrome reported by the majority of the 29 studies as the only indication to perform a renal biopsy and as we noted in many studies these two patterns are the commonest cause of nephrotic syndrome[29-32]. We detected that there were very few studies[33] which distributed the glomerulopathy in primary and secondary diseases. The most common secondary glomerulopathy found was lupus nephritis[34-35].

Few researchers that have attempted to study secondary diseases, such as amyloidosis have shown that the deposition of amyloid is appeared in the mesangium as well as along the basement membrane of the glomerular capillary wall and this accumulation of amyloid damages the glomerular basement membrane. The presence of amyloid in terms of its quantity correlates with the clinical behavior of the disease as well. Among the histomorphological patterns, in lupus erythematosus, mesangial and membranous abnormalities can be found such as focal proliferative or diffuse proliferative mesangial or membranous glomerulonephritis, interstitial nephritis, glomerular sclerosis, vascular sclerosis and necrotizing renal vasculitis[36].

Many different clinical characteristics and prognosis are associated with each of the morphological patterns. There is a possibility of occurrence of mesangial as well as focal proliferative lupus nephritis without the presence of any clinical feature and in general, they have a good prognosis. The progressive and irreversible renal functional abnormality and nephrotic syndrome are the manifestations of diffuse proliferative lupus nephritis. In one-sixth of the case, we observed milder forms could be transformed to diffuse proliferation. Although the nephrotic syndrome is the characteristic finding of membranous lupus nephritis, it may persistently present but the renal functional abnormality develops very slowly and its severity is least common[37].

During diffuse proliferative lupus nephritis, necrotizing vasculitis occurs oftentimes and produces the picture of malignant hypertension which may ultimately lead to uremia. Interstitial nephritis occurs in the association of many glomerular patterns but most of the time it may occur as the predominant lesion both histologically and clinically as well. Glomerular sclerosis may occur with hypertension and vascular sclerosis majorly develops during lupus nephritis, and proceed towards severe forms even though the active entity has remitted[38].

Hypertension also has its implications for kidney health and manifests important morphological signs as diseases progress. Microalbuminuria is a predictor of the clinical progression of the disease. Additionally, research also suggests that it is also a predictor of the clinical progression of diabetic nephropathy. The underlying histomorphological spectrum of both the disease is like that of the primary renal diseases[39].

We recognized basic limitations in most of the studies including unavailability of immunofluorescence and electron microscopic technology and lack of uniformity in depicting morphological patterns. For example, we noted, many of the series published earlier in 2000 reported diffuse proliferative and focal proliferative glomerulonephritis even without further description of the picture. Although the performance of renal biopsy is mandatory for the appropriate diagnosis of glomerular diseases, we acknowledge, that this is not an impossible technique or method to establish and perform the renal biopsy in developing countries where the resources and competence are confined. Therefore, there is an urgent need to make pillars for the improvement in this area to perform, proceed and interpret the renal biopsy.

Table 1: Distribution of morphological patterns among different regions of the world.

Research Study	Male	Female	Most Common PGN	Most Common SGN
Vuen et al. 2020[14]	28.7%	71.3%	MCD 38.9%	LN 87.2%
AlYousef et al. 2020 [15]	61.2%	38.8%	IgA nephropathy 23.9%	LN 41.8%
Asif et al.2017[33]	53.3%	646.6%	MN 71%	LN 60%
Ayach et al. 2011[34]	61%	39%	MCD 79.20%	Amyloidosis 2.6%
Nadium et al. 2013[36]	54.9%	45.1%	FSGS 29.6%	-----
Mohammad et al. 2012[39]	76%	24%	FSGS 22%	Amyloidosis 5%
Gunawardena et al. 2018[40]	17.43	82.5%	FSGS 24.8%	0%
Rath i et al. 2014[41]	60.2%	39.8%	FSGS 30.6%	LN 62.5%
PriyadarShiniet al. 2019[42]	62.97%	37.03%	MCD 47.63%	LN (no % found)

MCD: Minimal change disease
FSGS: Focal segmental glomerulosclerosis
LN: Lupus nephritis
IgA: Immunoglobulin-A nephropathy

CONCLUSION

Glomerular diseases are an area of demanding expertise and the establishment of renal biopsy registries as these is found to be crudely portraying due to a lack of data on morphology. The step towards the betterment of this area may offer a chance to depict the prevalence and patterns of glomerulopathies and this may impacts positively on chronic kidney disease evaluation and treatment since most glomerular diseases are liable to treatment.

ACKNOWLEDGEMENTS

We are grateful to Miss Ambreen Wasim for her statistical guidance.

CONFLICTS OF INTEREST

All the authors declare that there are no conflicting interests found in the preparation and publication of this research work.

AUTHORS' CONTRIBUTION

AS wrote the manuscript, TM and FL revised and edited the manuscript.

REFERENCES

1. World Health Organization. World health statistics 2016: monitoring health for the SDGs sustainable development goals [Internet]. World Health Organization; 2016 [cited 2020 June 18]. Available from: https://apps.who.int/iris/handle/10665/206498
2. Grams ME, Sang Y, Levey AS, Matsushita K, Ballew S, Chang AR, *et al*. Kidney-failure risk projection for the living kidney-donor candidate. New Eng J Med. 2016;374(5):411-421.
3. Mubarak M, Kazi JI, Naqvi R, Ahmed E, Akhter F, Naqvi SA, *et al*. Pattern of renal diseases observed in native renal biopsies in adults in a single centre in Pakistan. Nephrol. 2011;16(1):87-92.
4. Ali Jaffar Naqvi S. Nephrology services in Pakistan. Nephrol Dial Transplant. 2000;15(6):769-771.
5. Ullah K, Butt G, Masroor I, Kanwal K, Kifayat F. Epidemiology of chronic kidney disease in a Pakistani population. Saudi J Kidney Dis Transpl. 2015;26(6): 1307-1310.
6. Jha V. Current status of end-stage renal disease care in India and Pakistan. Kidney Int Suppl. 2013;3(2):157-160.
7. Africa N, Coast12 I, Faso15 B. Regional disparities in etiology of end-stage renal disease in Africa. Saudi J Kidney Dis Transpl. 2013;24(3):594-595.
8. Bethesda MD. United States Renal Data System: 2014 [internet]. Annual Data Report; 2014 [cited 2020 Nov 18]. Available from: https://adr.usrds.org/2020
9. Cai G, Chen X. Etiology, comorbidity and factors associated with renal function decline in Chinese chronic kidney disease patients. J Am Soc Nephrol. 2011;22: A 183-A184.
10. Ali ET, Abdelraheem MB, Mohamed RM, Hassan EG, Watson AR. Chronic renal failure in Sudanese children: aetiology and outcomes. Pediatr Nephrol. 2009;24(2):349-353.
11. Sumaili EK, Krzesinski JM, Cohen EP, Nseka NM. Epidemiology of chronic kidney disease in the Democratic Republic of Congo: review of cross-sectional studies from Kinshasa, the capital. Nephrol Ther. 2010;6(4):232-239.
12. Zhuo L, Wang H, Chen D, Lu H, Zou G, Li W. Alternative renal biopsies: past and present. Int Urol Nephrol. 2018;50(3):475-479.
13. Menn-Josephy H, Lee CS, Nolin A, Christov M, Rybin DV, Weinberg JM, *et al*. Renal interstitial fibrosis: an imperfect predictor of kidney disease progression in some patient cohorts. Am J Nephrol. 2016;44(4):289-299.
14. Vuen LA, Chee PH, Lojikip SL, Wei WK, Kheng G, Sia CF. Pattern of biopsy-proven renal disease in Sabah: A retrospective cross-sectional study over 3.5 years. Med J Malaysia. 2020;75(2):152-157.
15. AlYousef A, AlSahow A, AlHelal B, Alqallaf A, Abdallah E, Abdellatif M, *et al*. Glomerulonephritis histopathological pattern change. BMC Nephrol. 2020;21:1-7.
16. Ando D, Yasuda G. Circadian blood pressure rhythm is changed by improvement in hypoalbuminemia and massive proteinuria in patients with minimal change nephrotic syndrome. Cardiorenal Med. 2016;6(3):209-215.
17. Zhu P, Zhou FD, Wang SX, Zhao MH, Wang HY. Increasing frequency of idiopathic membranous nephropathy in primary glomerular disease: A 10-year renal biopsy study from a single Chinese nephrology centre. Nephrol. 2015;20(8):560-566.
18. Ratzel S, Cullinan SB. Nephrotic syndrome (NS), the association of gross proteinuria, hypoalbuminaemia, edema, and hyperlipidemia. Am J Hum Genet. 2015; 96(1):153-161.
19. Chembo CL, Marshall MR, Williams LC, Walker RJ, Lynn KL, Irvine J, *et al*. Long-term outcomes for primary glomerulonephritis: New Zealand Glomerulonephritis Study. Nephrol. 2015; 20(12);899-907.
20. Nylk J, Pullman JM, Campbell EC, Gunn-Moore FJ, Prystowsky MB, Dholakia K. Structured illumination microscopy as a diagnostic tool for nephrotic disease. In advanced biomedical and clinical diagnostic and surgical guidance systems XV. Int Soc Opt Photonics. 2017; 10054, p.1005417. Available from, https://www.spiedigitallibrary.org/conference-proceedings-of-spie/10054/1005417/
21. De Vriese AS, Sethi S, Nath KA, Glassock RJ, Fervenza FC. Differentiating primary, genetic, and secondary FSGS in adults: a clinicopathologic approach. J Am Soc Nephrol. 2018;29(3):759-774.
22. Krishnamoorthy S, Ramakrishnan SR, Vivek B, Suja L. Clinical, biochemical and histopathological profile of adult nephrotic syndrome patients in a

tertiary care hospital. J Evol Med Dent Sci. 2015;4 (71):12361-12376.
23. Ikram M, Muhammad S, Ali A, Muhammad N, Bahadur K, Haq MI. Frequency of histopathological pattern of renal disease in a tertiary care hospital. J Postgrad Med Inst. 2017;31(2):131-134.
24. Hommos MS, De Vriese AS, Alexander MP, Sethi S, Vaughan L, Zand L, *et al*. The incidence of primary vs secondary focal segmental glomerulosclerosis: A Clinicopathologic Study. Mayo Clin Proc. 2017;92 (12):1772-1781).
25. Wang H, Abajobir AA, Abate KH, Abbafati C, Abbas KM, Abd-Allah F, *et al*. Global, regional, and national under-5 mortality, adult mortality, age-specific mortality, and life expectancy, 1970-2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet. 2017;390(10100):1084-1150.
26. Joly D, Béroud C, Grünfeld JP. Rare inherited disorders with renal involvement—approach to the patient. Kidney Int. 2015;87(5):901-908.
27. Fencl F, Vondrak K, Rosík T, Zieg J, Chadimová M, Háček J, *et al*. Recurrence of nephrotic proteinuria in children with focal segmental glomerulosclerosis: early treatment with plasmapheresis and immunoadsorption should be associated with better prognosis. Minerva Pediatr. 2016;68(5): 348-354.
28. Cunningham A, Benediktsson H, Muruve DA, Hildebrand AM, Ravani P. Trends in biopsy-based diagnosis of kidney disease: a population study. Can J Kidney Health Dis. 2018;5:1-9.
29. Wijkström J, González-Quiroz M, Hernandez M, Trujillo Z, Hultenby K, Ring A, *et al*. Renal morphology, clinical findings, and progression rate in Mesoamerican nephropathy. Am J Kidney Dis.2017;69(5) :626-636.
30. Hu W, Chen Y, Wang S, Chen H, Liu Z, Zeng C, *et al*. Clinical–morphological features and outcomes of lupus podocytopathy. Clin J Am Soc Nephrol. 2016;11(4):585-592
31. Stanchev SS, Iliev AA, Malinova LG, Landzhov BV, Kotov GN, Hinova-Palova DV. Light microscopic study of renal morphological alterations in spontaneously hypertensive rats. J Biomed Clin Res. 2017;10(1):18-24.
32. Lionaki S, Marinaki S, Panagiotellis K, Tsoumbou I, Liapis G, Vlahadami I, *et al*. Glomerular diseases associated with malignancies: histopathological pattern and association with circulating autoantibodies. Antib. 2020;9(2):18.
33. Asif N, Ahsan MN, Khanzada SW. Spectrum of renal parenchymal diseases: An eleven year retrospective review of renal biopsy data from a tertiary care hospital in Pakistan. Ann King Edward Med Univ. 2017;23(1):13-17.
34. Ayach G, El-Filali H, Saidi S, El-Gharib S, Kaobai G, Noaur H, *et al*. Histopathological study of pure primary nephrotic syndrome in adolescents and young Moroccan adults. Arab J Nephrol Transplant. 2011;4(3):137-140.
35. Al-Sadoon EI, Rahim KA, Al-Anazi A, Faqeehi H, Al-Batati S. Spectrum of pediatricbiopsy-proven renal diseases: A single center experience. Saudi J Kidney Dis Transplant. 2020;31(1): 176-181.
36. Nadium WK, Abdelwahab HH, Ibrahim MA, Shigidi MM. Histological pattern of primary glomerular diseases among adult Sudanese patients: A single center experience. Indian J Nephrol. 2013;23 (3): 176-179.
37. Kandil H, Sosnin D, Mahmoud A, Shalaby A, Soliman A, Elmaghraby A, *et al*. Hypertension and correlation to cerebrovascular change: A brief overview. Cardiovas Imaging Image Anal. 2018:345.
38. Brück K, Stel VS, Gambaro G, Hallan S, Völzke H, Ärnlöv J, *et al*. CKD prevalence varies across the European general population. J Am Soc Nephrol. 2016;27(7):2135-2147.
39. Mohammad N, Khan TM, Orakzai AN, Imran M. Histological pattern of glomerulopathies. Gomal J Med Sci. 2012;10(1):7-11.
40. Gunawardena K, Wijewickrama E, Arambepola C, Lanerolle R. Descriptive analysis of glomerulonephritis by histological type and their progression among adults in a tertiary care center in Sri Lanka. Saudi J Kidney Dis Transplant. 2018;29(1): 136-139.
41. Rathi M, Bhagat RL, Mukhopadhyay P, Kohli HS, Jha V, Gupta KL, *et al*. Changing histologic spectrum of adult nephrotic syndrome over five decades in north India: A single center experience. Indian J Nephrol. 2014;24(2): 86-91.
42. PriyadarShini L, Pradhan SK. Pattern of renal histopathological findings in children: a single center study. J Clin Diagn Res. 2019;13(12)1-4.

REVIEW ARTICLE

Stem Cell Therapy: A Promising Treatment of Parkinson's Diseases

Humaira Ansari[1], Shumaila Usman[2], Syed Tousif Ahmed[1], Shazia Hashmat[1]
[1]Department of Physiology, Ziauddin University, [2]Department of Research, Ziauddin University, Karachi, Pakistan.

ABSTRACT

The neurodegenerative disorder is a prolonged persistence curse and effect on economic and physical challenges in an aging world. Parkinson has come in the second category of disability disorders and associated with progressive dopaminergic neuronal degeneration with severe motor complications. It is an observation that gradual disease progression causes 70% degeneration of striatal dopaminergic neurons. Globally there are around 7-10 million patients with Parkinson's disease, however, there are huge efforts for therapeutic improvement. According to studies, no single molecular pathway was pointed out as a single etiology to control disease progression due to a lack of targeted therapeutic strategies. Previously implemented symptomatic treatments include L-dopa (L-3,4-dihydroxyphenylalanine), deep brain stimulation, and the surgical insertion of a medical device. This leads to dyskinesia, dystonia and a higher risk of major surgical complications respectively. However, not all the above-mentioned therapies cannot regenerate the dopaminergic neurons in Parkinson's disease patients. Recent advances in the field of cellular therapy have shown promising outcomes by differentiation of multipotent mesenchymal stem cells into dopaminergic neurons under the influence of a regenerative substance. In this review, we have discussed the differentiation of dopaminergic neurons by using different cell types that can be used as a cellular therapeutic approach for Parkinson's disease. The information was collected through a comprehensive search using the keywords, "Parkinson Disease, Dopamine, Brain derived neurotrophic" factor and neuron from reliable search engines, PubMed, Google Scholar and Medline reviews from the year 2010 to 2020.

Keywords: Parkinson Disease; Dopamine; Brain Derived Neurotrophic Factor; Neuron.

Corresponding Author:
Dr. Humaira Ansari
Department of Physiology,
Ziauddin University, Karachi, Pakistan.
Email: humaira.ansari15@yahoo.com
https://doi.org/10.36283/PJMD10-1/013

INTRODUCTION

Parkinson's disease (PD) is a neurodegenerative progressive disorder affecting millions of people worldwide. It is characterized by typical movement disorder including rigidity, tremors and bradykinesia[1]. It is identified pathogenically, the Lewy bodies made up of misfolded forms of the protein a-syn[2].

Pharmacological treatment can be advantageous for many years but prolong use of pharmacological treatment had produced many side effects including, on-off fluctuations and wearing-off phenomenon[3], confusion, hypotension, hallucination, orthostatic hypotension, fatigue, brain hemorrhage, infarction, and seizures[4].

Other innovative modalities had been introduced to PD consisting of gene therapy and cellular therapy. Gene therapy produces enzymes which are responsible for non-synaptically, generating L-dopa (L-3,4-dihydroxyphenylalanine) and dopamine (DA) but this treatment had not proved to be fruitful because this therapy works without any feedback regulation of cellular specificity[5].

Another new modality is cell-based therapy that has attracted the attention of researchers as being the potentially feasible novel therapies for neurodegenerative diseases. It encompassed the derivation of specific neuronal subtypes lost in the disease and consequent transplantation into exaggerated areas of the nervous system cell types had been used for the differentiation of dopaminergic

neurons for the subsequent transplantation into the Parkinson's patient[6]. In this review, we have discussed the different strategies and cell types used for the differentiation of dopaminergic neurons.

DISCUSSION

Cellular therapy is considered as the elementary unit of regenerative medicine[7] in the upcoming fields. The goal is to restore the lost function rather than produce a new organ. The differentiated mesenchymal stem cells (MSCs) are plentifully used as a cellular therapy for degenerative diseases. Besides MSCs, other stem cells have also varying differential potential and can be obtained from different sources[9].

Embryonic Stem Cells (ESCs)

Embryonic stem cells (ESCs) are derived by the blastocyst. The major characteristics have a high capability for potency and self-renewal. ESCs are formed into three germ layers endoderm, mesoderm and ectoderm and the formation of the specific organs. Some progenitor cells retained the organ, can proliferate under injury repair, and can differentiate. These tissue stem cells are found in bone, blood, muscle, adipose tissue, liver, brain, skin, gastrointestinal tract and bone marrow. These cells have the potential to differentiate into three three primary germ layers and also sustain in an undifferentiated state and also survive in culture media for a long time[10].

The following transcriptional factors of ESCs e.g., Nanog and Oct4 maintain an undifferentiated state of the stem cells and self-regeneration ability. It was observed that no genetic aberrations are proliferated in the ESC line. Embryonic stem cells are cultured in a media containing the anti-differentiation cytokine in leukemia inhibitory factors (LIF). Withdrawal of the embryonic stem cells from the feeder film consequently caused the development of "embryoid bodies", having all three germs layer[10] however; their use is restricted due to ethical issues and high risk of teratoma formation after transplantation[10].

Induced Pluripotent Stem Cells (IPSCs)

Adult somatic stem cells can convert induced pluripotent stem cells because these cells are genetically reprogrammed and convert into embryonic stem cells (ESC). Now iPSCs are a very important tool for drug development, regenerative medicine and modeling diseases. Further research also showed other numerous benefits, a cell source for Parkinson's disease (PD) replacement as well as the capability to use patient's peculiar cells and consequently reduced the necessity for immuno-suppression. Furthermore, a clinical trial was initiated in Japan, Takahashi's lab, where PD patients have received human leukocyte antigen (HLA) matched iPSCs derived dopamine neurons. All these researches can be used as assistance for future approach[3]; however, it has limitations due to the high risk of teratoma formation after transplantation[9].

Differentiation of IPSCS

Bone marrow mesenchymal stem cells (BMSCs) have great potential to differentiate into neuron-like cell by the presence of the following factor such as hepatocyte growth factors (HGF), vascular endothelial growth factors (VEGF), epidermal growth factors (EGF). These differentiated cells were identified through immunocytochemistry; phase contrast inverted microscopy, immunocytochemistry and transcriptase-polymerase chain reactions to identify the neuronal specific markers[11].

Sympathetic neurons can also be derived from induced pluripotent stem cells and embryonic stem cells. Scientists used the helix-loop-helix, Achaete-scute homolog 1 (ASCL1), Paired-like homeobox (PHOX2B), homeodomain transcription factor as important factors for sympathetic neuronal development. However, they also showed the activation of Wingless-related integration site (WNT) signaling pathway[12]. Neural differentiation derived from iPSCs by the induction of brain derived growth factors and neuronal cells production with better dendritic, axonal growth and intracellular connectivity can be visible within seven days[13].

According to Cheng et al. rat bone marrow mesenchymal stem cells differentiate into dopamine neuron like cells by the induction of liver X receptor, causes well differentiation of BMSCs into dopamine neurons[14]. This research also showed that when carbonized substrate cultured on human neuronal stem cells, they could be differentiated into matured and specialized neuron cells. Literature also showed that carbon material like graphene oxide promotes the mouse embryonic stem cells differentiation into the dopaminergic neuron, with neuronal gene expression markers[15]. Another scientist Velasco et al. differentiated hESCs and iPSCs into mature neurons under the influence of transcription factor either NeuroD1 or Neurogenin 2 within two weeks[16].

Adult Stem Cells

Application of adult stem cell therapy is better compared to the utilization of aborted human embryo's ventral mDA[17] because of immune reactions causes dysfunction of transplanted DA neurons[18]. Adult stem cells are obtained from all tissues of the three germ layers and also from the placenta for example, MSCs can be derived from human amnion epithelial cells. These stem cells had various properties such as anti-inflammatory, no rejection issue, and limited differentiation ability. In vitro they are

differentiated into different germ cell layers. Numerous researches had proved in vivo transplantation of adult stem cell repairs of the injured organs, like bone tissue repair and new specialized cell generation. Revascularization of the ischemic cardiac tissue also proved that adult stem cells could release many molecular mediators, with immunomodulatory, angiogenic and anti-apoptotic chemoattractant properties, that stimulate the healing process in culture media[9]. The usage of hMSCs in cell based therapy had involved wide interest in the applications of numerous incurable diseases and had revealed several superior characteristics for therapeutic usage compared to other types of stem cells which are discussed below.

Mesenchymal Stem Cell (MSCs)

Mesenchymal stem cells have prodigious potential with a big source for cellular therapy for neuronal disorders. MSCs are the best example of adult multipotent progenitor cells, derivative from several adult tissues and in vitro they have the ability of self-renewal. The discovery of MSCs from both human and mouse origins can be differentiated into functional neurons and encouraged as a replacement of impaired neurons[19].

The mesenchymal stem cells are spindle-shaped morphologically. They can adhere to tissue culture plastic and grow in culture media and can expand and maintain the multipotent characteristics[19]. They are easy to separate, more multilineal differentiation integration into a strong functional shape[20] and are unrestricted from ethical problems but they have restricted replicative life span[9]. MSCs release the soluble factors that are significant for cell existence and proliferation, control immune responses and migration to the particular site of injury[21].

MSCs are obtained from many tissues: adipose tissues, bone, bone marrow, peripheral blood, Wharton's jelly, dental pulp, decidua basalis, amniotic fluid, umbilical cord, placenta, and amniotic membrane, chorionic villi from human placenta, menstrual blood, breast milk, and urine etc[22]. MSCs with their novel therapeutic properties are the most significant cell type in the era of tissue engineering and cell based therapy[23]. Besides their wide availability of proliferative capacity and multipotency, they are the most clinically practiced cell source[24]. They aid in the release of neurotrophic and angiogenic factors that stimulate neuronal growth-enhancing synaptic connections, promote angiogenesis, neurogenesis, differentiation and axonal remyelination[25].

MSCs is the best source for cell-based therapy hence it is safely used for autologous transplantation and showed high differentiation potential[26], no tumorigenicity due to paracrine secretion of these cells, their property exhibits wide clinical potential by regulating apoptosis, angiogenesis, cell differentiation, immunomodulation and extracellular matrix composition[19]. The importance of mesenchymal cells, their differentiation ability, potential regeneration of the desire cells, all these unique properties are discussed in this review.

Mesenchymal Stem Cells Based Therapies for Neurologlogical Diseases

Mesenchymal Stem Cells (MSCs) have unique features as compare to other stem cells. These cells have been used in different clinical trials including, Parkinson's disease, Spinal cord injury, Huntington's disease, multiple sclerosis and brain ischemia[27].

The human mesenchymal stem cells (hUMSCs) is a high source of progenitor and stem cells containing mesenchymal stem cells. hUMSCs are achieved either from cord blood or the cord tissue. hUMSCs have numerous benefits as compared to other sources of stem cells. MSCs show (Figure 1) various cell surface antigens including CD 105, CD 73, CD90, CD146, and various integrins and adhesion molecules. They can easily be harvested with no harm to the baby and mother. They exhibit high proliferative property and low immunogenicity[28]. Due to the abundant availability of the umbilical cord, its compatible and effective clinical application can be considered as one of the most plentiful sources of non-embryonic stem cell[29].

Differentiation of HUMSCS into Dopaminergic Neurons like Cells

Differentiation of dopaminergic neurons can be achieved by the exposure of extrinsic factors[30], including Wnt family members, soluble proteins, growth factors (FGF, GDNF, BDNF, TGFβ2 and EGFR) and chemical inducers (dbcAMP, AA and BHA)[31]. When hUMSCs were cultured in neural containing medium, they produced neural morphologies and expressed neural markers such as nestin, NeuN and glial fibrillary acidic protein[27]. The Wharton's jelly of the MSCs transdifferentiated into neuron-like cells and they expressed Nestin and Neuro-D1 as neuronal markers by using Valproic acid[32].

Previously MSCs from human Wharton jelly was successfully induced by using a glial cell-derived neurotrophic factor and brain derived neurotrophic factors into neurons and auditory hair cells during in vitro experiment[33]. Human umbilical cord tissues differentiated into neuron like cells by using nestin, GFAP and synaptic markers: SYN, PSD95, and GAP43[34]. Mallis et al.[35] observed WJ-MSCs differentiated into neural-like cells by using basic fibroblast growth factor and Forskolin, thus differentiating cells into neuron-like cells and astrocytes by cAMP-elevating agents such as Forskolin and 3-isobutyl-1-methylxanthine, and also without the presence of growth factors[36].

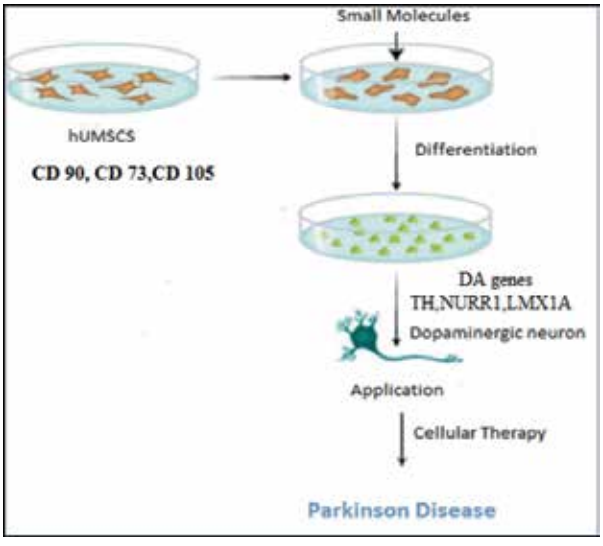


Figure 1: Human umbilical cord mesenchymal cells (huMSCs) differentiation into dopaminergic neurons.

Dopaminergic Neuron Differentiation from Other Sources

Many sources were identified from which dopaminergic neuron differentiation from stem cells was initiated for future applications in regenerative medicine. One of the promising sources is Human olfactory ectomesenchymal stem cells (OE-MSCs) which can differentiate into dopaminergic (DA) neurons by maintaining their plasticity. OE-MSCs were potentiated to differentiate into DA neuron-like cells in vitro by the induction of sonic hedgehog (SHH) signaling pathway, basic fibroblast growth factor (bFGF), fibroblast growth factor 8, Glial cell line-derived neurotrophic factor (GDNF) and brain derived neurotrophic factor (BDNF)[29]. Human dental pulp stem cell (hDPSCs) differentiation to dopaminergic neurons has been proved a high source. The hDPSCs are obtained noninvasively by deciduous teeth and sustain their multipotency with neuron-like cells and self-renewal properties. Knockout-embryonic stem cell (KO-ES) medium with leukemia inhibitory factor (LIF) was used in which the human pulp cells were expanded. Within 4 days, the neurosphere was formed and further transferred into ITS (human insulin transferrin sodium) and fibronectin media, which further confirmed the selection for Nestin-positive cells. In conclusion, the cells were moved into N-2/ascorbic acid media to promote dopaminergic neurons by the differentiation process and observing the expressions of mesenchymal stem cell markers and early neuronal markers at different stages[37].

According to Lairson et al, fluoxetine stimulates neurogenesis in neural stem cells within the hippocampus. This result showed that there is a link between the regeneration of neurons and their role in reducing the symptoms of depression[38]. Another research showed that MSCs can be differentiated into osteogenic cells and expressed the bone markers under the induction of osteogenic differentiation medium. In the stage of proliferation, MSCs release laminin, fibronectin and collagen type I in the matrix maturation stage. They secrete alkaline phosphate and during matrix, mineralization expressed the bone genes such as osteopontin proteins and sialoprotein[39].

It was also observed that statin molecules are effective in both neuronal differentiation and midbrain neuron specification. Other statin molecules are less potent in neuronal differentiation as compared to mevastatin such as simvastatin[40]. Neural progenitor cells upon differentiation into dopaminergic neurons by mevastatin had shown high expression of dopamine specific genes including TH, Nurr1 and LMX1a compared to other statin molecules. Statin had shown noteworthy effects in both promoting the midbrain neuron specification and neuronal lineage differentiation. Moreover, its efficient mechanism of apoptosis induction in the undifferentiated cells eliminates the chances of brain tumor cancer stem cells[41]. Gonzalez et al. reported that neural stem cell differentiation into dopamine neurons in the presence of guggulsterone would be a new approach for the treatment of Parkinson's disease[42]. According to Osborn in the area of transplantation, immune compatibility is very significant and these strategies have fewer advantages to the patients rather than an autologous approach.

Advantages of Autologous Transplantation for Parkinson's Disease

a) In Parkinson's patients, no immune suppression by autologous transplantation.
b) These are serious immunological reactions of allogeneic transplantation.
c) Neural cell autologous approach potentially is greater incorporated the axonal functional[3].
d) This therapy is effective for neurodegenerative diseases such as Parkinson diseases as shown in Table 1.

Table 1: Overview of advantages and disadvantages of different types of stem cell for cellular therapy of Parkinson's disease[43].

Stem Cells	Advantages	Disadvantages
ESCs	High proliferative capacity	High risk of teratoma formation
	High potential to differentiate ant cell types	High risk of immune rejection
		Ethical issues
NSCs	Limited differentiation potential	Restricted proliferative capacity
	Decrease the risk of tumor formation	Low neuronal differentiation capacity
		Ethical issues
		Risk of immune rejection
MSCs	High proliferative capacity	Differentiated into DA
	No ethical issues	
iPSCs	High proliferative capacity	Risk of teratoma formation
	No ethical issues	Differentiation of DA
	No immune rejection	

CONCLUSION

Therapeutic strategies for neurodegenerative disorders have been found mandatory requirement and have proven to be a major promising achievement for neurological diseases like Parkinson's disease. Damage to the dopaminergic neurons in the Substantia Nigra being the sole cause of movement disorders in PD patients has led to many innovative therapies like drug therapies acting on receptors but then wearing off side effects prevail. Deep brain stimulation also facing different limitations in the regeneration of dopaminergic neurons has led tone modalities to be investigated gradually. Emerging options of dopaminergic neuron regeneration by Human Mesenchymal Stem Cells differentiation has been proved the best source of cell-based therapies for neurological diseases. The source of MSC has been exhibited through other sources like Human Dental Pulp cells, Human Olfactory Ectomesenchymal stem cells and hUMSCs, statin through gene expression of dopamine. These examples are a high source of dopaminergic neuron differentiation, which were initiated for future application in regenerative medicine.

ACKNOWLEDGMENTS

I would like to thank Dr. Nabila for her valuable support towards experimental work support.

CONFLICT OF INTEREST

There was no conflict of interest among the authors.

AUTHORS' CONTRIBUTION

HA and SU performed the conceptualization of the study, literature search and prepared the manuscript. ST did the overall evaluation of the review and SH helped in the preparation of the manuscript.

REFERENCES

1. Engelender S, Isacson O. The threshold theory for Parkinson's disease. Trends Neurosci. 2017;40(1):4-14.
2. Moutinho M, Codocedo JF, Puntambekar SS, Landreth GE. Nuclear receptors as therapeutic targets for neurodegenerative diseases: lost in translation. Annu Rev Pharmacol Toxicol. 2019;59:237-261.
3. Osborn TM, Hallett PJ, Schumacher JM, Isacson O. Advantages and recent developments of autologous cell therapy for Parkinson's disease patients. Front Cell Neurosci. 2020;14:1-13.
4. Wang Y, Ji X, Leak RK, Chen F, Cao G. Stem cell therapies in age-related neurodegenerative diseases and stroke. Ageing Res Rev. 2017;34:39-50.
5. Herrington TM, Cheng JJ, Eskandar EN. Mechanisms of deep brain stimulation. J Neurophysiol. 2016;115(1):19-38.
6. Lunn JS, Sakowski SA, Hur J, Feldman EL. Stem cell technology for neurodegenerative Diseases. Ann Neurol. 2011;70(3):353-361.
7. Teo AK, Vallier L. Emerging use of stem cells in regenerative medicine. Biochem J. 2010;428(1):11-23.
8. Sánchez A, Schimmang T, García-Sancho J. Cell and tissue therapy in regenerative medicine. InStem Cell Transplantation: Springer, New York, NY; 2012. p. 89-102.
9. Kim HJ, Park JS. Usage of human mesenchymal

stem cells in cell-based therapy: advantages and disadvantages. Dev Reprod. 2017;21(1):1-10.
10. Kolios G, Moodley Y. Introduction to stem cells and regenerative medicine. Respiration. 2013;85(1):3-10.
11. Bae KS, Park JB, Kim HS, Kim DS, Park DJ, Kang SJ. Neuron-like differentiation of bone marrow-derived mesenchymal stem cells. Yonsei Med J. 2011; 52(3):401-412.
12. Oh Y, Cho GS, Li Z, Hong I, Zhu R, Kim MJ, *et al*. Functional coupling with cardiac muscle promotes maturation of hPSC-derived sympathetic neurons. Cell Stem Cell. 2016;19(1):95-106.
13. Lin MH, Chung CY, Chen KT, Yeh JC, Lee TH, Lee MH, et al. Comparison between polybutylcyanoacrylate nanoparticles with either surface-adsorbed or encapsulated brain-derived neurotrophic factor on the neural differentiation of iPSCs. Int J Mol Sci. 2019;20(1):1-23.
14. Cheng O, Tian X, Luo Y, Mai S, Yang Y, Kuang S, et al. Liver X receptors agonist promotes differentiation of rat bone marrow derived mesenchymal stem cells into dopaminergic neuron-like cells. Oncotarget. 2018;9(1): 576-590.
15. Ferraro RM, Ginestra PS, Giliani S, Ceretti E. Carbonization of polymer precursors substrates to direct human iPSC-derived neurons differentiation and maturation. Procedia CIRP. 2020;89:39-44.
16. Velasco I, Salazar P, Giorgetti A, Ramos-Mejía V, Castaño J, Romero-Moya D, *et al*. Concise review: generation of neurons from somatic cells of healthy individuals and neurological patients through induced pluripotency or direct conversion. Stem Cells. 2014;32(11):2811-2817.
17. Grealish S, Diguet E, Kirkeby A, Mattsson B, Heuer A, Bramoulle Y, *et al*. Human ESC-derived dopamine neurons show similar preclinical efficacy and potency to fetal neurons when grafted in a rat model of Parkinson's disease. Cell Stem Cell. 2014;15(5):653-665.
18. Studer L. Strategies for bringing stem cell-derived dopamine neurons to the clinic-the NYSTEM trial. InProgress in brain research: Elsevier; 2017. p. 191-212.
19. Volkman R, Offen D. Concise review: mesenchymal stem cells in neurodegenerative diseases. Stem Cells. 2017;35(8):1867-1880.
20. Green EM, Lee RT. Proteins and small molecules for cellular regenerative medicine. Physiol Rev. 2013;93(1):311-325.
21. Squillaro T, Peluso G, Galderisi U. Clinical trials with mesenchymal stem cells: an update. Cell Transplant. 2016;25(5):829-848.
22. Van Pham P, Truong NC, Le PT, Tran TD, Vu NB, Bui KH, *et al*. Isolation and proliferation of umbilical cord tissue derived mesenchymal stem cells for clinical applications. Cell Tissue Bank. 2016;17(2):289-302.
23. Bara JJ, Richards RG, Alini M, Stoddart MJ. Concise review: Bone marrow-derived mesenchymal stem cells change phenotype following in vitro culture: implications for basic research and the clinic. Stem Cells. 2014;32(7):1713-1723.
24. Marędziak M, Marycz K, Tomaszewski KA, Kornicka K, Henry BM. The influence of aging on the regenerative potential of human adipose derived mesenchymal stem cells. Stem Cells Int. 2016;2016: 1-16.
25. Castillo-Melendez M, Yawno T, Jenkin G, Miller SL. Stem cell therapy to protect and repair the developing brain: a review of mechanisms of action of cord blood and amnion epithelial derived cells. Front Neurosci. 2013;7:194-207.
26. Chang YH, Wu KC, Liu HW, Chu TY, Ding DC. Human umbilical cord-derived mesenchymal stem cells reduce monosodium iodoacetate-induced apoptosis in cartilage. Tzu Chi Med J. 2018;30(2):71-80.
27. Stoddard-Bennett T, Reijo Pera R. Treatment of Parkinson's disease through personalized medicine and induced pluripotent stem cells. Cells. 2019;8 (1):26-40.
28. Dalous J, Larghero J, Baud O. Transplantation of umbilical cord–derived mesenchymal stem cells as a novel strategy to protect the central nervous system: technical aspects, preclinical studies, and clinical perspectives. Pediatr Res. 2012;71(2):482-490.
29. Ali H, Bahbahani H. Umbilical cord blood stem cells–potential therapeutic tool for neural injuries and disorders. Acta Neurobiol Exp (Wars). 2010;70(3):316-324.
30. Pan G, Liu J. Small molecules and extrinsic factors promoting differentiation of stem cells into insulin-producing cells. Ann Endocrinol. 2019; 80(2): 128-133.
31. Kim JH, Auerbach JM, Rodríguez-Gómez JA, Velasco I, Gavin D, Lumelsky N, *et al*. Dopamine neurons derived from embryonic stem cells function in an animal model of Parkinson's disease. Nat. 2002;418(6893):50-56.
32. Ghorbani S, Tiraihi T, Soleimani M. Differentiation of mesenchymal stem cells into neuron-like cells using composite 3D scaffold combined with valproic acid induction. J Biomater Appl. 2018;32(6): 702-715.
33. Kil K, Choi MY, Park KH. In vitro differentiation of human Wharton's jelly-derived mesenchymal stem cells into auditory hair cells and neurons. J Int Adv Otol. 2016;12(1):37-42.
34. Shi Y, Nan C, Yan Z, Liu L, Zhou J, Zhao Z, *et al*. Synaptic plasticity of human umbilical cord mesenchymal stem cell differentiating into neuron-like cells in vitro induced by edaravone. Stem Cells Int. 2018;2018:1-12.
35. Mallis P, Papadopoulos G, Michalopoulos E. Wharton jelly mesenchymal stem cells derived from human umbilical cord capable to differentiate into neural-like cells and their potential use in neurological disorders. Electronic J Biol. 2017, 13(4): 442-461.
36. Shahbazi A, Safa M, Alikarami F, Kargozar S, Asadi MH, Joghataei MT, *et al*. Rapid induction of neural differentiation in human umbilical cord matrix mesenchymal stem cells by cAMP-elevating agents. Int J Mol Cell Med. 2016;5(3): 167-177.
37. Chun SY, Soker S, Jang YJ, Kwon TG, Yoo ES. Differentiation of human dental pulp stem cells into dopaminergic neuron-like cells in vitro. J Korean Med Sci. 2016;31(2):171-177.
38. Lairson LL, Lyssiotis CA, Zhu S, Schultz PG. Small molecule–based approaches to adult stem cell therapies.

Annu Rev Pharmacol Toxicol. 2013;53: 107-125.
39. Antebi B, Zhang Z, Wang Y, Lu Z, Chen XD, Ling J. Stromal-cell-derived extracellular matrix promotes the proliferation and retains the osteogenic differentiation capacity of mesenchymal stem cells on three-dimensional scaffolds. Tissue Eng Part C Methods. 2015;21(2):171-181.
40. Sucu S, Brunner-Lorieux S, Hommet Y, Binet V, Bideau E, Debroas E, *et al*. Statins induce both neuronal-like differentiation and programmed death in PC 12 cells [dissertation on the internet]. Universite de CAEN; 2016. [cited 2020 Sep 7]. Available from: https://www.researchgate.net/profile/Dominique_Duval/publication/307575962
41. heon Rhim J, Luo X, Xu X, Gao D, Zhou T, Li F, *et al*. A High-content screen identifies compounds promoting the neuronal differentiation and the midbrain dopamine neuron specification of human neural progenitor cells. Sci Rep. 2015;5:1-14.
42. Gonzalez R, Garitaonandia I, Abramihina T, Wambua GK, Ostrowska A, Brock M, *et al*. Deriving dopaminergic neurons for clinical use. A practical approach. Sci Rep. 2013;3:1-5.
43. Jensen P, Krabbe C, Meyer M. Cell Therapy for parkinson's disease: status and perspectives. Transl Neurodegener. 2011: 343-378. InTech-Open Access Publisher.

REVIEW ARTICLE

Therapeutic Value of Medicinal Mushroom *Agaricus blazei* Murill

Mehwish Murad Ali[1], Mirza Tasawer Baig[1], Aisha Jabeen[1], Muhammed Aslam[2], Uzma Shahid[3]
[1]Department of Pharmacy, Ziauddin University, [2]Department of Pharmacy, Sindh University,
[3]Department of Medicine, Ziauddin University, Karachi, Pakistan.

ABSTRACT

Agaricus blazei Murill (AbM) is a mushroom that has been utilized in alternative drug to anticipate cardiac disease, diabetes, arthritis, increased cholesterol, cancer, hepatitis and cancers. It contains β-glucan, which contributes to decreasing blood sugar in-vivo and acts against cancer. The extract of this mushroom exerts antioxidant action in-vivo. AbM contains agaritine that exerts antitumor effects against leukemic cells in vitro. This mushroom is also found to have immune-stimulatory function, tumor growth suppression, action against allergy, antiviral effects, antimicrobial function and immune modulatory effects. Researchers also studied its action in decreasing blood cholesterol. According to several studies, AbM containing (1→6)-β-D-glucan-exerts anti angiogenesis action. The purpose of this extensive review on the medicinal value of AbM mushroom was to highlight its significance and its traditional uses by scientific evidence to determine the effectiveness of the mushroom in various evidence-based uses. Google Scholar and PubMed search engines were used to browse articles from 1994-2019 on the therapeutic value of AbM. Initially, 74 articles were found related to the therapeutic value of AbM. After reviewing the available article, 42 were selected based on the medicinal uses of AbM. It was concluded that AbM possessed various bioactive compounds that are responsible for its therapeutic effects.

Keywords: Mushroom; Antidiabetic; Therapeutics.

Corresponding Author:
Dr. Mirza Tasawer Baig
Department of Pharmacy,
Ziauddin University, Karachi, Pakistan.
Email: mirzatasawerbaig@gmail.com
https://doi.org/10.36283/PJMD10-1/014

INTRODUCTION

Agaricus blazei Murrill (AbM) is a mushroom, basidiomycete brown fungus, origin to Brazil. It is extensively utilized for medicinal functions together with non-prescript as a nutritious mushroom as well as in extract form[1]. *Agaricus blazei* is a nutritious mushroom refer to the family Agaricaceae has been frequently utilized as a health nutriment additive to prevent arteriosclerosis, cancer, hyperlipidemia, chronic hepatitis together with diabetes[2]. In Japan *Agaricus blazei* Murrill (AbM) is familiar as Himematsutake, Agarikusutake or Kawarihiratake, in Brazil, it is known as *Cogumelo do sol* and in China as Ji Song Rong[3]. *Agaricus blazei* is thought to be a nutritious mushroom due to having nutritional value; also, the secondary metabolites of this mushroom are varied chemically and possess an extensive range of biological functions. Including the bioactive substances are minerals, glucan peptides, vitamins, polyphenols, polysaccharides, glycoproteins, polyunsaturated fatty acids and triterpenoids[4]. According to the culture of Brazil, it would be effective against several conditions, for example, hepatitis, atherosclerosis, increased blood sugar, heart disease, dyslipidemia etc[5]. This mushroom possesses immune modulating and antimicrobial effects both in-vivo and in-vitro and as well as it has been used to treat cancer, hepatitis, dermatitis and hyperlipidemia traditionally[6]. The purpose of this extensive review on the medicinal value of AbM mushroom was to highlight its significance and to build upon the existing work in its traditional uses by synthesizing the available information to determine the effectiveness of the mushroom in various

evidence-based uses. This will also support the growing focus on the medicinal value of mushrooms in general and AbM in particular.

DISCUSSION

Agaricus blazei Murill (AbM) is a nutritive mushroom origin in Brazil. It is cultivated in countries like Indonesia, Taiwan, China, Japan and Korea[7]. It is a basidiomycete generally familiar as sun mushroom. It is marketed as a natural form for utilization and to use in capsules, solutions, and syrups in Brazil. Several pieces of research have been revealed that the mushroom *Agaricus blazei* Murill contains polysaccharides, which are produced by mycelial fermentation, are the reason for its anti-tumor together with immune-modulating effects, and possess biological functions as discussed later in this review[8].

Diabetes Mellitus

Agaricus brasiliensis is the origin of Brazil. It is developed extensively in Japan. It is utilized for the healing of hepatitis, cancer, cardiac disease, dyslipidemia, dermatitis as well as polysaccharides, β-glucan also a-glucan have been found to exert antimutagenic also immunomodulating actions both in vitro as well as in vivo. The probable system of natural polysaccharides related to Diabetes Mellitus depends on 6 ways which include the development of plasma insulin (decrease of pancreatic glucagon), the elevation of insulin susceptibility (enhancement of insulin resistance), the control of alpha glycosidase enzymes in the bowel (decrease of carbohydrates decomposition and absorption), the elevation of hepatic glycogen (blockage of sugar dysplasia), the increment in the utilization of glucose by peripheral tissue, and the scavenging of free radicals (lipid peroxidation).

Antidiabetic also hypoglycemic effects of this mushroom have been noted. Researchers found that the extract of this mushroom exert marked antioxidant action in streptozotocin-induced diabetic rats showed reduced lipoperoxidation also iNOS expression in the lungs. It has been preferred by these findings that this mushroom markedly decreases oxidative stress also play role in tissue improvement in increased blood sugar. The clinical proofs showed that this mushroom together with antidiabetic agents could enhance insulin blockage in patients having Diabetes Mellitus type-II. One group has noted that antidiabetic action by this mushroom in diabetic rats is because of the suppression of proinflammatory cytokine generation, which leads to the betterment of pancreatic beta cells mass[9].

An extract of *Agaricus blazei* Murill can reduce blood glucose levels in sterptozotocin induced mice and exert an antidiabetic effect. It is composed of β-glucan that is an immunostimulatory compound and enhances insulin generation. One of the researchers noted that healing by beta glucan, which is contained in *Agaricus blazei* Murill as a chemical compound helps to reduce blood sugar in sterptozotocin-induced rats. The mechanism of action is to raise the secretion of insulin from islands of Langerhans also preserving and reproduction of islands of Langerhans cells of both healthy and diabetic mice. *Agaricus blazei* Murill can reduce blood glucose levels in a diabetic state while according to the current study the result indicated that intake of this mushroom by normal people showed no response on random blood sugar level[10].

Anti-inflammatory Activity

The nutritious mushroom *Agaricus blazei* Murill has been included in scientific studies due to having immunomodulatory actions. One of the studies has revealed that this mushroom suppresses inflammatory procedures caused by high fat diet in mice by lowering the generation of proinflammatory cytokines (TNF-a) [11]. *Agaricus blazei* Murill stimulates the immune system by enhancing natural killer cell action also activity of macrophage[12].

The extract of polysaccharides contained in mushroom *Agaricus blazei* Murill exerts antioxidant activity and immunomodulatory function in chicken spleens[13]. The biological functions and several pharmacological actions, which include anti-inflammatory, antitumor, anti-diabetes, immunomodulatory, anti-hypercholesterolemia, anti-oxidant and anti-heart disease effects of *Agaricus blazei* Murill have been noted[14].

Anti-cancer Effects

ABM is a traditional remedy against cancer. The active compounds contained in it are polysaccharides have been known to exert inhibitory effects against tumor indirectly by enhancing immune function[15]. *Agaricus blazei* Murill is used as a traditional diet. It is utilized for its antihypertensive, anticancer, lowering blood sugar, lowering cholesterol also immunostimulant effect. This mushroom is composed of β- (1-6) -; β- (1-3) –glucan, α- (1-4) -; β- (1-6) –glucan, β- (1-6) -; a- (1-3) –glucan, α- (1-6) -; α- (1-4) –glucan, riboglucan, ergosterol, lectins, glucomannan, RNA-protein complex, blazein, sodium pyroglutamate, Agaritin, ascorbic acid, agariblazepirol C, total phenol and a- and δ-tocopherol. *Agaricus blazei* Murill is used as a traditional diet.

It is utilized for its antihypertensive, anticancer, lowering blood sugar, lowering cholesterol also immunostimulant effect. This mushroom is composed of β- (1-6) -; β- (1-3) –glucan, α- (1-4) -; β- (1-6) –glucan, β- (1-6) -; a- (1-3) –glucan, α- (1-6) -; α- (1-4) –glucan, riboglucan, ergosterol, lectins, glucomannan, RNA-protein complex, blazein, sodium pyroglutamate, Agaritin, ascorbic acid, agariblazepirol C,

total phenol and α- and δ-tocopherol. Due to the presence of this composition, this mushroom can be utilized as an anticancer agent. According to the study *Agaricus Blazei* Murill extract with solvents such as chloroform, n-hexane, ethylacetate also dichloromethane possess an action against cancer in MCF-7 cells. This extract can be established as an anticancer agent in MCF-7 cells[16].

Glucan contained in *Agaricus blazei* Murill revealed anticancer effect also having a standardized quantity of beta glucans. Agaritine contained in *Agaricus blazei* Murill showed an antitumor effect against leukemic cells in vitro. It was quite different from beta glucan that inhibits the generation of tumor cells indirectly. The mechanism of action includes apoptosis. Agaritine exerts an antitumor effect directly against leukemic tumor cells in vitro. Moreover, a steroid known as blazein obtained from this mushroom was found to promote cell death also structural variation suggestive of apoptotic chromatin condensation in lung cancer cells of humans. This compound has similar activity on cancer cells of the stomach as lung cancer[17].

AbM is found to induce apoptosis or programmed cell death in ovarian cancer cells of humans[16]. In mouse models of fibrosarcoma, myeloma, prostate cancer, ovarian as well as lung cancer AbM showed antitumor activities, also in human studies against gynecological cancer (marked Natural Killer cell activity and quality of life) and leukemia[19].

Neuroprotective Effects

Administration of extract of *Agaricus blazei* Murill protects the myenteric plexus in animals. The administration of aqueous extract of *Agaricus blazei* Murill sufficiently sustained myenteric plexus homeostasis, which certainly persuades the physiology and inhibits the death of neurons as well as glial cells[18]. Aging mutates morpho-functional, biometric, blood factors in the jejunum, and induce morpho-quantitative variations in enteric NS. Long-term administration of aqueous extract of *Agaricus blazei* Murill greatly sustained myenteric plexus homeostasis that helps to maintain physiology also stops the death of neuron cells[21].

Hepatitis

The affirmative outcomes revealed by *Agaricus blazei* Murill in various affected models. According to several clinical evidence, it could be raising therapy for curing various infections also hepatitis specifically for patients having a resistant illness[22]. It was observed that Agaricus *brasiliensis* could suppress the processing of structural processes that form liver necrosis[23]. Agaricus *brasiliensis* is origin in Brazil also cultivated in China as well as Japan due to its medicinal effects. This mushroom has been utilized commonly to prevent several illnesses such as hepatitis. According to some studies, the extract of this mushroom can improve hepatic injury in rats caused by CCL_4 [24]. *Agaricus blazei is* commonly utilized to heal numerous conditions like high blood sugar, cardiac disease, increased cholesterol, cancer, and hepatitis and skin diseases. Several clinical types of research showed the possible significance of this mushroom in the therapy of chronic hepatitis.

Some researchers concluded that the polysaccharide extract from this mushroom in a dose of 1500 mg revealed a marked decrease in hepatic enzymes in 4 patients having hepatitis B over a twelve month duration, aspartate aminotransferase decreased from 246 to 61 IU per liter, alanine transaminase from 151 to 46 IU per liter. The extract of this mushroom given to rats was found to decrease the action of plasma alanine transaminase also aspartate aminotransferase increased by CCl4. Some researchers revealed that the extract containing active compounds work on liver cell membrane directly or indirectly to improve liver injury caused by CCl4 in rats. Oral intake of fermentation mycelia and broth of this mushroom in mice having liver injury caused by ethanol caused a marked decrease in liver fibrosis[25]. *Agaricus blazei* Murill is a mushroom origin to Brazil.

This mushroom has been used as nutritious food for cancer. It contains biological functions such as antiviral, antibacterial, anti genotoxic, antiparasitic and anti tumoral functions. In addition, it is useful in the therapy of high blood sugar and hepatitis in an animal model[26]. The study helped to guide about 2 probable ways to utilize basidiomycetes to treat hepatitis. One, different basidiomycetes containing compounds have been utilized as stimulants in vaccines. A DNA vaccine can stimulate CD8 (+) T-cell response however, this response is very less in maximum mammals. About this guidance, it was found that injection of DNA vaccine to treat hepatitis together with extract of mushroom *Agaricus blazei* Murill fortified with polysaccharides, which are used as adjuvants to mice markedly, improved humoral as well as cellular immune responses[27]. *Agaricus subrufescens* usually utilized for the therapy of several diseases including liver disease, cancer, increased blood sugar, cardiac disease, skin disease and increased cholesterol.

Immune Stimulatory Effects

The clinical effects from mushroom *Agaricus subrufescens* that have been reported include immune stimulatory function, tumor growth suppression, action against allergy, anti viral effects, anti microbial function and immune modulatory effects[28]. The fruiting body of *Agaricus blazei* Murill is enriched with β-glucans that helped to stimulate the innate immune system. This compound has been known to have antitumor action in both *in vitro* and *in vivo*. For in vitro it is useful for ovarian

cancer, leukemia cells, fibrosarcoma and hepatocarcinoma. For in vivo it is useful for lung cancer, fibrosarcoma and multiple myeloma. Hence, it has been thought that the clinical action of this mushroom is because of β-glucans having immunostimulatory action[29]. The mushroom *Agaricus blazei* Murill has been utilized to cure various illnesses such as cancer as well as infection. *Agaricus blazei* Murill enriched in antitumor protein-glucan complexes as well as β-glucans that are strong stimulators of natural killer (NK) cells, macrophages, dendritic cells and granulocytes.

This mushroom contains stimulatory actions on the formation of proinflammatory cytokines in immune cells as if monocyte-derived dendritic cells as well as monocytic cells[30]. Seeing that in 1960 the Japanese scholars have identified immune modulating together with antitumor functions of *Agaricus blazei* Murill in the mouse. *Agaricus blazei* Murill based mixed basidiomycete mushroom extract was known to be the particular one that markedly decreases bacteria in blood as well as improved survival estimate of the mouse in pneumococcal sepsis. After some time it was called as Andosan™ for trading and selected for more investigations that revealed its defense against allergy also G-negative sepsis in other mouse models. These functions and the antitumor effect of the mushroom presented in Andosan™ is most likely to be because of the immuno-modulatory relative shift stimulated by the mushroom[31].

Hypercholesterolemia

Agaricus blazei Murill is found to have clinical effects, is usually utilized in alternative drugs to anticipate cardiac disease, arthritis, increased cholesterol, cancer, hepatitis, and increased blood sugar[32]. One of the studies revealed that administration of *Agaricus brasiliensis* has enhanced the serum lipid levels in rats having high cholesterol by balancing the expression of the main gene associated with the metabolism of liver cholesterol[33]. In Wistarrats it is found that the supplements in *Agaricus brasiliensis* are significant modulators of lipid profile. It has been detected to decrease triglycerides, cholesterol together with lipid deposit on hepatic tissue together with enhancing excretion by faeces[34]. β–Glucan in *Agaricus blazei* Murill is found to reduce blood cholesterol however, the process associated with it is not clear. Mushroom *Agaricus blazei* Murill has been given to albino Fischer rats having hypercholesterolemia for two months. It has been revealed to reduce serum cholesterol as well as bring about marked variations in gene expression associated with cholesterol[35].

Moreover, studies have been recommended that *Agaricus blazei* Murill has an advantageous role in hyperlipidemia and diabetes together with the enhancement of insulin resistance as a vaccine adjuvant and has helped to stop tumor development as well as angiogenesis[36].

Angiogenesis

It has been notified that the food nutrients including *Agaricus blazei*-derived ergosterol possess anti angiogenic effect. Studies reported that substance containing (1→6)-β-D-glucan- could exert anti angiogenesis function. The antitumor function together with antitumor immunological effect of *Agaricus blazei* contains (1→6)-β-D-glucan protein complex has been notified. One of the methods has been suggested that the process of angiogenesis can be suppressed by extraction of this mushroom's fruiting body with lesser aliphatic alcohol or lesser aliphatic alcohol having twenty percent or less than twenty percent of water[37].

Some researchers notified that regular administration of extract of this mushroom for six months has markedly enhanced mental as well as physical constituents of traits of the patient's life. studies suggested that pyroglutamate also ergosterol together with their anti angiogenic effects can be derived from *Agaricus blazei* Murill also β-glucan[38]. Studies revealed that *Agaricus blazei* Murill possesses an effect against angiogenesis means it suppress the development of new blood vessels to the tumor. It also suppresses an enzyme known as aromatase related to the formation of breast cancer[39].

Nutraceutical and Cosmeceutical

One of the research investigated possible reutilization of large-scale dump *Agaricus blazei* Murill. Hence, the constituents of vital supplements as well as chemical constituents were derived. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) tetrazolium reduction assay, also with Lactate dehydrogenase assays were utilized to examine activity as well as cell death of human colorectal adenocarcinoma cell lines HT29 and Caco-2 of *Agaricus blazei* Murill ethanolic extract. *Agaricus blazei* Murill ethanolic extract was added in a semi solid base cream used cosmetically. The cell activity, functions of extracts and finished product ingredient on a keratinocyte cell line HaCaT were analyzed. Principal supplements like carbohydrates, proteins also with a low fat compound were derived for *Agaricus blazei* Murill. Furthermore, its pH was also desirable. The cell activity of HaCaT cells in the extract existence as well as the final product was sustained by the dependence of concentration that showed the safeness of *Agaricus blazei* Murill ethanolic extract for pharmaceutical cosmetic purposes. The outcomes recommend that *Agaricus blazei* Murill remnants can be utilized as a cheap as well as a worthwhile source of pharmaceutical nutrition and pharmaceutical cosmetic components[40]. To the best of our knowledge we are ending the discussion by presenting the summary of the pharmacological activities of Agaricus blazei Murill in Table 1.

Table 1: Summary on pharmacological activities on *Agaricus blazei* Murill.

Pharmacological Activity	Experimental Designs	Studies
Diabetes Mellitus[9,10]	*In-vivo*	Vitak et al. 2017
	In-vivo	Misgiati and Corebima, 2015
Anti-inflammatory activity[11-14]	*In-vivo*	Tontowiputro et al. 2018
	In-vivo	Kang et al. 2015
	In-vivo	Xie et al. 2017
	In-vitro	Hahne et al. 2014
Cancer[18,41-42,44]	*In-vitro*	Misgiati et al. 2017
	In-vitro	Itoh, Ito and Hibasami, 2008
	In-vivo	Hsu et al. 2008
	In-vivo	Førland et al. 2011
Neuroprotective[21]	*In-vivo*	Santi-Rampazzo et al. 2015
Hepatitis[23- 24,43]	*In-vivo*	de Souza et al. 2018
	In-vivo	Zhang et al. 2015
	In-vivo	Wang and Ma, 1994
Immune stimulatory effects[29-31]	*In-vitro and In-vivo*	Tangen et al. 2015
	In-vitro	Tangen et al. 2014
	In-vivo	Hetland et al. 2016
Hyperchol esterolemia[33-34]	*In-vivo*	de Miranda et al. 2017
	In-vivo	Henriques et al. 2016
Angiogenesis[37-38]	*In-vitro*	Ito et al. 2016
	In-vivo	Kimura et al. 2015
Nutraceutical and cosmeceutical applications[40]	*In-vitro*	Taofiq et al. 2019

CONCLUSION

Therapeutic use of Mushroom *Agaricus blazei* Murill in diseases such as Diabetes Mellitus, Neuroprotection, Cancer, Hepatitis, Immunomodulatory agent, Hypercholesterolemia, Angogenesis and Cosmetics was highlighted which contains constituents like β- (1-6) and Agaritine that helps to cure various diseases. Hence, *Agaricus blazei* Murill is a promising drug for improved health. AbM also possessed various bioactive compounds that are responsible for its therapeutic effects.

ACKNOWLEDGEMENTS

The authors wish to thank Dean Faculty of Pharmacy, Ziauddin University, and Prof. Dr. Ali Akbar Sial (Late), for his support and motivation to complete the project.

CONFLICT OF INTEREST

The authors proclaim that they have no conflict of interest.

AUTHORS' CONTRIBUTION

All authors contributed equally to this review article and were involved in drafting the manuscript. All authors read the manuscript and approved the final version.

REFERENCES

1. Wang H, Li G, Zhang W, Han C, Xu X, Li YP. The protective effect of *Agaricus blazei* Murill, submerged culture using the optimized medium composition, on alcohol-induced liver injury. Biomed Res Int. 2014;2014:01-9.
2. Shimizu T, Kawai J, Ouchi K, Kikuchi H, Osima Y, Hidemi R. Agarol, an ergosterol derivative from *Agaricus blazei*, induces caspase-independent apoptosis in human cancer cells. Int J Oncol. 2016;48(4):1670-1678.
3. Ni WY, Wu MF, Liao NC, Yeh MY, Lu HF, Hsueh SC, *et al*. Extract of medicinal mushroom *Agaricus blazei* Murill enhances the non-specific and adaptive immune activities in BALB/c mice. *In Vivo*. 2013;27(6):779-786.
4. Fanhani JC, Murakami AE, Guerra AF, do Nascimento GR, Pedroso RB, Alves MC. Effect of *Agaricus blazei* on the diet of broiler chickens on immunity, serum parameters and antioxidant activity. Semin Cienc Agrar. 2016;37(4):2235-2246.
5. Liu Y, Zhang L, Zhu X, Wang Y, Liu W, Gong W. Polysaccharide *Agaricus blazei* Murill stimulates myeloid derived suppressor cell differentiation from M2 to M1 type, which mediates inhibition of tumour immune-evasion via the Toll-like receptor 2 pathway. Immunol. 2015;146(3):379-391.
6. Lima CU, Gris EF, Karnikowski MG. Antimicrobial properties of the mushroom *Agaricus blazei*–integrative review. Rev Bras Farmacogn. 2016;26(6):780-786.
7. Yeh MY, Shang HS, Lu HF, Chou J, Yeh C, Chang JB, *et al*. Chitosan oligosaccharides in combination with *Agaricus blazei* Murill extract reduces hepatoma formation in mice with severe combined immunodeficiency. Mol Med Rep. 2015;12(1):133-140.
8. Wang HT, Yang LC, Yu HC, Chen ML, Wang HJ, Lu TJ. Characteristics of fucose-containing polysaccharides from submerged fermentation of *Agaricus blazei* Murill. J Food Drug Ana. 2018;26(2):678-687.
9. Vitak T, Yurkiv B, Wasser S, Nevo E, Sybirna N. Effect of medicinal mushrooms on blood cells under conditions of diabetes mellitus. World J Diabetes. 2017;8(5):187-201.
10. Misgiati M, Corebima AD. The effect of *Agaricus blazei* Murill on haematological parameter, random blood sugar, total cholesterol, and uric acid of wistar rats (Sprague Dawley). J Sci Res Stud. 2015;2(2):56-62.
11. Tontowiputro DK, Sargowo D, Tjokroprawiro A, Rifa'i M. Anti-inflammatory activity of *Agaricus blazei* Murill extract in the spleen of mice fed a high-fat diet. Trop J Pharm Res. 2018;17(3):483-489.
12. Kang IS, Kim RI, Kim GS, Kim NR, Shin JY, Kim C. Effects of *Agaricus blazei* Murill water extract on immune response in BALB/c mice. J Korean Soc Food Sci Nutr. 2015;44(11):1629-1636.
13. Xie W, Lv A, Li R, Tang Z, Ma D, Huang X, *et al*. *Agaricus blazei* Murill polysaccharides protect against cadmium-induced oxidative stress and inflammatory damage in chicken spleens. Biol Trace Elem Res. 2018;184(1):247-258.
14. Hahne JC, Meyer SR, Dietl J, Honig A. The effect of Cordyceps extract and a mixture of Ganodermalucidum/*Agaricus blazei* Murill extract on human endometrial cancer cell lines in vitro. Int J Oncol. 2014;45(1):373-382.
15. Cheng F, Yan X, Zhang M, Chang M, Yun S, Meng J, *et al*. Regulation of RAW 264.7 cell-mediated immunity by polysaccharides from *Agaricus blazei* Murill via the MAPK signal transduction pathway. Food Funct. 2017;8(4):1475-1480.
16. Misgiati M, Sukardiman S, Widyawaruyanti A. Anti-breast cancer potency of multistage extraction from jamur dewa (*Agaricus blazei* Murill) solvents on MCF-7 cells. Indonesian J Cancer Chemoprevent. 2017;8(2):68-73.
17. Paterson RR, Lima N. Biomedical effects of mushrooms with emphasis on pure compounds. Biomed J. 2014;37(6):357-368.
18. Schwartz B, Hadar Y. Possible mechanisms of action of mushroom-derived glucans on inflammatory bowel disease and associated cancer. Ann Transl Med. 2014;2(2):01-11.
19. Hetland G, Therkelsen SP, Nentwich I, Johnson E. Andosan-an anti-allergic and anti-inflammatory ingredient prepared from *Agaricus blazei* mushroom. J Clin Cell Immunol. 2015;6(2):01-10.
20. Qin DW, Gu Z, Guo JY. Medicinal mushroom for prevention of disease of modern civilization. Evid Based Complement Alternat Med. 2015:01-02.
21. Santi-Rampazzo AP, Schoffen JP, Cirilo CP, Zapater MC, Vicentini FA, Soares AA, *et al*. Aqueous extract of *Agaricus blazei* Murrill prevents age-related changes in the myenteric plexus of the jejunum in rats. Evid Based Complement Alternat Med. 2015;2015:01-13.
22. Lee C. Influences of the extract of *Agaricus blazei* Murill on hepatitis, cancer and immunity (doctoral dissertation). 2015. Available from: https://scholarworks.bridgeport.edu/xmlui/handle/123456789/1429
23. de Souza AC, de Almeida Goncalves G, Soares AA, de Sá-Nakanishi AB, de Santi-Rampazzo AP, Natali MR, *et al*. Antioxidant action of an aqueous extract of royal sun medicinal mushroom, *Agaricus brasiliensis* (Agaricomycetes), in rats with adjuvant-induced arthritis. Int J Med Mushrooms. 2018;20(2):101-117.
24. Zhang L, Yuan B, Wang H, Gao Y. Therapeutic effect of *Agaricus brasiliensis* on phenyl hydrazine-induced neonatal jaundice in rats. Biomed Res Int. 2015;2015:1-6.

25. Chatterjee A, Acharya K. Include mushroom in daily diet—A strategy for better hepatic health. Food Rev Int. 2016;32(1):68-97.
26. Huang HC, Hsu TF, Chao HL, Chen CC, Chiu SW, Chang TM. Inhibition of melanogenesis in murine melanoma cells by *Agaricus brasiliensis* methanol extract and anti-reactive oxygen species (ROS) activity. Afr J Microbiol Res. 2014;8(6):519-524.
27. Teplyakova T, Kosogova T. Fungal bioactive compounds with antiviral effect. J Pharm Pharmacol. 2015;3(8):357-371.
28. Pardo-Giménez AR, Pardo JE, Carrasco JA, Álvarez-Ortí MA, Zied DC. Use of Phase II mushroom compost in *Agaricus subrufescens* production. Mushroom Res. 2014:516-522.
29. Tangen JM, Tierens A, Caers J, Binsfeld M, Olstad OK, Trøseid AM, *et al*. Immunomodulatory effects of the AgaricusblazeiMurrill-based mushroom extract Andosan in patients with multiple myeloma undergoing high dose chemotherapy and autologous stem cell transplantation: a randomized, double blinded clinical study. Biomed Res Int. 2015;2015:1-11.
30. Tangen JM, Tryggestad AM, Hetland G. Stimulation of human monocytic cells by the medicinal mushroom *Agaricus blazei* Murill induces expression of cell surface markers associated with activation and antigen presentation. Appl Sci Rep. 2014;1(1):1.
31. Hetland G, Eide DM, Tangen JM, Haugen MH, Mirlashari MR, Paulsen JE. The *Agaricus blazei*-based mushroom extract, Andosan™, protects against intestinal tumorigenesis in the A/J Min/+ Mouse. PloS One. 2016;11(12):e0167754.
32. Rego BE, dos Santos FC, de Lima DS. Histopathological analysis of the hepatic parenchyma of Swiss mice subjected to Experimental therapy with *Agaricus blazei*. Pleiade Magzine. 2015;14(14):38.
33. de Miranda AM, Júnior JV, e Silva LS, Dos Santos RC, Silva ME, Pedrosa ML. *Agaricus brasiliensis* (sun mushroom) affects the expression of genes related to cholesterol homeostasis. Eur J Nutr. 2017;56(4): 1707-1717.
34. Henriques GS, Helm CV, Busato AP, Simeone ML. Lipid profile and glycemic response of rats fed on a semi-purified diet supplemented with *Agaricus brasiliensis* mushroom. Acta Sci Health Sci. 2016;38(1):71-79.
35. Gil-Ramírez A, Morales D, Soler-Rivas C. Molecular actions of hypocholesterolaemic compounds from edible mushrooms. Food Funct. 2018;9(1):53-69.
36. Therkelsen SP. Clinical effects and cytokine responses from ingestion of Andosan™ in Patients with ulcerative colitis and Crohn's disease. A randomized placebo controlled study. Series of dissertations submitted to the faculty of medicine, University of Oslo. 2018:189-183.
37. Ito H, Itoh H, Fujishima M, Arakawa Y, Nakada F, inventors; Powerful Healthy Food Corp, Sun Chlorella Corp, assignee. Method of inhibiting angiogenesis. United States patent US 9,308,229. 2016.
38. Kimura Y, Sumiyoshi M, Usuki KI. Effects of *Agaricus blazei* extract plus lactoferrin or lactoferrin alone on tumor growth and UFT-induced adverse reactions in sarcoma 180-or highly metastatic osteosarcoma LM8-bearing mice. Nat Prod J. 2015;5(1):57-69.
39. Gosavi Mahavir, C. Mushrooms of immortality: Anti cancerous use of mushrooms in Chinese medicine - A Review. Int J Life Sci. 2015;A5:1-6.
40. Taofiq O, Rodrigues F, Barros L, Peralta RM, Barreiro MF, Ferreira IC, *et al*. *Agaricus blazei* Murill from Brazil: an ingredient for nutraceutical and cosmeceutical applications. Food Func. 2019;10(2):565-572.
41. Itoh H, Ito H, Hibasami H. Blazein of a new steroid isolated from *Agaricus blazei* Murill (himematsutake) induces cell death and morphological change indicative of apoptotic chromatin condensation in human lung cancer LU99 and stomach cancer KATO III cells. Oncol Rep. 2008;20(6):1359-1361.
42. Hsu CH, Hwang KC, Chiang YH, Chou P. The mushroom *Agaricus blazei* Murill extract normalizes liver function in patients with chronic hepatitis B. J Altern Complement Med. 2008;14(3):299-301.
43. Wang L, Ma H. Observation on the treatment effect of *Agaricus blazei* to the liver function of chronic hepatitis patients. Lanzhou Med Coll. 1994;20:24-26.
44. Førland DT, Johnson E, Saetre L, Lyberg T, Lygren I, Hetland G. Effect of an extract based on the medicinal mushroom AgaricusblazeiMurill on expression of cytokines and calprotectin in patients with ulcerative colitis and Crohn's disease. Scand J Immunol. 2011;73(1):66-75.

KAP STUDY

Volunteering Activity as A Source of Life Satisfaction among Medical and Dental Students of Karachi, Pakistan

Shaista Emad[1,2], Faiza Nasir[2], Ayesha Akhlaq[2], Syed Raheel Pasha[2], Mohd Jahanzaib Noor[2], Rubina Ghani[1]

[1]Department of Biochemistry, Sohail University, [2]Jinnah Medical and Dental College, Karachi, Pakistan.

ABSTRACT

Background: Community Service is an integral component of undergraduate medical education. A volunteer is an individual who, without pay, reaches out beyond the confines of their normal responsibilities; freely and willingly contribute in different ways. Volunteering and serving others can help decrease tension, mentally empower volunteers and offer purpose in life. This study aimed to assess the motivation behind volunteering activities among medical students.

Methods: A cross sectional study was conducted comprised of a total of 450 students of different private and government sector medical colleges of Karachi, Pakistan. A volunteer functional inventory (VFI) questionnaire was used for the study. Predictability of motivation behind volunteerism was analyzed by student's t-test through SPSS software.

Results: Out of the 450 participants in this study, 286(64%) of respondents were found to be volunteers and 164(36%) were non-volunteers, the largest respondents constituting 154 (66.7%) had been volunteering for 1-5 months, 63(14%) spent time in volunteering for 6-10 months. It was found that 228(50%) of the students were influenced by their friends to start the act of volunteering. About 72(42%) of respondents were involved in community volunteering in health and emergency services. The various variables of the motivation (protective factor, value factor, career factor, social factor, understanding factor and enhancement factor) among medical students were determined significant ($p < 0.05$).

Conclusion: Medical students can effectively contribute in the training and healthcare initiatives. To the best of our knowledge, the involvement of healthcare volunteering is beneficial both at a personal and academic level for medical students.

Keywords: Community Service; Depression; Health; Medical Emergencies; Stress.

Corresponding Author:
Dr. Shaista Emad
Department of Biochemistry,
Sohail University, BMCHS Sharafabad,
Karachi, Pakistan.
Email: shaista.emad@yahoo.com
https://doi.org/10.36283/PJMD10-1/015

INTRODUCTION

Volunteerism is the principle of an individual offering their time, energy, services, knowledge and skills freely for the benefit of other people in the community as a social responsibility rather than for any financial reward[1]. The developing nations are still struggling in the field of volunteerism and it is not among the common activities in Pakistan. In Pakistan, the volunteer activity is said to be only 16% ranking and it is 78th in the world[2]. There is a lack of awareness about formal volunteering among students and the public. The concept of formal voluntary work is any unconditional donation or effort to organizations, practices[3]. Knowledge and understanding of volunteerism influence people to participate in volunteering programs despite the country's economic conditions[2].

The health care profession is emotionally demand-

ing as compared to other fields[4]. Medical students experience stress, depression and this is one of the reason that they participate in different activities to burnout stress[1,5]. The medical students commonly practice volunteering activity and they work individually or in groups and contribute their time, skills, energy, resources etc. Studies reported various fields of voluntary work as volunteers to offer their services in volunteering as per their field of interest[6]. Volunteer organizations are categorized as political, environmental, recreational, cultural, human services, educational and other[7]. Volunteering is a practice that may seem quite self-sacrificing, but it serves the volunteer for several purposes[8].

Volunteering offers vital assistance to people in need, valuable causes and society, but the rewards for the volunteer become even significantly higher[9,10]. Volunteering and serving others can help decrease tension, combat sadness; mentally empower volunteers and offer meaning and purpose in life[11]. Voluntary work may not only help the vulnerable, but also the volunteer[12]. There are six main functions served by volunteering services including values, understanding, social, career, enhancement and protective[13]. The values function works on those points that show how much we value the people in need.

The understanding function relates to the knowledge and competencies that a volunteer may have. The social function deals with the relationship between a volunteer and the society[14]. The career function is represented as the volunteers get to experience many things and it will help them in their profession. The enhancement function relates to personal growth. The last function is protection, which relates to the volunteers' problems. By getting involved in volunteering, they forget their troubles and protect themselves from negativity[15]. The Volunteer Function Inventory (VFI) was created to determine the degree to which each of these functions is served by volunteering[16]. This study aimed to provide insights into how medical students are involved in volunteering and how the act of volunteering helps to foster relationships that are more positive with patients.

METHODS

A cross sectional study was conducted comprising of a total of 450 students (male and female) of different private and government sector medical colleges of Karachi, Pakistan. The medical institutes, which were included in the study, are Dow University of Health Sciences, Liaquat National Medical and Dental College, Liaquat College of Medicine and Dentistry, Karachi Medical and Dental College, Jinnah Sind Medical University, Sir Syed College of Medical Sciences for Girls and Dow International Medical. Only MBBS and BDS students participated in the study. Students from other disciplines were excluded.

Ethical approval was obtained from the "Ethical Review Committee (ERC), Jinnah Medical and Dental College, (ERC.No.06-B19/30-11-2018). Volunteer functional inventory (VFI) scale that followed the international guidelines for volunteerism including comparability, feasibility, cost-effectiveness, efficiency and reliability was used for the study. VFI scale of volunteerism comprised of thirty items that measure motivational level for volunteering services. Participants were asked to answer each item on a scale of 7 points that ranges from 1 to 7 (strongly disagree/strongly agree). Volunteer functional inventory (VFI) questionnaire measures protective, value, career, social, enhancement and understanding functions[16]. Sociodemographic characteristics such as gender, age, education (MBBS or BDS), socioeconomic status (upper, middle or lower), marital status (single, married or engaged) and occupation (employed or unemployed) of each of the participant were also determined in the study. Questionnaire form was distributed among medical students of various medical colleges of Karachi. Formal approval was taken from each of the medical institutes participated in the study. The purpose of the study was described to each participant. Instructions and confidentiality issues were also described. Descriptive statistics of the measures were examined, as well as predictability of motivation behind volunteerism was analyzed by student's t-test through SPSS software version 20.0.

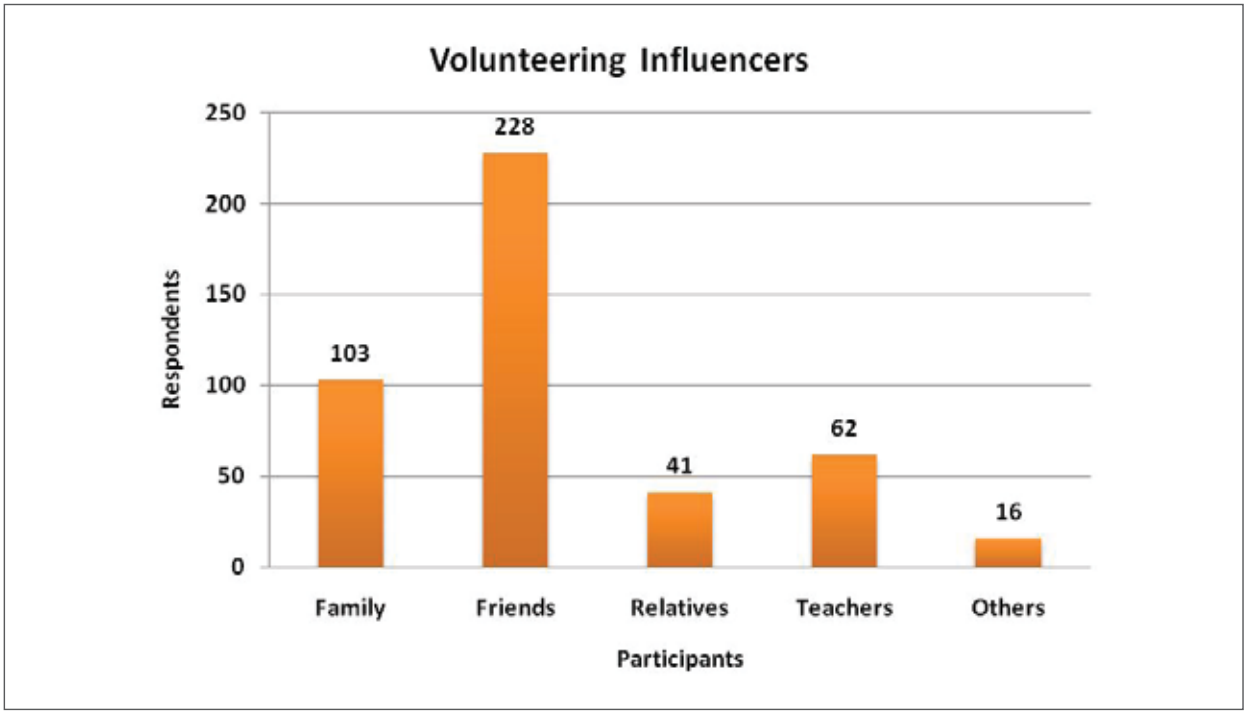
RESULTS

The study comprised 450 participants both male and female. The female participants constituted 65.8% (n=296) of the entire sample population, whereas male respondents were 34.2% (n=154) of the sample. The age group between 17-30 years of the respondents was included in the study. The maximum number of participants involved in the volunteering activity was 21 years old and it was found that 286(64%) of respondents were found to be volunteers and 164(36%) were non-volunteers (Table 1). Time was the major contributing factor in our study and the maximum number of respondents contributed their time for volunteering activity. However, it was also observed that majority of the respondent involved in volunteerism for the enhancement of their skills that enables them to critical thinking, communication, creativity, and problem solving.

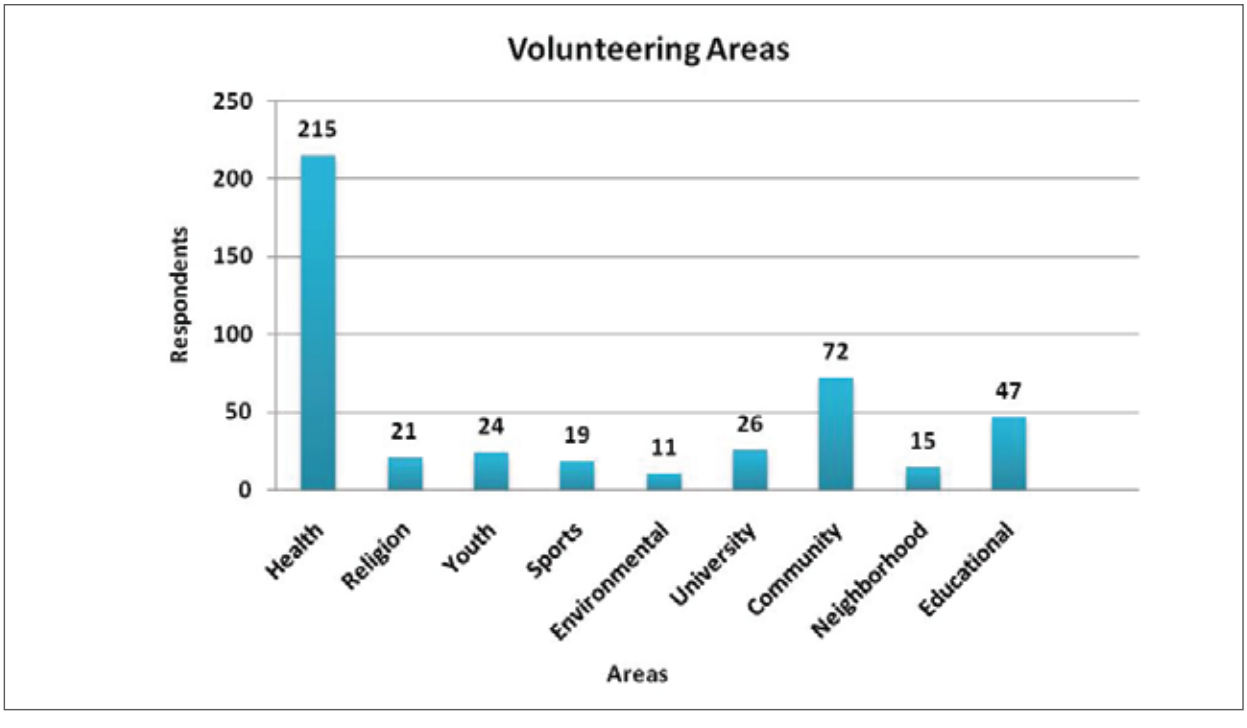
Table 1: Demographic characteristics of the study participants.

Variables	Category	Frequency (n)	Percent (%)
Gender	Male	154	34.2
	Female	296	65.8
Marital Status	Single	408	90.7
	Married	17	3.8
	Engaged	25	5.6
Age (years)	17 -20	179	40
	21 -24	**258**	**57**
	25 -30	13	3
Education	**MBBS**	**306**	**68**
	BDS	144	32
Socioeconomic Status	Upper	57	12.7
	Middle	380	84.4
	Lower	13	2.9
Volunteer	**-**	**286**	**64**
Non Volunteer	-	164	36
Duration of Volunteering	**1-5 months**	**154**	**66.7**
	6-10 months	63	14
	11 -15 months	23	5
	16 -20 months	18	4
	21 -25 months	10	2.22
	over 25 months	04	0.88

According to the findings, the largest respondents constituting 154(66.7%) had been volunteering for 1-5 months and the maximum time engaged in volunteering services among the n=176 respondents was 1-10 hours. The largest volunteer respondents constituted 228(49.4%) were influenced to start volunteering by their friends, whereas 72(42.0%) of the respondents worked as a community volunteer in health and emergency services (Figure 1a, 1b).



(a)



(b)

Figure 1: (a) sources that influence volunteering and (b) areas of volunteering.

The results of the present study showed a significant difference in the motivation, underlying factors, time, and areas and on the contributing factors. The six factors of motivation that lay the foundation for voluntary work includes protective factor, value factor, career factor, social factor, understanding factor and enhancement factor were found to be significant among the participants who participated in the study (Table 2).

Table 2: Variables of the motivation behind volunteerism among medical and dental students of Karachi.

Variables	Mean ± SD	t-test	*p*-Value
Protective factor	22.89 ± 7.49	-1.263	0.053
Value factor	25.92 ± 7.46	-2.872	0.004
Career factor	28.91 ± 7.25	-.948	0.036
Social factor	23.43 ± 7.04	-1.403	0.017
Understanding factor	30.51 ± 7.99	-2.236	<0.01
Enhancement factor	24.58 ± 7.33	-1.401	0.005

DISCUSSION

Medicine is a service-oriented profession that involves professional approaches and a sense of responsiveness and caring. Volunteering offers vital help to people in need, worthwhile causes, and the community, but the benefits can be even greater for the volunteer. This study aimed to examine patterns and attitudes toward volunteering activity among medical students in different institutes of Karachi, Pakistan. We found a high level of volunteerism among medical students who participated in the study. It is generally observed that young people volunteer for different reasons and benefits than older people[17,18].

There is always some motivation behind the act of volunteering. The foremost reason for young people is the opportunity to achieve work-related knowledge, skills, and qualifications that can help them in their education and careers[19]. Medical field is a service related job, which needs professional attitudes, the wisdom of sympathy and cares for the patients[1]. Volunteering activity decreases mortality, improves self-rated health, mental health, life satisfaction, the ability to carry out activities of daily living without functional impairment, social support and interaction, healthy behaviors and the ability to cope with one's illness[5,15]. It is generally observed that health care professionals from developed countries are regular volunteers[20]. Volunteering brings outcomes, or impacts, for students, communities, education institutions, and employers[10,21].

This study represents one of the first attempts to assess the sociodemographic characteristics of volunteerism activity among medical students of Karachi, Pakistan. We collected the data from 450 medical students in different medical colleges out of which 296 were female and 154 were male. In our study, we have attempted to cover all aspects of voluntary work to provide a detailed analysis of voluntary activities. The different categories in the areas of volunteering include health and emergency services, religious organizations, youth (mentor, tutor, coach, and counselor), sports or cultural activities, environmental, university clubs or organizations, community activities, neighborhood, educational and miscellaneous. The age of the respondents was between 18-26 years and the mean age was 20 years. Younger volunteers appear to be more motivated morally[15]. There is accelerated motivation among youth and they love the idea of helping the people in need[22]. Our results suggested that the maximum number of students had been volunteering for 1-5 months and 1-10 hours in the respective time. Medical students usually get semester break, which shows that they must be utilizing this time for some volunteer work. Friends play a major role in our life. Our friends influence us more easily. Volunteering is teamwork and friends together will make a better team. Friends are interlinked, which means interconnecting their opinions, emotions and attitudes are interrelated[23].

According to our study, friends were found to be a motivating factor for volunteering individuals. Medical students are more in contact with the patients and in one-way or another; they give their maximum time in health and emergency services[24]. Volunteering in health and emergency services is easy for them. Volunteering activities can include donation drives for patients who cannot afford their treatment, etc[25]. Our participants were found to be more interested in health and emergency services. This shows their contribution to direct patient care is likely to have substantial impacts on the patient's health and well-being. Time is also a major contributing factor in volunteering activity[24]. In our study 117 of the respondents contributed their time for volunteering activity. Spending time with patients, listen to those who have no one to talk or play with children who are physically or mentally unwell. Volunteering is not just about giving. We do not just give our services but also get so much in return[26] in a bilateral process.

Volunteering provides chances for college students to develop their social media platforms to improve their curricula and increase their personality-confidence through an exchange experience[26]. Volunteering requires skills, time, money and above all passion and courage. Some factors keep a volunteer motivated. Factors include support from family and friends, their profession and the most important factor in their field. One of the biggest motivators for volunteering is the simple pleasure of contributing something valuable to society, wanting to feel supportive[27,28]. In the present study motivation

factors that lay, the foundation for voluntary work including protective factor, value factor, career factor, social factor, understanding factor and enhancement factor were found to be significant. Findings of the present study suggest a more promising approach to promoting future involvement of students in volunteerism and to develop important values like humanitarianism, skills, the goal of gaining career-related experience, social relationships and to address personal problems.

CONCLUSION

Public health policy makers are motivated to encourage healthcare volunteering among the medical and dental students. They should promote a culture of volunteerism among students and explore potential mediators that link the relationship between volunteering and healthcare benefits.

ACKNOWLEDGEMENTS

We are thankful to the participants of government and private medical universities of Karachi for giving us their time and consent. We are also grateful to Jinnah Medical and Dental College for providing us facilities for this study.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICS APPROVAL

Ethical approval was obtained from the Ethical Review Committee (ERC) of Jinnah Medical and Dental College, Karachi, Pakistan.

PARTICIPANTS CONSENT

Verbal and written informed consent was obtained from all participants.

AUTHOR'S CONTRIBUTION

FN and AA did sample collection and wrote the manuscript. SRP and MJN did statistical analysis SE conceived the idea and overall supervised the project and finalized the manuscript. RG helped in sampling and designing of the project.

REFERENCES

1. Yeung JW, Zhang Z, Kim TY. Volunteering and health benefits in general adults: Cumulative effects and forms. BMC Public Health. 2018;18(1):1-8.
2. Mattiullah Butt BH, Kamran Ahmed Soomro. Volunteering activities in developing countries: A study of youth participation in Pakistan. Eur J Business Manag. 2015;7(13):317-326.
3. Stukas AA, Hoye R, Nicholson M, Brown KM, Aisbett L. Motivations to volunteer and their associations with volunteers' well-being. Nonprofit Volunt Sect Q. 2016;45(1):112-132.
4. Malby R, Boyle D, Crilly T. Can Volunteering Help Create Better Health and Care. An evidence review [Internet] London South Bank University, 2017 [cited 2020 Sep 16]. Available from https://openresearch.lsbu.ac.uk/download/
5. Atchley R, Baxter S, Blanchard J, Brady K, Comfort W, Egbert A. Working with seniors: Health, financial and social issues. Denver, CO: SCSA. 2009. p.10.
6. Francis JE. The functions and norms that drive university student volunteering. Int J Nonprofit Volunt Sect Mark. 2011;16(1):1-12.
7. Gage III RL, Thapa B. Volunteer motivations and constraints among college students: Analysis of the volunteer function inventory and leisure constraints models. Nonprofit Volunt Sect Q. 2012;41(3): 405-430.
8. Smith DH. A survey of voluntaristics: Research on the growth of the global, interdisciplinary, socio-behavioral science field and emergent inter-discipline. Voluntaristics Rev. 2016;1(2):1-81.
9. Brauchli R., Peeters, MC, van Steenbergen, EF., Wehner T, Hämmig O. The work–home interface: Linking work-related wellbeing and volunteer work.J Community Appl Soc Psychol, 2017; 27(1), 50-64.
10. Detollenaere J, Willems S, Baert S. Volunteering, income and health. PLoS One. 2017;12(3):e017 3139:1-11.
11. Salt E, Crofford LJ, Segerstrom S. The mediating and moderating effect of volunteering on pain and depression, life purpose, well-being, and physical activity. Pain Manag Nurs. 2017;18(4):243-249.
12. Al Saraidi AS, Awofeso N, Dolan TC. Volunteering in the United Arab Emirates' health system—motivations and challenges. Health. 2020;12(4):334-352.
13. Chacón F, Gutiérrez G, Sauto V, Vecina ML, Pérez A. Volunteer functions inventory: A systematic review. Psicothema. 2017;29(3):306-316.
14. Bauer S, Lim D. Effect of communication practices on volunteer organization identification and retention. Sustain. 2019;11(9):1-17.
15. Klug G, Toner S, Fabisch K, Priebe S. Characteristics and motivations of volunteers providing one-to-one support for people with mental illness: a survey in Austria. Soc Psychiatry Psychiatr Epidemiol. 2018;53(8):841-847.
16. Chacón F, Gutiérrez G, Sauto V, Vecina ML, Pérez A. Volunteer functions inventory: A systematic review. Psicothema. 2017;29(3):306-316.
17. McDougle LM, Greenspan I, Handy F. Generation green: understanding the motivations and mechanisms influencing young adults' environmental volunteering. Int J Nonprofit Volunt Sect Mark. 2011;16(4):325-341.
18. Dean J. Recruiting young volunteers in an area of selective education: A qualitative case study. Br J Sociol Educ. 2016;37(4):643-661.
19. Same A, McBride H, Liddelow C, Mullan B, Harris C. Motivations for volunteering time with older adults: A

qualitative study. Plos One. 2020;15(5):1-13.
20. Chatwin J, Ackers L. Volunteering and overseas placements in the NHS: a survey of current activity. BMJ Open. 2016;6(10):1-7.
21. Holdsworth C, Quinn J. The epistemological challenge of higher education student volunteering:reproductive" or "deconstructive volunteering? Antipode. 2012;44(2):386-405.
22. Schroeder T. Investigating barriers to volunteerism in a medical school volunteer patient program: a program development project. Univ Toronto Med J. 2017;94(2):42-46.
23. Dury S, De Donder L, De Witte N, Buffel T, Jacquet W, Verté D. To volunteer or not: The influence of individual characteristics, resources, and social factors on the likelihood of volunteering by older adults. Nonprofit Volunt Sect Q. 2015;44(6):1107-1128.
24. Tabassum F, Mohan J, Smith P. Association of volunteering with mental well-being: A lifecourse analysis of a national population-based longitudinal study in the UK. BMJ Open. 2016;6(8):1-9.
25. Casiday R, Kinsman E, Fisher C, Bambra C. Volunteering and health: what impact does it really have. London: Volunteer Eng. 2008;9(3):1-3.
26. Aranda M, Zappalà S, Topa G. Motivations for volunteerism, satisfaction, and emotional exhaustion: the moderating effect of volunteers' age. Sustain. 2019;11(16):1-16.
27. Greenfield EA, Marks NF. Continuous participation in voluntary groups as a protective factor for the psychological well-being of adults who develop functional limitations: Evidence from the national survey of families and households. J Gerontol B Psychol Sci Soc Sci. 2007;62(1):S60-S68.
28. Ainsworth, J. Feelings of ownership and volunteering: Examining psychological ownership as a volunteering motivation for nonprofit service organisations. J Retail Consum Serv. 2020;52,e101931.

KAP STUDY

Assessment of Knowledge, Attitudes and Practices towards Nutrition amongst Adolescents in Karachi

Kashaf Aqeel Zaidi[1], Wajeeha Mahmood[1], Farah Ahmad[2]
[1]Student, Ziauddin Medical College, Ziauddin University, [2]Department of Community Health Sciences, Ziauddin Medical College, Ziauddin University, Karachi, Pakistan.

ABSTRACT

Background: The association between health and nutrition are eminent. Quality nutrition is acknowledged as one of the most important determinants of optimal development, good health, and wellbeing. Therefore, the study aimed to determine the knowledge, attitudes, and practices amongst adolescents regarding nutrition.

Methods: It was a cross-sectional study conducted between 2016 to 2017 in Karachi. The sample size was 384; the purposive sampling technique was applied with a target population of adolescents between 14 and 19 years of age. Informed consent was taken before the questionnaire submission. Data were collected via self-administered questionnaires from students of various schools in Karachi. The Chi square test was applied for associations and a *p*-value of <0.05 was considered statistically significant.

Results: The mean age of 394 adolescents was (mean±SD) 17.08±1.63 years and found female predominance 282(71.6%). The average weight of the participants was 58.60 ± 18.58. Protein 305(77.4%) was the most widely consumed food. Breakfast was seen as the most important meal of the day among 213(54.1%) all participants. There was a significant association between healthy dietary habits and the frequency of exercise per week (*p*-value=0.001). Only 57(14.5%) followed a specific meal plan and the mostly 151(38.3%) not following a meal plan was due to lack of time.

Conclusion: Most of the adolescents 87(22.2%) understand the importance of various food nutrients. However, appropriate nutritional practices were not found in the majority of the adolescents, possibly due to the lack of time and sedentary lifestyle.

Keywords: Nutritional Quality; Adolescent Medicine; Non-Communicable Diseases.

Corresponding Author:
Kashaf Aqeel Zaidi
Ziauddin Medical College,
Ziauddin University, Karachi, Pakistan.
Email: kashafazaidi@gmail.com
https://doi.org/10.36283/PJMD10-1/016

INTRODUCTION

Nutrition is the intake of food, according to the body's dietary needs. Good nutrition comprises of an adequate, well balanced diet combined with regular physical activity; these are keystones of good health[1]. Body mass index (BMI) is an important indicator of health and nutrition in individuals and half of the Pakistani population has a BMI within the normal range, which is between 18.5-24.9kg/m^2 compared to South Asian adolescents[2]. Since 22.3% of the Pakistani population comprises adolescents, this makes nutritional awareness amongst adolescents critical to ensure that the young adults of the future have a healthy base[3]. Adolescence is characterized by increased energy and nutrient requirements along with changes in dietary habits, which introduces nutritional problems[4]. The number of overweight/obese adolescents has tripled in the last 30 years, particularly in females[5,6]. This has contributed

to an increase in the prevalence of various conditions including Type II diabetes mellitus, hypertension, and arteriosclerosis[7]. Moreover, micronutrient deficiencies including Vitamin A, Vitamin D, folate, iron and iodine are a major health concern in developing countries[8].

A comparative study has shown a remarkable increase in deranged BMI and associated problems owing to unhealthy eating habits and sedentary lifestyles[6]. According to a study conducted in Pakistan, 17% of the students were underweight, 65% were normal weight and 18% were overweight[9]. Even though breakfast is the most nutritious meal of the day, two studies conducted in Europe and Ireland showed half the study group did not consume breakfast[9]. A study conducted in Karachi identified that 85% of the students were leading a sedentary lifestyle but when they were asked about ways to lose weight, 96% listed exercise among their answers[10,11].

Many studies have been conducted to determine the prevalence and causes of nutritional deficiencies in Pakistan. However, very little research has been conducted to determine the source and accuracy of the nutritional information being delivered to adolescents, which will be an important aspect of this study. Since health education interventions in schools have been successful in reducing levels of obesity amongst adolescents and future risk of non-communicable diseases[12], therefore suitable measures can be taken to improve subsequent influence on health. The study aimed to determine the knowledge, attitudes, and practices amongst adolescents regarding nutrition.

METHODS

This was a cross-sectional study conducted from November 2016-October 2017. The sample size was n=384 based on a prevalence of 50% knowledge regarding nutrition with a bound of error of 1% and a confidence level of 95. The sample was selected using a purposive sampling technique. Institutional ethical approval was taken from Ziauddin University. The inclusion criteria were based on adolescents aged between 14-19 years, currently enrolled in a private school at similar levels of education. Exclusion criteria included those who were suffering from a medical condition that affected their dietary habits or spoke a language other than English.

A self-designed questionnaire was used to collect data. Students from various schools in Karachi were approached for data collection after obtaining consent from the schools. Moreover, consent was obtained from the participants before filling out the questionnaire. Data was entered and analyzed using SPSS version 20. Frequencies and percentages were calculated for categorical variables, mean and standard deviation (SD) was calculated for numerical data. Pearson Chi Square test was used to find associations between various qualitative variables of interest. For all purposes, a *p*-value of <0.05 was considered statistically significant.

RESULTS

The sample population consisted of n=394 adolescents between 14-19 years of age. Mean age of adolescents investigated was 17.08 ± 1.63 years. The sample was predominantly female n=282(71.6%) and the average weight recorded was 58.60 ± 18.58. The average height was 5 feet 6 inches ± 3 inches.

Table 1: Nutritional practices among adolescents

Dietary habits and practices of adolescents				
Most widely consumed food	**Proteins n (%)**	**Grains n (%)**	**Dairy products n (%)**	**Fruits and vegetables n (%)**
	305 77.4	n=205 52.0	n=245 62.2	n=215 54.6
Most important meal of the day	**Breakfast**	**Lunch**	**Dinner**	
	n=213 54.1	n=89 22.6	n=89 22. 6	
How often do you skip breakfast?	**Always**	**Sometimes**	**Never**	
	n=40 10.2	n=137 34.8	n=127 32.2	

Reason for skipping breakfast	**Running late for school**	**Not hungry**	**No help in preparing it**	
	n= 141 35.8	n=112 28.4	n=14 3.6	
Daily water consumption (number of glasses)	**Less than 5**	**At least 5 or more**		
	n=137 34.8	n=257 65.2		
Factors considered when choosing a meal	**Taste**	**Convenience**	**Price**	**Healthy food**
	n=259 65.7	n=35 8.9	n=34 8.6	n=66 16.8
What kind of lifestyle do you have?	**Traditional**	**Sedentary**	**Active**	
	n=152 38.6	n=92 23.4	n=150 38.1	
How often do you exercise per week?	**Everyday**	**Once a week**	**Twice a week**	**Thrice a week or more**
	n=42 10.7	n=87 22.1	n=67 17.0	n= 84 21.3

Proteins were the most widely consumed nutrient in 305(77.4%) participants (Table 1). Only 205(52%) of the adolescents had grains daily. Dairy products were consumed by 245(62.2%) every day. Fruits and vegetables were consumed daily by 215(54.6%) and 191(48.5%) of the population, respectively. Water was one of the most neglected portions of the daily intake in 137(34.8%) of the participants with consumption of fewer than 5 glasses of water every day whereas, 257(65.2%) of the adolescents were drinking at least 5 glasses of water each day.

The majority 222(56.3%) of the participants ate out once a week and most of the people denied eating out very frequently. Some important factors that people consider while choosing a meal were identified. Only 66(16.8%) opted for a health-based meal and 259(65.7%) chose taste as their priority. A small percentage 35(8.9%) and 34(8.6%) of the adolescents listed convenience and price as their utmost priority, respectively.

More than half the sample, 213(54.1%) identified breakfast as the most important. However, 89(22.6%) and 75(19%) considered lunch and dinner as their priority, respectively. Despite understanding the importance of breakfast, only 127(32.2%) were having it every day. On the other hand, 40(10.2%) always skipped breakfast and the majority 137(34.8%) mentioned skipping breakfast sometimes. 141(35.8%) mentioned skipping it because they are running late for school, whereas, 112(28.4%) claimed that they do not feel hungry in the morning.

A decent understanding was found in the adolescent age group regarding physical exercise as 280(71.1%) was exercising. Out of 280(71.1%), the highest percentage of 87(22.1%) was of who preferred exercising once a week. The majority of adolescents 129(32.7%) exercise for about half an hour to 1 hour and restricted themselves to less than half an hour and the rest worked out for more than an hour each time. However, when asked about lifestyles, the majority 152(38.6%) claimed to have a traditional lifestyle and 92(23.4%) sedentary.

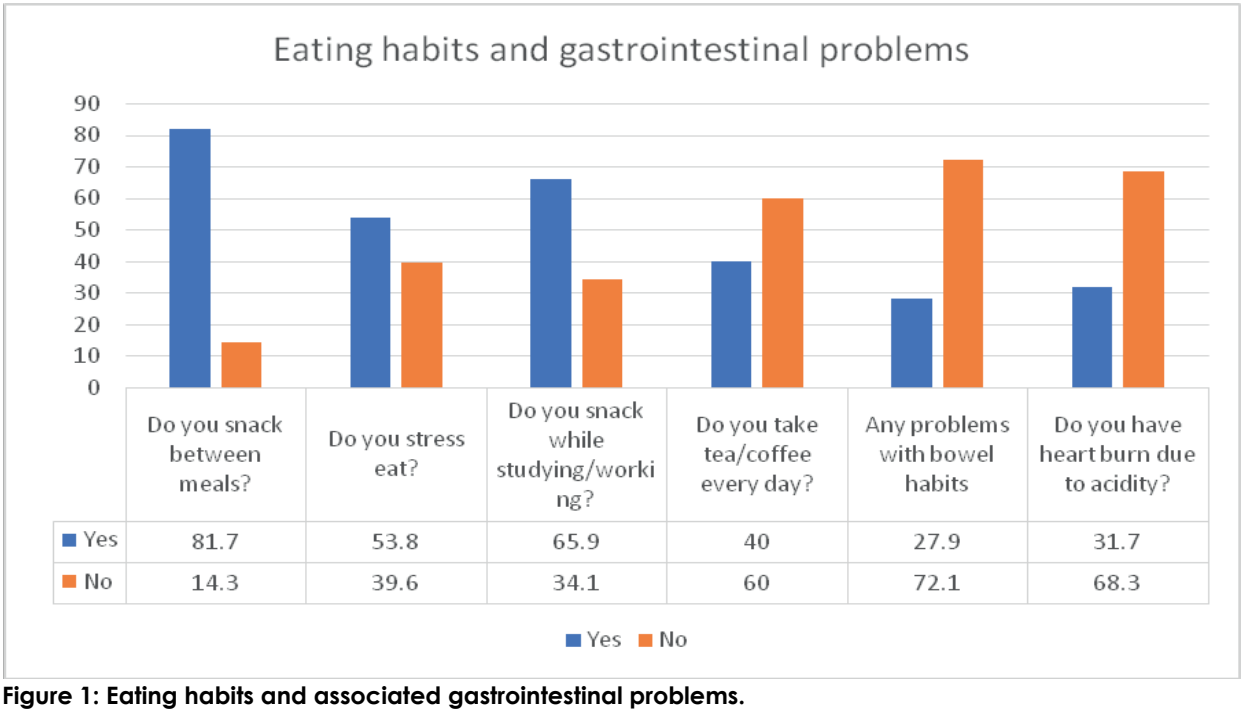


Figure 1: Eating habits and associated gastrointestinal problems.

Among eating habits and health related concerns, snacks, chips, cookies, and fried items were consumed by 117(29.7%), 64(16.2%) and 36(9.1%), respectively (Figure 1). On the other hand, fruits were consumed by 109(27.7%). The majority of the adolescents 125(31.8%) preferred working/studying in the evening which might explain the consumption of snacks. Whereas, 116(29.4%) preferred mornings followed by 93(23.5%) who preferred late night studying. The majority of the adolescents 208(52.9%) were sleeping for 7-8 hours every day. However, 130(32.9%) were getting only 5-6 hours of sleep or less. There was no significant association found between the hours of sleep they get and the level of physical activity.

Table 2: Association of diet, exercise, and medical problems.

How often do you exercise per week?	Do you think your habits are healthy?		*p*-Value
	Healthy	Unhealthy	
Everyday	25	17	0.001
Once a week	34	53	
Twice a week	26	41	
Thrice a week or more	42	42	
Never	30	84	
How often do you skip breakf ast?	**Do you consume snacks?**		
	Consumed	Unconsumed	
Never	104	27	0.438
Sometimes	115	22	
Very often	73	13	
Always	30	10	
How often do you exercise per week?	**Do you have any medical problems?**		
	Medical History	Healthy	
Everyday	9	33	0.484
Once a week	19	68	
Twice a week	11	56	
Thrice a week or more	10	74	
Never	20	94	

A significant association (Table 2) was found between the frequency of physical exercise and the participant perception of whether their dietary habits are healthy or not (*p*-value=0.001). The majority of the people who exercised daily believed they had healthy eating habits. Moreover, there was a rising trend in the perception of healthy habits with the number of days an individual exercised. Moreover, contrary to normal perception, a significant association was not found between snack consumption and the habit of skipping breakfast in adolescents (*p*-value=0.438). The association between exercise and experiencing medical problems was insignificant (*p*-value =0.484).

Following specific meal plans was not a common practice with only 57(14.5%). Amongst those following a plan, 37(9.4%) had made it for themselves, 21(5.3%) was following the one given by their parents and only 5(1.3%) were relying on one provided by a professional dietician. Out of 337(85.5%) of the population that was not following a specific plan, majority 151(38.3%) stated it was due to the lack of time. Moreover, 87(22.2%) stated that they felt healthy with their current style of eating and 99(25.1%) claimed that they have a balanced diet and do not feel the need for a specific diet plan. Most of the participants (Figure 2) correctly identified the functions of Vitamin A, D, C, zinc, sodium, and iron. However, the majority were unable to identify the functions of Vitamin B and K.

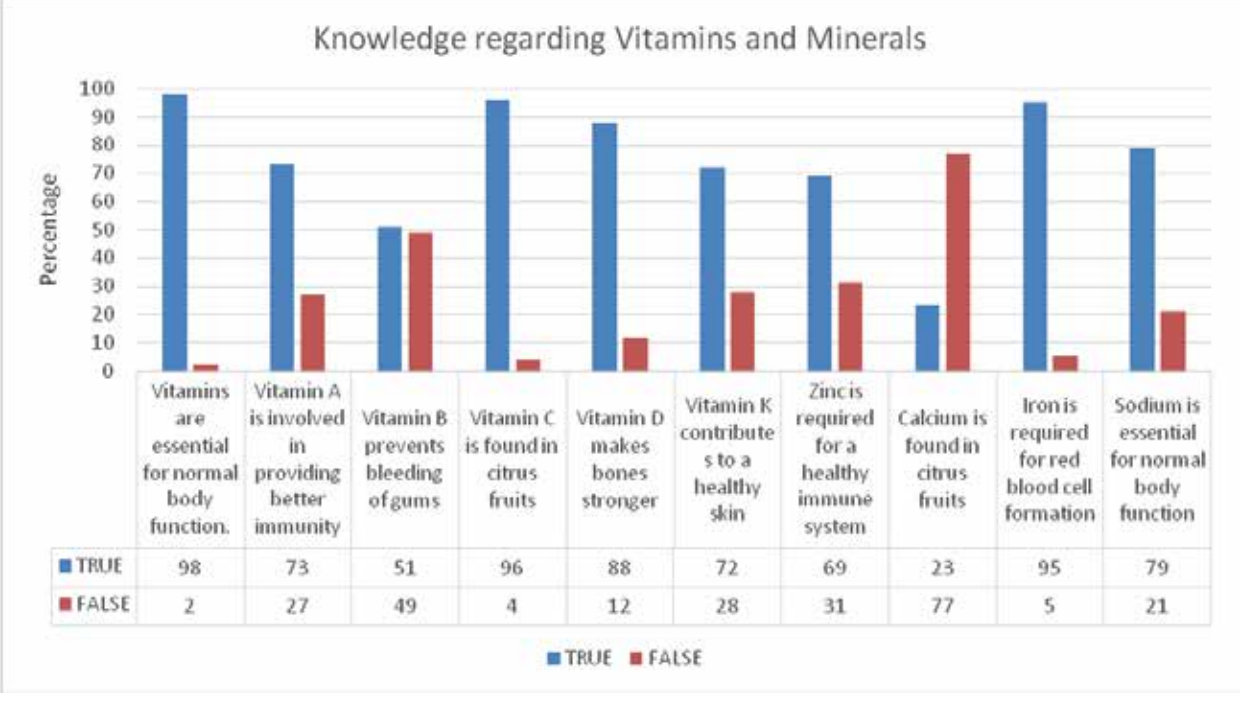


Figure 2: Knowledge of adolescents regarding the functions of vitamins and minerals.

DISCUSSION

Adolescence is a group that requires good nutritional practices as it is the final period of growth and development and it has been identified as a high-risk age group for the development of obesity[13]. Moreover, the eating habits developed at this age are more likely to persist throughout adulthood therefore; interventions in this age group prove to be more effective[14]. Thus, more attention needs to be paid to the adolescent age group to decrease the incidence of non-communicable, chronic diseases in the older population[15]. A study conducted in 2003 in Pakistan amongst the adolescent age group showed 17% people were underweight, 65% were in the normal range, 18% were overweight, and since then extremes of weight have been on a rise[10].

Various factors contribute to the development of eating habits, including family environment, cultural makeup and socioeconomic status of the adolescents[16]. These factors determine the rejection and acceptance of various foods and may even encourage unhealthy eating. Studies have confirmed that intake of fast food is associated with parents who directly attempt to control the dietary habits[17].

Furthermore, eating out was also recognized as a significant health hazard and hence majority (56.3%) ate only once a week and only 6.1% claimed to eat out every day. A study has reported that adolescents consume half the recommended number of fruits and vegetables, less than two-thirds of the recommended amount of milk products and consume more fats and sweets[18]. Our study also revealed similar results and showed dairy products consumption by a majority (62.2%) everyday.

The most consumed food nutrient was proteins as they were regarded as one of the richest sources of energy. Grains, despite being a part of the staple diet in Pakistan, were only consumed by 52% of adolescents. Therefore, emphasis needs to be laid on the consumption of fruits and vegetables. Similarly, a study has shown that there is more reliance on fruit juices and other sweetened beverages and the water was still consumed by the largest percentage of adolescents[19]. Our study is consistent with these findings, 65.2% of the study group was having at least 5 glasses of water a day.

Studies conducted in the West have shown that food choices were primarily based on convenience and hence the widespread availability of rich caloric foods with low nutritional value has led to their increased consumption[20]. Other major barriers identified to healthy eating comprised of cravings for unhealthy, rich food that predominantly contains fats[21]. However, in our study results, 65.7% of participants made food choices based on taste and only 16.8% opting for healthy food.

Studies have shown that adolescents who consume breakfast regularly have lower body fat content and healthy cardiovascular profile[22]. Thus, an attempt was made to understand the youth's opinion on the most important meal of the day, which was correctly identified as breakfast (54.1%). Previous studies have also shown similar results with half the population not consuming breakfast[23]. The most common reason listed for missing breakfast was being late for school or college as opposed to previous literature in which the main reason was not feeling hungry[16].

A significant proportion of the study group was found to be suffering from medical problems related to the alimentary tract, which included irregular bowel movements (27.9%), and chest burn due to acidity (31.7%). These problems occurring at such a young age and are directly associated with unhealthy, eating habits. Hence, there is a need to improve eating habits by providing appropriate knowledge and introducing effective interventions.

In accordance with previous studies, a reasonable understanding was seen amongst adolescents regarding exercise. The majority (71.1%) of the population claimed they were doing physical exercise on a regular basis[9]. However, only 10.9% were exercising every day, this is mainly due to an increased workload from schools and colleges at this age, which leaves lesser time for formal exercise.

Furthermore, sleep is known to have a major impact on the lifestyle of an individual. Studies have revealed that individuals who sleep at least 7 hours are more likely to be active in the hours that they are awake[24]. Our study showed similar results as amongst those who slept 5-6 hours, only 9.2% exercised daily. There was no significant association between the number of sleeping hours and engagement in physical activity. Therefore, interventions are required to inculcate healthy eating habits, encourage physical exercise regularly and improving sleeping habits[25].

According to the results, adolescents exhibited a variable understanding of important vitamins for instance role of Vitamin B and K in bleeding gums and healthy skin, respectively. However, they seemed to have more sound knowledge about various minerals as they correctly identified the roles of zinc, iron, and sodium. Majority of the study group did seem to have above average knowledge but further enhancement of it could improve their dietary practices. Previous studies have shown that the main source of information for adolescents were friends (71%) and television (52%) [14]. Various studies have established that an unhealthy lifestyle has multiple contributory factors to it. This includes the family setup, order of the child amongst siblings, socioeconomic status, frequency of various food groups' consumption and spending time on television and computers, which inevitably reduces time spent on outdoor activities[17].

CONCLUSION

Mostly adolescents understand the importance of food nutrients especially vitamins and minerals. However, appropriate nutritional practices were not found in the majority of the adolescents. This highlights the importance of health education and timely intervention in this age group with particular importance for stress reducing practices.

ACKNOWLEDGEMENTS

We would like to acknowledge Ziauddin University for allowing us to conduct this study.

CONFLICT OF INTEREST

There is no conflict of interest among the authors in this study.

ETHICS APPROVAL

The Ziauddin University granted an institutional approval for the study.

PATIENT CONSENT

There were no patients involved in the study. The study was conducted on healthy adolescents and consent was taken prior to filling of the forms.

AUTHORS' CONTRIBUTION

KAZ designed the questionnaire, collected data, analyzed data, and wrote the manuscript. WM suggested the topic, did literature review and partial analysis. FA supervised the research and helped with sampling and reviewing the manuscript.

REFERENCES

1. Nutrition [Internet]. Who.int. 2020 [cited 18 Sep 2020]. Available from: http://www.who.int/topics/nutrition/en/
2. Khan K, Jameel N, Khalil R, Gul S. Exploring nutritional status, physical activity and body mass index of Pakistani teens. Int Journal Res Med Sci. 2016;4:3563-3569.
3. UNICEF Pakistan [Internet]. Unicef.org. 2020 [cited 18 Sep 2020]. Available from: http://www.unicef.org/infobycountry/pakistan_pakistan_statistics.html
4. Das J, Salam R, Thornburg K, Prentice A, Campisi S, Lassi Z, *et al*. Nutrition in adolescents: physiology, metabolism, and nutritional needs. Ann N York Acad Sci. 2017;1393(1):21-33.
5. Obesity and overweight [Internet]. Who.int. 2020 [cited 18 Sep 2020]. Available from: https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight
6. Cureau F, Sparrenberger K, Bloch K, Ekelund U, Schaan B. Associations of multiple unhealthy lifestyle behaviors with overweight/obesity and abdominal obesity among Brazilian adolescents: A country-wide survey. Nutr Metab Cardiovasc Dis. 2018;28(7):765-774.
7. Bay J, Morton S, Vickers M. Realizing the potential of adolescence to prevent transgenerational conditioning of noncommunicable disease risk: multi-sectoral design frameworks. Healthc. 2016;4(3):39.
8. Mohd FMN, Devi NR, Suraiami M. Vitamin A in health and disease. Vitamin A [Internet]. 2019 [cited 18 Sep 2020]; Available from: https://www.researchgate.net/publication/332551578_Vitamin_A_in_Health_and_Disease
9. Hallström L, Vereecken CA, Labayen I, Ruiz JR, Le Donne C, Garcia MC, *et al*. Breakfast habits among European adolescents and their association with sociodemographic factors: the HELENA (Healthy Lifestyle in Europe by Nutrition in Adolescence) study. Public Health Nutr. 2012.15:1879-1889.
10. Rehman T, Rizvi Z, Siddiqui U, Ahmad S, Sophie A, Siddiqui M, *et al*. Obesity in adolescents of Pakistan. J Pak Med Assoc. 2003;53:315-319.
11. Wignall J. Supporting & promoting adolescent nutritional health equity. Adolesc Nutr. 2020. Springer, Cham. pp. 239-268.
12. Aziz S, Noorulain W, Zaidi UR, Hossain K, Siddiqui IA. Prevalence of overweight and obesity among children and adolescents of affluent schools in Karachi. J Pak Med Assoc. 2009;59(1):35-38.
13. Carrasco J, Gombert M, Carrasco-García Á, Codoñer-Franch P. Adolescent feeding: Nutritional risk factors. J Child Sci. 2018;08(01):e99-e105.
14. Dietz WH. Critical periods in childhood for the development of obesity. Am J Clin Nutr. 1994, 59(5):955-959.
15. Haynos A, Wall M, Chen C, Wang S, Loth K, Neumark-Sztainer D. Patterns of weight control behavior persisting beyond young adulthood: Results from a 15-year longitudinal study. Int J Eat Disord. 2018;51(9):1090-1097.
16. Nirmala A, Kanniammal C, Venkataraman P, Judie A. Predisposing factors associated with obesity among adolescents-A case control study. Biomed Res. 2018;29(18).
17. Kolodinsky J, Battista G, Roche E, Lee B, Johnson R. Estimating the effect of mobility and food choice on obesity in a rural, northern environment. J Transp Geogr. 2017;61:30-39.
18. Lipowska M, Lipowski M, Jurek P, Jankowska A, Pawlicka P. Gender and body-fat status as predictors of parental feeding styles and children's nutritional knowledge, eating habits and behaviours. Int J Environ Res Public Health. 2018;15(5):852-867.
19. Diethelm K, Jankovic N, Moreno LA, Huybrechts I, De Henauw S, De Vriendt T, *et al*. Food intake of European adolescents in the light of different food-based dietary guidelines: results of the HELENA (Healthy Lifestyle in Europe by Nutrition in Adolescence) Study. Public Health Nutr. 2012; 15:386-398.
20. Sui Z, Zheng M, Zhang M, Rangan A. Water and beverage consumption: Analysis of the Australian 2011–2012 national nutrition and physical activity survey. Nutrients. 2016;8(11):678-692.
21. Peltner J, Thiele S. Convenience-based food purchase patterns: identification and associations with dietary quality, sociodemographic factors and attitudes. Public Health Nutr. 2017;21(03):558-570.
22. Tiedje K, Wieland ML, Meiers SJ, Mohamed AA, Formea CM, Ridgeway JL, *et al*. A focus group study of healthy eating knowledge, practices, and barriers among adult and adolescent immigrants and refugees in the United States. Int J Behav Nutr Phys Act. 2014;11(1):63-76.
23. Hallström L, Labayen I, Ruiz JR, Patterson E, Vereecken CA, Breidenassel C, *et al*. Breakfast consumption and CVD risk factors in European adolescents: the HELENA (Healthy Lifestyle in Europe by Nutrition in Adolescence) Study. Public Health Nutr. 2013.16:1296-1305.
24. Ardeshirlarijani E, Namazi N, Jabbari M, Zeinali M, Gerami H, Jalili RB, *et al*. The link between breakfast skipping and overweigh/obesity in children and adolescents: A meta-analysis of observational studies. J Diabetes Metab Disord. 2019:1-8.
25. Gariépy G, Doré I, Whitehead R, Elgar F. More than just sleeping in: a late timing of sleep is associated with health problems and unhealthy behaviours in adolescents. Sleep Med. 2019; 56:66-72.
26. Pereira SM, Power C. Early adulthood determinants of mid-life leisure-time physical inactivity stability and change: Findings from a prospective birth cohort. J Sci Med Sport. 2018;21(7):720-726.

CASE REPORT

Balloon Assisted Endovascular Coiling in Wide Neck Basilar Tip Aneurysm

Inayat Ali Khan[1], Irfan Lutfi[2], Muhammad Ali[3], Atiq Ahmed Khan[4]

[1]Department of Neurosurgery, Ziauddin Medical University and Hospital (North), [2]Department of Interventional Radiology, Ziauddin Medical University and Hospital (North), [3]Department of Radiology, Ziauddin Medical University, [4]Department of Neurosurgery, Dow University of Health Sciences, Karachi, Pakistan.

ABSTRACT

Basilar tip aneurysms are the commonest aneurysms of the posterior circulation and constitute around 5-8% of all cerebral aneurysms. Ruptured basilar tip aneurysms may present with signs and symptoms of subarachnoid hemorrhage (SAH) with sudden, extremely severe headache, nausea and vomiting, stiff neck, blurred or double vision, sensitivity to light, seizure, drooping eyelid and loss of consciousness. At times, the hemorrhage could be fatal, reaching mortality beyond 20%. Basilar tip aneurysms have complex anatomy and have always been considered difficult to treat. Two well-known options to treat basilar tip aneurysms include the microsurgical approach and the endovascular approach. Endovascular treatment is now a cost-effective, preferred and logical rationale for the management of intracranial aneurysms. This case reports the first ever coiling procedure that was performed at Ziauddin Hospital (North Campus) for a basilar tip aneurysm and the other major challenge faced at our center was that the neck was wide, hence requiring a supporting balloon.

Keywords: Aneurysm; Subarachnoid Hemorrhage; Cerebral Aneurysms.

Corresponding Author:
Dr. Inayat Ali Khan
Department of Neurosurgery,
Ziauddin Medical University and Hospital (North),
Karachi, Pakistan.
Email: inayatbrain@yahoo.com
https://doi.org/10.36283/PJMD10-1/017

INTRODUCTION

Basilar tip aneurysms constitute around 5-8% of all intracranial aneurysms[1,2]. Surgical clipping is a difficult and risky option with a high risk of increased morbidity[3,4]. The option of endovascular approach for the treatment of basilar tip aneurysms using detachable coils is a straightforward procedure and does not differ from that of aneurysms in other locations[5,6]. Coiling procedures do have disadvantages like coil migration or compaction of the coil mesh itself [7]. Although the threat of reopening of the lumen may be there, still coiling especially with ballooning would always be the preferred choice[8,9].

CASE REPORT

A 46-year-old male patient was brought to the emergency room (ER) of Ziauddin hospital (North campus) with a history of severe/splitting headache, vomiting, double vision and altered level of consciousness. There was no history of head trauma. The patient was stabilized in the ER and an urgent CT (Computerized tomography) scan of the brain was done which revealed diffuse subarachnoid hemorrhage (SAH) with severe brain edema (Figure 1a).

The family was counseled regarding the chances of a suspected aneurysmal bleed and necessitating an urgent cerebral angiography. Hence, a 4-vessel digital subtraction angiography (DSA) was done which revealed a large wide neck basilar tip aneurysm (Figure 1b and 1c).

The anatomic location and the relationship of the aneurysm to neighboring structures were taken into consideration and the possibilities of clipping versus endovascular coiling were considered. Both options with their relative risks and benefits were discussed

with the family and the coiling option was accepted as the procedure of choice.

This was going to be the first ever coiling procedure for an aneurysm of the posterior circulation; hence, the interventional radiologist (IR) was wary of the challenge with the additional fact that this patient also needed a supporting device (balloon) for the wide neck without which the chances of successful coiling would not have been possible.

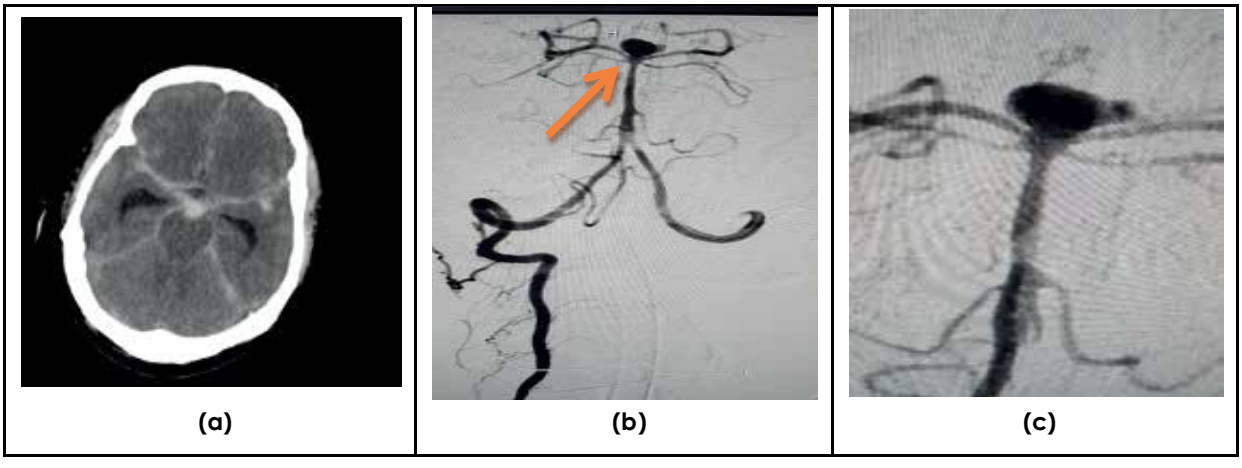


Figure 1a: CT brain showing diffuse subarachnoid hemorrhage (SAH); b: Digital subtraction angiography (DSA) reveals a basilar tip aneurysm (arrow); c: A zoom in image of the aneurysm.

As is already known, coiling of basilar tip aneurysms is not new to the world of neurosurgery and interventional radiology. Coiling of wide neck aneurysms of the posterior circulation have always been a daunting task. Coiling of basilar tip aneurysms (BTAs) is a procedure, which is not new to the world of neurosurgery or the interventional radiologist in general. This procedure (Figure 2a and 2b) was performed for the first time at Ziauddin hospital (North campus); it was a bit more challenging because this aneurysm in particular had a wide neck.

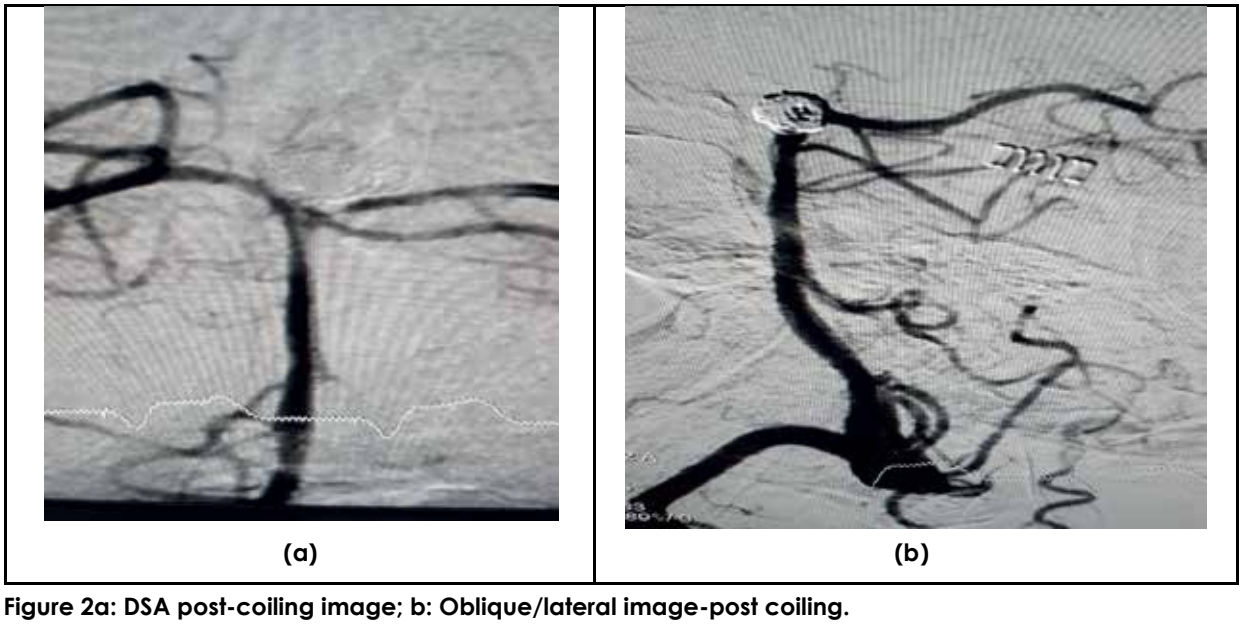


Figure 2a: DSA post-coiling image; b: Oblique/lateral image-post coiling.

It was performed under general anesthesia with an infusion of systemic heparinisation. The objective was to densely pack the aneurysm with coiling such that not a single additional coil could be placed to obtain complete occlusion. Our patient was discharged home on the third post-coiling day in good health and with stable vitals. Hence, a breakthrough at Ziauddin hospital (North) was registered with the first ever coiling of a posterior circulation aneurysm (in this case a basilar tip with a wide neck). The patient is being followed up regularly in the outpatient, fully ambulant, conscious, no symptoms of headache, excellent appetite.

DISCUSSION

The general principles of commonality, risk factors and ruptures that apply to basilar tip aneurysms are similar to the rest of the cerebral aneurysms. The main risk factors for basilar tip aneurysms include sedentary life, high calorie diet, sleeplessness, obesity, hypertension, chronic obstructive pulmonary disease, high cholesterol and atherosclerosis[10]. Basilar tip aneurysms are the commonest aneurysms of the posterior circulation and account for at least 5-8% of all intracranial aneurysms. Clinical findings usually are those associated with SAH, although bitemporal hemianopsia or an oculomotor palsy may occur along with a low Glasgow coma score[2].

Ruptured basilar aneurysms can at times be devastating as far as survival is concerned, they do however present with signs and symptoms of SAH. That may include sudden, extremely severe headache, which at times could be referred to as the "worst headaches of my life" by the patient, nausea and vomiting and blurred, or double vision sensitivity to light, seizure, drooping eyelid, loss of consciousness and confusion. 10% to 43% of patients with SAH report experiencing a sentinel headache in the 2 months preceding the rupture. Ruptured basilar tip aneurysms may result in fatal subarachnoid hemorrhage (SAH) and mortality could be as high as 23% [2].

At our center, diagnostic imaging for suspected aneurysms included CT (computerized tomography) angiography (CTA) and digital subtraction angiography (DSA). Once a SAH is noted on a plain CT scan, DSA becomes the first diagnostic modality of choice. In this particular patient, CT brain was done in the emergency department and after a clear suspicion of SAH secondary to a basilar tip aneurysm rupture, we proceeded for a DSA. Basilar tip aneurysms pose a special challenge. For a long time clipping, the aneurysm surgically had been the only practical solution but came with a price of significant morbidity[5]. However, the introduction of detachable coils has brought new hope to our patients and it has significantly facilitated the management of basilar tip aneurysms[11]. Detachable coils became available in the 1990s and with the arrival of detachable coils, surgical treatment for the same has become almost negligible, the reason being that technically coiling of basilar tip aneurysms is usually not different from that of other locations[10].

Owing to the proximity of the brainstem, difficulty in obtaining adequate exposure and crowding of arteries in this region, surgical clipping for basilar tip aneurysms remains a challenge and the procedure-related mortality and morbidity could be 9% and 19.4% [12]. Surgical techniques (for large and giant aneurysms, even those with wide necks) such as direct clipping under hypothermia with circulatory arrest and bypass surgery are no more serious treatment alternatives for coiling at most centers around the globe[8]. Whenever we talk about a large neck or a wide neck aneurysm, it is obvious that the aneurysm is a giant aneurysm, which was the case in our patient under discussion. Wide necks are always considered as an unfavorable anatomic feature for the plain reason that it becomes even more difficult to obtain satisfactory occlusion. Incomplete occlusions always have the potential of loose packing which may not fully prevent blood flow into the aneurysmal residual sac. Hence coiling of such patients is very challenging because it is not only technically very difficult in fact; coil herniation later could be a possibility[13].

The best way to overcome this difficulty would be to use supporting devices that would prevent the herniation of such coils. Supporting balloons have been available since the 1990s; the use of a supporting device may make the coiling process more complex, with a possibility of increased morbidity. The encouraging fact however is that studies have reported low morbidity for the same procedure when compared to basilar tip aneurysms of all sizes[9]. Still supporting balloons are the present day answer to this problem of imminent threat of coil herniation in the parent artery, the other option being intracranial stents[14].

After successful coiling, a major threat would be rebleeding from a coiled aneurysm and another possibility would be reopening of the aneurysm[15]. Studies however generally show that the vast majority of coiled aneurysms that have been adequately occluded tend to remain adequately occluded even at 6 months follow-up angiography[16]. Now basilar tip aneurysms with a wide neck and a size ≥ 10 mm may reopen late (even after 6 months), to tackle this problem extended follow up imaging may be considered[17].

Imaging follow-up is always an ongoing process. A follow-up imaging is recommended to be done at 6 months after the coiling procedure. This time is appropriate enough to catch up with an early coil herniation or reopening of the aneurysm. Besides the fact that an aneurysm may have been stable and completely occluded, reopening has still been reported after 8 years. Now instead of angiography, the patient can be followed up with a high resolution magnetic resonance angiography (MRA)[18, 19].

CONCLUSION

Coiling of Basilar tip aneurysms is a very effective and safe procedure even with all the anatomic challenges. The major confrontation is when this aneurysm has a wide neck, which may allow coil migration. Hence

ballooning, which helps to hold the coil in place is highly recommended. Balloon assisted coiling is very efficacious with a very low complication rate. Still, such patients need to be followed up by angiography to detect any reopening.

ACKNOWLEDGEMENTS

The authors fully acknowledge the entire team of the hospital that was involved in the special care of patient and facilitated us at all stages of patient management.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

PATIENT CONSENT

Detailed written (high-risk) consent was taken from the family of the patient (as the patient himself was on a ventilator) after fully counseling them about the risks and benefits of the procedure and the possible negative outcome that may follow.

AUTHOR'S CONTRIBUTION

All the authors fully contributed to the critical write-up of this case report.

REFERENCES

1. Brisman JL, Song JK, Newell DW. Cerebral aneurysms. N Eng J Med. 2006;355(9):928-939.
2. Schievink WI, Wijdicks EF, Piepgras DG, Chu CP, O'Fallon WM, Whisnant JP. The poor prognosis of ruptured intracranial aneurysms of the posterior circulation. J Neurosurg. 1995;82(5):791-795.
3. Ge H, Lv X, Jin H, Tian Z, Li Y, He H. The role of endovascular treatment in unruptured basilar tip aneurysms. Interv Neuroradiol. 2017; 23(1): 8-13.
4. Ogilvy CS, Hoh BL, Singer RJ, Putman CM. Clinical and radiographic outcome in the management of posterior circulation aneurysms by use of direct surgical or endovascular techniques. Neurosurg. 2002;51(1):14-22.
5. Lusseveld E, Brilstra EH, Nijssen PC, van Rooij WJ, Sluzewski M, Tulleken CA, *et al*. Endovascular coiling versus neurosurgical clipping in patients with a ruptured basilar tip aneurysm. J Neurol Neurosurg Psychiatry. 2002;73(5):591-593.
6. Sluzewski M, Van Rooij WJ, Beute GN, Nijssen PC. Balloon-assisted coil embolization of intracranial aneurysms: incidence, complications, and angiography results. J Neurosurg. 2006;105(3):396-399.
7. Sluzewski M, van Rooij WJ, Slob MJ, Bescós JO, Slump CH, Wijnalda D. Relation between aneurysm volume, packing, and compaction in 145 cerebral aneurysms treated with coils. Radiol. 2004;231(3): 653-658.
8. Evans JJ, Sekhar LN, Rak R, Stimac D. Bypass grafting and revascularization in the management of posterior circulation aneurysms. Neurosurg. 2004;55(5):1036-1049.
9. Van Rooij WJ, Sluzewski M. Coiling of very large and giant basilar tip aneurysms: midterm clinical and angiographic results. Am J Neuroradiol. 2007;28 (7):1405-1408.
10. International Study of Unruptured Intracranial Aneurysms Investigators. Unruptured intracranial aneurysms—risk of rupture and risks of surgical intervention. N Eng J Med. 1998;339(24):1725-1733.
11. Lozier AP, Connolly Jr ES, Lavine SD, Solomon RA. Guglielmi detachable coil embolization of posterior circulation aneurysms: a systematic review of the literature. Stroke. 2002;33(10):2509-2518.
12. Henkes H, Fischer S, Weber W, Miloslavski E, Felber S, Brew S, *et al*. Endovascular coil occlusion of 1811 intracranial aneurysms: early angiographic and clinical results. Neurosurg. 2004;54(2):268-285.
13. Sluzewski M, Van Rooij WJ, Beute GN, Nijssen PC. Balloon-assisted coil embolization of intracranial aneurysms: incidence, complications, and angiography results. J Neurosurg. 2006;105(3):396-399.
14. Kis B, Weber W, Berlit P, Kühne D. Elective treatment of saccular and broad-necked intracranial aneurysms using a closed-cell nitinol stent (Leo). Neurosurg. 2006;58:443-450.
15. Ferns SP, Sprengers ME, van Rooij WJ, Rinkel GJ, van Rijn JC, Bipat S, *et al*. Coiling of intracranial aneurysms: a systematic review on initial occlusion and reopening and retreatment rates. Stroke.2009; 40:523-529.
16. Sluzewski M, Menovsky T, van Rooij WJ, Wijnalda D. Coiling of very large or giant cerebral aneurysms: long-term clinical and serial angiographic results. Am J Neuroradiol. 2003;24:257-262.
17. Schaafsma JD, Sprengers ME, van Rooij WJ, Sluzewski M, Majoie CB, Wermer MJ, *et al*. Long-term recurrent subarachnoid hemorrhage after adequate coiling versus clipping of ruptured intracranial aneurysms. Stroke. 2009;40:1758-1763.
18. Majoie CB, Sprengers ME, van Rooij WJ, Lavini C, Sluzewski M, van Rijn JC, *et al*. MR angiography at 3T versus digital subtraction angiography in the follow-up of intracranial aneurysms treated with detachable coils. Am J Neuroradiol. 2005;26(6): 1349-1356.
19. Raymond J, Guilbert F, Roy D. Neck-bridge device for endovascular treatment of wide-neck bifurcation aneurysms: initial experience. Radiol. 2001;221:318-326.

CASE REPORT

Angioimmunoblastic T-cell Lymphoma: A Case Report

Sundus Nasim[1], Dua Azim[1], Farheen Malik[1], Faryal Tahir[1], Zehra Ashraf [2], Abdul Qayyum[3]
[1]Dow Medical College, Dow University of Health Sciences, Karachi,[2]Jinnah University for Women, Karachi,
[3]Department for Oncology, Dr. Ziauddin Hospital, Karachi, Pakistan.

ABSTRACT

Angioimmunoblastic T-cell lymphoma (AITL) is a rare, aggressive type of non-Hodgkin's lymphoma (NHL) that is mostly diagnosed in elderly patients. Its non-specific clinical presentation (lymphadenopathy, fever, night sweats, weight loss, generalized rash, and hepatosplenomegaly) often results in a delay in the diagnosis. The diagnosis is mainly established based on a detailed clinical evaluation and biopsy findings, and currently, available treatment options include corticosteroids, immunotherapy, and single- or multi-agent chemotherapy. Here, we report a case of a 61-year-old male who presented with complaints of easy fatigability, dyspnea, and fever along with inguinal lymphadenopathy and was diagnosed as a case of AITL. He was given multiple cycles of R-CHOP chemotherapy (Cytoxan, Hydroxyrubicin, Oncovin, and Prednisone chemotherapy regimen), which led to tumor eradication. The patient, however, expired due to unknown reasons. The case highlights the major diagnostic modalities and treatment strategies for AITL and sheds light on the poor prognosis of the disease despite adequate management.

Keywords: Angioimmunoblastic Lymphadenopathy; Lymphoma; T-Cell; Non-Hodgkin; Chemotherapy.

Corresponding Author:
Dr. Sundus Nasim
Dow Medical College,
Dow University of Health Sciences,
Karachi, Pakistan.
Email: sundusnasim9@gmail.com
https://doi.org/10.36283/PJMD10-1/018

INTRODUCTION

Angioimmunoblastic T-cell lymphoma (AITL) is a rare non-Hodgkin's lymphoma (NHL) in which the follicular T helper (TFH) cells undergo a malignant and aggressive transformation. This particular hematologic malignancy contributes to only 1-2% of all NHLs, and the prevalence of AITL in Pakistan is as low as 0.41%[1,2]. AITL typically presents in the sixth or seventh decade of life with constitutional symptoms, such as weight loss, fever, night sweats, hepatosplenomegaly, lymphadenopathy, and skin rash[3]. With a median 5-year survival of 32%, the disease prognosis is poor. These grim statistics could be due to frequent relapses or delay in the diagnosis of AITL, as most patients usually present at an advanced stage[1]. The present case report aimed to contribute to the knowledge and understanding of AITL's disease course and the development of post-treatment complications. No evidence of drug toxicity contributing to death was witnessed in this case. Thus, despite treatment, the fatal outcome of this case warrants further research about the exact cause of death.

CASE PRESENTATION

A 61-year-old male with type 2 diabetes mellitus presented to the emergency department of Dr. Ziauddin Hospital, Karachi, with complaints of easy fatigability, dyspnea, and low-grade fever accompanied by night sweats. The patient also reported significant weight loss. He had no significant history of cardiovascular disease, smoking, illicit drug intake, or chemical exposure; family history was non-contributory.

Physical examination revealed bilateral wheezes over the chest, unilateral left inguinal lymphadenopathy, and splenomegaly. Initially, pulmonary tuberculosis (TB) with dissemination to inguinal lymph node was suspected; however, the hard consistency of the lymph nodes pointed towards an underlying malignancy.

Complete blood count (CBC) with peripheral blood smear showed microcytic hypochromic anemia, bicytopenia, and hyper-segmented neutrophils. The Mantoux test was negative, ruling out Tuberculosis. Laboratory investigations during the initial hospital visit showed an elevation in the serum alkaline phosphatase (ALP) [160 IU/L, N=39-117 IU/L], beta-2 microglobulin (6.5 mg/L, N=<2.4), and lactate dehydrogenase (276 U/L, N=135-225 U/L). A chest x-ray revealed bilateral pulmonary infiltrates (Figure 1). Bone marrow aspiration was negative for extranodal involvement.

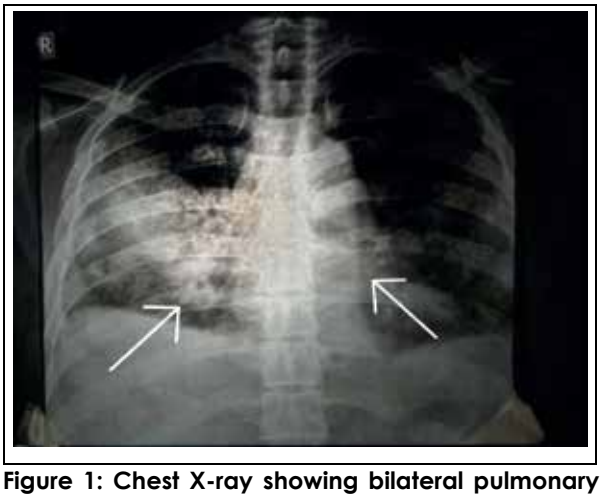


Figure 1: Chest X-ray showing bilateral pulmonary infiltrates.

Inguinal lymph node biopsy exhibited few scattered large cells with abundant cytoplasm, pleomorphic vesicular nuclei and prominent nucleoli. Immunohistochemical stains were positive for CD3 and CD4 in small cells, which highlighted a population of T lymphocytes with elevated Ki-67 (Mib-1). Additionally, stains for CD20 were positive in a few large cells; scattered B-cells also showed positive staining for CD10, CD21, PAX-5, BCL-6, and PD-1. It was thus decided to diagnose this as a case of AITL.

Pre-chemotherapy full-body positron emission tomography/computed tomography scan (PET/CT) showed hypermetabolic lymphadenopathy involving the cervical, supraclavicular, axillary, mediastinal, hilar, abdominal, retroperitoneal, pelvic, and inguinal regions bilaterally (Figure 2A). Axial PET/CT scan of mediastinum revealed hypermetabolic areas in the lungs, along with evidence of bilateral pleural effusion and mediastinal lymphadenopathy (Figure 3A). Overall, the imaging findings were suggestive of a diffuse lymphoproliferative disorder. Subsequently, the patient was started on R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone) chemotherapy, and he attained complete remission after two cycles of R-CHOP therapy. Post-chemotherapy full-body PET/CT scan showed no metabolic activity in the previously noted areas (Figure 2B). The axial PET/CT imaging of mediastinum (Figure 3B) showed no evidence of effusion as well as reduced metabolic activity. These imaging were indicative of a satisfactory response to the prescribed therapeutic regimen; hence, the patient was discharged.

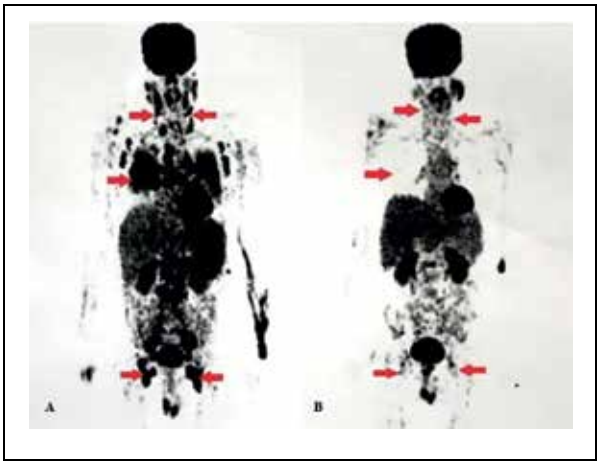


Figure 2: (A) Pre-chemotherapy positron emission tomography/computed tomography scan showing diffuse hypermetabolic activity; (B) Post-chemotherapy positron emission tomography/computed tomography scan showing reduced metabolic activity in previously noted areas.

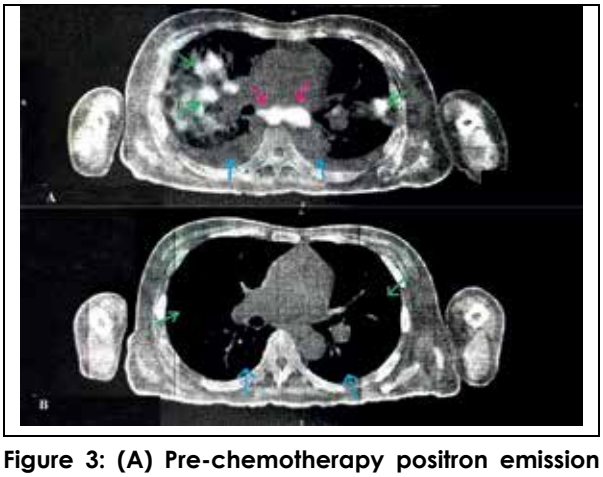


Figure 3: (A) Pre-chemotherapy positron emission tomography/computed tomography scan showing multiple hypermetabolic lesions in lungs (green arrows), bilateral pleural effusion (blue arrows) and mediastinal lymphadenopathy (pink arrows); (B) Post-chemotherapy positron emission tomography/-computed tomography scan showing reduced metabolic activity in previously noted areas (green arrows) with no evidence of pleural effusion (blue arrows).

After two weeks of remission, the patient again reported to the ED with complaints of fever and dyspnea. Laboratory investigation showed pancytopenia with significant leukopenia ($1.1x10^9$/L, N=4.0-10) and thrombocytopenia ($40x10^9$/L, N=150-440). After obtaining blood cultures, the patient received intravenous fluids and empiric antibiotics. Platelets were transfused for his worsening thrombocytopenia, and extensive blood workup was performed to exclude platelet transfusion reaction. Although blood cultures came out negative, the patient continued to deteriorate and eventually developed multiple organ failure (MOF). The patient also developed respiratory distress requiring endotracheal intubation and mechanical ventilation. Despite all possible efforts, the patient expired a week after readmission. The reason for sudden death remains unidentified, despite thorough imaging and laboratory investigations.

DISCUSSION

AITL is a rare hematological malignancy accounting for approximately 1-2% of NHLs, and almost 20% of all peripheral T-cell lymphoma (PTCL) cases diagnosed per year. It classically presents in the elderly with a mean age of sixty-five years. Unlike the other PTCL subtypes, the incidence of AITL is more common in Europe (28.7%) relative to Asia (17.9%) [1]. Diagnosis of AITL depends on the clinical picture, laboratory investigations, and biopsy report. Imaging techniques such as computerized tomography (CT) scan, magnetic resonance imaging (MRI), and positron emission tomography (PET) scan may help in the diagnosis of AITL[3].

The initial clinical presentation is usually ambiguous, which includes generalized lymphadenopathy, skin rash, hepatosplenomegaly, and development of B symptoms such as fever, night sweats, and weight loss. Moreover, autoimmune dysfunction is often the cause of many AITL symptoms, such as arthritis, vasculitis, thyroid abnormalities, autoimmune hemolytic anemia, and thrombocytopenia; these can easily misdirect the diagnosis towards an autoimmune disorder. Thus, prompt diagnosis of AITL is a significant challenge. Literature suggests that cutaneous manifestations, notably skin rash, are present in many cases. This rash resembles the morbilliform rash of measles, but erythematous and maculopapular appearances are not uncommon[3]. However, our patient was devoid of any cutaneous involvement. Furthermore, AITL may also spread to extra nodal sites that typically include bone marrow, spleen, lungs, and skin[4]. Nearly 70% of patients will have bone marrow involvement in the course of the disease[1]. While the patient did present with splenomegaly and pulmonary infiltrates, there was no bone marrow involvement in this case.

Apart from anemia and thrombocytopenia, laboratory investigations also show evidence of hypergammaglobulinemia, a positive Coombs test, elevated serum LDH level, and erythrocyte sedimentation rate (ESR) [1]. On an immunophenotypic analysis, neoplastic T-cells expressed CD3, CD4, and the TFH cell markers, which included CD10, CXCL13, and PD-1. The expression of CD10, BCL6, CXCL13, and PD1 represents an essential adjunct in the diagnosis of AITL, which helps in distinguishing AITL from other PTCLs. The consistent expression of CXCL13 has enhanced diagnostic confidence. Although the expression of these markers is variable, CXCL13 tends to be more specific relative to CD10 [1]. The pathologic markers expressed in this case were CD3, CD4, CD10, CXCL13, PD-1, and BCL-6. In addition, studies suggest that AITL is often associated with Epstein Barr virus (EBV) positive B-lymphocytes and can mimic viral illness[3]. This could be the result of a reactivation of EBV due to extreme immunosuppression. However, in this patient, the B cells were EBV negative.

The treatment of AITL includes corticosteroids, chemotherapy, radiotherapy, and stem cell transplantation[1,3]. Traditionally, both single-agent and combination chemotherapeutic regimens such as CHOP have been used. To this day, CHOP therapy remains the treatment of choice in patients with AITL[1]. Despite various trials, the addition of drugs like rituximab, alemtuzumab, bevacizumab, and belinostat to the CHOP regimen did not result in any therapeutic benefit[5]. In this case, CHOP therapy combined with rituximab (R-CHOP) was the first-line treatment. Following two cycles of R-CHOP therapy, the patient went into complete remission, indicating the efficacy of the said treatment modality.

The majority of the cases of AITL are complicated by superimposed infections rather than progressive lymphoma, since the affected individuals may develop immunosuppression due to both the disease and the chemotherapy treatment[3]. Thus, they are more prone to infections that can potentially cause severe, life-threatening complications and, consequently, death. Disease-specific death is more associated with older age and advanced stages of the disease (stage III and IV) [6].

Additionally, several studies have also identified toxicities secondary to the treatment as a common cause of mortality in patients with AITL. The main adverse effects of CHOP chemotherapy include immunosuppression, infections, cardiotoxicity, and hematological toxicities such as neutropenia and thrombocytopenia[5]. In this case, we did note hematological toxicities following chemotherapy; rituximab (anti-CD20 monoclonal antibody), in particular, has reported side effects of severe infusion-related reactions and thrombocytopenia requiring hospitalization. Apart from hemorrhagic cystitis (ruled out via urinalysis); the side effects of

cyclophosphamide, such as immune suppression and alopecia, were also evident in this case[7]. Doxorubicin can also cause significant cardiotoxicity that might prove fatal[8], but electrocardiography (ECG) and echocardiogram of this patient did not show any evidence of cardiotoxic side effects[8]. The exact cause of death in AITL is unknown; however, evidence reveals respiratory failure, cardiac failure, and infections due to immunosuppression may be significant contributors[9].

Hence, more research is required to establish the long-term safety and therapeutic efficacy of the aforementioned treatment strategies for patients with AITL. The potential success in treating AITL effectively and preventing its fatal complications depends on a thorough understanding of the disease pathophysiology and in developing improved therapeutic strategies with more tolerable side effects by conducting large-scale clinical trials.

CONCLUSION

A careful study of this case led us to conclude that the fatal outcome could be attributed either to the malignancy itself or to the side effects of the chemotherapy. Research that is more clinical is required to reduce the diagnostic delay of AITL and to devise better therapeutic strategies.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the Oncology Department of the Dr. Ziauddin Hospital for facilitating the study.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

PATIENT CONSENT

Patient's family have been informed regarding the study and written consent was taken.

AUTHOR'S CONTRIBUTION

All authors contributed equally in this case study.

REFERENCES

1. Lunning MA, Vose JM. Angioimmunoblastic T-cell lymphoma: the many-faced lymphoma. Blood. 2017;129(9):1095-1102.
2. Shahid R, Gulzar R, Avesi L, Hassan S, Danish F, Mirza T. Immunohistochemical profile of hodgkin and non-hodgkin lymphoma. J Coll Physicians Surg Pak. 2016;26(2):103-107.
3. Iannitto E, Ferreri AJ, Minardi V, Tripodo C, Kreipe HH. Angioimmunoblastic T-cell lymphoma. Crit Rev Oncol Hematol. 2008;68(3):264-271.
4. Mukherjee T, Dutta R, Pramanik S. Aggressive angioimmunoblastic T cell lymphomas (AITL) with soft tissue extranodal mass varied histopathological patterns with peripheral blood, bone marrow, and splenic involvement and review of literature. Indian J Surg Oncol. 2018;9(1):11-14.
5. Broccoli A, Zinzani PL. Angioimmunoblastic T-cell lymphoma. Hematol Oncol Clin North Am. 2017;31 (2):223-238.
6. Ermann DA, Vardell VA, Silberstein PT. Angioimmunoblastic T-cell lymphoma: patient characteristics and survival outcomes. Blood. 2019; 134 (Supplement_1):2194.
7. Webb H, Jaureguiberry G, Dufek S, Tullus K, Bockenhauer D. Cyclophosphamide and rituximab in frequently relapsing/steroid-dependent nephrotic syndrome. Pediatr Nephrol. 2016;31(4):589-594.
8. McGowan JV, Chung R, Maulik A, Piotrowska I, Walker JM, Yellon DM. Anthracycline chemotherapy and cardiotoxicity. Cardiovasc Drugs Ther. 2017;31(1):63-75.
9. Dong H, Jin N, Zhang H, Fan L, Zhan X, Li X. Rapid and fatal progression of Epstein-Bar virus-associated paracortical hyperplasia with T-cell oligoclones to overt angioimmunoblastic T-cell lymphoma: a case report. Int J Clin Exp Med. 2017;10(8):12889-12897.

MEDICAL EDUCATION

Repercussions of COVID-19 on Daily Life Routine and Psychological Attributes of Medical Students towards Online Classes

Akhtar Ali[1], Sehrish Ahmed[2], Syeda Mahnoor[3], Syed Shehryar[3], Sheh Zano[4], Sobia Naseem[5]

[1]Department of Pharmacology, Ziauddin University, [2]Department of Pathology, Ziauddin University, [3]Student, Ziauddin University, [4]Department of Biochemistry, Ziauddin University, Karachi, [5]Department of Oral and Maxillofacial Surgery, Jinnah Postgraduate Medical Centre, Karachi, Pakistan.

ABSTRACT

Background: The novel coronavirus disease or Covid-19 is caused by a virus of a strain named Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Countries enacted a strict self-isolation order as the authorities seek to clamp down on the coronavirus pandemic that has led to severe socio-economic disruptions to minimize close contact between individuals. One of them is the concept of work from home and the continuation of studies through online sessions. The study aimed to examine the psychological health of medical students in this quarantine period and the effect of this on their daily life activities and their attitude towards online classes.

Methods: It was a cross sectional study conducted in the Clifton campus of Ziauddin University Karachiin April 2020. Medical students studying in 1st, 2nd and 3rd year MBBS were included in the study. The total calculated sample size was n=105 however, 182 responses were received and data were analyzed. To assess psychological health depression, anxiety and stress scale (DASS) was used among study participants. In order to identify its effects on daily routine and attitude self-administered questionnaires were formulated. Data were analyzed using SPSS version 20.

Results: The DASS scale analysis showed that most of the students belonging to different educational years scored in severe or extremely severe depression 55(30.2%), anxiety 56(30.8%) and severe stress 32(24.2%) levels according to DASS criteria due to COVID-19. The current pandemic has affected their daily life routine however, 111(61%) favored the decision of conducting online classes.

Conclusion: The current situation has affected medical students psychologically and their daily life routine, students have shown a positive response to online sessions being delivered to them.

Keywords: Medical Students; Psychological Health; Daily Routine; Online Classes.

Corresponding Author:
Dr. Akhtar Ali
Department of Pharmacology,
Ziauddin University, Karachi, Pakistan.
Email: akhtar.ali@zu.edu.pk
https://doi.org/10.36283/PJMD10-1/019

INTRODUCTION

The coronavirus pandemic is a world-shattering event whose long last consequences we can only begin to fathom. The novel coronavirus disease called Covid-19 is caused by a virus named SARS-CoV-2[1]. This disease has expanded to touch nearly every corner of the globe since it first emerged at the beginning of the year in Wuhan, a city of China[2]. More than 1,013,000 people are known to be infected and more than 54,100 deaths have been recorded. In Pakistan, many confirmed cases have been reported to date [3]. With inadvertently rising numbers of confirmed cases of COVID-19 throughout the globe, the World Health Organization has declared the virus a global health emergency. Radical healthcare and preventive measures are advised for flattening the curve[4,5].

This pandemic [6] has affected every field of life. Countries enacted a strict self-isolation order as the authorities seek to clamp down on the coronavirus

pandemic that has led to severe socioeconomic disruptions, postponement or cancelation of cultural, religious and sports events globally, with methods included to minimize close contact between individuals[7]. These extreme measures are taken to stop or slow down the spread of the disease, implementing the concept of social and physical distancing. Mere closing of schools and universities in more than 160 countries has affected more than 1.5 billion students worldwide. The closure of malls and shopping centers has also resulted in panic buying[8]. Mental disorders especially depression and anxiety have received increasing global attention because of their negative effects on working ability and the performance of people[9]. Infectious disease pandemic aggravates this health issues[10]. During the SARS outbreak, it was estimated that 29% of those quarantined showed signs of Post Traumatic Stress Disorder, and 31% had symptoms of depression following isolation[11]. Similarly, the emergence of covid19 has caused serious unrest and upsurge the wave of anxiety globally[12].

Although quarantine measures have periodically been used for centuries to contain and control the spread of infectious diseases such as cholera, plague, SARS with some success[13]. But the history of invoking these measures is tarnished by threats, generalized fear, lack of understanding, discrimination, economic hardships, and rebellion[14]. Medical students have far higher rates of depression than the average person, their depression prevalence ranges from 9%-56%. According to a meta-analysis, 27% of medical students had depression or symptoms of it in nearly 200 studies of 129,000 medical students in 47 countries[15]. This may influence the student's health and quality of life[16-18]. With this devastating lock down many strategies have also been adopted to minimize the damage and carry on with the work in life. One of them is the concept of work from home and the continuation of studies through online sessions[19]. Based on the above findings, this study aimed to examine the psychological health of medical students in this quarantine period and the effect of this on their daily life activities and their attitude towards online classes.

METHODS

It was a cross sectional study conducted in Clifton campus of Ziauddin University Karachi from 1st to 30thApril 2020. Medical students studying in 1st, 2nd and 3rd year MBBS were included in the study. Ethical approval was taken from ERC of Ziauddin University (reference code 2400720AAPHA). The total calculated sample size was n =105, it was calculated by using a 50% proportion of the selected population. Non-probability Consecutive sampling technique was used to recruit the participants. To assess psychological health depression, anxiety and stress scale (DASS) was used among study participants. In order to identify its effects on daily routine and attitude self-administered questionnaires were formulated and which was pilot validated.

Initially, students were asked for their consent for participation in the study followed by demographic data, DASS and further questionnaires. The questionnaire was sent to students using a Google form link and responses were recorded. Total 184 responses were received in 10 days of link sharing out of the 2 participants who denied sharing the information and data was analyzed for n=182. Data were analyzed using SPSS version 20. Depression, anxiety and stress were identified using guidelines of DASS. Frequency and percentages were calculated to correlate the effects on the daily routine of medical students and attitude.

RESULTS

The participants of our study (100%) belonged to the age group 18-23 years, among them 66(36.37%) were males and 116(63.7%) were females. From 1st year MBBS 95(52.1%), 2nd year 39(21.4%) and 3rd year 48(26.4) students submitted the proforma. The DASS scale analysis showed that most of the students belonging to different educational years scored in severe or extremely severe depression 55(30.2%), anxiety 56(30.8%) and severe stress 32(24.2%) levels according to DASS criteria due to COVID-19 (Table 1).

Table 1: Responses of medical students according to depression, anxiety and stress scale (DASS) criteria.

Variables	Categories				
	Normal	Mild	Moderate	Severe	Extremely Severe
Depression	27 (14.8%)	26 (14.3%)	43 (23.6%)	29 (15.9%)	55 (30.2%)
Anxiety	29 (15.9%)	26 (14.3%)	29 (15.9%)	41 (22.5)	56 (30.8%)
Stress	41 (22.5%)	29 (15.9%)	35 (19.2%)	44 (24.2%)	32 (17.6%)

In order to assess the effect of covid-19 on the daily routine of medical students they were enquired about their activities during lockdown through a survey asking them whether a situation applied to them andto what degree. Most of the students replied that they are not waking early in the morning 79(43.4%), the majority of students were spending their time on social media applications 65(35.7%) and majorly favored the idea of sharing recorded lectures (Table 2).

Table 2: Daily routine of Medical students during COVID-19 lockdown.

Statement	Strongly Disagree	Disagree	Agree	Strongly Agree
1. I am waking up early during lockdown	79 (43.4%)	40 (22%)	33 (18%)	28 (15%)
2. I spend this lock down as vacations	44 (24.2%)	60 (33%)	44 (24.2%)	33 (18%)
3. I utilize time in watching movies	44 (24.2%)	65 (35.7%)	44 (24.2%)	28 (15.4%)
4. I read novels/magazines	83 (45.6%)	59 (32.4%)	29 (15.9%)	10 (5.5%)
5. I use social media apps mostly during lockdown	15 (8.2%)	39 (21.4%)	62 (34.1%)	65 (35.7%)
6. I read books related to my education year	20 (11%)	68 (37.4%)	63 (34.6%)	29 (15.9%)
7. I visit my friends / relatives during lockdown	148 (81.3%)	26 (14.3%)	6 (3.3%)	1 (0.5%)
8. I perform exercise in a routine fashion	61 (33.5%)	65 (30%)	40 (22%)	24 (13.2%)
9. I feel like my eating habits are affected during lockdown	35 (19.2%)	37 (20.3%)	57 (31.3%)	52 (28.6%)
10. I used to order food from outside quite often during lockdown	62 (34.1%)	46 (25.3%)	32 (17.6%)	41 (22.5%)
11. I find my sleeping pattern has been disturbed	19 (10.4%)	29(15.9%)	34 (18.7%)	99 (54.4%)
12. I use my free time to complete my assignments	38 (20.9%)	67 (36.8%)	42 (23.1%)	34 (18.7%)
13. I use my time in for my hobbies	26 (14.35)	69 (3 7.9%)	45 (24.7%)	40 (22%)
14. I use my time in playing computer games	97 (53.3%)	46 (25.3%)	28 (15.4%)	9 (4.9%)
15. I find myself often bored with nothing to do.	32 (17.6%)	51 (28.0%)	47 (25.8%0	51 (28%)

The attitude of medical students towards online classes during lockdown fifteen statements was asked and they were supposed to mark as strongly agree, agree, disagree and strongly disagree. Students favored the decision of continuing medical education online 111(61%) however; they reported that it is difficult to concentrate during online sessions. Many students 88(48.4%) (Table 3) found it difficult to ask questions during online sessions.

Table 3: Attitude of medical students towards online classes.

Statement	Strongly Agree	Agree	Disagree	Strongly Disagree
1. I favor the decision of online classes	33 (18.1%)	111 (61%)	30 (16.5%)	7 (3.8%)
2. It is a good way to deliver professional education online	23 (12.6%)	95 (52.2%)	54 (29.7%)	9 (4.9%)
3. Online sessions are helpful in creating our concepts	9 (4 .9%)	75 (41.2%)	75 (41.2%)	22 (12.1%)
4. It is helpful as the course will be completed in time	47 (25.8%)	78 (42.9%)	39 (21.4%)	16 (8.8%)
5. The supplemental online resource material available online is better than the traditional classroom one	27 (14.8 %)	47 (25.8%)	85 (46.7%)	19 (10.4%)
6. The facilitators are well trained for online sessions	15 (82%)	90 (49.5%)	64 (35.2%)	11 (6%)
7. I like the idea of not having to derive to school	58 (31.9%)	29 (15.9%)	62 (34.1%)	31 (17%)
8. I believe that high quality learning can take place without face-to-face interaction	18 (9.9%)	58 (31.9%)	70 (38.5%)	34 (18.7%)
9. I like the idea of flexibility in time and space in recorded lectures	73 (40.1%	89 (48.9%)	14 97.7%	(2.7%)
10. Learning at home does not give satisfactory feeling	38 (20.9%)	69 (37.9%)	65 (35.7%)	9 (4.9%)
11. Asking questions is difficult in online sessions	60 (33%)	88 (48.4)	25 (13.7%)	8 (4.4%)
12. I find it depressing to focus on the medical education	51 (28%)	64 (35.2%)	55 (30.2%)	11 (6%)
13. I attend the sessions just for the sake of attendance	28 (15.4%)	50 (27.5%	84 (46.2%)	19 (10.4%
14. I find it hard to concentrate in online sessions	64 (35.2%)	69 (37.9%)	43 (23.6%)	5 (2.7%)
15. As it was off for all other fields of life, scheduling online classes is not a good idea	10 (5.5%)	53 (29.1%)	87 (47.8%)	31 (17%)

DISCUSSION

Psychological morbidity in medical undergraduate students had been of great concern in the past few years due to an increase in the number of private medical schools all around the world. This has further been aggravated by a Covid-19 pandemic. Our study showed a prevalence of depression (30.2%). This survey indicated that 30.8% of college students were afflicted with severe anxiety because of the COVID-19. It results in an unintended pause in student's education due to global university closure. Similar to our study, significant psychological symptoms related to anxiety, stress, depression in university students have already been pointed out[20], and it is reported to get aggravated due to the current pandemic[21]. The anxiety of these students about COVID-19 might have been related to the effect of the virus on their educational studies[22]. It is not in accordance with Cao et al. in china who reported the same finding in their survey was conducted in China[21]. The findings of our study regarding stress highlighted that 24.2% of medical students suffering from severe stress during current situations are similar as reported in different studies across the globe[23].

Considering the depressed state of mind of the students, we measure the effect on their daily routine and their response towards the online sessions that are arranged by their respective institutes 35.7% of the respondents choose that they have started using social media apps during the lockdown. Most of the respondents (81.3%) did not visit their friends or relatives during the lockdown period which is in accordance with Balkhi et al. In addition, 31.3% of students feel that their eating habits are affected by lockdown and 34.1% of students responded they did not order food from outside during this time[24]. This might be because of the awareness that covid-19 affects mostly those who are not in a better state of health. Thus, 54.4% students' sleep pattern has been changed; it is in agreement to the study conducted in Italy in which it has been reported that the sleep difficulties were stronger for people with a higher level of depression, anxiety, and stress symptoms[25].

More than half of the students (53.3%) indulge in playing computer/console games. Playing video games has also been recommended by the WHO to prevent the spread of the COVID-19 pandemic[20]. Since, 28% of students felt bored because they had nothing to do during the lockdown period. This boredom could further aggravate the anxiety. Colleges shut across the world due to covid-19 have resulted in fast-paced shifts from face-to-face instruction to online environments. In our study, mixed results have been reported, 61% of respondents favor the decision of online classes taken by my medical school.

Half of the respondents agree that online education is a good way to deliver knowledge of professional education. Another important factor to consider, in terms of the effectiveness of online units, is student retention. There has been limited research regarding student attrition/retention in online psychology courses literature shows that higher rates of attrition are typically observed in online courses compared to face-to-face learning in a classroom[29]. In this study, 38.5% students disagreed and 18.7% strongly disagreed that high quality learning can take place without face to face interaction. However, Bowers et al. suggested that "carefully designed interactions, faculty student contact and ongoing instructor feedback" are critical for student retention[26].

In addition, 37.9% of respondents in this study found it hard to retain during online classes. The reason could be related to students' low perceived sense of connectedness and a perceived lack of instructor presence. However, equal responses (41.2% agreed and 41.2% have disagreed) came on online sessions helping create concepts. Furthermore, 35.2% of students found it depressing to focus on medical education during the current pandemic time. Due to the energy crisis (electricity issues), it is difficult in Pakistan to have persistent internet connectivity and continuous connection live interaction on online classes so students favored the decision of uploading recorded lectures to have access at any time. This could be because colleges have promptly implemented online educational activities; they were insufficient to ease students' minds in such uncertain times. Thus, if these students also consider themselves vulnerable to developing emotional disorders, institutions will need to implement prevention and intervention programs to mitigate the stress levels.

CONCLUSION

Covid-19, online classes, lockdown and social distancing have changed the medical student's daily life routine. Students' response regarding online classes is highly appreciable, as they have shown their concern and responsibility in continuing their medical education during the current pandemic. Moreover, the current situation has also affected their psychological health and responsible for the development of depression, anxiety and stress among medical students.

ACKNOWLEDGMENTS

We are thankful to all participants who participated in our study voluntarily. We also appreciate the efforts and valuable suggestions of Dr. Saeeda Baig (Head of the Biochemistry Department), Dr. Shehla Shaheen and Nisha Zahid (Department of Pharmacology).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICS APPROVAL

ERC approval was taken from Ziauddin University and ethics reference code 2400720AAPHA was issued.

PARTICIPANT CONSENT

Consent was taken prior to participation and it was allowed to participants that if they agreed on consent then there was access to other questions.

AUTHORS' CONTRIBUTION

AA did the concept of study, data analysis, drafting, and finalizing of the results. SA critically reviewed the article. Finally reviewed and approved by SM. Data collection and session organization was facilitated by SZ, and SS, assisted by SN. All authors read and approved the final manuscript.

REFERENCES

1. Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, *et al*. A novel coronavirus from patients with pneumonia in China, 2019. N Engl J Med. 2020. 382;8:727-733.
2. Lu R, Zhao X, Li J, Niu P, Yang B, Wu H, *et al*. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. Lancet. 2020;395(10224):565-574.
3. World Health Organization. Coronavirus disease (COVID-2019) situation reports [Internet]. World Health Organization, 2020 [cited 2020 Oct 18]. Available from: https:// www.who.int/emergencies/diseases/novel-coronavirus-2019/situationreports.
4. World Health Organization. Statement on the second meeting of the International Health Regulations (2005) [Internet]. Emergency Committee regarding the outbreak of novel coronavirus (2019-nCoV); 2020 [cited 2020 Oct 20]. Available from: https://www.who.int/news-room/detail/30-01-2020-statement-on-the-second-meeting-of-the-international-healthregulations-(2005)-emergency-committee-regarding-the-outbreak-of-novelcoronavirus-(2019-ncov).
5. Anderson RM, Heesterbeek H, Klinkenberg D, Hollingsworth TD. How will country-based mitigation measures influence the course of the COVID-19 epidemic? Lancet. 2020;395(10228):931-934.
6. Wilder-Smith A, Freedman DO. Isolation, quarantine, social distancing and community containment: pivotal role for old-style public health measures in the novel coronavirus (2019-nCoV) outbreak. J Travel Med. 2020;27(2):1-4.
7. BBC Coronavirus: which countries have evacuated their citizens? 2020 [cited 2020 Oct 20]. Available from: https://www.bbc.co.uk/news/world-asia-china-51312378
8. Mokhtar F, Gross S. Should schools close to fight virus? These places say no. 2020 [cited 2020 Oct 25]. Available from: https://www.bloomberg.com/news/articles/2020-03-27/should-schools-close-to-fight-virus-these-countries-say-no
9. Jack A. Why the panic? South Korea's MERS response questioned. BMJ. 2015:350: h3403.
10. Brooks SK, Webster RK, Smith LE, Woodland L, Wessely S, Greenberg N, *et al*. The psychological impact of quarantine and how to reduce it: rapid review of the evidence. The Lancet. 2020;395 (10227):14-20.
11. Hawryluck L, Gold WL, Robinson S, Pogorski S, Galea S, Styra R. SARS control and psychological effects of quarantine, Toronto, Canada. Emerg Infect Dis. 2004;10(7): 10(7): 1206-1212.
12. Bao Y, Sun Y, Meng S, Shi J, Lu L. nCoV epidemic: address mental health care to empower society. Lancet. 2020;395(10224): e37-e38.
13. Toronto Public Health. Severe acute respiratory syndrome (SARS), 2003 [cited 2020 Oct 30]. Available from: http://www.toronto.ca/ health
14. Mandavilli A. SARS epidemic unmasks age-old quarantine conundrum. Nat Med. 2003;9:487.
15. Rotenstein LS, Ramos MA, Torre M, Segal JB, Peluso MJ, Guille C, *et al*. Prevalence of depression, depressive symptoms, and suicidal ideation among medical students: A systematic review and meta-analysis. JAMA. 2016;316(21):2214-2236.
16. Moutinho IL, Maddalena ND, Roland RK, Lucchetti AL, Tibiriçá SH, Ezequiel OD, *et al*. Depression, stress and anxiety in medical students: A cross-sectional comparison between students from different semesters. Rev Assoc Med Bras. 2017;63(1):21-28.
17. Piumatti G. Motivation, health-related lifestyles and depression among university students: a longitudinal analysis. Psychiatry Res. 2018;260:412-417.
18. Auerbach RP, Alonso J, Axinn WG, Cuijpers P, Ebert DD, Green JG, *et al*. Mental disorders among college students in the World Health Organization world mental health surveys. Psychol Med. 2016;46 (14):2955-2970.
19. Wang C, Pan R, Wan X, Tan Y, Xu L, Ho CS, *et al*. Immediate psychological responses and associated factors during the initial stage of the 2019 coronavirus disease (COVID-19) epidemic among the general population in China. Int J Environ Res Public Health. 2020;17(5):1-25.
20. Cornine A. Reducing nursing student anxiety in the clinical setting: An integrative review. Nurs Educ Perspect. 2020;41(4):229-234.
21. Cao W, Fang Z, Hou G, Han M, Xu X, Dong J, *et al*. The psychological impact of the COVID-19 epidemic on college students in China. Psychiatry Res. 2020:e112934.
22. Odriozola-González P, Planchuelo-Gómez Á, Irurtia MJ, de Luis-García R. Psychological effects of the COVID-19 outbreak and lockdown among students and workers of a Spanish university. Psychiatry Res. 2020:e113108.
23. Vahedian-Azimi A, Moayed MS, Rahimibashar F, Shojaei S, Ashtari S, Pourhoseingholi MA. Compare

the severity of psychological distress among four groups of Iranian society in COVID-19 pandemic. BMC Psychiatry. 2020;20:1-7.
24. Balkhi F, Nasir A, Zehra A, Riaz R. Psychological and Behavioral Response to the Coronavirus (COVID-19) Pandemic. Cureus. 2020;12(5) : e7923.
25. Cellini N, Canale N, Mioni G, Costa S. Changes in sleep pattern, sense of time and digital media use during COVID-19 lockdown in Italy. J Sleep Res. 2020:1-5.
26. Bowers J, Kumar P. Students' perceptions of teaching and social presence: A comparative analysis of face-to-face and online learning environments. Int J Web-Based Learn Teach Technol. 2015;10(1):27-44.

LETTER TO EDITOR

Effect of COVID-19 on Undergraduate Medical Education: A Letter from Pakistan

Muhammad Fazal Hussain Qureshi, Muzna Shah, Mahira Lakhani
Student, Ziauddin University, Karachi, Pakistan.
https://doi.org/10.36283/PJMD10-1/020

Dear Editor,

First identified in Wuhan, China, on December 31 2019, coronavirus spread like bush fire. The WHO declared it as a Global Pandemic on March 11 2021. COVID-19 was termed novel based on newly identified pathogens, thus leading to a state of uncertainty regarding treatment and prevention[1]. This virus can present with an array of symptoms; dry cough, breathlessness, chest pain, myalgia, and diarrhea[2]. Undergraduate medical education has shifted to virtual learning systems to halt the transmission of the virus. This letter elaborates on the challenges faced and the impact of COVID-19 on the life of undergraduate medical students.

From the students to the working healthcare professionals, the coronavirus pandemic has affected the medical community the hardest; Clinical rotations were canceled with little or no warning while pre-clinical students moved to online classes[3]. The stress and uncertainty during this time are taking their toll on our psychological wellbeing[4]. Doctors are striving to deliver the same level of education while also battling the pandemic; exploring different ways of imparting knowledge as well as practical skills. While continuing with their education, medical students are helping combat this virus through volunteering their time at hospitals and with research.

Amidst this pandemic, the world is struggling to adapt to this new normal. Drastic changes have been brought to the medical education system as students now have online classes and clinical training. Although it is difficult to deliver information in this manner, online education has brought with it its advantages. Students save up on time, money, and energy, which can be put towards assignments and research projects. Online forums are an excellent way to prompt discussion amongst students as they pour in their opinions. Recorded lectures can be reviewed later for reinforcement. However, limited patient interaction has its challenges. Regardless, this adaptation over traditional university learning is safe and effective.

ACKNOWLEDGEMENTS

We appreciate the support of the Ziauddin University, Karachi.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTION

All authors contributed equally and approved the final manuscript.

REFERENCES

1. European Centre for Disease Prevention and Control [Internet] Coronavirus disease 2019 (COVID-19) pandemic: increased transmission in the EU/EEA and the UK–seventh update; 2020 [cited 2020 Oct 18]. Available from: https://www.ecdc.europa.eu/sites/default/files/documents/RRA-seventh-update-Outbreak-of-coronavirus-disease-COVID-19.pdf
2. Backer JA, Klinkenberg D, Wallinga J. Incubation period of 2019 novel coronavirus (2019-nCoV) infections among travellers from Wuhan, China, 20–28 January 2020. Euro Surveill. 2020;25(5):2000062.
3. Sandhu P, de Wolf M. The impact of COVID-19 on the undergraduate medical curriculum. Med Educ Online. 2020;25(1):1-3.
4. de Oliveira Araújo FJ, de Lima LS, Cidade PI, Nobre CB, Neto ML. Impact of Sars-Cov-2 And its reverberation in global higher education and mental health. Psychiatry Res. 2020:112977.

Corresponding Author:
Muhammad Fazal Hussain Qureshi
Ziauddin University,
Karachi, Pakistan.
Email: fazalhqureshi22@gmail.com

JOURNAL GUIDELINES

INSTRUCTIONS TO AUTHORS

Authors should consult the journal's instructions to authors before submitting their manuscripts. The manuscripts should be emailed on journal's address in the form of one document with tables and figures embedded in the text. The full manuscript has to be submitted online via email at pjmd@zu.edu.pk and http://ojs.zu.edu.pk/ojs/index.php/pjmd

PEPARATION OF MANUSCRIPTS

Manuscript should be typed in times roman font size 12, using double spacing throughout, including the title page, abstract, text, references, individual tables, and legends. This is a quick guidance to authors instruction, for detail information of declarations, acknowledgments, author's contribution, ethical approval visit our website https://pjmd.zu.edu.pk/instructions-to-authors/

PLAGIARISM

In order to have checks and balances against unethical practices, the Higher Education Commission of Pakistan has developed a Plagiarism Policy which is applicable to all the Higher Educational Institutions of the country. Ziauddin University is a strong proponent of academic integrity and intellectual honesty. The Plagiarism Policy of HEC is strictly applied in all the academic concerns of the university including PJMD. The policy should be adhered to when preparing a manuscript of original work to be submitted to the PJMD. A covering letter should also be provided, certifying that the content of the manuscript is not plagiarized.

COVER LETTER

A cover letter that includes the information:

- An explanation of why your manuscript should be published in Pakistan Journal of Medicine and Dentistry.
- An explanation of any issues relating to journal policies.
- Confirmation that all authors have approved the manuscript for submission.
- Confirmation that the content of the manuscript has not been published, or submitted for publication elsewhere.

TITLE PAGE

The title page should carry

1. The title of the article (not more than 10 words)
2. The name of each author, with designation
3. The name of the department(s) and institution(s) to which the work should be attributed
4. The name and office address with email of the corresponding author.
5. Source(s) of support in the form of grants, equipment, drugs, or all of these.
6. A short running head or foot line of no more than 40 characters.

AUTHORSHIP

Should only be given to those who have participated sufficiently in the work to take public responsibility for the content. The contribution of each author must be described in detail.

ORIGINAL ARTICLE

An original article should have minimum word limit of about 2000 words with two tables and two figures supported by 30 references.

ABSTRACT AND KEYWORDS

A structured abstract of 250 words is required for original articles while other types of articles require unstructured abstracts of 150 words each. The content of the abstract should be structured under the headings of Background (which should include the Objective), Methods, Results and Conclusion with 3 to 10 relevant keywords from the Medical Subject Headings (MeSH).

INTRODUCTION

The introduction should provide the background and the rationale, along with brief literature review on the topic. The section should highlight the purpose of the article using only strictly pertinent references.

METHODS

In this section, the sampling procedure for the research should be described (patients or laboratory animals,

including controls). The age, sex and other important characteristics should be clearly defined with name of ethical review committee of your organization who has reviewed the manuscript. The methods should be written with references, apparatus (the manufacturer's name and address in parentheses), and procedures in sufficient detail to allow others workers to repeat the procedure. In addition, the statistical methods used to analyze them should also be specified.

RESULTS
Important observations and findings should be highlighted. The data in this section can take the form of text, tables, and illustrations, all of which should be organized and presented in a logical sequence.

DISCUSSION
In this section only the new and important aspects of study should be highlighted. Repetition and redundancy of data should be avoided and references given in the Introduction should not be repeated. The inferences of findings and their limitations, including implications for future research should be discussed with references from latest relevant studies.

CONCLUSION
The conclusion should be matched with the objectives of the study and unqualified statements should be.

ACKNOWLEGEMENTS
All participants who do not justify authorship should be acknowledged for their contributions. Such participants can take the form of departmental chair, technical help, financial and material support, data collection, scientific.

REFERENCES
All work cited and researched should referred to in the Vancouver referencing style. A minimum of 25 references should be cited.

REVIEW ARTICLE
A review should have unstructured Abstract (200 words), an introduction (200 words), and Discussion (up to 2000 words), with 40 to 60 references.

SHORT COMMUNICATION AND COMMENTARY
Short Communications or commentaries should have unstructured Abstract (150 words), and general text (1500 words) with 20 references.

CASE REPORTS
The structure of the case report should include an Abstract (150 words), an Introduction (100 words), a Case Report section (500 words) and Discussion (1000 words), with 10 references.

STUDENT CORNER
Students contributions of CHS research based on KAP study is also accepted. The general format of the Original Article should be followed 1000 to 1500 words of content.

LETTERS TO EDITORS
Letters to Editors should be concise and not exceed 400 words with a maximum of 5 references.