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EDITORIAL

AN OVERVIEW OF INTEGRATED MODULAR CURRICULUM FOR UNDERGRADUATE MEDICAL PROGRAMMES IN SOME IMPORTANT COUNTRIES

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Several medical schools in the world including but not limited to the University of California, San Francisco (UCSF), the University of Michigan Medical School, the University of Liverpool, the University of Manchester, the University of Dundee, the University of Western Australia (UWA), and the University of Adelaide first adopted and then moved to a more integrated curriculum because of the concerns about the effectiveness of the integrated modular curriculum (IMC) and because IMC was not providing the necessary depth of knowledge. The All-India Institute of Medical Sciences (AIIMS), and the Christian Medical College (CMC) in Vellore, adopted but later reverted to the traditional curriculum. Other medical schools that have adopted and then left the IMC include the Armed Forces Medical College (AFMC) in Pune and the Maulana Azad Medical College (MAMC) in Delhi. History of IMC in medicine in some important countries is summarized here.

Keywords: Integrated modular curriculum, Traditional curriculum, more integrated curricula
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The curriculum is an essential component of education, as it provides a framework for learning and helps to ensure that students acquire the knowledge and skills they need to succeed in their future careers. The components of a curriculum include the goals and objectives, content, instructional strategies, assessment, and evaluation. Goals and objectives define what students should know and be able to do by the end of the course or program. Content refers to the subject matter and materials used to teach the curriculum. Instructional strategies are the methods and techniques used to deliver the content. Assessment measures students' learning and progress, while evaluation assesses the effectiveness of the curriculum. These components are interrelated and must be aligned to ensure that students achieve the desired learning outcomes.[1,2]

The traditional curriculum, which is based on a subject-wise approach, has been criticized for being outdated, lacking in clinical relevance, and failing to prepare students for the practical aspects of clinical practice. An integrated modular curriculum (IMC), on the other hand, is designed to provide a more holistic and comprehensive approach to medical education, with a focus on problem-solving, critical thinking, and clinical skills. The Pakistan Medical and Dental Council (PMDC) has recommended the adoption of an integrated modular curriculum because the World Health Organization (WHO) has emphasized the importance of an integrated approach to medical education, stating that it can lead to better health outcomes and more effective healthcare systems.[3]

In Pakistan, some medical Institutions have adopted this system while others are reluctant, especially the teachers are showing reservations on the implementation of this system.[4] IMC facilitates contextual and applied learning thus it enhances students' levels and depths of knowledge, but also develops learners' critical thinking to perceive in a diverse and wide range of situations.[5] We take an overview of this system in some important countries.

United States of America (USA)

Several medical schools in the United States have adopted and then left the integrated modular curriculum. One example is the University of California, San Francisco (UCSF),[6] which implemented an integrated curriculum in 2004 but later abandoned it in 2013 due to concerns about the effectiveness of the approach. The University of Michigan Medical School[7] adopted an integrated curriculum in 2006 but later shifted back to a traditional curriculum in 2015. Other medical schools that have experimented with integrated curricula include the University of Pennsylvania[8], and the University of Virginia[9]. These schools have cited various reasons for abandoning the integrated approach.

These medical schools provide students with a strong foundation in basic medical sciences and clinical skills. MD program at Harvard Medical School has 'Pathways' curriculum[10], Johns Hopkins University School of Medicine uses 'The Genes to Society Curriculum'[11] and University of Pennsylvania Perelman School of Medicine uses 'IMPaCT Curriculum'[9].

United Kingdom (UK)

Several medical schools in the UK have adopted and then left the integrated modular curriculum. The University of Liverpool adopted the curriculum in 1996 but later abandoned it in 2002 due to concerns about the lack of clinical exposure for students. The University of Manchester also adopted the curriculum in 1996 but later reverted to a traditional curriculum in 2004 due to concerns about the lack of integration between basic and

clinical sciences. The University of Dundee adopted the curriculum in 2001 but later abandoned it in 2007 due to concerns about the lack of clinical exposure and the need for more flexibility in the curriculum.[12] Some other schools which experimented it are: University of Nottingham adopted it in 1999 and left in 2008; the University of Leeds, adopted in 2000 and left in 2008; the University of Sheffield, adopted in 2000 and left in 2008; the University of Bristol, adopted in 2002 and left in 2008; the University of Southampton, adopted in 2002 and left in 2008; the University of Warwick, adopted in 2003 and left in 2008; the University of Aberdeen, adopted in 2005 and left in 2010. It is noteworthy that some of these schools have since adopted new versions of IMC or other innovative teaching methods.

Australia

The University of Western Australia[13] introduced the IMC in 2006, but later it was abandoned in 2012 due to concerns about effectiveness of the approach. The school returned to a more traditional curriculum structure, which is now focused on a problem-based learning approach-PLACES curriculum. The University of Adelaide[14,15] which adopted the IMC in 2007, moved away from this approach in 2016 and returned to a more traditional curriculum structure. The decision was made after a review found that the IMC was not meeting needs of the students and was not providing necessary depth of knowledge.

India

Several medical schools in India have adopted and then left the integrated modular curriculum. One such example is the All-India Institute of Medical Sciences[16] which introduced the IMC in 1997 but later abandoned it in 2009 due to various reasons, including the lack of trained faculty and inadequate infrastructure. Another example is Christian Medical College[17] in Vellore, which adopted the integrated modular curriculum in 2005 but later reverted to traditional curriculum in 2014 due to concerns about the quality of education and the need for more clinical exposure. Other medical schools that have adopted and then left the integrated modular curriculum include the Armed Forces Medical College in Pune and Maulana Azad Medical College in Delhi.

Curriculum is a dynamic document that is updated according to the technological advancement, changing socio-cultural and economic conditions of a society. While the above-mentioned medical schools were leaving the integrated modular curriculum, these medical schools never lost their international recognition.

REFERENCES

1. Marsh CJ, Willis G. (Eds). Curriculum: Alternative approaches, ongoing issues. Upper Saddle River (NJ): Pearson/Merril Prentice Hall, 2007. Retrieved from: https://uaeu.on.worldcat.org/oclc/62741449. [Accessed: 29 Aug 2023]
2. Posner GJ. (Ed). Analyzing the curriculum. McGraw-Hill Education; 2004.
3. World Health Organization. Transforming and scaling up health professionals' education and training. 2013. Retrieved from: https://iris.who.int/bitstream/handle/10665/93635/9789241506502_eng.pdf. [Accessed: 16 Jul 2023]
4. Anwar MI, Kiani JA, Nadeem N. Integrated modular curriculum: design, delivery and assessment during first two years of medical education —a review at AJK Medical College, Muzaffarabad, Pakistan. Pak J Med Health Sci 2018;12(4):1591–5.
5. Waqar T, Khalid T. Integrated-modular system for under graduate medical students: faculty's perception. Pak Armed Forces Med J 2019;69(3):465–71.
6. UCSF School of Medicine. School of medicine revamps curriculum to reflect changing health practice 2013. Retrieved from: https://www.ucsf.edu/news/2013/06/106036/school-medicine-revamps-curriculum-reflect-changing-health-practice. [Accessed: 28 Jul 2023]
7. University of Michigan Medical School (2015). Medical school curriculum shifts back to traditional model. Retrieved from: https://medicine.umich.edu/medschool/node/3076. [Accessed: 28 Jul 2023]
8. University of Pennsylvania Perelman School of Medicine. IMPaCT (Integrating Medicine Patient Care Transitions) Curriculum. Retrieved from: https://www.med.upenn.edu/student/curriculum/. [Accessed: 29 Aug 2023]
9. University of Virginia School of Medicine. Curriculum. (Cells to Society Curriculum). Retrieved from https://med.virginia.edu/md-program/ume-md-curriculum. [Accessed: 29 Aug 2023]
10. Harvard Medical School. Curriculum. Retrieved from https://meded.hms.harvard.edu/education/md-program-curriculum [Accessed: 29 Aug 2023]
11. Johns Hopkins University School of Medicine. Curriculum. Retrieved from: https://www.hopkinsmedicine.org/som/curriculum/ [Accessed: 28 Aug 2023]
12. Straus SE. Geriatric medicine. BMJ 2001; 13;322(7278):86–9.
13. The University of Western Australia. Medical School-2017-18 Guidebook. Retrieved from: https://www.uwa.edu.au/schools/-/media/Faculties/HMS/Docs/MD-Guidebook-2023.pdf. [Accessed: 31 Aug 2023]
14. Australian IPE Curriculum Renewal. Retrieved from: https://nexusipe-resource-exchange.s3.amazonaws.com/AustralianIPECurriculumRenewal_2014.pdf. [Accessed: 31 Aug 2023]
15. The University of Adelaide. Bachelor of Medicine and Bachelor of Surgery degree. Retrieved from: https://www.adelaide.edu.au/degree-finder/bmbbs_bmbbs.html. [Accessed: 31 Aug 2023]
16. All India Institute of Medical Sciences. MBBS Curriculum. Retrieved from: https://aiimsbhubaneswar.nic.in/admin/Document/Notices/MBBS%20Curriculumbe99644b-1bd1-462c-9534-b7f5c941118f.pdf. [Accessed: 31 Aug 2023]
17. Christian Medical College Vellore reverts to traditional curriculum. https://www.cmch-vellore.edu/SinglePage.aspx? [Accessed: 31 Aug 2023]

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ORIGINAL ARTICLE

SERUM C REACTIVE PROTEIN AS AN INFLAMMATORY MARKER IN GESTATIONAL DIABETES MELLITUS

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Background: Gestational diabetes mellitus is the most prevalent metabolic disorder of pregnancy. A number of factors are implicated in its causation. Inflammation may play a role in development of diabetes. This study aimed to look for an association between serum C reactive protein and gestational diabetes mellitus. **Methods:** This cross-sectional analytical study was carried out at Physiology Department, Army Medical College in collaboration with Pak Emirates Military Hospital from July 2019 to March 2020. Thirty healthy pregnant females and 30 gestational diabetes mellitus patients with 24–32 weeks of gestation were selected. The diagnosis of gestational diabetes was made on the basis of abnormal glucose tolerance test according to International Association for Diabetes and Pregnancy Study Group (IADPSG) criteria. Blood samples were evaluated for serum CRP. Data were analyzed on SPSS-22. Data were expressed as mean and standard deviation. Comparison of data was performed using independent samples *t*-test. Pearson correlation coefficient was determined to find association between numerical variables. Results were considered significant at $p \leq 0.05$. **Results:** Mean Serum C reactive protein level was significantly higher in gestational diabetes group compared with healthy control group and had significant positive correlation with plasma glucose levels as well as glycosylated haemoglobin. **Conclusion:** High CRP levels in GDM had positive correlation with blood sugar levels. Early diagnosis of inflammation with raised CRP level may be helpful in identifying the subjects at risk.

Keywords: Gestational diabetes, inflammation, C-reactive protein

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INTRODUCTION

Normal pregnancy is associated with significant anatomical, physiological and biochemical changes. Sometimes, these changes may lead to pathological conditions and diseases. One of such diseases is gestational diabetes mellitus (GDM). GDM is the most prevalent metabolic disorder in pregnancy that affects more than 14% pregnancies in Southeast Asia.[1] Prevalence of diabetes is highly variable in different populations and is partially affected by the criteria used for its diagnosis.[2] A comprehensive data regarding its prevalence in Pakistan is not available. However, different local studies have shown a prevalence of 8% to 26% in different areas of Pakistan.[3] Increased prevalence of GDM in recent years is in direct proportion to high prevalence of obesity and type 2 diabetes mellitus.[4] Further, GDM can lead to type 2 diabetes both in mother and offspring in addition to causing complications like foetal macrosomia, shoulder dystocia and respiratory distress.[5]

GDM is an independent type of diabetes in which glucose intolerance is first recognized after 24th week of pregnancy.[6] Both genetic and environmental factors are implicated in aetiology of GDM. Pregnancy is associated with changes in carbohydrate, protein and lipid metabolism owing to placental hormones like estrogens, progesterone, human placental lactogen and cortisol etc. This alteration in metabolism serves the purpose of foetal development.[7] Even during the normal pregnancy, insulin sensitivity can be decreased up to 70%. This insulin insensitivity is compensated by increased insulin secretion by beta cells of pancreas.[8] However, an insufficient insulin secretion in the presence of insulin resistance state during pregnancy results in gestational diabetes.

Inflammation is involved in causation of many diseases including cardiovascular diseases and type 2 diabetes.[9] Pregnancy is a pro-inflammatory condition and maternal immune response is disrupted during pregnancy. As a matter of fact, inflammation plays a significant role in early pregnancy during the process of implantation and decidualization.[10,11] However, uncontrolled inflammation may be a cause of gestational as well as neonatal complications.[12] Increased cytokine production in inflammation interacts with post synaptic insulin receptors and interferes with the normal physiological tyrosine phosphorylation of insulin receptor substrate thereby affecting the normal glucose metabolism. The subclinical inflammation results in development of insulin resistance.[6]

Many studies are conducted to device methods and tests for early diagnosis of gestational diabetes. Considering the role of inflammation in pathogenesis of

insulin resistance, studies are carried out to find association between inflammatory markers and diabetes type 2.[13] The increased level of CRP and other inflammatory mediators like interleukin-6 and plasminogen is found to be associated with development of insulin resistance and diabetes.[14] Increased level of inflammation is found to be an additional factor for GDM development.[15] Identifying the association between inflammation and GDM can help in early diagnosis and timely management of GDM and thus can prevent many gestational and neonatal complications. We measured serum CRP levels in GDM patients and compared them with those in normal pregnant women during same gestational period to find any association between inflammation and gestational diabetes.

PATIENTS AND METHODS

The comparative cross-sectional study was conducted at Physiology Department in association with Pak Emirates Military Hospital after formal approval from Ethical Review Board of the Institution. Sample size was calculated using WHO calculator[16] for which confidence level was taken at 95% and margin of error as 5%. With an estimated GDM prevalence of 3.5% in Pakistan, sample size of 54 individuals was calculated. After written informed consent, a total of 60 pregnant women were included in study, 30 were diagnosed having gestational diabetes while 30 were healthy pregnant women. All pregnant women were within 24 to 32 weeks of gestation.[6] The diagnosis of gestational diabetes was made on basis of International Association for Diabetes and Pregnancy Study Group (IADPSG) criteria approved by American Diabetes Association (ADA).[17] Detailed history was taken from all subjects and relevant clinical examination was carried out. Women with acute or chronic infection or inflammation as discovered during history and clinical examination were excluded from study.

Blood samples for glucose estimation were collected under aseptic technique in collection tubes containing sodium fluoride and potassium oxalate. Analyses for glucose were performed within two hours of sample collection using Roche Cobas C Analyzer. For CRP estimation, blood samples were collected in serum collection tubes. The test was performed using immunoturbidimetric assay on Roche Cobas C Analyzer. For glycosylated haemoglobin (HbA1c), blood samples were collected in tubes containing anti-coagulant. The concentration of both haemoglobin and HBA1c was determined and HbA1c/haemoglobin ratio was expressed as percentage HbA1c (%HbA1c). Data were analyzed on SPSS-22. The numerical variables were compared for their Mean±SD value by employing independent samples *t*-test whereas correlation between different numerical variables was interpreted using Pearson's correlation coefficient. The results were regarded significant at $p \leq 0.05$.

RESULTS

Thirty patients of GDM and 30 healthy pregnant women were included in the study. The subjects were matched for age and gestational age. There were no significant differences in GDM and control group regarding BMI and parity. A significant difference was found in mean serum CRP value between the two groups (p=0.005) (Table-1). Further analysis revealed a significant positive correlation of CRP with fasting, 1 hour postprandial, 2 hour postprandial glucose, as well as with HbA1c. (Table-2)

Table-1: Comparison of OGTT, CRP and HbA1c between two groups. (Independent Samples *t*-test)

Variable	Control group	GDM group	*p*
BMI (Kg/m^2)	27.34±1.56	27.46±1.94	0.79
Fasting plasma glucose (mmol/L)	4.52±0.47	5.98±1.04	<0.000*
1 hour plasma glucose (mmol/L)	6.87±1.22	10.07±1.95	<0.000*
2 hour plasma glucose (mmol/L)	5.58±0.81	8.29±1.41	<0.000*
CRP (mg/dL)	4.75±2.6	7.93±5.7	0.005*
HbA1c (%)	5.3±0.59	5.8±1.05	0.012*

*Significant

Table-2: Correlation of CRP with different parameters

Parameter correlated	*r*	*p*
Fasting plasma glucose	0.393	0.001*
1 hour plasma glucose	0.389	0.001*
2 hour plasma glucose	0.238	0.050*
HbA1c	0.258	0.033*

*Significant

DISCUSSION

We compared serum CRP levels in healthy pregnant women and patients of GDM. Serum CRP levels were significantly raised in GDM patients compared to healthy pregnant women. CRP levels also correlated positively with fasting plasma glucose, postprandial glucose and HbA1c levels.

Our findings are consistent with other studies.[18] Mostafa *et al* conducted a study recruiting 60 pregnant women divided into two groups of healthy women and GDM patients with 30 subjects in each group. They found a significant difference in CRP level in both groups with high CRP levels in GDM group (p=0.04).[19] That study was carried out in Egypt. Sample size of both studies is comparable and similar diagnostic criteria were implicated for diagnosis of GDM. In addition, a significant positive correlation of CRP was shown with BMI. In our study, positive correlation between CRP and BMI was not significant. This difference may be due to the fact that in our study, both groups did not have significant differences in mean

BMI. Inflammation as depicted by raised CRP levels appears to be a significant risk factor for GDM.

Farghaly *et al* conducted a prospective cohort study including 496 women. They measured serum CRP of all the women in 1st trimester and followed them for development of GDM by performing OGTT at 24–28 week of gestation, and found that CRP was higher in GDM group (p=0.000). Moreover, CRP levels correlated positively with glucose load test (GLT) as well as 1 hour and 2 hour GLT. They also found that sensitivity, specificity and accuracy of CRP in diagnosing GDM were acceptable.[15]

Another study carried out by Xiang *et al* at China included 96 healthy and 95 GDM patients. They measured levels of CRP along with other inflammatory markers at 24–28 weeks gestation. Their findings are also comparable to ours and showed a significant positive association between CRP and GDM in addition to a significant positive correlation of CRP with plasma glucose levels and HbA1c.[20] Their findings also emphasized the role of inflammation in GDM as a number of inflammatory markers were assessed and found to be raised in GDM group.

Siddiqui *et al*[21] conducted a study in India with similar study design. They recruited 50 healthy pregnant and 53 GDM patients. They compared CRP levels in both groups and found a significant difference in IL-6 levels but the differences in CRP levels in both groups were not significant. However, CRP levels correlated positively with FBS and postprandial blood sugar levels. In their study, GDM was positively correlated with BMI, age and positive family history of diabetes.

In contrast, a study carried out by Hezareh *et al*[22] in Iran did not reveal a significant difference in CRP levels in GDM and normal pregnant women. They also measured CRP levels in early pregnancy and followed them with OGTT at 24–28 weeks of gestation.

CRP has appeared as sensitive indicator of inflammation in cardiovascular diseases.[23] Moreover, sufficient evidence is available that suggests the role of inflammation in type 2 diabetes and insulin resistance.[24] A number of cross-sectional studies have depicted the role of CRP and other inflammatory markers in GDM. Results of a multitude of prospective cohort studies also implicate the role of inflammation in developing GDM.[15]

The results of the current study further support the hypothesis that inflammation plays a role in the pathogenesis of glucose intolerance and gestational diabetes. Thus measurement of CRP levels may be helpful in identifying women at risk of developing GDM and early diagnosis of GDM.

The small sample size and a cross-sectional study design are the limitations of our study. Future prospective studies involving a large number of subjects may prove helpful.

CONCLUSION

CRP levels are significantly higher in GDM and were positively correlated with blood sugar level. Early diagnosis of inflammation with raised CRP level may be helpful in identifying patients at risk of developing GDM.

REFERENCES

1. Nguyen CL, Pham NM, Binns CW, Duong DV, Lee AH. Prevalence of gestational diabetes mellitus in eastern and southeastern Asia: a systematic review and meta-analysis. J Diabetes Res 2018;2018:6536974.
2. Behboudi-Gandevani S, Amiri M, Bidhendi Yarandi R, Ramezani Tehrani F. The impact of diagnostic criteria for gestational diabetes on its prevalence: a systematic review and meta-analysis. Diabetol Metab Syndr 2019;11:11.
3. Gul S, Rafiq K, Nesar S, Bokhari SWA, Mughal MA, Naveel HT, *et al*. Management of Gestational Diabetes mellitus with Risk Factors and Trans-Generational Prevention. RADS J Biol Res Appl Sci 2021;12(2):146–53.
4. Simmons D, Sacks DA, McCance DR, Maresh M. Epidemiologic context of diabetes in pregnancy. In: A practical manual of diabetes in pregnancy. West Sussex: Wiley-Blackwell; 2010.1–16.
5. Johns EC, Denison FC, Norman JE, Reynolds RM. Gestational diabetes mellitus: mechanisms, treatment, and complications. Trends Endocrinol Metab 2018;29(11):743–54.
6. Omazić J, Viljetić B, Ivić V, Kadivnik M, Zibar L, Müller A, *et al*. Early markers of gestational diabetes mellitus: what we know and which way forward? Biochem Med 2021;31(3):030502.
7. Sharma AK, Singh S, Singh H, Mahajan D, Kolli P, Mandadapu G, *et al*. Deep insight of the pathophysiology of gestational diabetes mellitus. Cells 2022;11(17):2672.
8. Kopylov AT, Papysheva O, Gribova I, Kotaysch G, Kharitonova L, Mayatskaya T, *et al*. Molecular pathophysiology of diabetes mellitus during pregnancy with antenatal complications. Sci Rep 2020;10(1):19641.
9. Tsalamandris S, Antonopoulos AS, Oikonomou E, Papamikroulis GA, Vogiatzi G, Papaioannou S, *et al*. The role of inflammation in diabetes: current concepts and future perspectives. Eur Cardiol 2019;14(1):50–9.
10. Chavan AR, Griffith OW, Wagner GP. The inflammation paradox in the evolution of mammalian pregnancy: turning a foe into a friend. Curr Opin Genet Dev. 2017;47:24–32.
11. Ramanjaneya M, Butler AE, Alkasem M, Bashir M, Jerobin J, Godwin A, *et al*. Association of complement-related proteins in subjects with and without second trimester gestational diabetes. Front Endocrinol (Lausanne) 2021;12:641361.
12. Vecchié A, Bonaventura A, Carbone F, Maggi D, Ferraiolo A, Carloni B, *et al.* C-reactive protein levels at the midpregnancy can predict gestational complications. Biomed Res Int 2018;2018:1073151.
13. Oguntibeju OO. Type 2 diabetes mellitus, oxidative stress and inflammation: examining the links. Int J Physiol Pathophysiol Pharmacol 2019;11(3):45–63.
14. Kianpour M, Saadatmand F, Nematbakhsh M, Fahami F. Relationship between c-reactive protein and screening test results of gestational diabetes in pregnant women referred to health centers in Isfahan in 2013–2014. Iran J Nurs Midwifery Res 2019;24(5):360–4.
15. Farghaly TA, Helmy NA, Abbas AM, Ahmed AGM. C-reactive protein as a screening test for gestational diabetes mellitus in first-trimester of pregnancy: a prospective cohort study. Int J Reprod Contracept Obstet Gynecol 2018;7(12):4798–803.
16. Qadir SY, Yasmin T, Fatima I. Maternal and foetal outcome in gestational diabetes. J Ayub Med Coll Abbottabad 2012;24(3-4):17–20.
17. Saeedi M, Cao Y, Fadl H, Gustafson H, Simmons D. Increasing prevalence of gestational diabetes mellitus when implementing

the IADPSG criteria: a systematic review and meta-analysis. Diabetes Res Clin Pract 2021;172:108642.
18. Alamolhoda SH, Yazdkhasti M, Namdari M, Zakariayi SJ, Mirabi P. Association between C-reactive protein and gestational diabetes: a prospective study. J Obstet Gynaecol 2020;40(3):349–53.
19. Mostafa MK, Ouf TF, Abd AL-Aziz AF, Hegazy HA. The relation between serum C-reactive protein level and gestational diabetes. Egypt J Hosp Med 2019;75(2):2149–53.
20. Xiang LL, Chen C, Wang QY, Zhu YT, Chen YJ, Zeng Y. Impact of inflammatory factors, hemoglobin A1c, and platelet parameters in gestational diabetes mellitus. Arch Gynecol Obstet 2023;307(2):439–46.
21. Siddiqui S, Waghdhare S, Panda M, Dubey S, Jha S. Association of IL-6 and CRP levels with gestational diabetes mellitus. Diabetes 2018;67(Suppl 1):2417–PUB.
22. Hezareh F, Moghaddam-Banaem L, Shahali S. The relationship between maternal serum Interleukin-6 and CRP levels at first trimester of pregnancy and gestational diabetes occurrence. Iran J Obstet Gynecol Infertil 2021;24(5):59–68.
23. Avan A, Tavakoly Sany SB, Ghayour-Mobarhan M, Rahimi HR, Tajfard M, Ferns G. Serum C-reactive protein in the prediction of cardiovascular diseases: Overview of the latest clinical studies and public health practice. J Cell Physiol 2018;233(11):8508–25.
24. Kanmani S, Kwon M, Shin MK, Kim MK. Association of C-reactive protein with risk of developing type 2 diabetes mellitus, and role of obesity and hypertension: a large population-based Korean cohort study. Sci Rep 2019;9(1):4573.

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ORIGINAL ARTICLE

SAFETY AND EFFICACY OF N-BUTYL-2-CYANOACRYLATE FOR MANAGEMENT OF GASTRIC VARICES

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Background: Gastric varices (GVs) bleed less frequently than oesophageal varices. However, gastric variceal bleeding tends to be more severe with higher mortality and re-bleed frequently after spontaneous haemostasis. This study was conducted to determine the safety and efficacy of N-Butyl-2-Cyanoacrylate for the management of gastric varices in patients presenting to tertiary care setting. **Methods:** This descriptive case study was performed at Department of Gastroenterology and Hepatology, Hayatabad Medical Complex, Peshawar between Jan and July 2022. Seventy-three patients of either gender were included in the study. Screening and interventional endoscopy were done and patient were observed for efficacy and safety of N-Butyl-2-Cyanoacrylate injection. Data was analysed using SPSS-22. **Results:** The mean age and BMI of the patients were 54.96±2.64 years and 28.98±246 Kg/m^2 respectively. There were 75.78% male and 24.21% female (M:F=31:1) patients. The aetiological cause of gastric varices was Hepatitis-B virus in 62.10%, alcohol in 23.15% and other causes in 14.73% patients. Child Pugh Class was A in 37.89% patients, B in 50.52% and C in 11.57% patients. Adequate haemostasis was achieved in 95.78% patients and re-bleeding was observed in 30.52% patients. Fever was recorded in 5.26% patients, abdominal pain in 3.15% and diarrhoea in 1.05%. Spontaneous bacterial peritonitis was not reported in any case (0.0%). **Conclusion:** N-Butyl-2-Cyanoacrylate is efficacious and safe for the management of gastric varices.

Keywords: N-Butyl-2-Cyanoacrylate, Gastric varices, efficacy, Peshawar, Pakistan

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INTRODUCTION

Dilated submucosal veins in the stomach known as gastric varices (GV) can cause life-threatening upper gastrointestinal bleeding. Patients with portal hypertension or elevated pressure in the portal vein system, which may be a complication of cirrhosis, are most likely to have them. After oesophageal varices (EV), GV are the most frequent cause of upper gastrointestinal (UGI) bleeding in patients with portal hypertension.[1] The gastro-esophageal (azygous) venous system and the gastro-phrenic venous system are two different types of portosystemic collateral drainage systems that can be used to drain gastric varices that originate at hepatofugal collateral pathways into the systemic vein. Occasionally, localized portal hypertension brought on by splenic vein occlusion results in gastric varices at the hepato-petal collateral pathway. These varices drain through the stomach veins.[2] Gastric varices may also be found in patients with splenic vein thrombosis into which the short gastric veins drain stomach flow. Gastric varices and bleeding are a possible complication of schistosomiasis caused by portal hypertension.[3] Hematemesis, melena, or rectal bleeding are all symptoms that patients with bleeding gastric varices may exhibit. The patients may experience rapid bleeding and may go into shock soon.[4]

Approximately 10–30% of variceal haemorrhage is caused by GV. They have a higher mortality rate and a propensity to bleed more heavily. After spontaneous haemostasis, between 35 and 90% of patients re-bleed. Around 50% of people with liver cirrhosis have gastro-esophageal varices.[5] Acute upper gastrointestinal bleeding is one of the leading causes of hospitalization worldwide, with an annual incidence of 50–150 episodes per 100,000 people. Acute upper gastrointestinal bleeding is associated with a 10–14% mortality rate.[6] Recent studies reported the incidence of gastric varices due to various causes about 15% in Pakistani population. In 40–50% patients, variceal bleeding stops on its own, but within the first six weeks, the incidence of early re-bleeding varies between 30–40%, and about 40% of all re-bleeding episodes happen within the first five days.[7] The most accepted classification of GV differentiates them into two types, i.e., those caused by portal hypertension (cirrhotic or non-cirrhotic) and those caused by isolated splenic vein thrombosis (SVT). GV caused by portal hypertension is much more common than GV caused by SVT.[8]

Gastric varices bleeding must be controlled or managed using a combination of treatment strategies. Patients with cirrhosis or high portal blood pressure are more likely than patients with SVT to experience gastric variceal bleeding.[9] The bleeding causes significant blood loss, which must be compensated for by blood transfusion to maintain circulation of blood. New endoscopic treatment options and interventional radiological procedures have recently expanded the therapeutic arsenal for GV.[10] Cyanoacrylate glue therapy, trans-jugular intrahepatic portosystemic shunt

(TIPSS), balloon-occluded retrograde transvenous obliteration (BRTO), and devascularisation surgery are all treatment options for bleeding GV.[11]

For gastric variceal obturation, tissue adhesives have been used, such as N-butyl-2-cyanoacrylate (NB2-CY), a monomer that quickly undergoes exothermic polymerization upon contact with the hydroxyl ions present in water. A cyanoacrylate ester known as N-butyl cyanoacrylate (n-BCA, NBCA) is a butyl ester of 2-cyano-2-propenoic acid.[12] It has a sharp, offensive smell and is a clear, colourless liquid. It does not dissolve in water and its primary function is as the foundational element of cyanoacrylate medical glues.[13] Past studies reported higher efficacy and safety for controlling GV using cyanoacrylate but these studies haven't been performed on our local population. The current study's goal was to assess the safety and efficacy of N-butyl-2-octyl-cyanoacrylate in treatment of patients with gastric varices in local population.

PATIENTS AND METHODS

This was a descriptive case studies performed at Department of Gastroenterology and Hepatology, Hayatabad Medical Complex, Peshawar from 21st January to 20th July 2022. Permission and approval of the study were taken from the Hospital Ethics Committee. Sample size was calculated through World Health Organization online sample size calculator using 95% confidence level with anticipated population proportion of 96.9% and absolute precision of 4%. Non-probability consecutive sampling technique was used. Patient of either gender with ages 18–75 years with high risk of gastric varices, i.e., varices of more than 1 Cm size, history of bleeding from gastric varices and those with cirrhosis of Child Turcotte Pugh Score greater than 5 were included in our study. Pregnant and lactating mothers, patients with shock or Grade III/IV hepatic encephalopathy, bleeding secondary to oesophageal varices or portal hypertensive gastropathy and those having sensitivity to N-butyl-2-octyl-cyanoacrylate were excluded from the study. Written informed consent was obtained from the patients.

Screening and interventional endoscopy were done in Gastroenterology Ward, Lady Reading Hospital, Peshawar. Patients were observed for hematemesis and vital record, and full blood count were obtained six hourly for 48 hours. The procedure was performed using a standard forward-viewing video endoscope (Pentax EG 29910). N-Butyl-2-Cyanoacrylate (Histoacryl, B Braun, Germany) was mixed with lipiodol ((Lipiodol Ultra Fluid, Therapex, Canada) in 1:1 ratio and injected into bleeding fundal varices. The volume used was 0.5–4 mL injection, decided according to size of varix. Data was recorded on a pre-designed proforma and analysed on SPSS-22. Numerical variables, i.e., age, BMI and number of patients included were summarized as Mean±SD. Qualitative variables like aetiology of GV, Child Pugh class, re-bleeding, homeostasis, fever, abdominal pain, diarrhoea and SBP were presented as frequency and percentage. Effect modifiers like age, gender, BMI, aetiology of GV and Child Pugh class were controlled by stratification. Post-stratification Chi-square test was applied keeping $p<0.05$ as significant.

RESULTS

There were total 95 patients included in this study. The mean values for numeric variables of the patients who received N-butyl-2-cyanoacrylate are shown in Table-1. The mean values for qualitative variables are shown in Table-2.

The mean BMI of patients who received N-butyl-2-cyanoacrylate for management of gastric varices was 28.98±2.46 Kg/m^2. Out of 95 patients with gastric varices, there were 1 (1.05%) patient of BMI <18.5 Kg/m^2, there were 19 (20.0%) patients of BMI range of 18.5–24.9 Kg/m^2, 51 (53.68%) patients of BMI range of 25–29.9 Kg/m^2 and 24 (25.26%) patients of BMI range of ≥30 Kg/m^2. Stratification of age and gender with respect to efficacy and safety of N-Butyl-2-Cyanoacrylate is shown in Table-3.

Stratification by child Pugh score and aetiology of varices is shown in Table-4.

Table-1: Distribution of patients (n=95)

Variables		Patients (n)	Percentage (%)	Mean±SD
Age Groups (Years)	18–20	2	2.10	54.96±2.64 (Years)
	21–30	4	4.21	
	31–40	7	7.36	
	41–50	17	17.89	
	51–60	36	37.89	
	61–70	20	21.05	
	71–75	9	9.47	
Gender	Male	72	75.78	–
	Female	23	24.21	

Table-2: Qualitative variables distribution (n=95)

	Distribution		Patients (n)	Percentage (%)
Aetiology of gastric varices	Viral		59	62.10
	Alcoholic		22	23.15
	Others		14	14.73
Child Pugh class	A		36	37.89
	B		48	50.52
	C		11	11.57
Efficacy	Haemostasis	Yes	91	95.78
		No	4	4.21
	Re-bleeding	Yes	29	30.52
		No	66	69.47
Safety	Fever	Yes	5	5.26
		No	90	94.73
	Abdominal pain	Yes	3	3.15
		No	92	96.84
	Diarrhoea	Yes	1	1.05
		No	94	98.94
	Spontaneous bacterial peritonitis	Yes	0	0
		No	95	100

Table-3: Stratification of Groups

	Efficacy					Safety								
	Haemostasis (n=95)		Re-bleeding (n=95)			Fever		Abdominal		Diarrhoea		Spontaneous bacterial peritonitis		
	Yes n (%)	No n (%)	Yes n (%)	No n (%)	p*	Yes n (%)	No n (%)	Yes n (%)	No n (%)	Yes n (%)	No n (%)	Yes n (%)	No n (%)	p*
Stratification by Age														
18–20 (n=2)	2 (100.0)	0 (0,0)	0 (0.0)	2 (100.0)		0 (0.0)	2 (100.0)	0 (0.0)	2 (100.0)	0 (0.0)	2 (100.0)	0 (0.0)	2 (100)	
21–30 (n=4)	4 (100.0)	0 (0.0)	0 (0.0)	4 (100.0)		0 (0.0)	4 (100.0)	0 (0.0)	4 (100.0)	0 (0.0)	4 (100.0)	0 (0.0)	4 (100)	
31–40 (n=7)	7 (100.0)	0 (0.0)	0 (0.0)	7 (100.0)		0 (0.0)	7 (100.0)	0 (0.0)	7 (100.0)	0 (0.0)	7 (100.0)	0 (0.0)	7 (100)	
41–50 (n=17)	16 (94.11)	1 (5.88)	4 (23.52)	13 (76.47)	0.821**	1 (5.88)	16 (94.11)	1 (5.88)	16 (94.11)	1 (5.88)	16 (94.11)	0 (0.0)	17 (100)	0. 630**
51–60 (n=36)	35 (97.23)	1 (2.78)	18 (50.0)	18 (50.0)		3 (8.34)	33 (91.67)	1 (2.78)	35 (97.24)	0 (0.0)	36 (100.0)	0 (0.0)	36 (100)	
61–70 (n=20)	19 (95.0)	1 (5.0)	3 (15.0)	17 (85.0)		1 (5.0)	19 (95.0)	1 (5.0)	19 (95.0)	0 (0.0)	20 (100.0)	0 (0.0)	20 (100)	
71–75 (n=9)	8 (88.89)	1 (11.12)	4 (44.45)	5 (55.56)		0 (0.0)	9 (100.0)	0 (0.0)	9 (100.0)	0 (0.0)	9 (100.0)	0 (0.0)	9 (100)	
Total	91 (95.78)	4 (4.21)	29 (30.52)	66 (69.47)		5 (5.26)	90 (94.73)	3 (3.15)	92 (96. 84)	1 (1.05)	94 (98. 94)	0 (0.0)	95 (100)	
Stratification by Gender														
Male (n=72)	70 (97.23)	2 (2.78)	21(29.16)	51 (70.83)	0. 319**	4 (5.56)	68 (94.45)	3 (4. 16)	69 (95.83)	1 (1.38)	71 (98.61)	0 (0.0)	72 (100)	0. 267**
Female (n=23)	21 (91.30)	2 (8.69)	8 (34.78)	15 (65.21)		1 (4.34)	22 (95.65)	0 (0. 0)	23 (100.0)	0 (0.0)	23 (100.0)	0 (0.0)	23 (100)	
Total	91 (95.78)	4 (4.21)	29 (30.52)	66 (69.47)		5 (5.26)	90 (94.73)	3 (3.15)	92 (96.84)	1 (1.05)	94 (98.94)	0 (0.0)	95 (100)	

Table-4: Stratification of Groups

	Efficacy					Safety								
	Haemostasis (n=95)		Re-bleeding (n=95)			Fever		Abdominal		Diarrhoea		Spontaneous bacterial peritonitis		
	Yes n (%)	No n (%)	Yes n (%)	No n (%)	p*	Yes n (%)	No n (%)	Yes n (%)	No n (%)	Yes n (%)	No n (%)	Yes n (%)	No n (%)	p*
Stratification by Child Pugh Class														
A (n=36)	35 (97.23)	1 (2.78)	9 (25.0)	27 (75.0)		2 (5.56)	34 (94.45)	1 (2.78)	35 (97.23)	1 (2.78)	35 (97.23)	0 (0.0)	36 (100)	
B (n=48)	46 (95.83)	2 (4.16)	18 (37.5)	30 (62.5)	0.214**	3 (6.25)	45 (93.75)	2 (4.16)	46 (95.83)	0 (0.0)	48 (100.0)	0 (0.0)	48 (100)	**0 . 577 * ***
C (n=11)	10 (90.90)	1 (9.09)	2 (18.18)	9 (81.81)		1 (9.09)	10 (90.90)	0 (0.0)	11 (100.0)	0 (0.0)	11 (100.0)	0 (0.0)	11 (100)	
Total	91 (95.78)	4 (4.21)	29 (30.52)	66 (69.47)		5 (5.26)	90 (94.73)	3 (3.15)	92 (96.84)	1 (1.05)	94 (98.94)	0 (0.0)	95 (100)	
Stratification by Aetiology of Varices														
Viral (n=59)	57 (96.61)	2 (3.38)	23 (38.98)	36 (61.01)		2 (3.38)	57 (96.61)	1 (1.69)	58 (98.30)	1 (1.69)	58 (98.30)	0 (0.0)	59 (100)	
Alcoholic (n=22)	21 (95.45)	1 (4.54)	6 (27.27)	16 (72.72)	0. 982**	3 (13.63)	19 (86.36)	2 (9.09)	20 (90.90)	0 (0.0)	22 (100.0)	0 (0.0)	22 (100)	0.743**
Others (n=14)	14 (100.0)	0 (0.0)	0 (0.0)	14 (100.0)		1 (7.14)	13 (92.58)	0 (0.0)	14 (100.0)	0 (0.0)	14 (100.0)	0 (0.0)	14 (100)	
Total	91 (95.78)	4 (4.21)	29 (30.52)	66 (69.47)		5 (5.26)	90 (94.73)	3 (3.15)	92 (96.84)	1 (1.05)	94 (98.94)	0 (0.0)	95 (100)	

DISCUSSION

Gastric varices (GVs) are known for massive bleeding and are frequently challenging to treat using standard methods. Approximately half of all cirrhosis patients have gastric varices. It usually happens after oesophageal varices and is the most frequent cause of upper gastrointestinal tract bleeding with high portal blood pressure.[14] Knowledge of the pathophysiology and treatment options for patients with gastric varices has significantly changed over the past 20 years. The majority of GV patients in the United States have underlying portal hypertension rather than splenic vein thrombosis, but ruling out the latter is still a crucial first step in the assessment.[15] Varices that develop in the stomach's fundus are particularly troublesome. Fundal varices can sometimes appear as polypoid masses occasionally resembling a cluster of grapes.[16] In the current study on 95 patients, we determined the safety and efficacy of N-Butyl-2-Cyanoacrylate for the management of gastric varices in the patients presenting to tertiary care setting.

In a study by Lizardo-Sanchez L *et al*[17], there were 9 (56%) males and 7 (44%) females, while in another study conducted by Jun CH *et al*[18], there were 379 males (83.3%) and 76 females (16.7%). In a study of Mosli MH *et al*[19], the majority of patients were males 79.3% (95% CI, 63.6–95%). In the current study, there were 75.78% male and 24.21% female patients (M:F ratio 31:1). It was reported that immediate haemostasis was achieved in 93% of patients with GV and no complication was observed following 2-octyl-cyanoacrylate injection.

A study by Jun CH *et al*[18], evaluated the efficacy and safety of N-Butyl-2-Cyanoacrylate for treatment of GV and they found that haemostasis achieved initially in 96.9% (441/455) of patients; re-bleeding occurred in 35.2% (160/455), and the bleeding-related death rate was 6.8% (31/455) during follow-up. Complications recorded following NB2-CYA therapy were included fever (6.8%), abdominal pain (3.7%), diarrhoea (1.3%), spontaneous bacterial peritonitis (0.7%), bacteremia (0.4%), and embolism (0.2%).[18] In the current study, it was found that majority of patients who achieved adequate haemostasis (100%) were in younger age groups, i.e., 18–20, 21–30 and 31–40 years, followed by older age groups, i.e., 51–60, and 61–70 years age groups, i.e., 97.23% and 95.0% respectively. Lower number of patients who achieved haemostasis was found in older age group (71–75 years). Re-bleeding was not observed in younger age groups however it was higher in middle age groups, i.e., 51–60 years followed by older age groups, i.e., 71–75 years (44.45%). Efficacy of N-Butyl-2-Cyanoacrylate for the management of gastric varices was observed more in younger age groups in our study but results were statistically not significant.

The safety with age was also stratified and found that majority of patients with fever (8.34%), abdominal pain (5.88%) and diarrhoea (5.88%) were observed in middle age group (51–60 years and were not observed in younger and older age groups. However, spontaneous bacterial peritonitis was not observed in any patient. On the other hand, safety of N-Butyl-2-Cyanoacrylate for the management of gastric varices was observed more in younger and older age

groups and less in middle age groups. However, the results were not statistically significant.

The safety of N-Butyl-2-Cyanoacrylate with effect gender was also compared and found that majority of patients with fever (5.56%) were males followed by females (4.34%). All patients with abdominal pain (4.16%) were male. All patients with diarrhoea (1.38 %) were male. Spontaneous bacterial peritonitis was not observed in any patient. Safety of N-Butyl-2-Cyanoacrylate for the management of gastric varices was observed more in females and less in males, but the results were not statistically significant. On the other hand, safety and efficacy of N-Butyl-2-Cyanoacrylate for the management of gastric varices was found more in Child Pugh class A and less in class B in our study but results were statistically not significant. The safety and efficacy of N-Butyl-2-Cyanoacrylate for the management of gastric varices was found more in patients who had gastric varices aetiology other than virus and alcohol but results were statistically not significant.

This was a single centred study carried out at a single tertiary facility. A multicentre randomized controlled trial ought to be carried out to see the potential clinical effects of cyanoacrylate.

CONCLUSION

N-Butyl-2-Cyanoacrylate is efficacious and safe for the management of gastric varices. However, large, multicentered study is required to generalize the results in local population.

REFERENCES

1. Henry Z, Patel K, Patton H, Saad W. AGA clinical practice update on management of bleeding gastric varices: expert review. Clin Gastroenterol Hepatol 2021;19(6):1098–107. e1.
2. Lesmana CRA, Raharjo M, Gani RA. Managing liver cirrhotic complications: Overview of esophageal and gastric varices. Clin Mol Hepatol 2020;26(4):444–60.
3. Lee EW, Shahrouki P, Alanis L, Ding P, Kee ST. Management options for gastric variceal hemorrhage. JAMA Surg 2019;154(6):540–8.
4. Shen D, Qian X, Wang J, Li F, Fang Q, Zhao J, *et al.* Risk factor analysis of non-acute symptomatic portal vein thrombosis after endoscopic gastric variceal injection. J Chin Physician 2021;23(3):338–42.
5. Philips CA, Ahamed R, Rajesh S, George T, Mohanan M, Augustine P. Beyond the scope and the glue: update on evaluation and management of gastric varices. BMC Gastroenterol 2020;20(1):361.
6. Jakab SS, Garcia-Tsao G. Evaluation and management of esophageal and gastric varices in patients with cirrhosis. Clin Liver Dis 2020;24(3):335–50.
7. Ahmed A, Ali L, Shehbaz L, Nasir S, Rizvi SR, Aman MZ, *et al.* The prevalence of acute upper gastrointestinal bleeding and the factors causing hemorrhage as observed at a tertiary health care centre in Karachi, Pakistan. Pak J Surg 2017;33(1):36–40.
8. Shatzel JJ, O'Donnell M, Olson SR, Kearney MR, Daughety MM, Hum J, *et al.* Venous thrombosis in unusual sites: a practical review for the hematologist. Eur J Haematol 2019;102(1):53–62.
9. Vine LJ, Subhani M, Acevedo JG. Update on management of gastric varices. World J Hepatol 2019;11(3):250–60.
10. McCarty TR, Bazarbashi AN, Hathorn KE, Thompson CC, Ryou M. Combination therapy versus monotherapy for EUS-guided management of gastric varices: A systematic review and meta-analysis. Endosc Ultrasound 2020;9(1):6–15.
11. Goral V, Yılmaz N. Current approaches to the treatment of gastric varices: glue, coil application, TIPS, and BRTO. Medicina (Kaunas) 2019;55(7):335.
12. Al-Bawardy B, Gorospe EC, Saleem A, Buttar NS, Wong Kee Song LM. Outcomes and predictors of rebleeding after 2-octyl cyanoacrylate injection in acute gastric variceal hemorrhage. J Clin Gastroenterol 2016;50(6):458–63.
13. Thiruvengadam SS, Sedarat A. The role of endoscopic ultrasound (EUS) in the management of gastric varices. Curr Gastroenterol Rep 2021;23(1):1.
14. Lee HA, Chang JM, Goh HG, Kim TH, Lee YS, Suh SJ, *et al.* Prognosis of patients with gastric variceal bleeding after endoscopic variceal obturation according to the type of varices. Eur J Gastroenterol Hepatol 2019;31(2):211–7.
15. Al-Khazraji A, Curry MP. The current knowledge about the therapeutic use of endoscopic sclerotherapy and endoscopic tissue adhesives in variceal bleeding. Expert Rev Gastroenterol Hepatol 2019;13(9):893–7.
16. Yang W, Yusufu Y, Wang C. Gastrointestinal: A rare case of upper gastrointestinal bleeding: Splenosis mimicking gastric varices. J Gastroenterol Hepatol 2022;37(9)1651.
17. Lizardo-Sanchez L, Burdick J, Trotter JF, editors. Safety and efficacy of 2-octyl-cyanoacrylate in the management of patients with gastric and duodenal varices who are not candidates for transjugular intrahepatic portosystemic shunts. Proc (Bayl Univ Med Cent) 2016;29(4):371–3.
18. Jun CH, Kim KR, Yoon JH, Koh HR, Choi WS, Cho KM, *et al.* Clinical outcomes of gastric variceal obliteration using N-butyl-2-cyanoacrylate in patients with acute gastric variceal hemorrhage. Korean J Intern Med 2014;29(4):437–44.
19. Mosli MH, Aljudaibi B, Almadi M, Marotta P. The safety and efficacy of gastric fundal variceal obliteration using N-butyl-2-cyanoacrylate; the experience of a single Canadian tertiary care centre. Saudi J Gastroenterol 2013;19(4):152–9.

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MB: Manuscript writing
DN: Sample collection and Laboratory Work
SF: Sample collection and laboratory work
KH: Results analysis
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ORIGINAL ARTICLE

STRESS HORMONE LEVELS AMONG CHILDREN BEREAVED BY TERROR ATTACK

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Background: Terrorism is characterized by the use of violence against civilians, with the expressed desire of causing terror or panic in the population. Since the Soviet invasion of Afghanistan in 1979, the Khyber Pakhtunkhwa (formerly NWFP) province of Pakistan has suffered the most from unrest and terrorist activities. Terror related bereavement is more stressful and difficult to recover compared from that of bereavement from natural death. **Methods:** This retrospective study was carried out in the Department of Physiology, IBMS, Khyber Medical University, Peshawar, Pakistan. Salivary Cortisol Levels were estimated in Non-Bereaved and Bereaved Groups of children. **Results:** Children in the Bereaved Group showed a statistically significant (p=0.005) higher levels of salivary cortisol in the morning sample (Log Cortisol M=14.43) as compared with the children in the Non-Bereaved Group (Log Cortisol M=6.44). Children in the Bereaved Group showed a statistically non-significant (p=0.164) decrease in the levels of salivary cortisol in the post-dexamethasone sample (Log Cortisol M=8.85) as compared with the children in the Non-Bereaved Group (Log Cortisol M=4.25). **Conclusion:** Terror bereavement causes long-term activation of the Hypothalmo-Pituitary-Adrenal (HPA) axis, elevating the cortisol levels even 3–4 years after the traumatic bereavement. Chronic elevation of cortisol results in the manifestations of depression related signs and symptoms in bereaved children.

Keywords: Terrorism, Bereavement, Salivary Cortisol levels, Depression

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INTRODUCTION

Terrorism is characterized by the use of violence against civilians, with the expressed desire of causing terror or panic in the population.[1] The Global Terrorism Index (GTI) defines terrorism as 'the threatened or actual use of illegal force and violence by a non-state actor to attain a political, economic, religious, or social goal through fear, coercion, or intimidation.' This definition recognizes that terrorism is not only the physical act of an attack but also the psychological impact it has on a society for many years after. The intensity of global terrorism has decreased since the peak of terror activity in 2014. Pakistan was among five countries which accounted for three quarters of all deaths from terrorism in 2016. As regards the deaths related to terrorism, 2016 was the third deadliest year since 2000.[2]

Since the Soviet invasion of Afghanistan in 1979, the Khyber Pakhtunkhwa (formerly NWFP) province of Pakistan has suffered the most from unrest and terrorist activities.[3] Bereavement is a state of sadness or mourning after the death of some loved one, close relative, sibling or even a friend.[4] Terror related bereavement is more stressful and difficult to recover compared from that of bereavement from natural death.[5] Childhood bereavement due to terrorism is associated with increased emotional and behavioural problems, depression, suicide, post-traumatic stress disorder (PTSD), alcohol or substance use in adulthood.[6]

Trauma and psychological stressors are some of the most potent stimuli of the endocrine stress response, i.e., non-specific activation of the hypothalamic-pituitary-adrenal (HPA) and sympatho-adreno-medullary (SAM) axes occur.[7] In humans, both high and low levels of HPA activity had been related to stress sensitive mental disorders, which responded well while treating some patients by exogenously giving glucocorticoid.[8]

Dexamethasone, a corticosteroid, shows similar effects to those of cortisol and provides an estimate of corticosteroid feedback sensitivity. In normal individuals, the morning salivary cortisol levels were diminished due to negative feedback exerted on HPA axis by giving exogenous dexamethasone. However, this level will remain higher in chronically depressed persons due to dysfunctional HPA axis negative feedback mechanism.[9] It was hypothesized that bereaved children due to terror related deaths would have greater HPA axis activation as compared with the non-bereaved children. That hyper-activation in bereaved children would be evidenced by higher basal salivary cortisol levels. It was also hypothesized that there would be less dexamethasone suppression of basal salivary cortisol in bereaved children.

PARTICIPANTS AND METHODS

This study was conducted in the Institute of Basic Medical Sciences (IBMS), Khyber Medical University

(KMU), Peshawar, Pakistan during 2015–18. Approval was taken from the Institutional Ethical Review Committee of the Khyber Medical University. Written informed consent was obtained from all participants. A planned questionnaire was used for interview which was done face to face to obtain the correct psychological and behavioural information. A General Physical Examination was also carried out to obtain the anthropometric data.

Eighty-eight children were randomly recruited from the local population. They were divided into two groups, i.e., Bereaved Group and Non-bereaved Group. Bereaved Group had 49 children from 35 families and Non-bereaved Group had 39 children from 33 families. The bereaved participants were inducted through local '*Jirga*' (assembly of some elders) of the North-West part of Khyber Pakhtunkhwa province which was badly affected area of terrorist activities. Non-bereaved children were selected from the same socio-demographic background from settled areas of Peshawar. Ages of the participants ranged between 8 and 18 years. The bereaved children included 46 (93.9%) boys and 3 (6.1%) girls. Time was calculated as time between the terror event (incidence) and time of filling questionnaire and taking blood. In the case of controls (non-bereaved) time was calculated as time after natural death of parent or any other loved one.

Bereaved children were included if they suffered from bereavement due to terror attack associated loss of parents or close loved ones or if they were themselves injured in the attack. Time since bereavement was about 3–5 years in the Bereaved Group. In case of loss (death) of multiple loved ones, nearest one was reported in data as the cause, e.g., if one parent and uncle were lost, only parent was reported as cause of bereavement.

All the non-bereaved children were apparently healthy, normal subjects and did not experience terror attack related sudden loss of their parents or loved one. Bereaved children having psychopathology before the terror attacks were excluded from the study; children showing symptoms of any disease on physical examination were also excluded from the study. Children using any drug which alters hormonal levels such as antidepressants, antipsychotic and anti-anxiety drugs were also excluded from this study.

For salivary cortisol collection microfuge tubes and a real cotton balls were used. At the time of sample collection children were instructed to pop the cotton ball into their mouths and roll it with the help of their tongue and gently chew to saturate the cotton ball completely with the saliva for one minute. After complete saturation, the saliva from the cotton ball was squeezed in to the microfuge tube. The saliva thus collected was kept in ice box and rapidly transferred and stored at -80 °C till analysis. All the participants were restricted to exercise or eat for at least 30 minutes prior to sample collection. Three salivary samples were collected at different time intervals, i.e., morning (M, 8.00 AM), evening (E, 6.00 PM) and post-dexamethasone sample (PD at 7.00 PM). A dose of 1 mg dexamethasone tablet was given orally to each child in the presence of trained medical person soon after collecting the evening samples at 6.00 PM and post-dexamethasone samples were collected after one hour of dexamethasone treatment, i.e., at 7 PM. The analysis of sample was done within one month of collection.

Salivary cortisol concentration was measured by Enzyme Linked Immunosorbent Assay (ELISA) method using human cortisol saliva kit (Calbiotech Inc., 10461 Austin Dr, Spring Valley, CA) according to manufacturer's instructions. Duplicate samples were run for analysis and mean values for each sample were calculated. Cortisol values were log-transformed to correct for deviations from normality. Assay detection limit for cortisol was 0.01 ng/ml. Statistical analyses were carried out by using SPSS-19. The normality of the data was tested using Kolmogorov-Smirinov and Shapiro-Wilk tests and histograms. Summary statistics were calculated and data were expressed as Mean±SD. Comparison of categorical data between bereaved and non-bereaved was analysed using Independent Sample *t*-test, and $p \leq 0.05$ was accepted as significant.

RESULTS

Table-1 shows the anthropometric and behavioural parameters in children of the Non-Bereaved and the Bereaved groups. Time since bereavement was about 3–5 years in the Bereaved Group. Time since bereavement or death of any relative in Non-Bereaved was significantly different in bereaved (4.49±1.71 years) as compared to Non-Bereaved (0.28±1.23) ($p<0.001$). Ages of the participants in the two groups showed non-significant ($p=0.97$) differences. Body Mass Index (BMI) showed a statistically significant ($p=0.001$) lower values among children of Bereaved Group as compared with the Non-Bereaved group. All behavioural parameters showing signs of depression (Isolation, Suicidal Thought, Crying and Sighing, Difficulty in Sleeping, Fatigue, Headache, Appetite Loss) were present in higher number of children among the Bereaved Group as compared with the Non-Bereaved Group and the difference was statistically significant ($p=0.001$).

Children in the Bereaved Group showed a statistically significant ($p=0.005$) higher levels of Salivary Cortisol in the morning sample (Log Cortisol M=14.43) as compared with the children in the Non-Bereaved Group (Log Cortisol M=6.44). Children in the Bereaved Group showed a statistically non-significant ($p=0.512$) higher levels of Salivary Cortisol in the evening sample (Log Cortisol M=11.46) as compared

with the children in the Non-Bereaved Group (Log Cortisol M=8.96). Children in the Bereaved Group showed a statistically non-significant (p=0.164) decrease in the levels of Salivary Cortisol in the Post-Dexamethasone sample (Log Cortisol M=8.85) as compared with the children in the Non-Bereaved Group (Log Cortisol M=4.25). Although the dexamethasone suppressed the cortisol level (Log Cortisol PD=8.85) in the Bereaved Group as compared with their Morning levels (Log Cortisol M=14.43), their cortisol levels were higher than the morning levels in the Non-Bereaved Group (Log Cortisol M=6.44). (Table-2).

Table-1: Anthropometric and behavioural parameters in Non-bereaved and Bereaved Groups

Parameter	Non-Bereaved Control (n=39)	Bereaved Cases (n=49)	*p*
Time since Bereavement (Yrs)	0.28±1.23	4.49±1.71	<0.001
Age SQRT*	14.76 (15.60–13.83)	14.78 (15.58–13.90)	0.97
BMI (Kg/m^2)	20.26±3.40	17.72±3.28	0.001
Isolation	3 (7.7%)	41 (83.7%)	<0.001
Suicidal thought	0 (.0%)	23 (46.9%)	<0.001
Crying & sighing	0 (.0%)	47 (95.9%)	<0.001
Difficulty in sleeping	2 (5.1%)	45 (91.8%)	<0.001
Fatigue	3 (7.7%)	41 (83.7%)	<0.001
Headache	2 (5.1%)	40 (83.3%)	<0.001
Appetite loss	1 (2.6%)	44 (89.8%)	<0.001

SQRT=*Squared for analysis, values are geometric means

Table-2: Salivary Cortisol Levels in Non-Bereaved and Bereaved Groups [Mean (Range)]

Parameter	Non-Bereaved Control (n=39)	Bereaved Cases (n=49)	*p*
Log cortisol M*	6.44 (4.60–8.99)	14.43 (9.71–21.44)	0.005
Log cortisol E*	8.96 (6.38–12.56)	11.46 (5.80–22.63)	0.512
Log cortisol PD*	4.25 (1.84–9.80)	8.85 (4.58–17.11)	0.164

*Log transformed for analysis, values are geometric mean.

DISCUSSION

Over the past more than a decade, terrorists killed an average of 26,000 people worldwide each year. The global death toll from terrorism over the past decade ranged from 8,200 in 2011 to a high of 44,600 in 2014.[10] In Pakistan, terrorism is increasing during the last three years.[11] Pakistan has already endured over US $ 126 billion economic losses and 83,000 people, including armed forces personnel, policemen, and other martyred.[12]

Although bereavement is always difficult, most children and young people learn to adjust and live with their loss over time. However, some are unable to process their grief if the traumatic way they perceive the death leaves them feeling profoundly unsafe. As a result, they are more likely to develop mental health problems and to have difficulties in areas such as relationships and school attainment. These children need significant support.[13]

This study reports the neuroendocrine alteration in parentally bereaved children who have participated in this retrospective study of the impact of sudden unexpected parental or close relative death during terrorist attacks during the global war against terrorism in the North-West part of Pakistan.

Bereaved children had significantly higher rates of psychiatric problems compared to non-bereaved children. Our results are in line with previous longitudinal studies on the levels of cortisol response and stress induced depression in bereaved children.[14,15] BMI of Bereaved Group children showed a lower value as compared with the Non-Bereaved Group. This may be because bereaved children had decreased appetite.

Salivary cortisol levels remained high in Bereaved as compared with the Non-Bereaved children. The level of morning salivary cortisol in bereaved children was significantly higher compared to non-bereaved children (p=0.005). The levels of evening and post dexamethasone salivary cortisol response were also high in bereaved children to non-bereaved control. The salivary cortisol levels remained high despite dexamethasone suppression suggesting an impaired central feedback. Our results are in line with previous longitudinal studies on the levels of cortisol response and stress induced depression in bereaved children.[14,15] The non-significant change in salivary cortisol response in the evening and post-dexamethasone group might be due to the small number of subject included in our study. However, discrepancies still exist in different studies and showed inconsistent salivary cortisol levels measured at different time intervals. Another study observed a significant increase in the evening salivary cortisol level rather than morning cortisol among children exposed to stressful events compared to control children not exposed to such events.[16] Similar variations in salivary cortisol measurement were also reported to be dependent upon gender, age and time of sampling.[17]

CONCLUSION

Terror bereavement causes long-term activation of the HPA axis, elevating the cortisol levels even 3–4 years after the traumatic bereavement. Chronic elevation of cortisol results in the manifestation of depression related signs and symptoms.

RECOMMENDATION

Further studies are warranted to examine changes in the HPA axis after exposure to chronic stress to understand how the evolving change in stress response and neuroendocrine alteration correlate to mental and physical health of bereaved children.

REFERENCES

1. Teaching guide on international terrorism: definitions, causes, and responses. Retrieved from: https://www.usip.org/publications/2001/11/files/resources/terrorism.pdf. [Accessed: 2 Dec 2022]
2. Global Terrorism Index 2017. Retrieved from: https://reliefweb.int/report/world/global-terrorism-index-2017. [Accessed: 26 Jan 2023]

3. Support to Pakistan's Action to Counter-Terrorism with a Special Reference to Khyber Pakhtunkhwa (PACT KP) project. URL: https://www.eeas.europa.eu/sites/default/files/pact-kp_fiche.pdf. [Accessed: 6 Dec 2022]
4. Kaltman S, Bonanno GA. Trauma and bereavement: Examining the impact of sudden and violent deaths. J Anxiety Disord 2003;17(2):131–47.
5. Nakajima S, Ito M, Shirai A, Konishi T. Complicated grief in those bereaved by violent death: the effects of post-traumatic stress disorder on complicated grief. Dialogues Clin Neurosci 2012;14(2):210–4.
6. Harris T, Brown GW, Bifulco A. Loss of parent in childhood and adult psychiatric disorder: the role of lack of adequate parental care. Psychol Med 1986;16(3):641–59.
7. Henley DE, Kaye JM, Lightman SL. The endocrine response to stress. In: Oxford Textbook of Endocrinology and Diabetes [Internet]. Oxford University Press; 2011. pp.287–94. Retrieved from: https://academic.oup.com/book/25144/chapter/189453762. [Accessed: 12 Dec 2022]
8. Brent DA, Perper JA, Moritz G, Liotus L, Richardson D, Canobbio R, *et al*. Posttraumatic stress disorder in peers of adolescent suicide victims: Predisposing factors and phenomenology. J Am Acad Child Adolesc Psychiat 1995;34(2):209–15.
9. Dietz LJ, Stoyak S, Melhem N, Porta G, Matthews KA, Payne MW, *et al*. Cortisol response to social stress in parentally bereaved youth. Biol Psychiat 2013;73(4):379–87.
10. Ritchie H, Hasell J, Mathieu E, Appel C, Roser M. Terrorism. URL: https://ourworldindata.org/terrorism. [Accessed: 1 Feb 2023]
11. Annual security report-2022. Available at: https://crss.pk/annual-security-report-2022/. [Accessed: 30 Jan 2023]
12. NSC to decide on 'decisive' operation against terrorists. URL: https://tribune.com.pk/story/2398632/nsc-to-decide-on-decisive-operation-against-terrorists-asif. [Accessed: 1 Feb 2023]
13. Traumatic bereavement. A guide for schools and colleges to help children and young people who are struggling. UK Trauma Council. URL: https://uktraumacouncil.org/wpcontent/uploads/2021/02/210226_UKTC_TB_animationguide_draft_v2.pdf. [Accessed: 1 Feb 2023]
14. Pfeffer CR, Altemus M, Heo M, Jiang H. Salivary cortisol and psychopathology in children bereaved by the September 11, 2001 terror attacks. Biol Psychiat 2007;61(8):957–65.
15. Luecken LJ, Appelhans BM. Early parental loss and salivary cortisol in young adulthood: the moderating role of family environment. Dev Psychopathol 2006;18(1):295–308.
16. Hart J, Gunnar M, Cicchetti D. Altered neuroendocrine activity in maltreated children related to symptoms of depression. Dev Psychopathol 1996;8(1):201–14.
17. Jessop DS, Turner-Cobb JM. Measurement and meaning of salivary cortisol: A focus on health and disease in children: Rev Stress 2008;11(1):1–14.

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ORIGINAL ARTICLE

RELATIONSHIP OF SLEEP QUALITY WITH MENTAL WELL-BEING AND ACADEMIC PRODUCTIVITY

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Background: Better sleep quality is associated with better mental health. This study aimed to link sleep quality of medical students with their psychiatric health and academic performance. **Methods:** Sleep quality of 104 medical students (52 each from 1st and 5th year of MBBS with equal gender distribution) was measured on Pittsburgh Sleep Quality Index (PSQI) while Hamilton Depression Rating Scale (HDRS) along with Hamilton Anxiety Rating Scale (HAM-A) was used to assess their psychiatric health, both of which were later correlated with their academic scores. **Results:** Sleep quality of 1st year medical students was significantly better as compared to their final year counterparts (p=0.001) and (p=0.000) respectively which affected indices of psychiatric health in such a way that 1st year medical students scored significantly lower on scales of depression as well as anxiety as compared to final year medical students (p=0.001), (p=0.000) and (p=0.001, p=0.000). Within 1st and final year (male and female) medical students, sleep quality had a strong positive correlation with scores of depression and anxiety (r=0.547, p=0.004), (r=0.587, p=0.002), (r=0.66, p=0.000) ,(r=0.490, p=0.011) and (r=0.518, p=0.007), (r=0.527, p=0.006), (r=0.541, p=0.004), (r=0.596, p=0.001) respectively, which in turn had a negative correlation with academic performance of theirs (r=-0.400, p=0.043), (r=-0.614, p=0.001) and (r=-0.550, p=0.004), (r=-0.573, p=0.002) respectively. **Conclusion:** Medical students with poor sleep quality, harbour higher degrees of depression and anxiety and perform poorly on academic front as compared to those with better sleep quality.

Keywords: Depression, Anxiety, Medical students, Sleep quality, Academic performance

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INTRODUCTION

Since better sleep quality has a specific neuro-protective role[1], its deterioration as an uptrend within a wide spectrum of society as well as medical fraternity under influence of a number of triggering factors such as long academic hours, hectic professional duties and heightened social expectations is being considered a matter of high concern as it can induce inflammatory as well as neuro-conductive anomalies reflecting in terms of inter-connected cognitive and psycho-psychiatric impairments.[2]

It is believed that psycho-psychiatric upheaval, which thrives within the boon of poor sleep quality, originates as a result of dysregulated bio-physiology of crucial neurological factors such as that of nuclear factor kappa-B which coupled with a continuous overstimulation of hypothalamo-pituitary axis (HPA) within a sleep deprived status leads to overexpression of a whole spectrum of Reactive Oxygen Species (ROS) such as IL-6 and TNF-α within neurological centers that primarily regulate mood and behaviour.[3] These dysregulations not only enhance presynaptic reuptake of monoamines and speed up their degradation but enhance glutamate associated excito-toxicity within circuits aligned with emotional control also[4] which then initiate a syndrome of emotional volatility that leads to evolution of depression (apsycho-psychiatric disorder highlighted by feelings of dejection, desolation, passivity and suicidal intentions)[5] and anxiety (psychosomatic condition marked by stressful notions that manifest themselves in form systemic upheavals).[6]

In addition to dysregulation of HPA, sleep deprivation also brews a chaos for neuro-biochemical levels of Vascular Endothelial Growth Factor (VEGF), Brain Derived Neurotropic Factor (BDNF) as well as cortisol that not only initiates a long term disruption of circadian rhythm where sleep deprivation and altered circadian biology keep potentiating each other in a complementary fashion but also enhances the probability of mood disorders presenting within the diaspora of depression.[7] Since sleep deprivation also leads to poor serotonin levels within various components of limbic cortex that gets linked with an altered neurophysiological status of cAMP associated 5HT-4 receptors within CA1 area of the hippocampus, an area primarily associated with retaining and learning capacities of the brain, hence sleep deprivation not only paves way for the establishment of depressive states but also for a consistent memory decline.[8]

An unchecked poor sleep leads to an enhanced cholinergic, glutaminergic as well as α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and N-methyl-D-aspartate (NMDA) receptor activity within the prefrontal cortex which is coupled with dysregulation of neuropeptide S (NPS) within central and lateral portions of amygdala, both of which in

unison strengthen the establishment of anxiety though an elevated neuronal activity within a myriad of limbic centres working in close association with hippocampal nuclei. This limbic overstimulation later initiates neuronal degeneration within hippocampal nuclei and this than depicts itself in terms of a long lasting decline of cognitive performance. Thus, sleep deprivation through above stated intricate pathways sets a fertile neuronal environment for evolution of depressive as well as anxiety disorders complimenting a consistent memory decline.[9]

Considering the importance of sleep quality for mental harmony of an individual, as has been stated in the above presented case, this study tried to extend a scientific proof regarding importance of proper sleep quality for mental wellbeing as well as academic productivity while working with a young population of the medical students of south Punjab who are considered to be the most brilliant lot of the country and are observed to experience a gradual decline of sleep quality as they ascend towards their graduation year.

METHODOLOGY

Sample size of 26 participants for each group of this cross-sectional, observational study was calculated with a power of 90% and an alpha level of 5% through utilization of mean differences of sleep quality between first and final year medical students.[10]

$$\frac{n= \sigma 2(Z1-\alpha/2+Z1-\beta)2}{(\mu 0-\mu 1)2}$$

The study was conducted after getting official approval from Institutional Review Board (IRB) of Nishtar Medical University, Multan. To ensure that obesity did not present itself as a confounder within psycho-psychiatric indices of our study, we only included non-obese subjects, declared so by the WHO 2000 and 2015 criteria (males with a BMI<24.9 and a WHR≤0.9 while females having a BMI<24.9 and a WHR≤0.8) respectively. Subjects considered obese, those with previous history of psycho-psychiatric and/or endo-metabolic ailment(s) and those with history of recreational drug use and/or family abuse were excluded from the paradigm of this study.

Consent in writing was obtained from medical students of 1st and 5th year of medicine. BMI and WHR were calculated to segregate a general pool of non-obese subjects, out of which 52 students from 1st year of medicine and 52 from 5th year of medicine were randomly selected through lottery ticket method (observing an equal gender representation). Group 1 and 2 thus consisted of 1st year males (1st YMs) and females (1st YFs), while Group 3 and 4 consisted of 5th year males (5th YMs) and females (5th YFs) respectively. The total population, hence consisted of 104 medical students with 26 subjects falling into each of the above mentioned four study groups.

Pittsburgh Sleep Quality Index (PSQI) scale with a Cronbach's alpha value of 0.83[11] was then administered to participants of all groups which, while running on a Likert scale, where a score higher than 5 represents a poorer shade of sleep quality while a score less than 5 depicts a better sleep quality, helped us deduce the sleep quality of the students from both 1st and 5th year of medicine. To assess degree of depression within medical students of each of the study groups, Hamilton Depression Rating Scale (HDRS), having a Cronbach's alpha of 0.86[12], was used. This tool runs on a Likert scale as well and here a score of 0–7 represents non-existence of depression, a score of 8–16 indicates presence of mild depression, a score of 17–23 implies existence of a moderate degree of depression while a score more than 24 indicated severe depression. To establish degree of anxiety of subjects, Hamilton Anxiety Rating Scale (HAM-A) with a Cronbach's alpha value of 0.87[13] was used. This too is a Likert scale associated tool where a score of <17 indicates non-existent to mild shade of anxiety, scores of 18–24 marks the presence of mild to moderate degree of anxiety while score of 25–30 represents moderate to severe form of anxiety. In addition to these scales, percentage of total score achieved by students of each of the four categories in the last professional examination was considered an index of academic performance.

Collected data was entered into SPSS-26 where it was analysed for normality distribution via Shapiro-Wilk's and Kolmogorov Smirnov's tests. Since most of study parameters were found to have a normal distribution, hence parametric inferential statistics was applied for data analysis. ANOVA, paired with Post-hoc Tukey's test was applied to draw a comparison of sleep quality, depression, anxiety and academic performance between study groups while Pearson's correlation was applied to determine correlation between various quantitative variables.

RESULTS

Since majority of our study parameters were normally distributed, hence our data has been represented as Mean±SD. It was noted that, 1st YM had an age of 19.538±0.760, weight of 60.069±5.903 Kg, squared height of 2.899±0.199 m^2, body mass index of 20.727±1.675 Kg/m^2, waist circumference of 76.788±5.665 Cm, hip circumference of 92.846±4.763 Cm and waist hip ratio of 0.826±0.030. 1st YF had an age of 18.807±0.693, weight of 52.926±5.677 Kg, squared height of 2.497±0.220 m^2, body mass index of 21.205±1.476 Kg/m^2, waist circumference of 72.038±4.268 Cm, hip circumference of 92.076±5.491 Cm and waist hip ratio of 0.779±0.024. 5th YM had an age of 22.884±0.816, weight of 65.042±7.931 Kg, squared height of 2.985±0.276 m^2, body mass index of 21.765±1.437 Kg/m^2, waist circumference of

75.384±9.073 Cm, hip circumference of 89.538±8.892 Cm and waist hip ratio of 0.840±0.028. The 5th YF had an age of 23.538±0.581, weight of 55.807±5.151 Kg, squared height of 2.645±0.183 m^2, body mass index of 21.121±1.610 Kg/m^2, waist circumference of 71.730±6.494 Cm, hip circumference of 93.038±7.372 Cm and waist hip ratio of 0.770±0.027.

After application of ANOVA to determine the existence of differences for PSQI, HDRS, HAM-A and AS (p=0.000, p=0.000, p=0.000 and p=0.000 respectively), we (through Post Hoc Tukey's Test) found that the sleep quality (deduced through PSQI) along with scores of depression (calculated on HDRS) and anxiety (estimated via HAM-A) within 1st YMs and 1st YFs were significantly lower than 5th YMs and 5th YFs and that 1st YM and 1st YF performed significantly better in their academia than 5th YMs and 5th YFs (Table–1).

It was also observed that sleep quality, both within 1st and 5th year male and female medical students, had a significant positive correlation with the degree of depression and anxiety while a negative one with academic aces. Moreover, the intensity of psycho-psychiatric instability that reflected itself in terms of the scores of depression and/or anxiety had an independent negative correlation with academic scores too. This data has been represented in Table-2 and 3 respectively.

The correlation of sleep quality and the indices of psycho-psychiatric and academic stability, for the whole of the study population combined together, has been represented in Figure-1.

Table-1: Comparison (Post Hoc Tukey's) of PSQI, HDRS, HAM and Academic Scores in study groups

Variable	Groups In Comparison (n=26)		p
PSQI Scores	1st YMs 3.320±2.035	1st YFs 4.840±2.211	0.641
	5th YMs 8.520±5.591	5th YFs 12.320±6.283	0.024
	1st YMs 3.320±2.035	5th YMs 8.520±5.591	0.001
	1st YFs 4.840±2.211	5th YFs 12.320±6.283	0.000
HRDS Scores	1st YMs 4.800±3.201	1st YFs 7.200±3.628	0.647
	5th YMs 12.240±8.733	5th YFs 14.680±8.591	0.607
	1st YMs 4.800±3.201	5th YMs 12.240±8.733	0.001
	1st YFs 7.200±3.628	5th YFs 14.680±8.591	0.001
HAMA Scores	1st YMs 12.800±7.388	1st YFs 16.680±9.910	0.549
	5th YMs 23.800±10.839	5th YFs 31.080±9.784	0.041
	1st YMs 12.800±7.388	5th YMs 23.800±10.839	0.000
	1st YFs 16.680±9.910	5th YFs 31.080±9.784	0.000
Academic Scores	1st YMs 85.840±5.171	1st YFs 83.820±5.798	0.667
	5th YMs 77.180±6.130	5th YFs 72.404±6.094	0.034
	1st YMs 85.840±5.171	5th YMs 77.180±6.130	0.000
	1st YFs 83.820±5.798	5th YFs 72.404±6.094	0.000

Table-2: Correlation of sleep quality with indices of depression, anxiety, and academia

	Sleep Quality							
	1st YMs		1st YFs		5th YMs		5th YFs	
Variable	***r***	***p***	***r***	***p***	***r***	***p***	***r***	***p***
HDRS	0.547	0.004	0.667	0.000	0.518	0.007	0.541	0.004
HAMA	0.587	0.002	0.490	0.011	0.527	0.006	0.596	0.001
AS	-0.400	0.043	-0.614	0.001	-0.550	0.004	-0.573	0.002

Table-3: Correlation of sleep quality with academic score for all the study groups

	Academic Score							
	1st YMs		1st YFs		5th YMs		5th YFs	
Variable	***r***	***p***	***r***	***r***	***r***	***p***	***r***	***p***
HDRS	-0.544	0.004	-0.393	0.047	-0.632	0.001	-0.660	0.000
HAMA	-0.631	0.001	-0.390	0.049	-0.570	0.003	-0.292	0.148

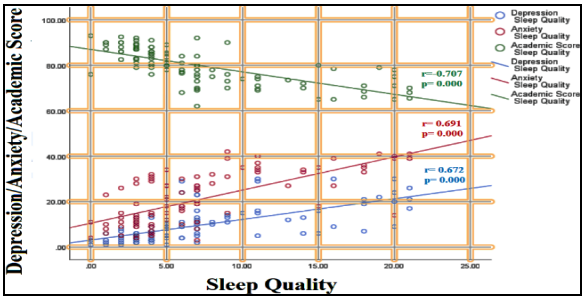

Figure-1: Correlation of sleep quality with indices of depression, anxiety and academia for whole of study population combined together

DISCUSSION

We observed that sleep quality of both male and female medical students attending their first year of medical school was significantly better than their final year counterparts, a fact which is supported by projections of earlier studies[14] and could be attributed to an ever more competency demanding academics and to non-availability of viable guidelines to tackle this pressure that ultimately leads to evolution of academic uncertainty within students of clinical years that affects their sleep quality by acting as a cutaneous stressor.[15]

Final year male and female medical students with poor sleep quality experienced a higher degree of depression than students in 1st year of medicine having better sleep quality. This finding is in accordance with those being reported by recent research papers[16] and could be justified on proposition that sleep deprivation leads to an increased degradation of tryptophan within kynurenine pathway which not only reduces serotonin levels in key mood controlling areas like hypothalamus and frontal cortex but also leads to their neurodegeneration due to accumulation of neurotoxic kynurenine metabolites which than becomes a prelude over which depression can thrive.[17]

Regardless of their gender final year medical students harboured a far more intense shade of anxiety than their first year counterparts which is a finding echoed within the projections of contemporary research papers[18] where it is suggested that sleep reduction not only increases the levels of inflammatory cytokines, i.e., IL-6, TNF-α, and IFN-γ, but also enhances the level of stress hormones like cortisol, both of which combined together lead to reduced production of neuro-calming chemicals like serotonin and melatonin as well as an increased accumulation of excitotoxins within basal ganglia and its associated cortical mood controlling areas and this provides an environment for stress disorders like anxiety to brew.[19]

Within students of final year of medicine, females did experience poor sleep quality and heightened anxiety even compared to their age and ethnicity matched male counterparts. This again is a finding that is inline with those which portray that an intense shade of anxiety syndrome expresses itself in females approaching postgraduate practical rung of life because they have to face higher degree of marital as well as domestic expectations[20] which when gets a unison with regularly fluctuating endo-menstrual status of theirs is able to alter expression of dopamine, serotonin, acetylcholine and GABA within cortico-hypothalamo-amygdaloid regions that disrupts harmony of HPA to create emotional volatility.[21]

Sleep deprivation, both within 1st and final year medical students regardless of gender, had a positive correlation with degree of depression as well as anxiety and a negative with academic performance, a finding that is supported by data projected by recently.[15] It is suggested that sleep deprivation leads to insensitivity of serotonin-1A receptors within the fronto-cortical circuitry associated with mood stability which than paves way for emergence of mood disturbances like depression and anxiety that disrupts intellectual focus and becomes the base of poor academia.[22]

Regardless of gender distribution, both within 1st and final year medical students, intensity of depression and anxiety that evolved over sleep deprived status showed an independent negative correlation with academic performance too. This stands in accord with projections of neuro-biochemical studies[23] which suggest that sleep deprivation, through glutathione-kynurenic acid imbalance, creates an environment of oxidative stress within hippocampus, basal amygdala as well as lateral prefrontal cortex that leads to their neuro-degeneration which consequently exacts a toll on the sleep deprived students' memory as well as on fact retrieval capacity and provides a prelude for poor academic focus and productivity.[24]

CONCLUSION

As the medical students move to their graduation year, their sleep quality declines while their degree of depression and anxiety plummets which leads to an academic deterioration. We advocate for better sleep quality, specifically for the students whose studies become ever more demanding towards the end of the degree courses, so that the most productive strata of the society which has to be the custodian of our nation's future could extend us its best instead of slipping into the clutches of psycho-psychiatric disorders. Future researchers who intend to further explore this domain of neurophysiology are recommended to carry out cohort studies which do observe a selected population over its entire period of graduation.

REFERENCES

1. Schneider L. Neurobiology and neuroprotective benefits of sleep. Continuum (Minneap Minn) 2020;26(4):848–70.
2. Vaseghi S, Arjmandi-Rad S, Eskandari M, Ebrahimnejad M, Kholghi G, Zarrindast MR. Modulating role of serotonergic signaling in sleep and memory. Pharmacol Rep 2022;74:1–26.
3. Agrawal S, Kumar V, Singh V, Singh C, Singh A. A review on pathophysiological aspects of sleep deprivation. CNS & Neurological Disorders-Drug Targets (Formerly Current Drug Targets-CNS & Neurological Disorders). 2023;22(8):1194–208.
4. Begdache L, Kianmehr H, Sabounchi N, Marszalek A, Dolma N. Principal component regression of academic performance, substance use and sleep quality in relation to risk of anxiety and depression in young adults. Trends Neurosci Educ 2019;15:29–37.
5. Benazzi F. Various forms of depression. Dialogues Clin Neurosci. 2006;8(2):151–61.
6. Daviu N, Bruchas MR, Moghaddam B, Sandi C, Beyeler A. Neurobiological links between stress and anxiety. Neurobiol Stress 2019;11:100191.
7. Vaseghi S, Mostafavijabbari A, Alizadeh MS, Ghaffarzadegan R, Kholghi G, Zarrindast MR. Intricate role of sleep deprivation in modulating depression: focusing on BDNF, VEGF, serotonin, cortisol, and TNF-α. Metab Brain Dis 2023;38(1):195–219.
8. Eydipour Z, Nasehi M, Vaseghi S, Jamaldini SH, Zarrindast MR. The role of 5-HT4 serotonin receptors in the CA1 hippocampal region on memory acquisition impairment induced by total (TSD) and REM sleep deprivation (RSD). Physiol Behav 2020;215:112788.
9. Xie JF, Shao YF, Wang HL, Wang C, Cui GF, Kong XP, *et al.* Neuropeptide S Counteracts Paradoxical Sleep Deprivation-Induced Anxiety-Like Behavior and Sleep Disturbances. Front Cell Neurosci 2018;12:64.
10. Choueiry N, Salamoun T, Jabbour H, El Osta N, Hajj A, Rabbaa Khabbaz L. Insomnia and Relationship with Anxiety in University Students: A Cross-Sectional Designed Study. PLoS One 2016;11(2):e0149643.

11. Morris JL, Rohay J, Chasens ER. Sex differences in the psychometric properties of the Pittsburgh sleep quality index. J Women's Health (Larchmt) 2018;27(3):278–82.
12. Obeid S, Abi Elias Hallit C, Haddad C, Hany Z, Hallit S. Validation of the Hamilton Depression Rating Scale (HDRS) and sociodemographic factors associated with Lebanese depressed patients. Encephale 2018;44(5):397–402.
13. Barbar S, Haddad C, Sacre H, Dagher D, Akel M, Kheir N, *et al.* Factors associated with problematic social media use among a sample of Lebanese adults: The mediating role of emotional intelligence. Perspect Psychiatr Care 2021;57(3):1313–22.
14. Bhatti AA, Khan UA, Khan HF. Sleep habits of first year and final year medical student. Rawal Med J 2012;37(2):148–51.
15. Shao R, He P, Ling B, Tan L, Xu L, Hou Y, *et al.* Prevalence of depression and anxiety and correlations between depression, anxiety, family functioning, social support and coping styles among Chinese medical students. BMC Psychol 2020;8(1):38.
16. Al-Khani AM, Sarhandi MI, Zaghloul MS, Ewid M, Saquib N. A cross-sectional survey on sleep quality, mental health, and academic performance among medical students in Saudi Arabia. BMC Res Notes 2019;12(1):665.
17. Bhat A, Pires AS, Tan V, Babu Chidambaram S, Guillemin GJ. Effects of Sleep Deprivation on the Tryptophan Metabolism. Int J Tryptophan Res 2020;13:1178646920970902.
18. Quek TT, Tam WW, Tran BX, Zhang M, Zhang Z, Ho CS, *et al.* The global prevalence of anxiety among medical students: A meta-analysis. Int J Environ Res Public Health 2019;16(15):2735.
19. Chanana P, Kumar A. Possible involvement of nitric oxide modulatory mechanisms in the neuroprotective effect of Centella Asiatica against sleep deprivation induced anxiety like behaviour, oxidative damage and neuro inflammation. Phytother Res 2016;30(4):671–80.
20. Pokhrel NB, Khadayat R, Tulachan P. Depression, anxiety, and burnout among medical students and residents of a medical school in Nepal: a cross-sectional study. BMC Psychiatry 2020;20(1):298.
21. Giannini A, Caretto M, Genazzani AR, Simoncini T. Neuroendocrine changes during menopausal transition. Endocrines 2021;2(4):405–16.
22. Frau R, Traccis F, Bortolato M. Neurobehavioural complications of sleep deprivation: Shedding light on the emerging role of neuroactive steroids. J Neuroendocrinol 2020;32(1):e12792.
23. Singh R, Suri JC, Sharma R, Suri T, Adhikari T. Sleep Pattern of Adolescents in a School in Delhi, India: Impact on their Mood and Academic Performance. Indian J Pediatr 2018;85(10):841–8.
24. Liaqat H, Parveen A, Kim SY. Neuroprotective Natural Products' Regulatory Effects on Depression via Gut-Brain Axis Targeting Tryptophan. Nutrients 2022;14(16):3270.

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ORIGINAL ARTICLE

FREQUENCY OF SARCOPENIA IN CIRRHOTIC PATIENTS DETERMINED ON ABDOMINAL COMPUTED TOMOGRAPHY

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Background: Fibrosis of the liver progresses to cirrhosis resulting in hepatocellular dysfunction. Sarcopenia is an early sign of liver malfunction. Early detection of sarcopenia may provide a chance to cure or delay deterioration of liver function. This study aimed to detect sarcopenia on abdominal computed tomography (CT). **Methods:** This descriptive, cross-sectional study was carried out at the Department of Diagnostic Radiology, Fauji Foundation Hospital Rawalpindi from 1st October 2022 to 31st March 2023. All patients suffering from Hepatitis B or C referred for evaluation of complications were included in the study. After informed consent and examination, at LV3 level an axial CT image in abdominal window was assessed on each scan using Radiant® software. Muscles including internal and external obliques and transversus abdominus, rector spinae, quadratus lumborum, and psoas were identified and evaluated. A threshold of -29 to -150 HU, and -29 to 150 HU for fatty tissue and skeletal muscles respectively was used. Cross-sectional area of each muscle was calculated on the vitrea and skeletal muscle index was calculated. Cut-off value for men was taken as 52.4 Cm^2/m^2, and for women it was 38.5 Cm^2/m^2. **Results:** A total 120 chronic liver disease patients (21 males and 99 female) were studied. All (100%) men, and 82 (83.8%) women had sarcopenia. **Conclusion:** Sarcopenia was a common finding with male predominance in patients suffering from Hepatitis B and/or C. CT evaluation is an effective and non-invasive tool for evaluation of sarcopenia as an indicator of liver dysfunction.

Keywords: Sarcopenia, Cirrhosis, Liver, Hepatitis, Hepatocellular carcinoma, Computed tomography

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INTRODUCTION

Cirrhosis is the last stage of progressive liver fibrosis due to viral, non-alcoholic, or alcoholic steatohepatitis.[1] Its consequences include liver damage leading to fibrosis, necroinflammation, vascular remodelling and hepatocellular dysfunction. Liver transplantation can be a curative treatment, but it cannot be done in many cases.[2] Management is directed towards prevention or delaying complications like variceal bleeding, ascites, hepatorenal syndrome, hepatic encephalopathy, or hepatocellular carcinoma (HCC).[3]

Sarcopenia is defined as loss of skeletal muscle mass, and appears in patients with cirrhosis of liver.[4] It indicates bad prognosis like hepatic decompensation, poor quality of life, longer intensive care unit and hospital stay, higher incidence of infection, high overall healthcare cost, and high mortality in cirrhotic patients who are considered and evaluated for liver transplantation.[5] Skeletal muscle area of cross-section calculated on computed tomography (CT) is an index of diminished muscle mass (myopenia), skeletal muscle depletion, and increased muscle fat deposition (myosteatosis) which are associated with decreased muscle quantity and quality.[6–8] The role of CT scan to establish sarcopenia syndrome in cirrhotic patients can be of value to help improve the outcomes and decision making of vital surgery.

This study aimed at looking for prevalence of sarcopenia using abdominal CT in patients of cirrhosis liver presenting to Radiology Department of Fauji Foundation Hospital Rawalpindi, Pakistan.

MATERIAL AND METHODS

After approval from Hospital Ethical Review Committee this study was conducted from 1st October 2022 to 31st March 2023 in CT Scan Department of Diagnostic Radiology, Fauji Foundation Hospital, Rawalpindi on patients of Hepatitis B and/or C referred for evaluation of hepatic complications. The sample size was calculated[9] as 113 using RAOSOFT calculator at 95% confidence level. Written informed consent was given by all patients under study. Detailed data of each patient was recorded including age, height, gender, Hepatitis B and C profile, substance abuse (alcohol etc.), and history of known chronic medical diseases (e.g., chronic hypertension, diabetes mellitus). Hepatitis B and C negative patients were not included in the study.

Image on axial CT at the level of LV3 in abdominal window was assessed on each scan. Radiant® software was used for analysis of the images. Muscles including internal oblique, transversus abdominus, external oblique, psoas, quadratus lumborum, and rector spinae at LV3 were identified and evaluated. Threshold for fatty tissue was kept as -29 to -150 HU, and for skeletal muscle it was -29 to 150 HU. Cross-sectional

area of each muscle was manually calculated on the vitrea and normalised for height and skeletal muscle index was calculated as total measured area at LV3 divided by patient's height in meter squared. Cut-off value for men was taken as 52.4 Cm^2/m^2, and for women it was 38.5 Cm^2/m^2.[10]

Data was entered and analysed using SPSS-21. Quantitative variables like age, height and skeletal muscle index were presented as Mean±SD. Qualitative variables like hepatitis profile, hepatocellular carcinoma, history of known chronic medical diseases (chronic hypertension, diabetes mellitus) were presented as frequencies and percentages. Student's *t*-test was used for data interpretation and $p \leq 0.05$ was considered as statistically significant.

RESULTS

A total of 120 chronic liver disease patients (21 males and 99 female) were included in the study. Mean age of male patients was 50.05±6.57 years while that of female patients was 50.05±5.85 years. There were no significant mean differences between males and females ($p>0.05$). Mean height of males was 1.73±0.04 m while that of females was 1.55±0.08 m with significant differences between males and females ($p<0.05$). The mean skeletal mass index in men was 31.75±9.20, and in women it was 32.32±6.90 with non-significant differences between males and females ($p>0.05$). The relationship between height of males and females was compared with mean skeletal mass index using Pearson correlation, and it was non-significant ($p>0.05$). The correlation between age and skeletal muscle index was also non-significant ($p>0.05$). (Table-1).

All 21 (100%) male patients, and 82 (83.8%) female patients had sarcopenia, showing male predominance having sarcopenia in Hepatitis. Gender differences in the frequency of sarcopenia were significant statistically ($p<0.05$). (Table-2).

Table-1: Age and height of the patients (Mean±SD)

Patients	Age (Years)	Height (m)	Skeletal Muscle Index
Male (n=21)	50.048±6.569	1.732±0.042	31.747±9.200
Female (n=99)	50.051±5.853	1.554±0.075	32.315±6.901
p	$p>0.05$	$p<0.05$	$p>0.05$

Table-2: Frequency of sarcopenia in patients with Hepatitis B and/or C

Patients	Sarcopenia	No sarcopenia	*p*
Male (n=21)	21	0	<0.05
Female (n=99)	82	17	

DISCUSSION

Significant association of sarcopenia in patients with cirrhosis was revealed in this study. With a few exceptions the findings of the present study were in agreement with previous work.

Puneeta Tandon *et al*[11] conducted a study on sarcopenia and frailty in decompensated cirrhosis and found that 43% (57% men and 25% women) patients with cirrhosis had sarcopenia. Liu J *et al*[12] observed sarcopenia in 113 of 159 (71%) men, and 32 of 65 (49%) women with cirrhosis. We also found higher percentage of sarcopenia in men than women. Our finding of 100% cases having sarcopenia in males may be attributed to a comparatively lesser number of males in our patients.

Jaya Benjamin *et al*[13] in a computed tomography-based study on patients having cirrhosis due to alcoholism found 68.2% patients having sarcopenia assessed through skeletal muscle index. Elizabeth *et al*[14] found sarcopenia in 62% of their patients. Tatsunori Hanai *et al*[15] conducted a retrospective study and observed that sarcopenia was found in 68% of their patients. They concluded that it was more likely in females and Hispanic patients; this could be due to any racial and/or ethnic factors in their study population.

Xin Xing Tantai *et al*[16] observed a that sarcopenia was present in 37.5% cirrhotic patients, more in males, those with alcohol related liver diseases, and child Pugh grade C cirrhosis. Toshiro Masuda *et al*[17] observed sarcopenia collectively in 47.1% cases, 58.3% in males, and 35.65% in females. Findings of our study are in agreement with these studies.

Topan MM *et al*[18] found liver cirrhosis in 55.2% alcoholics and sarcopenia in 57.2% cases with no significant differences between males and females. They found association of sarcopenia with higher risk of HCC besides other complication. They concluded that sarcopenia was a frequent complication of cirrhosis liver and was associated with adverse effects on health, and poor survival rates. They recommended prioritization of this simple test for early detection of complications to increase survival rate and decrease hospital burden. Similar results have been reported by many other workers; some are summarised in Table-3.

Table-3: Comparison of Skeletal Muscle Index (Cm^2/m^2) in men at the level of L3–4 vertebrae with other studies

Study	n	Men [n (%)]	Cut-off values for Sarcopenia	Prevalence
This study	120	21 (17.5)	**Men:** ≤52.4 Cm^2/m^2 **Women:** ≤38.5 Cm^2/m^2	85.83% (men 100%, women 83.8%)
Cruz *et al*[19]	234	157 (67)		70% (men 76%, women 58%)
DiMartini *et al*[20]	338	223 (6)		68% (men 76%, women 51%)
Hanai *et al*[4]	130	76 (58)		68% (men 82%, women 50%)
Montano-loza *et al*[21]	112	78 (70)		40% (men 50%, women 18%)
Tandon *et al*[22]	142	85 (60)		41% (men 54%, women 21%)
Meza-Junco *et al*[23]	116	98 (84)	**Men:** BMI ≥25 Kg/m^2: ≤53 Cm^2/m^2, BMI <25 Kg/m^2: ≤43 Cm^2/m^2	30% (men 31%, women 28%)
Montano-loza *et al*[24]	248	169 (68)	**Women:** ≤41 Cm^2/m^2	45% (men 52%, women 30%)

Sarcopenia can result after loss of appetite due to liver malfunction. It can be taken as an indication of deteriorating liver function, and an early warning sign. Appropriate treatment measures to delay the progress of the disease can be practiced which can help in prolonging lives of the patients. It is of significant importance in our set-up because of the widespread liver disease in Pakistan, especially due to Hepatitis B and C.

CONCLUSION

Sarcopenia is common in patients with Hepatitis B and/or C with male predominance for sarcopenia evaluated non-invasively on CT. Sarcopenia may be taken as an important diagnostic and prognostic tool in patients with cirrhosis or hepatocellular carcinoma to start early measures for management especially those considered for liver transplantation.

REFERENCES

1. Zambrano DN, Xiao J, Prado CM, Gonzalez MC. Patient-generated subjective global assessment and computed tomography in the assessment of malnutrition and sarcopenia in patients with cirrhosis: Is there any association? Clin Nutr 2020;39(5):1535–40.
2. Kim HY, Jang JW. Sarcopenia in the prognosis of cirrhosis: Going beyond the MELD score. World J Gastroenterol 2015;21:7637–47.
3. Tsochatzis EA, Bosch J, Burroughs AK. Liver cirrhosis. Lancet 2014;383(9930):1749–61.
4. Hanai T, Shiraki M, Nishimura K, Ohnishi S, Imai K, Suetsugu A, *et al*. Sarcopenia impairs prognosis of patients with liver cirrhosis. Nutrition 2015;31(1):193–9.
5. Carey EJ, Lai JC, Sonnenday C, Tapper EB, Tandon P, Duarte-Rojo A, *et al*. A North American expert opinion statement on sarcopenia in liver transplantation. Hepatology 2019;70(5):1816–29.
6. Dasarathy S, Merli M. Sarcopenia from mechanism to diagnosis and treatment in liver disease. J Hepatol 2016;65(6):1232–44.
7. Fujiwara N, Friedman SL, Goossens N, Hoshida Y. Risk factors and prevention of hepatocellular carcinoma in the era of precision medicine. J Hepatol 2018;68(3):526–49.
8. Dennison EM, Sayer AA, Cooper C. Epidemiology of sarcopenia and insight into possible therapeutic targets. Nat Rev Rheumatol 2017;13(6):340–7.
9. Tandon P, Low G, Mourtzakis M, Zenith L, Myers RP, Abraldes JG, *et al*. A model to identify sarcopenia in patients with cirrhosis. Clin Gastroenterol Hepatol 2016;14(10):1473–80.
10. Lee JT. Sarcopenia at abdominal CT in patients with cirrhosis. Radiology 2022;303(3):720–1.
11. Tandon P, Montano-Loza AJ, Lai JC, Dasarathy S, Merli M. Sarcopenia and frailty in decompensated cirrhosis. J Hepatol 2021;75(Suppl 1):S147–162.
12. Liu J, Ma J, Yang C, Chen M, Shi Q, Zhou C, *et al*. Sarcopenia in patients with cirrhosis after transjugular intrahepatic portosystemic shunt placement. Radiol 2022;303(3):711–9.
13. Benjamin J, Shasthry V, Kaal CR, Anand L, Bhardwaj A, Pandit V, *et al*. Characterization of body composition and definition of sarcopenia in patients with alcoholic cirrhosis: A computed tomography based study. Liver Int 2017;37(11):1668–74.
14. Aby ES, Lee E, Saggi SS, Viramontes MR, Grotts JF, Agopian VG, *et al*. Pretransplant sarcopenia in patients with NASH cirrhosis does not impact rehospitalization or mortality. J Clin Gastroenterol 2019;53(9):680–5.
15. Hanai T, Shiraki M, Imai K, Suetugu A, Takai K, Shimizu M. Usefulness of carnitine supplementation for the complications of liver cirrhosis. Nutrients 2020;12(7):1915.
16. Tantai X, Liu Y, Yeo YH, Praktiknjo M, Mauro E, Hamaguchi Y, *et al*. Effect of sarcopenia on survival in patients with cirrhosis: a meta-analysis. J Hepatol 2022;76(3):588–99.
17. Masuda T, Shirabe K, Ikegami T, Harimoto N, Yoshizumi T, Soejima Y, *et al*. Sarcopenia is a prognostic factor in living donor liver transplantation. Liver Transpl 2014;20(4):401–7.
18. Topan MM, Sporea I, Dănilă M, Popescu A, Ghiuchici AM, Lupuşoru R, *et al*. Impact of sarcopenia on survival and clinical outcomes in patients with liver cirrhosis. Front Nutr 2021;8:766451.
19. Cruz Jr RJ, Dew MA, Myaskovsky L, Goodpaster B, Fox K, Fontes P, *et al*. Objective radiological assessment of body composition in patients with end-stage liver disease: going beyond the BMI. Transplantation 2013;95(4):617–22.
20. DiMartini A, Cruz RJ Jr, Dew MA, Myaskovsky L, Goodpaster B, Fox K, *et al*. Muscle mass predicts outcomes following liver transplantation. Liver Transpl 2013;19(11):1172–80.
21. Montano-Loza AJ, Meza–Junco J, Prado CM, Lieffers JR, Baracos VE, Bain VG, *et al*. Muscle wasting is associated with mortality in patients with cirrhosis. Clin Gastroenterol Hepatol 2012;10:166–73.
22. Tandon P, Ney M, Irwin I, Ma MM, Gramlich L, Bain VG, *et al*. Severe muscle depletion in patients on the liver transplant wait list: its prevalence and independent prognostic value. Liver Transpl 2012;18(10):1209–16.
23. Meza-Junco J, Montano-Loza AJ, Baracos VE, Prado CM, Bain VG, Beaumont C, *et al*. Sarcopenia as a prognostic index of nutritional status in concurrent cirrhosis and hepatocellular carcinoma. J Clin Gastroenterol 2013;47(10):861–70.
24. Montano-Loza AJ, Meza-Junco J, Baracos VE, Prado CM, Ma M, Meeberg G, *et al*. Severe muscle depletion predicts postoperative length of stay but is not associated with survival after liver transplantation. Liver Transpl 2014;20(6):640–8.

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ORIGINAL ARTICLE

CORRELATION AND PREVALENCE OF MEGALOBLASTIC ANAEMIA IN TYPE 2 DIABETIC PATIENTS USING METFORMIN

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Background: Metformin is the most widely used drug in the management of type 2 diabetic patients but its use is associated with vitamin B_{12} deficiency induced megaloblastic anaemia. However few studies in Pakistan have assessed this risk. This study was conducted to determine correlation and prevalence of megaloblastic anaemia in type 2 diabetics treated with metformin. **Methods:** This cross-sectional study was carried out in the Department of Medicine, Hayatabad Medical Complex, Peshawar from Dec 2021 to Nov 2022. A total of 156 type 2 diabetic patients who were taking metformin at least for the last 2 years, were included in the study. All these patients were evaluated for anaemia, raised Mean Corpuscular Volume (MCV) and vitamin B_{12} levels. **Results:** Out of 156 patients who participated in the study, 94 (60.25%) were male and 62 (39.75%) were female with a mean age of 57.56±8.5 years. Mean duration of diabetes was 7.37±3.61 years, whereas mean duration of metformin use was 5.85±2.9 years. Vitamin B_{12} deficiency was identified in 51 (32.69%) patients. These B_{12} deficient patients demonstrated a mean Hb of 9.8±1.4 g/dL. Hyper-segmented neutrophils and raised MCV (mean MCV 98.7±6.90 fl was also identified in them. There was a no correlation between serum B_{12} levels and dose and duration of metformin. **Conclusion:** Long term metformin use is significantly associated with B_{12} deficiency associated megaloblastic anaemia.

Keywords: Diabetes Mellitus, Metformin, $HbA1_C$, Vitamin B_{12}, Megaloblastic Anaemia

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INTRODUCTION

The predominant variant of diabetes worldwide is type 2 diabetes mellitus, impacting 85–90% of the total diabetic population. While type 2 diabetes mellitus primarily affects older adults and young individuals, it can also manifest in children. This condition arises from the interplay of genetic and environmental factors. A significant genetic predisposition exists, and the risk escalates substantially when coupled with lifestyle factors such as hypertension, excessive weight or obesity, insufficient physical activity, and poor dietary habits. Initially, type 2 diabetes often responds to interventions like a healthful diet and regular physical exercise.[1,2] Nevertheless, with time, most of the type 2 diabetes patients require adjunctive oral medications, and a considerable portion necessitates insulin therapy.

Metformin, a member of the biguanide class of oral anti-diabetic medications, stands as the primary first-line choice for managing type 2 diabetes.[3] Among the array of anti-hyperglycaemic agents, metformin demonstrates a positive correlation with enhanced cardiovascular outcomes, mitigating the elevated cardiovascular risk that contributes to mortality in patients with type 2 diabetes mellitus.[4]

Metformin usage presents several drawbacks. It triggers an impairment in vitamin B_{12} absorption, consequently elevating the susceptibility to vitamin B_{12} deficiency.[5] Metformin therapy has the potential to induce a reduction in folic acid levels.[6] The concurrent reduction in both vitamin B_{12} and folate concentrations results in an escalation of homocysteine levels, an autonomous risk factor for cardiovascular ailments.[7] Compromised cognitive function and the onset of Alzheimer's disease have been associated with diminished serum vitamin B_{12} levels among elderly patients.[8] Connections have been established between vitamin B_{12} deficiency and microangiopathic haemolysis as well as narcolepsy.[9]

Numerous studies have substantiated the correlation between metformin consumption and vitamin B_{12} deficiency in individuals with type 2 diabetes. Nevertheless, only a limited subset of these investigations has evaluated the prevalence of vitamin B_{12} deficiency in type 2 diabetes patients undergoing metformin treatment.

The prevalence of megaloblastic anaemia caused by B_{12} deficiency among patients receiving long-term treatment with metformin was 41% in a study conducted by Owhin SO *et al*[10]. This anaemia related with prolonged metformin use is almost always overlooked and seldom investigated by physicians, usually attributing it to the disease itself.[11]

On account of the revealed correlation between use of metformin and megaloblastic anaemia due to vitamin B_{12} deficiency and the lack of data in north-western region of Pakistan, the objective of this

study was to establish the prevalence of megaloblastic anaemia among Type 2 Diabetes Mellitus (T2DM) patients taking long-term metformin, who were followed-up at a medical out-patient clinic in a tertiary healthcare setup in north-western Pakistan.

MATERIAL AND METHODS

This cross-sectional study was done over a period of one year, from Dec 2021 to Nov 2022, at the Department of Medicine, Hayatabad Medical Complex (HMC), Peshawar. Ethical approval from the institutional ethical committee was granted before initiating this study. The study included a sample of 156 individuals aged 40–70 years who had been diagnosed with type 2 diabetes and taking 2,000 mg metformin for a minimum of 4 years.[12] The participants were selected using non-probability consecutive sampling and were asked to provide informed, written consent before being included in study.

Any patient with a previous history of anaemia, malabsorption, history of alcohol use, renal failure, patients currently on parenteral or enteral nutritional supplements, prior transfusion (during the last 3 months), previous gastric or intestinal surgery, and thyroid illness were excluded from the study. After a thorough clinical examination and detailed review of existing medical record, patients with strict vegetarian diet, neurological, or psychiatric diseases, unstable cardiopulmonary, and any neoplastic disorder were also excluded from the study. Additionally, subjects taking amino-salicylic acid, proton pump inhibitors, calcium supplements, H_2 receptor antagonists, vitamin B_{12}, or colchicine during the previous three months were disqualified from study.

The medical records of every patient were assessed and a specially designed proforma which was made after thorough literature review, was utilized to record the demographic information like name, age, gender, duration of DM and metformin use. Blood samples were collected by trained phlebotomists applying standard WHO phlebotomy protocols and transported to the main laboratory of HMC for investigations.

A complete blood count (CBC) was performed on blood samples, employing Sysmex pocH-100i® Automated Hematology Analyzer to look for anaemia (here defined as Hb<11 g/dL) and raised MCV (>96 fl). Parallel to this, the levels of serum vitamin B_{12} were determined using the Abbot Architect 1000 SR 239930 and the chemiluminescence technique. Serum B_{12} levels and haematological parameters were noted on the same proforma. Vitamin B_{12} levels below 200 pmol/L in patients were considered deficient, whereas levels above 300 pmol/L were regarded as normal.

SPSS-23 was used to analyse the gathered data and various tables, results, and calculations were made. To summarize the information, descriptive statistics were used, the variables of age, serum vitamin B_{12} levels, and duration of metformin use were considered as quantitative measures, and their Mean±SD were calculated. To evaluate the correlation between duration and dosage of metformin and serum vitamin B_{12} levels, Chi-square test was used with $p \leq 0.05$ considered as statistically significant.

RESULTS

All the participants were analysed based on age and gender distribution. The age ranged from 40–70 years with a mean age of 57.56±8.5 years. Male preponderance was noted with 94 (60.25%) male and 62 (39.75%) female. Mean duration of DM was 7.37±3.61 years whereas, mean duration of metformin use was 5.85±2.9 years.

The subjects demonstrated a mean $HbA1_C$ (glycosylated haemoglobin) of 8.85±1.5% while mean MCV was 93±4.67 fl/dL. Serum B_{12} levels ranged from 52 pmol/L to 920 pmol/L with mean B_{12} levels of 306±188.9 pmol/L, whereas vitamin B_{12} deficiency was identified in 51 (32.69%) patients.

Mean haemoglobin concentration of the patients under study was 12.65±2.4 g/dL whereas, B_{12} deficient patients (n=51) demonstrated a mean Hb of 9.8±1.4 g/dL.

Hyper-segmented neutrophils and raised MCV (mean MCV=98.7±6.90 fl) were also identified in them. The effect of metformin dose on cobalamin levels was assessed in patients taking less than 1.5 g and more than 1.5 g daily. Patients with low B_{12} levels received a mean dose of 2,100±510 mg, whereas patients with normal B_{12} levels received a mean dose of 1,570±628 mg. Serum B_{12} levels had inverse association with duration and dose of metformin.

Table-1: Demographic and laboratory features of study participants

Parameters	Frequency
Number of patients	156
Female	62 (39.75%)
Male	94 (60.25%)
Age (Years, Mean±SD)	57.56±8.50
Duration of DM (Years, Mean±SD)	7.37±3.61
$HbA1_C$ (%, Mean±SD)	8.85±1.5%
MCV (fl, Mean±SD)	93±4.6
Hb (g/dL, Mean±SD)	12.65±2.4
Vitamin B_{12} levels (pg/mL, Mean±SD)	306±190.5
Vitamin B_{12} deficiency	51 (32.69%)

Table-2: Clinical and laboratory characteristics of the individuals in relation to vitamin B_{12} levels

Demographics/ Parameters	Serum Vitamin B_{12} Levels		*p*
	Deficient <220 pg/ml	Normal >220 pg/ml	
Number of patients	51 (32.69%)	105 (67.31%)	
Age, (Years, Mean±SD)	56.01±7.65	55.98±8.11	0.04
Male	34 (66.66%)	60 (57.14%)	0.23
Female	17 (33.34%)	45 (42.86%)	0.42
Vitamin B_{12} levels, pg/ml (Mean±SD)	125±43.01	389±181.50	0.00001
MCV, fl (Mean±SD)	98.7±6.90	91±5.99	0.04
Hb, g/dL (Mean±SD)	9.8±1.4	12.5±1.8	0.04
Duration of DM, (Years, Mean±SD)	8.88±5.67	5.69±4.83	0.075
Duration of metformin use (Years, Mean±SD)	3.6±1.5	1.60±0.8	0.001
Dose of metformin, (mg, Mean±SD)	2042±540.73	1605±632.80	0.0003
$HbA1_C$, (%, Mean±SD)	8.83±0.90	8.33±0.88	0.405

DISCUSSION

Type 2 diabetes is a prevailing endocrine disorder on a global scale. Metformin is a widely prescribed drug for diabetes. Megaloblastic anaemia due to cobalamin (vitamin B_{12}) deficiency is one of the documented adverse effects of long-term metformin usage.[13] The transportation of vitamin B_{12}-intrinsic factor complex across the membrane of ileal cells is reliant on calcium, and the action of metformin on this calcium-dependent process can result in B_{12} deficiency and megaloblastic anemia.[14]

We found a significant number of diabetic patients with megaloblastic anaemia due to vitamin B_{12} deficiency (serum folate levels within normal limits). These patients were using metformin at least for the last two years and the blood smear showed hypersegmented neutrophils and raised MCV. Statistically significant correlation was observed between anaemia and B_{12} deficiency (p=0.045). These findings are supported by those of a study conducted on 34 Indian patients in 2018, identifying megaloblastic anaemia in 41% diabetics using metformin; similar results were obtained by other studies.[12,15,16]

We also correlated the effects of metformin dose and duration of its use with B_{12} deficiency. A significant inverse correlation was observed between B_{12} levels and dose and duration of metformin use (p=0.0003 and 0.001 respectively). These observations are comparable to another study by Marar *et al*, which showed a high prevalence of vitamin B_{12} deficiency in patients with T2DM on metformin therapy.[17] An inverse relationship was found between vitamin B_{12} levels and the dose and duration of metformin use as seen in study by Kim *et al*[18].

Demographic characteristics of our study subjects are comparable to that identified by other studies. Megaloblastic anaemia was more common in elderly diabetics probably due to imbalanced diet in elderly as compared to younger patients. Similar positive correlations between advancing age and B_{12} deficiency were found in another study by Kang *et al*[16].

Gender, $HbA1_C$, and B_{12} deficiency-related megaloblastic anaemia did not significantly correlate with one another.

CONCLUSION

There was a significant correlation between prolonged metformin usage and the development of secondary megaloblastic anaemia resulting from vitamin B_{12} deficiency. It is crucial for healthcare providers to acknowledge this important information and perform annual screenings for type 2 diabetic patients who have been using metformin for over two years. These screenings should involve laboratory tests such as complete blood count (CBC), peripheral smear examination, and serum vitamin B_{12} level assessment.

REFERENCES

1. Tinajero MG, Malik VS. An update on the epidemiology of type 2 diabetes: a global perspective. Endocrinol Metab Clin 2021;50(3):337–55.
2. Reed J, Bain S, Kanamarlapudi V. A review of current trends with Type 2 Diabetes epidemiology, aetiology, pathogenesis, treatments and future perspectives. Diabetes Metab Syndr Obes 2021;14:3567–602.
3. Kirpichnikov D, McFarlane SI, Sowers JR. Metformin: an update. Ann Intern Med 2002;137(1):25–33.
4. Zhang K, Yang W, Dai H, Deng Z. Cardiovascular risk following metformin treatment in patients with type 2 diabetes mellitus: Results from meta-analysis. Diabetes Res Clin Pract 2020;160:10800.
5. Aroda VR, Edelstein SL, Goldberg RB, Knowler WC, Marcovina SM, Orchard TJ, *et al*. Long-term metformin use and vitamin B_{12} deficiency in the diabetes prevention program outcomes study. J Clin Endocrinol Metab 2016;101(4):1754–61.
6. Owen MD, Baker BC, Scott EM, Forbes K. Interaction between metformin, folate and vitamin B_{12} and the potential impact on fetal growth and long-term metabolic health in diabetic pregnancies. Int J Mol Sci 2021;22(11):5759.
7. Ma Y, Peng D, Liu C, Huang C, Luo J. Serum high concentrations of homocysteine and low levels of folic acid and vitamin B_{12} are significantly correlated with the categories of coronary artery diseases. BMC Cardiovasc Disord 2017;17:37.
8. Cho HS, Huang LK, Lee YT, Chan L, Hong CT. Suboptimal baseline serum vitamin B_{12} is associated with cognitive decline in people with Alzheimer's disease undergoing cholinesterase inhibitor treatment. Front Neurol 2018;9:325.
9. Osman H, Alwasaidi TA, Al-Hebshi A, Almutairi N, Eltabbakh H. Vitamin B_{12} deficiency presenting with microangiopathic hemolytic anemia. Cureus 2021;13(1):e12600.
10. Owhin SO, Adaja TM, Fasipe OJ, Akhideno PE, Kalejaiye OO, Kehinde MO. Prevalence of vitamin B_{12} deficiency among metformin-treated type 2 diabetic patients in a tertiary institution, South-South Nigeria. SAGE Open Med 2019;7:2050312119853433.
11. Iftikhar R, Qadir A, Iqbal Z, Usman H. Prevalence of vitamin B_{12} deficiency in patients of type 2 diabetes mellitus on metformin: a case control study from Pakistan. Pan Afr Med J 2014;16:67.
12. Ko SH, Ko SH, Ahn YB, Song KH, Han KD, Park YM, *et al*. Association of vitamin B_{12} and metformin in type 2 diabetes. J Korean Med Sci 2014;29(7):965–72.

13. Infante M, Leoni M, Caprio M, Fabbri A. Long-term metformin therapy and vitamin B_{12} deficiency: An association to bear in mind. World J Diabetes 2021;12:916–31.
14. Bauman WA, Shaw S, Jayatilleke E, Spungen AM, Herbert V. Increased intake of calcium reverses vitamin B_{12} malabsorption induced by metformin. Diabetes Care 2000;23(9):1227–31.
15. Koduri VL, Nori SNS, Aditya SRKK, Madaboina S, Sindhuja ABK, Swati S. Haematological parameters and vitamin B_{12} levels in type II diabetic patients on metformin —a prospective case control study. J Evid Based Med Healthc 2018;5(31):2317–23.
16. Kang D, Yun JS, Ko SH, Lim TS, Ahn YB, Park YM, *et al.* Higher prevalence of metformin-induced vitamin B_{12} deficiency in sulfonylurea combination compared with insulin combination in patients with type 2 diabetes: a cross-sectional study. PLoS One 2014;9(10):e109878.
17. Marar O, Senturk S, Agha A, Thompson C, Smith D. The prevalence of vitamin B_{12} deficiency in patients with type 2 diabetes mellitus on metformin. R Coll Surg Irel Stud Med J 2011;4(1):16–20.
18. Kim J, Ahn CW, Fang S, Lee HS, Park JS. Association between metformin dose and vitamin B_{12} deficiency in patients with type 2 diabetes. Medicine (Baltimore) 2019;98(46):e17918.

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ORIGINAL ARTICLE

CORRELATION OF STATURE WITH MAXIMUM HEAD LENGTH OF MALE ADULTS OF UPPER PUNJAB

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Background: In the field of forensic medicine, stature estimation in unknown skeletonized bodies is one of the most significant biological parameters. It is done many times when highly mutilated or decomposed bodies or fragmentary remains of skull are brought for forensic examination. The objective of this study was to correlate stature with maximum head length in male adults of 21–30 years in Upper Punjab. This study was designed to evaluate the effectiveness of correlation as a tool to predict relationship of stature from maximum length of head. **Methods:** This was a cross-sectional, quantitative study. Data was collected from the 382 males of Kharian City in Upper Punjab selected with non-probability, purposive sampling. Head measurements were taken using blunt ended spreading callipers and the stature of individual was measured standing erect in anatomical position using a stadiometer. SPSS-25 software was used for data analysis. **Results:** The mean stature was 170.4±6.85 Cm. Mean head length was 16.95±0.6 Cm. Prediction accuracy tests were performed which indicated that regression models of this study can estimate the height with accuracy. **Conclusion:** This study established a correlation between stature and maximum head length. It will help the forensic experts to estimate stature from measuring head length, especially when only head is available as a result of some natural or human caused calamity.

Keywords: Stature, Head length, Correlation, Estimation, Male adults, Punjab, Forensic

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INTRODUCTION

Anthropometry is mostly used in the forensic sciences to assist law enforcement authorities in determining the identification of unidentified human remains. Routine techniques fail to work well on severely decayed and disfigured corpses, making it impossible to identify the deceased. In these circumstances, assessment of height is just as essential as other characteristics such as age, gender, and race.[1] Every component of the human body, including the head, face, trunk, and extremities, has a proportionate biological connection with stature, which is important in forensic investigation when estimating stature from dismembered and damaged corpse parts.[2] Estimation of standing height of an individual help to ascertain distinctiveness to someone.[3]

Estimation of stature has successfully been calculated by using regression equation developed by measuring various dimensions of different bones and body parts.[4] Human stature is calculated as measurement from highest part of head to the inferior most part of the foot. Height is a characteristic which is studied the most, as it is measurable quite readily, at negligible cost and with least consumption of time. Height measurement not only gives useful information about health status of someone or the nation but also indicates the financial condition of individuals as well as country.[5]

Height is such an observable characteristic, which depends upon inborn as well as internal and external environmental factors. It is influenced by environmental temperature, sunlight, personal hygiene, repeated infections, access to medical facilities, financial status and food.[5] In late 19th century a French criminologist, Alphonse Bertillon, developed certain principles of identification depending upon measurements of different parts of body. In this way anthropometry became one of the basic tools in criminology for identification of offenders.[6]

People constantly face dangers of instinctive and unnatural calamities; such calamities may lead to human body disintegration into pieces and disfigurement in such a way that individualization becomes difficult.[7] The possession of even fragmented or mutilated dead body gives the opportunity to the relatives to perform their loved one's funeral ceremony as per directions of their binding.[8] There are two different ways to measure height of fragmented or mutilated bodies. When only fragmented body is available, still height can be estimated by measuring any dimension of bone or any part of the body. Then either a statistical equation is used or some multiplication factor is used, which gives estimated living height. On the other hand, when all bones whose summation results in formation of height, are available then height of individual bones is measured and added up. A few centimetres are added in it to compensate the soft tissue thickness. Second method gives more authentic results.[9]

Among regression and multiplication methods error rate is less in regression method as compared to

multiplication method.[10] Many studies have been carried out throughout the world to form their own regression equations as development of height largely depends upon environmental factors along with inborn factors. As environmental factors vary from one geographical area to the other. So, a single regression equation cannot be universally applied.[11] Every country must have its own regression equation.[12]

This pioneering study aimed at developing regression equation with head length which can be applied to the adults of Punjab. As Pakistan has faced terrorist activities since long so fragmented bodies are brought to get identified. This regression equation will help in estimation of height which will further help to get the bodies to be individualized.

METHODOLOGY

This cross-sectional study was carried out in the Department of Forensic Medicine and Toxicology, PGMI/AMC, Lahore, for one-year duration after approval of the synopsis by the ethical committee of the institute. A total of 382 male adults of 21–30 years age from the Kharian city were included. The sampling technique was non-probability sampling method (Purposive sampling). The patients with dwarfism, gigantism, skeletal, spine and long bone deformities (acquired or congenital), persons with obvious head deformity and persons with surgical correction after injury to facial bones were excluded.

Measurements were taken using blunt ended spreading callipers by placing the anterior tip of calliper on glabella while allowing the posterior calliper tip to slide inferiorly along the median plane of occipital bone until the maximum length was reached. Undue pressure was avoided while taking the measurements. When measuring head length or any other body part, the skin thickness is typically not compensated for directly in most standard measurements and the skin and subcutaneous fat layer are included in the measurement.

The stature of individual was measured standing erect in anatomical position using a stadiometer. It was measured as the vertical distance between the vertex and the floor. All readings (in Cm.) were recorded on a proforma.

Three hundred and eighty-two male Punjabi adults of age group 21–30 years in Kharian city were included in this study. Informed consent was taken after giving full explanation of the purpose of this study to the subjects. By non-probability purposive sampling, 21–30 years old male adult attendants of patients coming to OPD of Civil Hospital, Kharian were selected.

RESULTS

Data collected was subjected to numerical extrapolation through SPSS-25. Mean height was 170.4±6.85 Cm (Range: 148–188 Cm). Mean head length was 16.9±0.6 Cm (Range: 15.1–18.8 Cm). (Table-1).

Numerical description of coefficients of regression (independent variable=head length) is depicted in Table-2.

Cross validation testing is shown in Table-3 when independent variable was head length.

Table-1: Numerical values of stature and head length

Parameter	Minimum	Maximum	Mean±SD
Standing Height	148	188	170.4±6.85
Head Length	15.1	18.8	16.95±0.6

Table-2: Numerical description of coefficients of regression (independent variable was head length)

Parameter	B	S.E.	*t*	*p*
Constant	104.5	9.32	11.206	0.000
Head length	3.88	0.55	7.07	0.000

Table-3: Correlation between actual and predicted values when independent variable was head length

Sample			Height	Predicted
20% Sample (Observed)	Height	Pearson Correlation	1	0.413**
		Sig. (2-tailed)		0.000
		N	75	75
	Predicted	Pearson Correlation	0.413**	1
		Sig. (2-tailed)	0.000	
		N	75	75
80% Sample (Extrapolated)	Height	Pearson Correlation		0.324**
		Sig. (2-tailed)		0.000
		N	307	307
	Predicted	Pearson Correlation	0.324**	1
		Sig. (2-tailed)	0.000	
		N	307	307

**Significant at $p<0.01$

DISCUSSION

Establishment of personal identity of an individual, alive or dead, is one of his/her fundamental rights which has been assigned to the Forensic experts. The ever-increasing brutality of human beings like dismembering bodies after murder, suicide bombing, blasts, accidents, and natural disasters like earthquakes are very common in this era, and in this region. The main aim of forensic experts is positive identification of human bodies at disaster sites by different methods including anthropometry.

Measurements in anthropometry are collected through careful and organized processes with the primary aim of providing data which is useful in the fields of forensic medicine, ergonomics, reconstructive surgery, and prosthesis. Most of the time, head and neck is presented for identification or is the only part of body available. The facial features of such unidentified persons are usually mutilated. Height is one of the most useful parameters for establishment of identity of any individual. In many countries, anthropometric

measurements of face have been correlated with height and to narrow down the number of suspected victims in establishment of personal identity.

Standing mean height of adult males in the current research was 170.4±6.85 Cm. Many researches carried out in Pakistan give almost the same male height on average. In a research carried out in University of Health Sciences, Lahore, average height of males was 170.5 Cm.[13] In another study the male height noted was 173.16 Cm.[14] A study carried out in Rawalpindi found that average male height was 171 Cm.[15] Average height in males in India was 174.74 Cm.[1] In another research in India male height was 165.5 Cm.[16] Male height in Kosovo was found to be 178.79 Cm.[17]

Mean head length in this study was 16.95±0.6 Cm. It varied between 15.10 Cm and 16.95 Cm. Some studies show variance in numerical values of variables used in this study. Numerical quantity of correlation coefficient between standing height and head length was 0.335. Although it was a positive but a weak correlation. The strength of correlation depends on age, gender, environmental factors, ethnic and genetic variations. Since this positive correlation, albeit weak, was with statistically significant *p*-value, it will have significant practical implications in the field of forensic medicine. A study carried out in Kathmandu Nepal showed that there was strongly positive correlation between height and head length with r=0.734.[18] Another study carried out in India showed almost same value of r (0.715) between height and head length.[19] Adults from Haryana showed very weak correlation between head length and height. In that study value of r was 0.174.[20]

According to the findings of this study, the best correlation coefficients between head length and body height are seen in men. Patil and Mody[21] found that head length was the greatest predictor of height. This is quite similar to our work. The most accurate estimates of antero-posterior head length and circumference were made by Chiba and Terazawa.[22] High correlation coefficients between cephalometric measures and stature have been recorded by Krishan and Kumar[23] who found that the diameter of the head was the most accurate predictor of stature. It is possible that the classification of the lower limb bones has some influence on the assessment of height. By the time a person reaches the age of around 18–20 years, the femoral condyles, proximal end of the tibia, first metatarsal base, and heads of the 2nd and 5th metatarsals fuse completely. Because the ossification process is dependent on a variety of variables, ossification activities begin earlier in Indians than in Westerners.[24]

The findings of this study are comparable to those of other research conducted on diverse people throughout the world. In Japanese cadavers, Chiba and Terazawa[22] found a SEE of 6.97 Cm when they used regression models to estimate stature from the sum of the skull diameter and circumference. Except for head length, which demonstrated a high level of dependability in assessing height (SE=3.71), Patil and Mody[21] found slightly larger standard errors for most variables. In their study of Koli male adolescents from north India, Krishan and Kumar[23] found a 4.41–7.21 Cm SEE from 16 cephalo-facial measures. In their research on the assessment of height from the skulls of indigenous South Africans from Raymond Dart's collection, Ryan and Bidmos[25] reported SEE ranging from 4.37 to 6.24 Cm. The SEE value in this study is lower than in previous investigations, ranging from 3.726 to 5.820 Cm. To put it another way, the height estimation using cephalo-facial measurements used in this study was shown to be more accurate than in previous ones of its kind. An endogamous (or genetically diverse) population provides a more reliable sample for this study because of the higher degree of homogeneity. Krishan and Kumar[23] used study samples from a mixed population, as did the others.

CONCLUSION

This study discovered a positive correlation between maximal head length and stature in Upper Punjab male adults aged 21–30 years. In forensic cases requiring the prediction of stature from head length, this regression equation is a useful tool. It is essential for estimating height from incomplete and deteriorated skull remains in forensic and anthropological studies. Regression modelling can be used to determine the other variables if one of the parameters is known.

REFERENCES

1. Khan MA, Bashir SI, Khan MA, Shahdad S. Determination of stature from measurements of hand length and hand breadth: an anthropometric study of Kashmiri population. Int J Anat Res 2017;5(2.3):3968–75.
2. Vadell J, Brutto GL, Leite AC. The Chinese South-South development cooperation: an assessment of its structural transformation. Rev Bras Polit Int 2020;63:e001.
3. Kamal R, Yadav PK. Estimation of stature from different anthropometric measurements in Kori population of North India. Egypt J Forensic Sci 2016;6(4):468–77.
4. Shrestha R, Shrestha PK, Wasti H, Kadel T, Kanchan T, Krishan K. Craniometric analysis for estimation of stature in Nepalese population —a study on an autopsy sample. Forensic Sci Int 2015;248:187–e1.
5. Stulp G, Barrett L. Evolutionary perspectives on human height variation. Biol Rev Camb Philos Soc 2016;91(1):206–34.
6. Basu S. Hundred years of forensic science in India (1849–1947): A historical perspective. Indian J Hist Sci 2020;55(1):80.
7. Shanbhag VK. Significance of dental records in personal identification in forensic sciences. J Forensic Sci Med 2016;2(1):39–43.
8. Cordner S, Ellingham ST. Two halves make a whole: both first responders and experts are needed for the management and identification of the dead in large disasters. Forensic Sci Int 2017;279:60–4.
9. Pablos A, Gomez-Olivencia A, García-Pérez A, Martínez I, Lorenzo C, Arsuaga JL. From toe to head: Use of robust regression methods in stature estimation based on foot remains. Forensic Sci Int 2013;226(1–3):299. e1–7.

10. Krishan K, Kanchan T, Sharma A. Multiplication factor versus regression analysis in stature estimation from hand and foot dimensions. J Forensic Leg Med 2012;19(4):211–4.
11. Jelenkovic A, Sund R, Yokoyama Y, Latvala A, Sugawara M, Tanaka M, *et al*. Genetic and environmental influences on human height from infancy through adulthood at different levels of parental education. Sci Rep 2020;10(1):7974.
12. Gualdi-Russo E, Bramanti B, Rinaldo N. Stature estimation from tibia percutaneous length: new equations derived from a Mediterranean population. Sci Justice 2018;58(6):441–6.
13. Anwar F, Alimgeer KS, Kumar R, Somrongthong R. Comparing log-based and exponent-based functions to predict human height by foot length. Int J Med Toxicol Forensic Med 2021;11(2):30902.
14. Malik AR, Akhter N, Ali R, Aziz K. A study on estimation of stature from foot length: a study on estimation of stature from foot length. Professional Med J 2015;22(5):632–9.
15. Qayyum R, Arif M, Majeed AI. Pattern of anthropometric measurements in the population of Rawalpindi. Ann Pak Inst Med Sci 2016;12(4):215–8.
16. Varu PR, Manvar PJ, Mangal HM, Kyada HC, Vadgama DK, Bhuva SD. Determination of stature from hand dimensions. J Med Res 2015;1(3):104–7.
17. Popovic S, Gardasevic J, Masanovic B, Arifi F, Bjelica D. Standing height and its estimation utilizing foot length measurements in adolescents from western region in Kosovo. Sport Mont 2017;15(3):3–7.
18. Manandhar B, Shrestha R. Estimation of body height from head length among dental students of a dental college. JNMA: J Nepal Med Assoc 2018;56(213):861–5.
19. Reddy M, Reddy V, Wadhwan V, Venkatesh A. Correlation and estimation of stature from cephalofacial measurements: A study on Western Uttar Pradesh population. J Forensic Dent Sci 2018;10(2):101–6.
20. Kumar M, Gopichand PV. Estimation of stature from cephalo-facial anthropometry in 800 Haryanvi adults. Int J Plant Anim Environ Sci 2013;3(2):42–6.
21. Patil KR, Mody RN. Determination of sex by discriminant function analysis and stature by regression analysis: a lateral cephalometric study. Forensic Sci Int 2005:147(2–3):175–80.
22. Chiba M, Terezawa K. Estimation of somatometry of skull. Forensic Sci Int 1998:97(2–3):87–92.
23. Krishan K, Kumar R. Determination of stature from cephalo-facial dimensions in a North Indian population. Leg Med (Tokyo) 2007:9(3):128–33.
24. Wube B, Seyoum G, Taye G. Estimation of stature by anatomical anthropometric parameters in first-year regular undergraduate students at Debre Markos University, North West Ethiopia. Ethiop J Health Dev 2019;33(3):188–97.
25. Ryan I, Bidmos MA. Skeletal height reconstruction from measurements of the skull in indigenous South Africans. Forensic Sci Int 2007:167(1):16–21.

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ORIGINAL ARTICLE

PROTECTIVE ROLE OF OLIVE OIL IN THE DOXORUBICIN INDUCED OVARIAN TOXICITY IN FEMALE RATS

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Background: The pathophysiological effect of chemotherapy on female reproductive system is complex. The current study aimed to determine the protective effect of olive oil extract against doxorubicin induced ovarian toxicity in female rats. **Methods:** This was an analytical experimental randomized control study carried out in the Department of Anatomy, Peshawar Medical College. Twenty-four female rats weighing 200–250 g were included and were divided into 3 groups, i.e., control group, experimental group-I and experimental group-II. Animals were weighed at the beginning of the experiment and before their sacrifice. The ovaries were obtained after their sacrifice. Five (5) µm thick sections were cut and stained with H & E stain, and Massan Trichome to see the changes the ovarian follicles. Data was entered and analysed on SPSS-20. Independent sample *t*-test and Chi-square test were used to measure the difference between and within groups, and $p \leq 0.05$ was considered statistically significant. **Results:** A significant increase in the mean weight of rats in control group and experimental group-II was observed ($p<0.05$) while a post-experiment significant decrease in the mean body weight of rats in experimental group-I was seen ($p>0.05$). There was no significant difference in number of primordial and secondary ovarian follicles within the groups ($p>0.05$) while a statistical significant difference in number on primary ovarian follicles, atretic follicles and nuclear fragments within the groups ($p<0.05$). **Conclusion:** The co-administration of olive oil has shown a protective effects against the doxorubicin induced ovarian toxicity in female rats.

Keyword: Doxorubicin, Ovarian toxicity, Female rats, Chemotherapy, Olive oil

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INTRODUCTION

Female ovary contains all the germ cells (oocytes) which are required for the whole life span by the time of their birth. These oocytes are kept in follicular cells which are surrounded by pre-granulosa cells. There are approximately 2 million follicles inside the ovary which are reduced to 300,000 at the time of puberty.[1] The premenopausal phase in women's reproductive life starts when less than a thousand follicle cells are left, normally around 45 years of age. The complete loss of ovarian follicles in healthy woman usually occurs around 50 years of age when all the ovarian follicles are vanished. However, early menopause can also result due to apoptosis or destruction caused by cancer chemotherapy. This phenomenon is known as 'chemotherapy-induced infertility', usually in young women. The cancer survivor women's ovary contain follicles same as those of post-menopausal women.[2] The pathophysiological effects of chemotherapy on female reproductive system is complex and depends upon its potency and frequency of administration, the age of the patient and her medical history also play their role in manipulating the functions of ovary.[3] Many anti-cancer drugs like cyclophosphamide, cisplatin, and doxorubicin (DOX) cause ovarian toxicity by direct damage to ovarian follicles, oocytes, granulosa, and cumulus cells, or may indirectly renders damage to ovarian parenchymal cells and vascular system which leads to inhibition of nutrients supply to the ovarian follicular cells.[4] In both cases, the ultimate result will be follicular atresia reducing the ovarian reserve. For this purpose, gonads preservative strategies are followed nowadays, i.e., cryopreservation of reproductive cells which can be implanted later especially in female cancer patients who are prone to chemotherapy-induced premature ovarian failure (POF).[5]

DOX is a cytotoxic agent from the anthracycline class of anti-tumour antibiotics which inhibits DNA synthesis by targeting topoisomerase II. The DOX cytotoxicity mechanism involves the induction of DNA intercalation between guanine and cytosine base pairs which interferes with DNA and RNA synthesis.[6] DOX is identified as an intermediate risk group member for causing ovarian toxicity, and causes both follicle-dependent and independent ovarian toxicity. A possible mechanism for DOX-induced ovarian toxicity could be due to activation of ataxia-telangiectasia mutated (ATM) kinase, which is an important downstream mediator of the RH-DNA damage response. The deregulation of ATM activity can influence cell survival. Studies in mouse models have shown that inactivation of ATM can lead to a suppression of apoptosis and an acceleration of tumour

development.[7] DOX could damage oocyte's DNA integrity in the ovarian follicle, but it has also been reported to induce damage to granulosa cells, the capillary system, and also induce oxidative stress.[8] Increasing evidence from studies in animal model has shown that DOX targets DNA and also mitochondria where it reduces mitochondrial functions and possibly triggers apoptotic signalling pathways by increasing the expression of pro-apoptotic mediators such as caspase-3 and caspase-12 in egg and granulosa cells.[9]

The leaves of the olive tree (*Olea europaea L.*) have been used as traditional therapies for many years in the Mediterranean countries. Several experimental studies have shown that olive leaves are useful in lowering blood pressure. Besides this, they have anti-atherogenic, anti-inflammatory, sugar and cholesterol lowering activities. These effects are attributed to the antioxidant components of the olive leaves. Oleuropein and its derivatives such as hydroxytyrosol and tyrosol are the most important phenolic compounds of olive leaves that are suspected to be responsible for their pharmacological properties. In addition, olive leaves contain caffeic acid, p-coumaric acid, vanillic acid, vanillin, Luteolin, Diosmetin, Rutin, Verbascosid, Luteolin-7-glucoside, Apigenin-7-glucoside and Diosmetin-7-glucoside. OLE or its constituents, especially oleuropein, protect tissues as an antioxidant when administered for therapeutic purposes.[10] Several researchers have examined the protective potential of OLE or its components, in various pathologies induced by oxidative stress such as arteriosclerosis, diabetes, the brain ischemia and lead-induced neuropathy.

The current study aimed to determine the protective effect of oral olive oil extract against doxorubicin-induced ovarian toxicity in female rats.

MATERIAL AND METHODS

This analytical experimental randomized control trial was carried out in the Department of Anatomy, Peshawar Medical College, Peshawar. The duration of study was six months from 1st January to 30th June 2021. The sample size and number of groups were calculated through the one-way ANOVA formula for animal studies. Twenty-four female rats weighing 200–250 g, were procured from the animal house of Peshawar Medical College, Peshawar and divided into three groups. The animal house facility of Pakistan Council of Scientific and Industrial Research (PCSIR), Peshawar, was used. An independent room was allocated for this research which was well ventilated and 12 hours light and dark cycle was maintained. The temperature was maintained as 18 to 26 °C. Healthy female rats were selected which had been previously pregnant. Rats with any disease prior to the onset of experiment, or the ones that developed disease during the study were excluded from the study. All animals were weighed at the beginning of the experiment. This weight was abbreviated as W_i (initial weight). They were then weighed just before their sacrifice and this weight was abbreviated as W_f (final weight). Doxorubicin injection (10 mg/dL) (ONCODOX-10 by AJ Mirza Pharmaceuticals, Lahore Pakistan) were procured. Olive oil extract was purchased from the local market. The animals were kept in solid bottom polypropylene cages and fed on commercial standard mash feed and water for rat. Experiments were conducted as per the protocol approved by the Institutional Animal Ethics Committee. The animals were divided into 3 groups comprising of eight (8) female rats each, i.e., control group, experimental group-I and experimental group-II (Table-1). On 28th day, animals were euthanized, and organs were collected in 10% neutral buffered formalin for micro-pathological changes and were processed for paraffin embedding, 5 µm thick sections were cut on rotary microtome and stained with H and E, for routine microscopy. Sections were also stained with Massan Trichome to see the changes in the connective tissue elements of the ovarian follicles. The follicles having visible nuclei were counted. Follicles containing 20 or more apoptotic granulosa cells (appearance of apoptotic bodies in granulosa cell layers), disorganized granulosa cells, a degenerating oocyte, or fragmentation of oocyte nucleus were categorized as atretic follicles.

Data was entered and analysed on SPSS-20. Quantitative variables were presented as Mean±SD. Categorical variables were computed as frequencies and percentages. Independent sample *t*-test and Chi-square test was used to measure the difference between and within groups, and $p \leq 0.05$ was considered statistically significant.

Table-1: Groups Demographics

Groups	Description
Control Group	Received 12 mL/L olive oil mixed in water once a week for 4 weeks.
Experimental Group-I	Doxorubicin injected intraperitoneally at 7.5 mg/Kg body weight once weekly for 4 weeks.
Experimental Group-II	Doxorubicin injected intraperitoneally at 7.5 mg/Kg once weekly for 4 weeks and then treated with olive oil, i.e., 12 mL/L of water for 4 weeks

RESULTS

The mean weight of rats in control group was 107.25±1.83 g before the experiment, while there was a significant increase in the mean weight of rats in control group after the experiment ($p<0.05$). The mean weight of rats in experimental group 1 before and after the experiment was 117.13±3.56 g and 93.0±6.41 g respectively and a significant decrease in the mean body weight of rats in experimental group 1 was found ($p<0.05$). The mean weight of rats in experimental group 2 before the experiment was 112.75±4.80 g. A significant increase in mean body weight of rats in

experimental group 2 was found after the experiment (p<0.05). The results are summarized in Table-2.

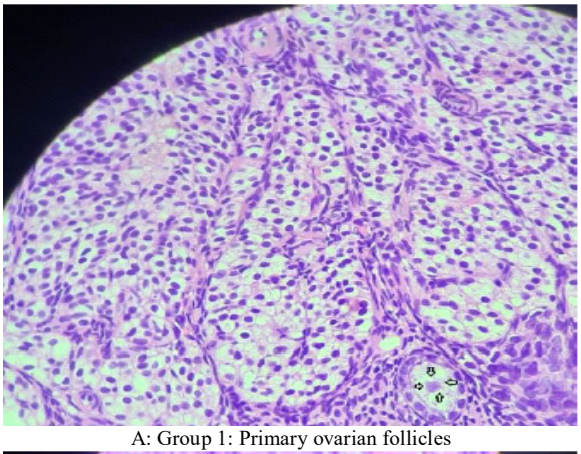
The H and E stain results are shown in Figure-1. There was no statistical significant difference between primordial and secondary ovarian follicles within the groups (p>0.05). However, a statistical significant difference was observed in number on primary ovarian follicles (right ovary), atretic follicles (in both ovaries) and nuclear fragments (left ovary) within the groups (Table-3).

Table-2: Mean body weight of animals (g)

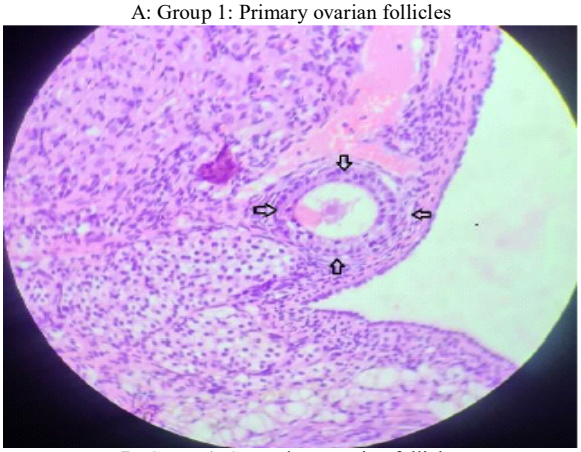
Group	Before experiment	After experiment	Weight change	*p*
Control group	107.25±1.83	111.50±4.63	-4.25±3.28	<0.05
Experimental Group-I	117.13±3.56	93.0±6.41	24.13±5.19	<0.05
Experimental Group-II	112.75±4.8	155.25±25.5	42.50±21.86	<0.05

Table-3: Follicle analysis within groups (Mean±SD)

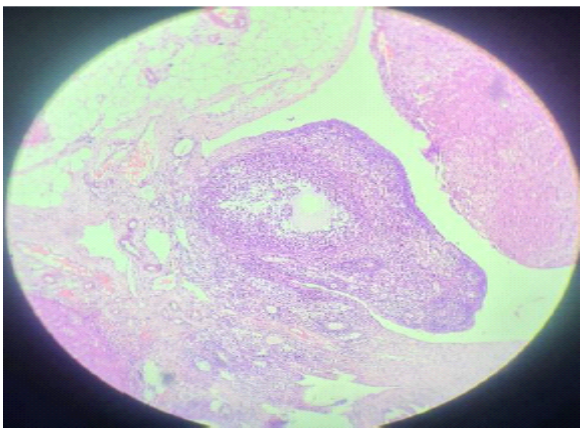
Follicles	Ovary	Control Group	Experimental Group-1	Experimental Group-2	*p*
Primordial	Right	1.75±1.65	2.13±2.1	2.37±1.92	0.78
	Left	1.63±1.99	1.63±1.77	1.63±1.77	1.00
Primary	Right	1.0±1.07	2.13±1.82	3.25±2.12	**0.05**
	Left	1.13±1.36	1.13±1.25	1.38±1.06	0.89
Secondary	Right	1.5±1.93	3.13±2.36	3.75±2.43	0.14
	Left	1.75±1.28	1.25±0.46	1.63±0.74	0.52
Atretic	Right	0.000	1.75±0.88	4.00 ±2.98	**0.001**
	Left	0.000	1.25±0.46	1.50±0.93	**0.002**
Nuclear Fragment	Right	0.000	0.50±0.75	0.25±0.46	0.173
	Left	0.000	1.50±0.93	0.75±0.89	**0.002**



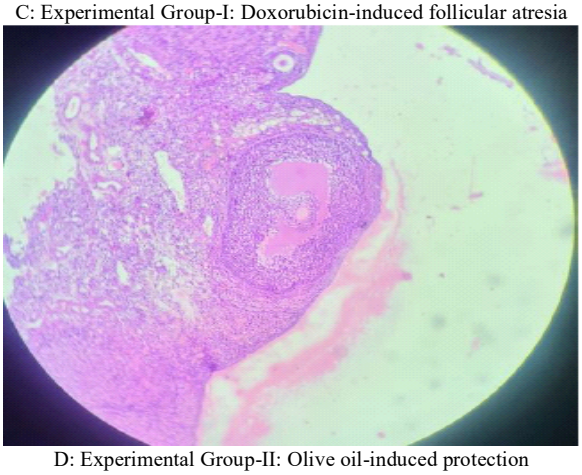
A: Group 1: Primary ovarian follicles



B: Group 1: Secondary ovarian follicles



C: Experimental Group-I: Doxorubicin-induced follicular atresia



D: Experimental Group-II: Olive oil-induced protection

Figure-1: Ovarian follicles in different experimental groups

DISCUSSION

Although chemotherapeutic medicines have been effective in the treatment of cancer, toxic effect from chemotherapy is indeed a common and unfortunate side-effect of treatment that can occur even at standard doses. One of the most serious long-term consequences of cytotoxic drug treatment is infertility, which is caused by premature ovarian failure (POF) or insufficiency (POI). As a result, the preservation of ovarian reserve and the prevention of infertility has risen to the top of the priority list for patients and their doctors. The specific causes of ovarian toxicity are unknown, and they vary depending on the medication and the cell type.

In the current study, the 28-day treatment period was chosen to test the effects of the drug on the ovulatory phase. It was observed that the 28 days doxorubicin-treated rats had reduced body weight (p<0.05), which suggests the loss of weight associated to enhanced oxidative stress. Previously, Swamy *et al*, also found that there was a significant reduction in the body weight of doxorubicin-treated albino rats as compared to animals in the normal control group.[11] However, a significant increase in body weight was observed in the rats included in control group and the

group which was receiving doxorubicin along with oral olive oil (p<0.05). The body weight of the rats in this group was increased since olive oil contain antioxidant compounds, it had a protective role against the toxic effects of doxorubicin treatment.

The density of primordial follicles determines female fertility. Chemotherapy survivors' ovarian reproductive function is investigated by monitoring menstrual behaviour or certain serum indicators as well as the number of primordial follicles to accurately predict fertility. Primordial follicular oocytes are very vulnerable to chemotherapeutic substances. Damage to the DNA causes the deposition of p53 in the cell. p53 is a transcription factor or tumour suppressor protein that plays a role in cellular stress and development. Doxorubicin has been reported to increase the expression of the p53 protein, which triggers apoptosis in the injured cells. The same mechanism may be assumed to be responsible for the enhanced p53 activity in primordial follicles of doxorubicin-treated rats. As per our study, there was no significant difference in the mean number of primordial follicles between the control group and the experimental groups (p>0.05). Morgan *et al*[12] have observed a significant decrease in number of primordial follicles in doxorubicin-treated mice group.

Doxorubicin can cause DNA damage in larger follicles with more granulosa cells, such as primary, secondary, and antral follicles. Bar-Joseph *et al*, observed that doxorubicin can pass through the follicular basement membrane and deposits in the oocyte's DNA and mitochondria.[13] This causes serious damage to DNA and oxidative stress in nuclei and mitochondria leading to decrease count of targeted ovarian follicles. Besides targeting primordial follicles, doxorubicin also affects later phases of follicle development, resulting in a decrease in primary and secondary ovarian follicles.[14] In our study, it was found that there was a significant difference between the mean number of primary ovarian follicles in right ovaries of rats in control group and the experimental groups (1 and 2) (p=0.05). However, no significant difference was found between the mean number of primary ovarian follicles in left ovaries in control group and the experimental groups (1 and 2) (p>0.05). Similarly, no significant difference was observed between the mean number of secondary ovarian follicles in right and left ovaries of rats in control group and the experimental groups (1 and 2) (p>0.05). Doxorubicin was formerly thought to be only mildly toxic to reproductive organs especially the female reproductive organs. However new evidence contradicts this previous assumption.[15]

Doxorubicin therapy results in dose-dependent acute ovarian toxicity characterized by decreased ovarian size and weight and may be associated to ischemia and parenchymal fibrosis. Single dose treatment resulted in increased follicular atresia in the rat ovaries, which could be caused by significant oxidative stress.[16,17] In our study, a significant difference was found in the mean number of atretic ovarian follicles in both right and left ovaries of control group as compared to the experimental groups (1 and 2) (p<0.05). The similar observation was seen by Samare-Najaf *et al*, in rat model, i.e., doxorubicin produced a dose-dependent follicular atresia which was prevented by concomitant administration of antioxidant therapy.[18] Several studies had been published indicating doxorubicin-induced DNA damage in several normal tissues, resulting in dysregulation of apoptotic signalling pathways such as decreased Bcl-2, caspase activation, p53 accumulation, and eventually cell apoptosis.[19–21] In our study, due to severe ovarian atresia and apoptosis, nuclear fragments were seen in the atretic follicles, and a significant difference was found in mean number of nuclear fragments in both left and right ovarian atretic follicles in experimental groups (1 and 2) as compared to the control group (p<0.05).

Doxorubicin induced toxicity arises because of its large, repeated doses and production of reactive oxygen species (ROS) or free radicles. Several adjuvant antioxidant therapies have been studies with significant advantages. Roti *et al*, found that Dexrazoxane —an iron chelating agent, significantly protected the overall survival rate of ovarian follicles against the toxic effects of doxorubicin in female mice till the course of chemotherapy.[22] Phytochemicals are promising supplements having significant antioxidant activities against the toxic effects of various chemotherapeutic agents including doxorubicin. Samare-Najaf *et al*[18], proved that quercetin (plant derived flavonoid) and vitamin E (antioxidant) have protective effect against the ovo-toxic effects of doxorubicin in rat model. Mohajeri *et al*[23], also demonstrated the protective effects of curcumin against the toxic effects of doxorubicin.

In the current study, olive oil was used as an antioxidant to study its potential to protect the ovarian follicular reserves against the toxic effects of doxorubicin in female rat model. After 28 days of the study, the mean body weight of experimental group 2 was increased by 42.50±21.86 gm as compared to experimental group 1 in which the mean body weight was decreased by 24.13±5.19 gm. Because of protective effect of olive oil, an increase in number of ovarian follicles was observed in those rats which were receiving doxorubicin and olive oil simultaneously. On the other hand, a decrease in number of ovarian follicles was seen in rats to which only doxorubicin was administered.

Our results have confirmed the protective role of olive oil against the toxic effects of doxorubicin in ovary in animal models. The results of our study coincide with the results of a study by Nishi *et al*[24], which also demonstrated the protective effect of olive

oil against the doxorubicin induced ovarian toxicity in Wistar rats.

CONCLUSION

The co-administration of olive oil can be a new adjuvant therapy which has shown promising effects against the doxorubicin induced ovarian toxicity in animal models.

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REFERENCES

1. Kim J, Park EY, Kim O, Schilder JM, Coffey DM, Cho CH, *et al*. Cell origins of high-grade serous ovarian cancer. Cancers 2018;10(11):433.
2. Anderson RA, Cameron D, Clatot F, Demeestere I, Lambertini M, Nelson SM, *et al*. Anti-Müllerian hormone as a marker of ovarian reserve and premature ovarian insufficiency in children and women with cancer: a systematic review. Hum Reprod Update 2022;28(3):417–34.
3. Kim S, Kim SW, Han SJ, Lee S, Park HT, Song JY, *et al*. Molecular mechanism and prevention strategy of chemotherapy-and radiotherapy-induced ovarian damage. Int J Mol Sci 2021;22(14):7484.
4. Bedoschi G, Navarro PA, Oktay K. Chemotherapy-induced damage to ovary: mechanisms and clinical impact. Future Oncol 2016;12(20):2333–44.
5. Blumenfeld Z. Fertility preservation using GnRH agonists: rationale, possible mechanisms, and explanation of controversy. Clin Med Insights Reprod Health 2019;13:1179558119870163.
6. Khongorzul P, Ling CJ, Khan FU, Ihsan AU, Zhang J. Antibody–drug conjugates: a comprehensive review. Mol Cancer Res 2020;18(1):3–19.
7. Kciuk M, Gielecińska A, Kołat D, Kałuzińska Ż, Kontek R. Cancer-associated transcription factors in DNA damage response. Biochim Biophys Acta Rev Cancer 2022;1877(4):188757.
8. Onder GO, Balcioglu E, Baran M, Ceyhan A, Cengiz O, Suna PA, *et al*. The different doses of radiation therapy-induced damage to the ovarian environment in rats. Int J Radiat Biol 2021;97(3):367–75.
9. Wu Y, Chen M, Jiang J. Mitochondrial dysfunction in neurodegenerative diseases and drug targets via apoptotic signalling. Mitochondrion 2019;49:35–45.
10. Visioli F, Davalos A, López de las Hazas MC, Crespo MC, Tomé-Carneiro J. An overview of the pharmacology of olive oil and its active ingredients. Br J Pharmacol 2020;177(6):1316–30.
11. Swamy AV, Gulliaya S, Thippeswamy A, Koti BC, Manjula DV. Cardioprotective effect of curcumin against doxorubicin-induced myocardial toxicity in albino rats. Indian J Pharmacol 2012;44(1):73–7.
12. Morgan S, Lopes F, Gourley C, Anderson RA, Spears N. Cisplatin and doxorubicin induce distinct mechanisms of ovarian follicle loss; imatinib provides selective protection only against cisplatin. PloS One 2013;8(7):e70117.
13. Bar-Joseph H, Ben-Aharon I, Rizel S, Stemmer SM, Tzabari M, Shalgi R. Doxorubicin-induced apoptosis in germinal vesicle (GV) oocytes. Reprod Toxicol 2010;30(4):566–72.
14. Zhang T, He WH, Feng LL, Huang HG. Effect of doxorubicin-induced ovarian toxicity on mouse ovarian granulosa cells. Regul Toxicol Pharmacol 2017;86:1–10.
15. Brayboy LM, Oulhen N, Long S, Voigt N, Raker C, Wessel GM. Multidrug resistance transporter-1 and breast cancer resistance protein protect against ovarian toxicity, and are essential in ovarian physiology. Reprod Toxicol 2017;69:121–31.
16. Al-Kawlani B, Murrieta-Coxca JM, Chaiwangyen W, Fröhlich K, Fritzsche A, Winkler S, *et al*. Doxorubicin induces cytotoxicity and miR-132 expression in granulosa cells. Reprod Toxicol 2020;96:95–101.
17. Renu K, Pureti LP, Vellingiri B, Valsala Gopalakrishnan A. Toxic effects and molecular mechanism of doxorubicin on different organs–an update. Toxin Rev 2022;41(2):650–74.
18. Samare-Najaf M, Zal F, Safari S. Primary and secondary markers of doxorubicin-induced female infertility and the alleviative properties of quercetin and vitamin E in a rat model. Reprod Toxicol 2020;96:316–26.
19. Granados-Principal S, Quiles JL, Ramirez-Tortosa CL, Sanchez-Rovira P, Ramirez-Tortosa M. New advances in molecular mechanisms and the prevention of adriamycin toxicity by antioxidant nutrients. Food Chem Toxicol 2010;48(6):1425–38.
20. Jabłońska-Trypuć A, Krętowski R, Kalinowska M, Świderski G, Cechowska-Pasko M, Lewandowski W. Possible mechanisms of the prevention of doxorubicin toxicity by cichoric acid—antioxidant nutrient. Nutrients 2018;10(1):44.
21. Tocchetti CG, Cadeddu C, Di Lisi D, Femmino S, Madonna R, Mele D, *et al*. From molecular mechanisms to clinical management of antineoplastic drug-induced cardiovascular toxicity: a translational overview. Antioxid Redox Signal 2019;30(18):2110–53.
22. Roti Roti EC, Salih SM. Dexrazoxane ameliorates doxorubicin-induced injury in mouse ovarian cells. Biol Reprod 2012;86(3):96.
23. Mohajeri M, Sahebkar A. Protective effects of curcumin against doxorubicin-induced toxicity and resistance: A review. Crit Rev Oncol Hematol 2018;122:30–51.
24. Nishi K, Gunasekaran VP, Arunachalam J, Ganeshan M. Doxorubicin-induced female reproductive toxicity: an assessment of ovarian follicular apoptosis, cyclicity and reproductive tissue histology in Wistar rats. Drug Chem Toxicol 2018;41(1):72–81.

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ORIGINAL ARTICLE

HEPATOPROTECTIVE EFFECT OF ZINC COMPLEX OF BETULINIC ACID ON PYRAZINAMIDE INDUCED HEPATOTOXICITY IN MICE

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Background: Hepatotoxicity is a common side effect of pyrazinamide, a first line anti-tuberculous drug. Objective of this study was to determine the hepatoprotective effect of zinc complex of Betulinic acid (BA) on pyrazinamide induced hepatotoxicity in mice. **Methods:** This experimental**,** randomized control study was conducted at Islamic International Medical College, Rawalpindi, and National Institute of Health Islamabad from 1 Sep 2020 to 31 Aug 2021. Thirty male Balb/c albino mice were divided into three groups each having 10 mice. Group 1 received normal diet with no medication. Group 2 (NC) received pyrazinamide 500 mg/Kg daily for 28 days. Group 3 (DC) received Zinc complex of Betulinic acid 1 mg/Kg/day per oral once daily along with pyrazinamide. Final sampling was done on day 28 by intracardiac puncture for estimation of serum ALT and bilirubin level. Data was analysed on SPSS-21. Comparisons between the groups were analyzed using one way ANOVA (Post Hoc Tuckey test), and $p<0.05$ was considered significant. **Results:** Zinc complex of Betulinic acid treated group had significantly decreased serum ALT (58.00±5.639) and bilirubin (0.617±0.601) in comparison with pyrazinamide treated group. **Conclusion:** Zinc complex of Betulinic acid exerted significant hepatoprotective activity in pyrazinamide induced hepatotoxicity.

Keywords: Hepatoprotective, Pyrazinamide, Betulinic Acid, Zinc

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INTRODUCTION

Liver is an indispensable organ in the human body which executes a myriad of crucial tasks, e.g., metabolism of fat, proteins and carbohydrates, coagulation factor production, metabolism of xenobiotics, drugs and various nutritional substances.[1] Due to the presence of liver between the site of absorption and systemic circulation, it can be the target of toxic metabolites.[2]

Tuberculosis is the 9th leading cause of deaths throughout the world. Every year 10 million new cases of tuberculosis are notified.[3] Pakistan is ranked at 5th position among first 22 high burden countries of tuberculosis. WHO report about TB incidence published in 2016 states that incidence in Pakistan is 270 per 100,000 population.[4] Treatment given for TB includes a combination regime which involves isoniazid, rifampicin, pyrazinamide, and ethambutol. Among these, isoniazid, rifampicin and pyrazinamide are hepatotoxic and their most common side effect is drug induced liver injury. The incidence of drug induced liver damage in tuberculosis patients taking ATT drugs is about 2–28%.[5] Various studies has documented that pyrazinamide is more hepatotoxic than isoniazid and rifampicin among first line anti-TB hepatotoxic drugs.[6]

Pyrazinamide (PZ) is a nicotinamide pyrazine analogue. It has bacteriostatic as well as bactericidal activity against mycobacterium tuberculosis. Studies on rat model have shown that PZ causes a change in activity of enzymatic as well as non-enzymatic antioxidants. Some of these antioxidants include superoxide dismutase (SOD), Glutathione (GSH), and malondialdehyde. Oxidative stress can be involved in pyrazinamide induced hepatotoxicity. These findings are further supported by the studies which have shown the down regulation of PPAR-α along with its target genes by pyrazinamide and improvement of pyrazinamide induced hepatotoxicity by fenofibrate which is a PPAR-α agonist.[7]

Betulinic acid (BA) is a lupane-type triterpenoid with a natural pentacyclic structure. It is very common and has a broad distribution in the plant kingdom. There are many different plants which contains BA such as *Betulacea* (birch tree), *Ziziphus* (Indian jujube), *Syzygium* (black plum)*, Diospyros* (date plum)*, Paeonia* (garden pony).[8] There are number of biological activities associated with this compound such as anti-tumour activity, anti-oxidant activity, hepatoprotective activity, cardio-protective activity, anti-inflammatory activity as well as nephroprotective effects.[9] Hepatoprotective effects of BA are attributed to the beneficial effects of this compound on antioxidant enzymes such as glutathione, glutathione peroxidase and superoxide dismutase along with betulinic acids ability to curb lipid peroxidation.[10]

Zinc is a trace element which is most common in human body. Zinc reduces oxidative stress by enhanced induction of metallothioneins. Zinc also produces its anti-oxidant effects by enhancing the activation of certain enzymes which are the component

of antioxidant system such as glutathione and catalase. It also causes stabilization of protein sulfhydryl groups against oxidation and also has antagonistic action against the chemical reactions which are catalysed by transition metals. Antioxidant effects of Zn are further enhanced by the ability of Zn to cause exchange of redox actives metals, e.g., copper and iron on certain binding sites.[11] Zn has an established hepatoprotective action.[12] Studies have shown that the hepatoprotective actions of Zn are attributed partly to their above mentioned antioxidant effects.[13]

Studies have shown the hepatoprotective effects of both the betulinic acid[10] and zinc[14] due to their antioxidant actions but limited data is available about their combined effect. This study was done to see the hepatoprotective effects of zinc complex of BA keeping in mind anti-oxidant effects of the compound so that it can be used as an adjunct drug therapy.

METHODOLOGY

It was an experimental randomized control study which was conducted at Pharmacology Department, Islamic International Medical College, Rawalpindi in collaboration with Riphah Institute of Pharmaceutical Sciences and Animal House of National Institute of Health, Islamabad. After approval of the Institutional Review Committee, the study was carried out in one year from 1 Sep 2020 to 31 Aug 2021.

A total of 30 healthy male albino Balb/C mice weighing 30–50 g, and aged 8 weeks, were included in the study. All mice were kept under room temperature of 22±2 °C and 12-hour light-dark cycle for 1 week. Zinc complex of Betulinic acid was prepared at Riphah Institute of Pharmaceutical Sciences, Islamabad. The mice were randomly divided into three groups each containing 10 mice. Group 1, i.e., normal control group was provided with tap water and normal diet. Group 2, was given pyrazinamide in dose of 500 mg/Kg.[15] Group 3 was given zinc complex of Betulinic acid 1 mg/Kg in 1% starch jelly along with pyrazinamide.[10]

On day 0 blood samples were taken from 2 mice in each group for baseline evaluation. Second blood sampling was done from 2 mice in each group on day 15. Final sampling was done on day 28. Sampling was done through cardiac puncture by using 3 cc syringes. Samples were allowed to clot. Serum was separated with centrifugation at 3,000 RPM for 5 minutes. Serum was aspirated in sterile tubes and ALT was estimated using ALT kit (Merck) and bilirubin was estimated using serum Bilirubin kit (Merck) on Chemistry Analyser, MicroLab 200 (Merck).

Data was analysed using SPSS-20. Mean and standard error of mean was calculated for all groups and Post-hoc test was done for comparison between the different groups. Results were considered significant at $p<0.05$.

RESULTS

Table-1 shows the comparison of Mean±SEM of all groups. The results of group 3 are comparable to group 2 and significant of $p<0.05$ was seen. The significant results are verified which are certainly comparable with the disease control group. In zinc complex of Betulinic acid treated group, there was a substantial drop in hepatic enzymes which shows the positive effect of zinc complex of Betulinic acid in the treatment of pyrazinamide induced hepatotoxicity.

Table-1: Comparison of ALT and Bilirubin in all groups (Mean±SEM)

Serum (U/L)	Group 1	Group 2	Group 3	*p*
ALT	41.17±4.238	165.67±7.592	58.00±5.639	0.000
Bilirubin	0.217±0.0477	1.000±0.0577	0.617±0.0601	0.000

DISCUSSION

In the present study it is observed that zinc complex of Betulinic Acid ameliorates the hepatotoxic effect induced by Pyrazinamide. Hepatoprotective effect of zinc complex of Betulinic acid was seen in group 3. Damage to the hepatocellular membrane leads to its disruption and release of hepatic enzymes. ALT and bilirubin are located in the cytosol and increased serum level of these enzymes suggests that the hepatocytes have been damaged.

Use of anti-oxidants has been proposed to combat liver injury caused by oxidative stress. Improvement in the biochemical markers like ALT and bilirubin in this study is supported by the study of Yi J *et al*[10] who studied the hepatoprotective activity of betulinic acid on alcohol induced liver damage in Kunming mice. They established that betulinic acid can cause improvement in the hepatic enzyme levels and a decrease in micro vesicular steatosis in mice administered with alcohol through improvement of tissue redox system, maintenance of antioxidant system and decrease lipid peroxidation in liver.

Reduction in the hepatic enzyme activity by betulinic acid was also studied by Zheng *et al*[16], who proved the hepatoprotective effect of betulinic acid on D-GalN/LPS-induced acute liver damage in mice. They showed that BA pretreatment improved the survival rate of mice administered with D-GalN/LPS, and attenuated serum transaminases. BA administration caused an increase in GSH and CAT activity, and decreased MDA level, which indicates that one of the hepatoprotective mechanisms of BA might be via the antioxidant defence system.

Abdullah *et al*[17] concluded that root extract of *Ziziphus oxyphylla*, a herb traditionally used for the treatment of hepatic diseases in Pakistan, showed improvement in hepatic enzyme levels. It also improved the antioxidant enzymes and decreased the lipid peroxidation in carbon tetrachloride induced hepatic damage in BALB/c mice. That study determined that the

hepatoprotective effects of *Ziziphus oxyphylla* are through improvement in antioxidant defense system and stabilization of membrane. The study also concluded that active components of *Ziziphus oxyphylla* responsible for its hepatoprotective action are pentacyclic triterpines the most important being Betulinic acid.[17]

Al-Jawad FH *et al*, studied the hepatoprotective effects of zinc by subjecting the rats to thallium poisoning. In his study he revealed that zinc caused a significant reduction in hepatic enzyme level such as ALT and AST indicating the hepatoprotective effects of zinc against hepatic injury caused by thallium. There was also preservation of normal hepatic architecture in zinc treated mice.[18]

Improvement in serum ALT and bilirubin by administration of zinc was also documented by Wardah Siddique, *et al*. They documented that administration of zinc to isoniazid and rifampicin induced hepatotoxicity in mice leads to the improvement in not only the biochemical but histological parameters as well.[19]

In the present study improvement in biochemical parameter in group 3, was observed indicating that zinc complex of Betulinic acid can be used in the treatment of pyrazinamide induced hepatotoxicity.

CONCLUSION

Zinc complex of Betulinic acid significantly lowers hepatic enzyme in pyrazinamide induced hepatotoxicity in mice. Zinc complex of Betulinic acid can be used as an adjunct in the prevention of pyrazinamide induced hepatotoxicity.

REFERENCES

1. Shehata AA, Farrag AA, Elhady EE, Elsheikh HH. Effect of Mushroom Fungus Feeding on Induced Hepatotoxicity Rats. Egypt J Appl Sci 2020;35(11):127–42.
2. Thuwaini MM, Al-Derawi KH, Kadhem H. The possible protective effect of carthmustinctorius (Leaves) on anti-tuberculosis (rifampin & isoniazid) drugs-induced hepatotoxicity in rats. Int J Pharm Res 2018;10(4):516–22.
3. Khan MD, Ahmad B, Hussain A, Ishaq T, Haider O, Murtaza HG. Frequency of drug-resistant mycobacterium tuberculosis in Chiniot, Pakistan. Life Sci J Pak 2021;3(1):3–7.
4. Abebe G, Bonsa Z, Kebede W. Treatment outcomes and associated factors in tuberculosis patients at Jimma University Medical Center: A 5-Year retrospective study. Int J Mycobacteriol 2017;6(3):239–45.
5. Marjani M, Fahimi F, Sadr M, Dizaji MK, Moniri A, Khabiri S, *et al*. Evaluation of Silymarin for management of anti-tuberculosis drug induced liver injury: A randomized clinical trial. Gastroenterol Hepatol Bed Bench 2019;12(2):138–42.
6. Hussain Z, Zhu J, Ma X. Metabolism and hepatotoxicity of Pyrazinamide, an antituberculosis drug. Drug Metab Dispos 2021;49(8):679–82.
7. Zhang Y, Guo H, Hassan HM, Ding PP, Su Y, Song Y, *et al*. Pyrazinamide induced hepatic injury in rats through inhibiting the PPARα pathway. J Appl Toxicol 2016;36(12):1579–90.
8. Ríos JL, Máñez S. New pharmacological opportunities for betulinic acid. Planta Med 2018;84(1):8–19.
9. Silva FSG, Oliveira PJ, Duarte MF. Oleanolic, ursolic and betulinic acids as food supplements or pharmaceutical agents for type 2 diabetes: promise or illusion? J Agric Food Chem 2016;64(15):2991–3008.
10. Yi J, Xia W, Wu J, Yuan L, Wu J, Tu D, *et al*. Betulinic acid prevents alcohol-induced liver damage by improving the antioxidant system in mice. J Vet Sci 2014;15(1):141–8.
11. Marreiro DD, Cruz KJ, Morais JB, Beserra JB, Severo JS, de Oliveira AR. Zinc and oxidative stress: Current mechanisms. Antioxidants (Basel) 2017;6(2):24.
12. Aftab K. Hepatoprotective role of zinc sulphate in carbon tetrachloride induced liver toxicity. J Pharmacol Clin Res 2016;1(3):1–4.
13. Liu J, Zhou ZX, Zhang W, Bell MW, Waalkes MP. Changes in hepatic gene expression in response to hepatoprotective levels of zinc. Liver Int 2009;29(8):1222–9.
14. Jarosz M, Olbert M, Wyszogrodzka G, Młyniec K, Librowski T. Antioxidant and anti-inflammatory effects of zinc. Zinc-dependent NF-κB signaling. Inflammopharmacology 2017;25(1):11–24.
15. Taziki S, Khori V, Jahanshahi M, Seifi A, Babakordi F, Nikmahzar E. Protective role of taurine against hepatotoxicity induced by pyrazinamide in rats. Natl J Physiol Pharm Pharmacol 2018;8(6):824–8.
16. Zheng ZW, Song SZ, Wu YL, Lian LH, Wan Y, Nan JX. Betulinic acid prevention of D-galactosamine/lipopolysaccharide liver toxicity is triggered by activation of Bcl-2 and antioxidant mechanisms. J Pharm Pharmacol 2011;63(4):572–8.
17. Abdullah, Khan MA, Ahmad W, Adhikari A, Ibrar M, Rehman MU, *et al*. Exploration of hepatoprotective potential and phytochemicals of ziziphus oxyphylla edgew. Pak Vet J 2020;40(4):431–6.
18. Al-Jawad FH, Sharquie KE, Abu Raghif, Nashtar SB. Hepatoprotective effects of zinc sulphate and silymarin against thallium-induced poisoning in rats. Iraqi Acad Sci J 2016;1:42–57.
19. Siddique W, Rashid N, Warraich NY, Talat H, Talha MU, Siddique H, *et al*. Comparison of hepatoprotective effect of silymarin and zinc sulfate against hepatotoxicity induced by isoniazid and rifampicin in albino rats. Pakistan J Med Heal Sci 2020;14(1):113–9.

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ORIGINAL ARTICLE

ACTIVE ROLE OF POTASSIUM NITRATE TOOTHPASTE FOR TREATING DENTINE HYPERSENSITIVITY AND MAINTAINING THEIR NORMAL PHYSIOLOGY

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Background: Prevalence of dentine hypersensitivity in individuals is increasing globally due to the intake of erosive acidic food beverages and improper brushing protocols in turn adversely affecting the tooth surfaces. Toothpastes containing various potassium salts are very effective in treating the dentine sensitivity when used properly for some time. Herein, we have investigated the association between the dentine sensitivity and 5% potassium nitrate toothpaste usage for a period of one month. **Methods:** For this interventional study, one hundred and forty participants with dentine sensitivity were analysed before and after using 5% potassium nitrate toothpaste. Visual Analog Scale (VAS) scoring tool was employed where sensitivity to Air among the participants was calculated by VAS-Air scoring and sensitivity to cold was tested by VAS-Cold scoring. Paired *t*-test was incorporated for performing the statistical analysis through the IBM SPSS-22. **Results:** There was a strong association between the dentine sensitivity and usage of 5% potassium nitrate toothpaste. After using the 5% potassium nitrate toothpaste for one month, the calculated VAS-Air scoring and VAS-Cold scoring reduced significantly against both the air and cold stimuli which was found to be 2.67±1.12 and 1.86±0.83 (p=0.001). **Conclusions:** The 5% potassium nitrate toothpaste was potent enough to reduce the dentine hypersensitivity in the participants against both the thermal and air stimuli.

Keywords: Dentine Hypersensitivity, Erosive food, Thermal stimuli, Toothpaste, Visual Analog Scale

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INTRODUCTION

Recently recorded prevalence of dentin hypersensitivity globally has been ranging between 1.34–98% which is quite alarming.[1,2] Dentin Hypersensitivity is quite a painful sensation produced and is commonly also referred as Tooth sensitivity or common cold of dentistry.[3] This tooth pain experienced by a patient might be of short tenure but acutely excruciating in nature leading to the prolonged discomfort eventually.[4] This problem often occurs because of eating erosive acidic beverages and using hard, improper brushing techniques that can possibly affect the quality of any individual's life.[5] These factors further deteriorated the tooth's structure eventually leading to the destruction of entire tooth surfaces.[6] Sometimes, these routine practices promote the thinning of the outer enamel surfaces of teeth and exposing the dentine prominently via attrition, abrasion, wear, abfraction, erosion, gingival recession and scratches.[7] Eventually, the widening and opening of dentinal tubules lead to their expansion.[6] These dentinal tubules get more responsive to many stimuli such as tactile, chemical, mechanical, thermal, osmotic, and physical irrespective of any pathology in them.[7] Furthermore, these dentinal tubules also allow the absorption of stimulants excessively inside the tooth pulp resulting in its severe irritation, pain and discomfort.[6] Therefore, it is necessary to block the dentinal tubule openings for the elimination of these negative hypersensitivity responses in the teeth.

Various toothpastes are exploited to overcome the dentinal hypersensitivity by occluding the widened dentinal tubules on the large scale. These toothpastes include fluorides, chloride hexahydrate, strontium, stannous fluoride, aluminium ferric-oxalates, and potassium ferric-oxalates, and fluorides in their compositions.[8] Moreover, the most easily available, cost effective and simplest type of toothpaste categories that could be helpful in declining the dentine hypersensitivity incorporated the potassium salts and their compounds. Additionally, the ions present in these potassium salts might be capable of hindering the transmission of pain signals and their impulses through the intra-dental nerves.[9] Previous studies showed that the longer partakers used toothpastes, the greater reduction in DH.[10] Previously, a toothpaste containing 3% potassium nitrate salt reduced the dentine hypersensitivity to some extent but after the usage for longer duration.[11] Still, there is gap in literature regarding the availability of useful potassium salt percentage in the toothpaste that could be potent enough to reduce the dentine hypersensitivity in short time application. The current study focused on treating the chronic dentine hypersensitivity in patients with the help of 5% potassium nitrate containing toothpaste.

METHODOLOGY

This study was conducted for a period of one month, during June–July 2023 in School of Dentistry, Shaheed Zulfiqar Ali Bhutto Medical University Islamabad under Ethical Approval Letter No. SOD/ERB/2023/32.

Participants aged 18 years or older whose Visual Analog Scale (VAS) score for dentine hypersensitivity was in the ranges between 3−8 were included in this study.

Participants whose Visual Analog Scale (VAS) score for dentine hypersensitivity was not in the ranges between 3−8 were excluded from this study. Further, patients with full mouth rehabilitation were also excluded.

One-hundred-forty participants volunteered to take part in this study after their written consents. The tool used to measure the dentine hypersensitivity was Visual Analog Scale (VAS) scoring scale.[13,14] VAS scoring scale was used in patients before and after using the 5% potassium nitrate toothpaste. Visual Analog Scale (VAS) consisted of a calliper containing 10 mm scale representing the 'No Pain' at 0 end and 'Worst Possible Pain' at 10 end showing the intensity of pain. Teeth of the participants were tested for sensitivity to air pressure with the help of air dental syringe kept away at distance of one cm from participant's sensitive teeth involved in the study and air was blasted at least for one second (30 PSI pressure, 23±30 °C temperature) in order to calculate the VAS-Air score. After 5.0 minutes, VAS-Cold for sensitivity to cold stimulus was investigated, by air blasting the cold frozen water for one-second again on the participant's sensitive teeth involved in the study.

VAS-Air and VAS-Cold score was calculated before providing 5% potassium nitrate toothpaste to these participants. This data was recorded as the Baseline readings. Then, participants were provided with 5% potassium nitrate toothpaste (Sensed toothpaste, Gentle Care Corporation, Pakistan) and soft-bristled toothbrush for brushing their teeth twice a day. They were instructed for maintaining the strict oral hygiene and proper brushing for at least one month with the same toothpaste and toothbrush.

These participants were again recalled for calculating their VAS-Air and VAS-Cold scores after one month to evaluate the effectiveness of toothpaste containing 5% potassium nitrate in reducing the dentine's hypersensitivity in the participants to both air and Cold stimuli.[15]

SPSS-22 was used for the Descriptive analysis of means and standard deviation. Comparison between participant groups before and after was done using Paired *t*-test at 95% confidence interval, and $p \leq 0.05$ was taken as statistically significant.

RESULTS

The results conclude that the level of dentine hypersensitivity significantly reduced in participants using 5% potassium nitrate toothpaste. Sensitivity to air among these participants was calculated by VAS-Air scoring, whereas VAS-Cold scoring tested sensitivity to cold among the same participants. The mean value for the VAS-Air scoring among these participants at baseline before using the 5% potassium nitrate toothpaste was found to be 5.80±1.72 whereas mean value for VAS-Cold scoring was calculated to be 6.89±1.32 which was statistically significant ($p<0.05$). After using the same 5% potassium nitrate toothpaste for one month, the calculated mean VAS-Air scoring and mean VAS-Cold scoring among the same participants reduced significantly against both the air and cold stimuli. The reduction in the mean VAS-Air score among the study participants after using the toothpaste was found to be 2.67±1.12 and the declination in mean VAS-Cold score was observed to be 1.86±0.83 which was statistically significant ($p<0.05$) (Figure-1).

Before using toothpaste, participant's VAS-Air scores were significantly different from those after using toothpaste for a month (Mean±SD 3.129±1.967; $p=0.001$). As seen by the mean difference of 3.129 (95% CI [2.800, 3.457]), the usage of toothpaste was associated to a significant drop in VAS-Air scores. Similarly, there was a statistically significant difference in the VAS-Cold evaluations of participants before and after using toothpaste (Mean±SD 5.021±1.548; Mean±SD 0.131±4.763; $p=0.001$). According to the mean difference of 5.021 (95% CI [4.763, 5.280]), using toothpaste was associated with a significant decrease in VAS-Cold ratings (Table-1).

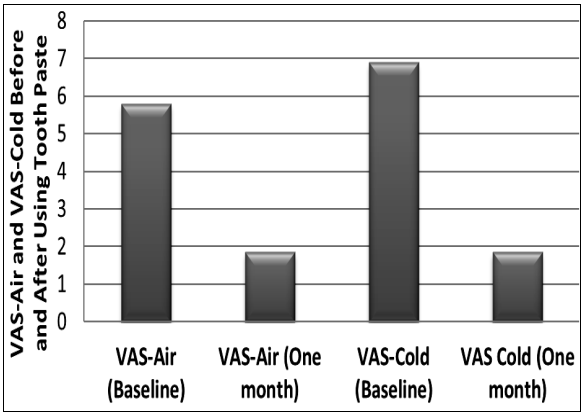

Figure-1: Mean difference of VAS-Scoring against sensitivity to Air (VAS-Air) and Cold (VAS-Cold) before and after using the 5% potassium nitrate toothpaste

Table-1:- Paired *t*-test comparative analysis of VAS-Scoring vs sensitivity to air and cold (VAS-Cold) pre and post using 5% potassium nitrate toothpaste.

VAS-Scoring groups	Comparison of VAS-Air score among participants at baseline and after one month of using 5% potassium nitrate toothpaste	Mean Difference with SD and (SE)	95% CI of the Difference		*p*
			Lower	Upper	
VAS-Air scoring group	Comparison of VAS-Air score among study participants before and after using 5% potassium nitrate toothpaste	3.12±1.96 (0.16)	2.800	3.457	0.001
VAS-Cold scoring group	Comparison of VAS-Cold score among study participants before and after using 5% potassium nitrate toothpaste	5.02±1.54 (0.13)	4.763	5.280	0.001

DISCUSSION

Dentine hypersensitivity has been a major problem affecting the community on the larger extent.[11] Previously, the reported minimum prevalence for dentine hypersensitivity was calculated to be 14.5% whereas the maximum value was found to be 57%.[15,16] Multiple factors acting simultaneously enhance dentine hypersensitivity. This severe problem of dentine hypersensitivity could be resolved easily with the help of potassium salt/compound-oriented toothpaste that could be capable of treating this common issue prevailing in our daily routine.

The current study investigated the effect of 5% potassium nitrate toothpaste on dentine's hypersensitivity of patients. The results of our study displayed statistically significant deduction in the dentine hypersensitivity of participants against both air and cold stimuli after the usage of this toothpaste for the duration of one month. The findings in this study were similar to a previous research[17] but with a potassium citrate toothpaste and not potassium nitrate salt. The reasons for dentine's hypersensitivity reduction in our study could possibly be the increased amounts of potassium ion absorption in the dentinal tubules that might have prohibited them from sending the sensitivity occupying signals to the nerves[18]. Secondly, this viable entrance of potassium nitrate ions in the widened dentinal tubules might have brought them to their natural biological morphology by narrowing them, thus, reducing the dentine's hypersensitivity to a statistically significant level.

Preferable tool for measuring the clinical dentine's hypersensitivity is named as Visual Analog Scoring (VAS) system because of its ability to reproduce more valid and reliable results regarding tooth's sensitivity measurements.[19,20] Others have confirmed that this system is cost-effective, more sensitive and quite easy for recording the perception of pain and sensitivity in individuals appropriately.[20,21] Hence, accurate results attained in the current study were due to harnessing the authentic operating system of Visual Analog Scoring (VAS) system.

The most reliable methods employed by the dental practitioners to evaluate the dentine's hypersensitivity are through thermal[19] and air in the Value Analog Scoring system[22]. The major cause for obtaining the reliable results by using the Thermal (hot and cold) and air blasting was because of the short-time application of these stimuli for only one second on the sensitive tooth surfaces.[19,23] The current study incorporated both thermal and air blast techniques to calculate the effects of 5% potassium nitrate toothpaste on the dentine's hypersensitivity. The induction of two different tools at the same time in our research might have increased the validity and reliability of results respectively. The statistically significant reduction in both VAS-Air scoring and VAS-Cold scoring was noticed in participants of this study with toothpaste containing 5% potassium nitrate. These results were similar to another study where potassium citrate was used instead of potassium nitrate.[17]

Different toothpastes that include potassium-chloride and potassium-citrate in their compositions are clinically acceptable for treating the hypersensitivity of dentine.[24,25] On the other hand, potassium-nitrate containing toothpastes are approved by American Dental Association Council on Dental Therapeutics and Drugs. The ions released by potassium-nitrate are well-known for reducing the excitation of nerves especially intra-dental nerves, thereby resulting in pain and sensitivity cessation quite actively.[26] Antibiotics are also prescribed in dental pain and some factors should be considered when prescribing them.[27,28] Potassium salts, specifically potassium-citrate and potassium-nitrate, possess the upgraded qualities of reducing the hypersensitivity of dentine.[28] Dentinal hypersensitivity is a common symptom of many dental diseases[18], hence the dental experts should help provide the patients with necessary education on oral hygiene.[29]

Further studies are required to investigate the positive role of different types of toothpaste salts in the reduction of tooth's hypersensitivity other than only using the potassium salts and compounds.

CONCLUSIONS

Utilization of 5% potassium nitrate toothpaste on regular basis for complete one month is potent enough to reduce the hypersensitivity of dentine which can be treated easily by just adopting normal brushing protocols with toothpastes containing potassium nitrate salt.

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REFERENCES

1. Guerra F, Corridore D, Cocco F, Arrica M, Rinaldo F, Mazur M, *et al*. Oral health sentinel-based surveillance: a pilot study on dentinal hypersensitivity pain. Clin Ter 2017;168:e333–7.
2. Katirci G, Celik EU. The prevalence and predictive factors of dentine hypersensitivity among adults in Turkey. BMC Oral Health 2023;23(1):474.
3. John DS, Shenoy N, Bhandary R. Dentinal hypersensitivity: A clinical dilemma. J Datta Meghe Inst Med Sci Univ 2022;17(3):786–91.
4. Douglas-de-Oliveira DW, Vitor GP, Silveira JO, Martins CC, Costa FO, Cota LO. Effect of dentin hypersensitivity treatment on oral health related quality of life —A systematic review and meta-analysis. J Dent 2018;71:1–8.
5. Douglas-De-Oliveira DW, Lages FS, Paiva SM, Cromley JG, Robinson PG, Cota LO. Cross-cultural adaptation of the Brazilian version of the Dentine Hypersensitivity Experience Questionnaire (DHEQ-15). Braz Oral Res 2018;32:e37.
6. Pradeep AR, Sharma A. Comparison of clinical efficacy of a dentifrice containing calcium, sodium phosphosilicate to a dentifrice containing potassium nitrate and to a placebo on dentinal hypersensitivity: a randomized clinical trial. J Periodontol 2010;81(8):1167–73.
7. Canadian Advisory Board on Dentin Hypersensitivity. Consensus-based recommendations for the diagnosis and management of dentin hypersensitivity. J Can Dent Assoc 2003;69(4):221–6.
8. Dowell P, Addy M. Dentine hypersensitivity—a review. Aetiology, symptoms and theories of pain production. J Clin Periodontol 1983;10(4):341−50.
9. Wara-aswapati N, Krongnawakul D, Jiraviboon D, Adulyanon S, Karimbux N, Pitiphat W. The effect of a new toothpaste containing potassium nitrate and triclosan on gingival health, plaque formation and dentine hypersensitivity. J Clin Periodontol 2005;32(1):53−8.
10. Jang JH, Oh S, Kim HJ, Kim DS. A randomized clinical trial for comparing the efficacy of desensitizing toothpastes on the relief of dentin hypersensitivity. Sci Rep 2023;13(1):5271.
11. Shakoor A, Rana SS, Khan AS, Raja HZ, Saleem MN, Munir N. Frequency of various intensities of dentine hypersensitivity versus good and bad oral hygiene practices within Pakistani population. J Oral Health Oral Epidemiol 2022;11(3):164–8.
12. Hall C, Sufi F, Wang N, Goyal CR. Efficacy of an experimental 3% potassium nitrate mouthwash in providing long-term relief from dentinal hypersensitivity: an 8-week randomized controlled study (Study 1). Am J Dent 2017;30(6):27–34.
13. Pamir T, Ozyazici M, Baloğlu E, Onal B. The efficacy of three desensitizing agents in treatment of dentine hyper sensitivity. J Clin Pharm Ther 2005;30(1):73−6.
14. Noor N, Mujtaba H, Humayoun S, Umer MF, Javaid MM, Rehman A. Comparison of endodontic pain observed with sodium Hypochloride, hydrogen peroxide and Norman saline based root canal irrigants. Ann Pak Inst Med Sci 2022;18:3–8.
15. Shen SY, Tsai CH, Yang LC, Chang YC. Clinical efficacy of toothpaste containing potassium citrate in treating dentin hypersensitivity. J Dent Sci 2009;4(4):173–7.
16. Graf H, Galasse R. Morbidity, prevalence and intraoral distribution of hypersensitive teeth. J Dent Res 1977;56(2):162–5.
17. Sen S, Meena A, Prathebha PK. Comparative desensitizing effect of a toothpaste & mouthwash-containing potassium nitrate: An *in vivo* & *in vitro* scanning electron microscopic study. Adv Dent Oral Heal 2018;8(5):12–8.
18. Khairat RU, Jan SM, Behal R, Baroo AA. Effectiveness of potassium nitrate as desensitizing toothpaste and mouthwash in the treatment of dentin hypersensitivity. J Adv Med Dent Sci Res 2022;10(8):20–4.
19. Pashley DH. Mechanisms of dentin sensitivity. Dent Clin North Am 1990;34(3):449−73.
20. Clark GE, Troullos ES. Designing hypersensitivity clinical studies. Dent Clin North Am 1990;34(3):531−44.
21. Tolentino AB, Zeola LF, Fernandes MRU, Pannuti CM, Soares PV, Aranha AC. Photobiomodulation therapy and 3% potassium nitrate gel as treatment of cervical dentin hypersensitivity: A randomized clinical trial. Clin Oral Investig 2022;26(12):6985–93.
22. Salvato AR, Clark GE, Gingold J, Curro FA. Clinical effectiveness of a dentifrice containing potassium chloride as a desensitizing agent. Am J Dent 1992;5(6):303−6.
23. West NX. Dentine hypersensitivity: preventive and therapeutic approaches to treatment. Periodontol 2000. 2008;48:31−41.
24. Silverman G, Gingold J, Curro FA. Desensitizing effect of a potassium chloride dentifrice. Am J Dent 1994;7(1):9−12.
25. Sowinski J, Ayad F, Petrone M, DeVizio W, Volpe A, Ellwood R, *et al*. Comparative investigations of the desensitizing efficacy of a new dentifrice. J Clin Periodontol 2001;28(11):1032−6.
26. Jawa ZA, Jawa LA, Jamil BD, Sajid M, Javaid MM. Knowledge of antibiotic prescription for endodontic treatment in final year students and house officers. Pak J Public Health 2018;8(1):18–21.
27. Basharat S, Kumar R, Mushtaq M, Mohsin M. Understanding regarding antibiotic resistance among healthcare professionals in government sector of Tehsil Gujar Khan. Pak J Public Health 2019;9(3):144–8.
28. Grover V, Kumar A, Jain A, Chatterjee A, Grover HS, Pandit N *et al*. ISP good clinical practice recommendations for the management of dentin hypersensitivity. J Indian Soc Periodontol 2022;26(4):307–33.
29. Mylonas P, Milward P, McAndrew R. Denture cleanliness and hygiene: an overview. Br Dent J 2022;233(1):20–6.

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KH: Statistical analysis

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ORIGINAL ARTICLE

COMPARATIVE EFFECTS OF HIGH FAT AND CAGED CHICKEN DIET ON BODY WEIGHT GAIN IN ALBINO RATS

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Background: Consumption of high fat diet, caged chicken meat and sedentary lifestyle have seriously caused weight gain in developed countries resulting in hyperlipidemias and imbalance in the steroidal sex hormones ultimately. Objective of this study was to compare the effects of high fat and caged chicken diet on body weight of female albino rats. **Methods:** This randomized control trial was conducted in collaboration with National Institute of Health and Anatomy Department of Islamic International Medical College after approval from the Ethics Review Committee, from Sep 2021 to Sep 2022. This study was performed on 30 Albino Sprague Dawley adult female rats weighing 250–300 gm with no gross abnormality. They were divided into 3 equal groups of 10 rats in each group. Control group A was given standard rat diet. Experimental group B animals were given 60% fat while experimental group C animals were given cubes of caged chicken diet (20 gm/rat in the raw form orally) for 9 weeks. At the end of the experiment, animals were observed for relative body weight changes among groups. **Result:** The mean weight of the animals increased normally in control group A from 220 to 236 gm. Group B gained weight from 220 to 268 gm, and group C grew from 220 to 333 gm during 9 weeks. The weight gain was significantly higher in group C. **Conclusion:** Caged chicken diet as compared to high fat diet affects animals' weight more harmfully, and hence the health.

Keywords: High fat diet, polycystic ovaries, caged chicken, ovary, steroidal sex hormones

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INTRODUCTION

Excessive weight gain and obesity causes many disorders. Some of them may be quite serious like hypertension, diabetes, and other metabolic disorders including polycystic ovary syndrome (PCOS). PCOS is a complex endocrine disorder characterized by weight gain, insulin resistance, hyperandrogenism, menstrual abnormalities, polycystic ovaries, chronic anovulation, and decreased fertility.[1] It occurs after menarche in teenage girls or young adults who present with oligomenorrhea, hirsutism, infertility, and obesity.[2,3]

Food containing excess of oils and fats triggers the emergence of excessive deposition of adipose tissue and this leads to excessive weight gain.[4,5]

With the change in the eating habits, intake of chicken meat has markedly increased now-a-days. Chicken meat is rich in cholesterol and fats.[6] The increased cholesterol consumed through meat is absorbed in the intestine, where it is packaged as triacylglycerol-rich particles known as chylomicrons.[7] The deleterious effects of high fat diet (HFD) and caged chicken meat consumption is leading to changes in body weight.[8]

The present study was designed to compare relative body weight changes of female albino rats on feeding normal, high fat diet, and caged chicken diet.

MATERIAL AND METHODS

This randomized control trial was conducted in collaboration with National Institute of Health (NIH) and Department of Anatomy, Islamic International Medical College, Rawalpindi after approval by the Ethics Review Committee of the College. The study duration was 12 months from 15th September 2021 to 15th September 2022.

Simple random sampling was done by assigning numbers. The study was performed on 30 Albino Sprague Dawley adult female rats. Standard pellet animal diet was used for group A, 60% HFD for group B, and Caged Chicken Cubes 20 gm per rat were given to group C. The body weight of all the animals was recorded at the start of the study as well as before the sacrifice of animals.

Data was analysed on SPSS-21. Mean and standard error of mean were calculated. ANOVA was applied and results expressed as Mean±SD. Post hoc Tukey's test was applied for multiple comparisons among groups.

RESULTS

The control group A showed normal gain of body weight from 220 to 236 gm during 9 weeks of study. Experimental group B (High Fat Diet) showed weight gain from 220 to 268 gm. In experimental group C

(Caged chicken diet) the rats significantly gained body weight from 220 to 333 gm. Inter group comparison showed a significant increase in final body weight of experimental group C (Caged chicken diet) as compared to experimental group B (HFD) and experimental group A (Control) (Table-1).

Multiple comparison (Post hoc Tuckey's test) showed significant gain in body weight of caged chicken diet group C (p<0.001) as compared to high fat diet group B and Control group A (Table-2).

Table-1: Mean values for change in relative body weight of rats (ANOVA) in control and experimental groups (Mean±SD)

Groups	Group A (n=10)	Group B (n=10)	Group C (n=10)	*p*
Initial animal weight (g)	220±8.16	220±8.16	220±8.16	1.000
Final animal weight (g)	236±9.66	268±38.24	333±51.22	<0.001*

*Significant

Table-2: Comparison of the mean values for change in relative final body weight of rats among control and experimental groups

Final Body weight (Grams)		
Groups	**Mean differences**	***p***
A *vs* B	32	0.153
A *vs* C	97	<0.001*
B *vs* C	65	0.002*

*Significant

DISCUSSION

Excessive consumption of high fat diet, caged chicken meat and sedentary lifestyle have seriously increased the weight gain in developed countries.[8] Chicken meat is selected and consumed largely by general population of Pakistan as it is cheap, easily available and considered to be rich in dietary nutrient. Therefore, the population at large is consuming more fats and cholesterol rather than proteins hence gaining weight gain. Subsequently, numerous studies indicate that increased incidence of weight gain especially in young girls has caused many metabolic diseases from systemic malfunctions to reproductive disorders.

Weight gain is shown to reduce percentage of the pregnancy among the women through triggering abdominal fat accumulation and decreasing ovulation rates. Consequently, studies concerning the negative effects of weight gain on fertilization have gained more importance in recent years.

Female rats with diet-induced weight gain exhibit infertility and thus can serve as a model for human infertility models like polycystic ovaries. Weight gain is associated with adipogenesis, metabolic syndrome and abnormal accumulation of abdominal fat triggering the emergence of PCOS.[7–10]

This study revealed that caged chickens reared on commercial feed led to increase weight gain and growth rates in the experimental animals of group C. This was however, not seen in control animals A and high fat diet group B. Weight gain and high growth rates in Groups C was subjected to commercial feed and poultry grown on such feed. It was reported previously that chicken meat and chicken feed are rich of proteins, fats and cholesterol. This can be attributed to the recent increase in the rate of weight gain and obesity in the Pakistani population consuming chicken meat on daily basis. Caged chicken diet group gained more body weight due to hyperlipidemia and imbalance in the steroidal sex hormones as compared to control group and high fat diet group.[11]

The current study is the first one providing a comparison that shows feeding of rats with HFD and caged chicken meat bringing changes in animal body weight. This probably is attributed to the contents that are included in the feed provided to caged chickens to grow upon. Similar effects are anticipated in the humans who consume caged chicken on routine basis, hence bringing the deleterious effects on their health in terms of weight gain, growth, obesity and hormonal irregularities levels that may lead to progression of PCOS.[9]

CONCLUSION

Present study revealed marked increase in relative body weight of group C fed on caged chicken, as compared to HFD group B, and Control group A. Caged chicken diet is more harmful than HFD for weight gain and its consequences.

RECOMMENDATIONS

Excessive use of caged chicken must be discouraged, especially in young girls who are vulnerable to much weight gain and resulting PCOS. Further large scale studies including humans are recommended.

REFERENCES

1. Hilal G, Fatma T, Ferruh Y, Sabire G, Yüksel A. Effect of high-fat diet on the various morphological parameters of the ovary. Anat Cell Biol 2020;53(1):58–67.
2. Akram M, Roohi N. Endocrine correlates of polycystic ovary syndrome in Pakistani women. J Coll Physicians Surg Pak 2015;25(1):22–6.
3. Rostamtabar M, Esmaeilzadeh S, Tourani M, Rahmani A, Baee M, Shirafkan F, *et al*. Pathophysiological roles of chronic low-grade inflammation mediators in polycystic ovary syndrome. J Cell Physiol 2021;236(2):824–38.
4. Zulfiqar S, Noor S, Rafique H, Rehman B, Babar A, Shahid T, *et al*. Investigation of prevalence and awareness of polycystic ovary syndrome among Pakistani females: Proceedings of the Pakistan Academy of Sciences: Part B. Life Environ Sci 2022;59(1):77–83.
5. Sidra S, Tariq MH, Farrukh MJ, Mohsin M. Evaluation of clinical manifestations, health risks, and quality of life among women with polycystic ovary syndrome. PLoS One 2019;14(10):e0223329.
6. Saara Ahmad. Observation of various blood biochemical changes and their effects on ovaries secondary to administration of various diets in female albino Wistar rats. (PhD Thesis). Baqi Medical University, Karachi, Pakistan; 2015.
7. You W, Henneberg M. Meat consumption providing a surplus energy in modern diet contributes to obesity prevalence: an ecological analysis. BMC Nutr 2016;2(1):22.
8. Ahmad S, Ahmed I, Haider S, Batool Z, Ahmed F, Tabassum S, *et al*. Effects of consumption of caged and un-caged chicken meat on

ovarian health of female wistar rats. Pak J Zool 2018;50(2):487–93.
9. Rajan RK, Siva Salva Kumar M, Balaji B. Soy isoflavones exert beneficial effects on letrozole-induced rat polycystic ovary syndrome (PCOS) model through anti-androgenic mechanism. Pharm Biol 2017;55(1):242–51.
10. Sharma H, Zhang X, Dwivedi C. The effect of ghee (clarified butter) on serum lipid levels and microsomal lipid peroxidation. Ayu 2010;31(2):134–40.
11. Rocha AL, Oliveira FR, Azevedo RC, Silva VA, Peres TM, Candido AL, *et al*. Recent advances in the understanding and management of polycystic ovary syndrome. F1000Res 2019;8:F1000 Faculty Rev-565.

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ORIGINAL ARTICLE

PERCEPTION OF FIRST-YEAR MEDICAL STUDENTS REGARDING SELF-DIRECTED LEARNING AND CONVENTIONAL LECTURING IN PHYSIOLOGY THROUGH ACTIVITY FEEDBACK

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Background: In order to transform the healthcare professionals into lifelong learners, it is important to develop self-directed learning (SDL) skills in their early study years. Students engaged in SDL can complete their learning assignments and can be transformed into lifelong learners. The objective of this study was to evaluate the improvements in perception of students by integrating principles of SDL activity in conventional curricular system in a 4-week first-year MBBS respiratory module. **Methods:** It was a descriptive cross-sectional survey conducted in Ayub Medical College Abbottabad on First Year MBBS (2020–2021) batch, where the module coordinator assigned respiratory insufficiency case-based studies to 1st Year medical students. Students were asked to give response to 20 questions on standard questionnaire proforma. Students were asked to reflect on how this assignment affected their perception of SDL skills and improvement in learning. Students strongly agreed, agreed, neither agree nor disagree, disagree, and strongly disagree about the 20 responses on Likert scale. For simplification, descriptive parameters were modified to numerical values which were then used to analyze responses on MS Excel. **Results:** Feedback response rate was 80% (177, 99 males and 78 females). Frequency of responses was recorded both for conventional teaching (CT) and SDL. There was marked variation in responses, both for SDL and conventional method of teaching. **Conclusions:** The students positively perceived the activity as a valuable learning experience. SDL assignments can be successfully implemented in pre-clinical courses and show improvements in their perception and learning skills.

Keywords: Self-directed learning, Conventional teaching, Medical education, Physiology teaching

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INTRODUCTION

The emerging trends in field of medical education require a shift from traditional lecture generally involving a single teacher delivering lecture to a large group of students with the older methods of delivery like audio-visual aids, blackboard or Power-Point presentation etc. In concordance with this while the students primarily taking the initiative in their self-learning, new learning strategies like directed-self learning (DSL), problem-based learning (PBL), collaborative learning (CL) and self-directed learning (SDL) have been integrated in medical curricula over the past two- decades.[1] Furthermore, the blend of newer techniques like lecture, CL and self-directed methods are also in practice where the role of conventional lecture has been reduced.[2] SDL has been signified as a process in which the students take initiative and bear responsibility for their own learning. SDL also permits health professionals to continuously learn and update their knowledge throughout their professional careers.[1–3] SDL is defined as a process in which a learner takes the initiative, bears responsibility, identifies their learning needs, creates learning goals, organizes time and resources for learning, applies appropriate learning strategies and then evaluates their learning outcomes.[3]

The introduction of Integrated Modular System (IMS) now requires medical institutes to provide adequate opportunities for medical students to participate in self-learning activities and time for independent study to inculcate the skills of lifelong learning in medical students like the ability to recognize the gaps in one's knowledge, to know how to fill these gaps and find reliable sources needed, to gather the relevant information, and finally to apply this information to one's lifelong clinical practice.[4] Thus SDL is considered a key competency to be achieved in Integrated Modular System of medical school's curriculum which is actually a higher order active learning technique to promotes self-efficacy and higher level of cognitive skills of the students which has been strongly commended for effective medical training.[3,4] This helps the trainees in the diagnosis of their learning needs and goals, identification of available human and other resources for learning, in choosing and implementation of appropriate strategies and at the end evaluation of their overall learning outcomes. With the developmental evolution in the field of science and technology and medicine of course, the learning tools, educational technologies, and teaching methods have been gradually variegated.[5]

In this challenging era of medical education, medical students can complete learning assignments and remain informed by effectively engaging with self-directed teaching tools and be prepared for the future as a consistent lifelong learner.[6] It also covers all domains of learning: cognitive (knowledge), psychomotor (skills), and affective (attitude). In the field of medicine medical professionals need to acquire the newest medical expertise and means to sustain their specialized skilled competencies and comparatively serve their patients and community in a better way.[7] It is recommended that doctors need to learn throughout their careers.[6,7] It is increasingly documented that there should be early implementation of medical education strategies that should transform students into lifelong learners. SDL is assumed as an encouraging approach that can support this continuous learning in medical education.[8,9] It is a purposeful mental process, usually escorted with many behavioural accomplishments, affected by many social, cultural and educational setups, past experiences, study skills, self-awareness and motivation involved in search of information.[9]

Despite the importance of active learning and current progress in the field of integrated modular system, the medical education in Pakistan is still dubious and needs a lot of adaptations like probing and consideration of impelling factors.[10] Students orientation, teachers training, resource management, and time allocation may be helpful in augmenting students' SDL capacity.[11] The present study aimed to determine students' responses about SDL activity, and discover which factors can improve or affect students' active learning by implementing SDL in their study courses.

PARTICIPANTS AND METHODS

It was an analytical quasi experimental research conducted in the Department of Physiology, Ayub Medical College Abbottabad from June to August 2021 during Respiratory Module teaching to evaluate the performance of 1st Year MBBS students (2020–2021 batch) by self-directed learning, and responses were compared with conventional method of teaching. Ethical approval of Institutional Review Committee was obtained. Non-probability convenience sampling was used and written informed consent was obtained.

The potential study participants were 221 students of 1st Year MBBS. They were first taught the topic of respiratory insufficiency in a lecture hall as a large group discussion using PowerPoint presentation. At the end of module, they were assigned case-based studies whose topics were selected randomly, learning objectives of topic were made clear to the students and a list of reference books was provided beforehand.

After giving proper instructions about SDL and then given two-week time to gather clinical data regarding a specific topic and prepare for discussions. They were grouped into 10–15 students each and were advised to visit clinical wards to take history and examination of patients with respiratory diseases and relate them to the basic physiological concepts learnt in conventional classroom teaching. At the completion of task, the team leaders had to compile the summary of activity and present before class with PowerPoint presentation and teachers as facilitators.

The students individually assessed their perception, knowledge gaps of the cases, identified scholarly sources to fill their knowledge gaps, shared the information with their teammates, and reflected on their ability to guide their own learning. The facilitator encouraged their free self-expression and interaction among group during discussion but keeping the discussion limited to the learning objectives.

The Likert scale questionnaire was developed and modified using extensive literature review for the utilization of homogenous Likert scale. Students' responses were distributed on 5-point Likert scale as 5=strongly agree, 4=agree, 3=neither agree nor disagree, 2=disagree and 1=strongly disagree. Two such questionnaires were distributed among students one for evaluation of responses after SDL activity and one for conventional teaching/learning technique. Students were asked to record their responses on given questionnaire. The questionnaire was returned in one week. All those who returned filled proformas were included in study and the rest were excluded from study. Data were analysed on MS Excel.

RESULTS

The potential study participants were 221 students consisted of 124 males (56.1%) and 97 females (43.89%), of 1st Year MBBS aged 18–21 (19±0.5) years. The 98% (217) students participated in the SDL activity. Feedback response rate was 80% (177) 99 males and 78 females (Table-1).

In the proforma the frequency of responses was recorded both for Conventional Teaching (CT) and SDL. There was difference in the responses of students both for SDL and CT. Majority of the students favoured SDL. Some responses were intermediate between agree, neutral neither agree nor disagree, disagree and strongly disagree in the performance evaluation of SDL and CT method by feedback method. (Table-2).

Table-1: Distribution and response rates of male and female students of class (n=221)

Variables	N (%)
Males	124 (56.1%)
Females	97 (43.89%)
Age (Years, Mean±SD)	19±0.8
Activity response rate (Total)	217 (98%)
Males	119 (54.83%)
Females	98 (45.16%)
Feedback response rate (Total)	177 (80%)
Males	99 (55.93%)
Females	78 (44.06%)

Table-2: Students' response on Likert scale for SDL and CT

Questions	Number of participants Responses (n)									
	Self-directed learning (SDL)					Conventional teaching (CT)				
	SA	A	Neither A/nor DA	DA	SD	SA	A	Neither A/nor DA	DA	SD
1. The activity hold my interest	57	48	9	3	--	50	43	15	8	1
2. The trainer used relevant and convenient tool	45	40	15	10	7	63	52	1	1	--
3. The activity increased the students-teachers interaction	75	35	6	1	--	20	25	35	30	7
4. The activity was easy to conduct and relevant to students learning	27	23	44	13	10	65	22	16	10	7
5. The activity was time bound	75	24	6	12	1	80	23	10	4	--
6. Helped to integrate my basic knowledge in physiology	61	35	17	4	--	65	32	20	--	--
7. Gave me a chance to self-study	72	31	10	2	2	23	33	25	17	10
8. Broke the uniformity of class room lecture	53	36	11	13	4	25	27	24	20	21
9. Increased my communication skills	58	36	12	5	6	4	9	18	53	33
10. Increased my problem solving skill	45	31	32	5	4	3	2	25	43	44
11. Made me more responsible in studies	69	23	16	6	3	32	29	40	7	9
12. Increased my leadership skills planning and implementing.	42	31	20	13	11	--	--	--	51	66
13. Increased my confidence	45	37	21	13	1	4	11	60	23	19
14. Instilled learning motivation in students	55	46	8	3	5	35	49	28	5	--
15. Permitted Self-monitoring/evaluation	36	35	21	12	13	15	19	44	23	16
16. The trainer had a desired professional skill	24	42	38	7	6	46	40	29	2	--
17. Helped in creating learning goals	53	32	28	3	1	23	37	46	6	5
18. Endorsed self-efficacy and higher level of cognitive skills	59	44	10	1	3	19	24	45	20	9
19. Assisted in identifying available resources and adopt relevant strategy	61	28	11	10	7	17	19	22	37	22
20. Enabled me to apply this information to lifelong clinical practice	70	39	6	--	2	23	25	30	28	11

DISCUSSION

The literature on SDL in medical education reports multiple scopes of SDL and the purpose of present study was to determine the perception of students after introducing the SDL activity in a preclinical medical institute course. Results of this study show that including SDL activity in a preclinical course successfully improves students' skills and learning and can be practiced successfully and integrated into the curriculum. Several studies report the use of clinical cases in lecture-based courses to enhance self-directed learning similar to our study.[5–7] Taking and giving feedback to and from the students is an essential part of SDL activity that can be applied in lecture-based courses with involvement of minimal teaching faculty[9,10] and is an effective way of performance evaluation of students in such SDL skills as evidenced by activity response rate and feedback response rate in this study.

The SDL assignment was basically planned to encourage the quality of search skills needed to spot present and future gaps in students' knowledge. There are other studies which show a comparison of knowledge acquisition[8], learning evaluation and self-motivation by students using an SDL approach against conventional methods of course delivery.[9,10] Hill M *et al*[10] showed similar results as our study. They noted that SDL activities result in more gains in knowledge in contrast to traditional teaching methods as assessed by greater variation in the responses of students regarding two types of teaching strategies as Conventional Teaching (CT) and SDL.

Metacognition, the ability to think about one's thinking and self-awareness is a vital skill for SDL and is another issue which we identified by students response which showed remarkable improvement as evidenced by our students' answers. In fact, continual self-assessment or reflection is one of the frequently registered policies for teaching metacognitive skills in the health professions.[11] Reflection and feedback develop clinical reasoning skills and provide evidence-based care and is critical in the training of future physicians as we assessed by responses 10–14 in our study. The inability to reflect on one's own clinical practice often results in diagnostic and treatment errors.[12] There are three components of metacognition: planning, monitoring, and evaluating which we noticed in students' feedback. The valuable component of this study was a reflection of their perception because the students were able to identify their own strengths and weaknesses. The reflection upon this study shows us that there is need to include more opportunities for SDL in our preclinical coursework in order to polish their professional skills as stated by Galvin *et al*[14].

The SDL in medical education supports multiple aspects of students' learning like leadership and management skills, personality traits like self-confidence of the learner, the learning environment as it assists in identifying the available resources and adopt relevant strategy, and the metacognitive process and self-efficacy[15], as we observed by improved responses of students in their feedback. Overall, the students' reflective comments provide insight into how this activity affected their SDL abilities beyond what we could observe by simply grading their assignments.[16,17] We should not assume that all enrolled medical students

established, well-developed self-directed learning skills, the students revealed reflections like the ability to recognize one's knowledge gaps, to know where and how to find acceptable sources with the relevant information, and then to synthesize this information and apply it to one's clinical practice are lifelong learning skill that is an observation in our study which is consistent with the literature[18] on SDL.

The primary aim of medical educators is to adopt SDL to produce learners who can manage their own learning in their careers and have a continuous search for knowledge through critical thinking that will improve recall and retention of information to promote better learning and decision making.[19] SDL is the requirement of health professionals to increase motivation, independence, self-confidence in practice, self-discipline and goal orientation due to knowledge explosion, initiative, creativity, love of learning and independence in learning, learning opportunities, acceptance of responsibility to one's own learning and the constantly developing medical information during their careers as shown by responses of the students on Likert scale, and strongly depicted by many studies.[20–22]

Our study indicated that SDL can be enhanced by providing students with updated recent information about the assignment, specific performance goals, grading at the end of task completion, flexible time frame that allows sufficient time for task completion, support for student learning such as personal educators or supervisor, feedback and assessment consistent with other worldwide studies. Mentoring of students by faculty and peers, might improve the learning environment for students.[13,19] The inability of students to hold with the academic workload discourage SDL is indicating that the curriculum needs re-evaluation. Studies[13,16] have shown that curriculum plays a major role in SDL performance.

Our study indicates that students require support for SDL. Students need assistance to improve their self-management skills so as to take control over their own learning, especially in respect to time, resources and learning strategies due to the packed curriculum. Various strategies for SDL can be strengthened so that students can improve on their SDL skills.[21] As similarly stated by a studies[19,20] that students require more case or problem-based studies, clinical orientations, innovative teaching program group discussions and tutorials in regular teaching so as to improve their performance in exams and to make them more self-directed.

In the current situation of continuously emerging advances in teaching environment of medical institutes and field of medicine, SDL and especially self-motivation is crucial to enable medical students to become more independent and develop self-directed learning skills, show increased sense of responsibility, self-confidence and aggressiveness and answerability to one's own actions which are hallmark to a career of medical professionals.[21] It is a primary responsibility of the medical educators to implement SDL with the sole aim of producing medical professionals and lifelong learners who can significantly manage their own personal development and learning in this era of competition.[22,23] Throughout their careers they should have a continuous quest for learning knowledge through imagination and critical thinking that will enhance their decision making in terms of retention and recall of their remote knowledge to promote better application and life time learning.[19,24] Health professionals all the time, need to be self-directed, independent, self-confident in their practice, whether teaching or clinical skills. They should be motivated, self-disciplined and goal oriented due to changing environment, explosion of information and the continuously developing skills in medical fields during their professional lives.[25]

CONCLUSION

SDL assignments can be successfully implemented in pre-clinical courses and are perceived by the students as contributing to their skills for transforming them into effective seven star doctors of future. The limitation of this study is that it was single-point based, conducted at one institution and in 1st Year medical students only. Further activities and studies that enhance their SDL skills throughout their undergraduate teaching program should be evaluated.

REFERENCES

1. Sawatsky AP, Ratelle JT, Bonnes SL, Egginton JS, Beckman TJ. A model of self-directed learning in medicine residency: a qualitative study using grounded theory. BMC Med Educ 2017;17(31).
2. Khiat H. Academic performance and the practice of self-directed learning: the adult student perspective. J Furth High Educ 2017;41(1):44–59.
3. Kidane HH, Roebertsen H, van der Vleuten CPM. Students' perceptions towards self-directed learning in Ethiopian medical schools with new innovative curriculum: a mixed-method study. BMC Med Educ 2020;20(1):7.
4. Bhandari B, Chopra D, Singh K. Self-directed learning: assessment of students' abilities and their perspective. Adv Physiol Educ 2020;44(3):383–6.
5. du Toit-Brits C. A focus on self-directed learning: The role that educator' expectations play in the enhancement of students' self-directedness. South African J Educ 2019;39(2),1–11.
6. Robinson JD, Persky AM. Developing self-directed learners. Am J Pharm Educ 2020;84(3):847512.
7. Kaulback MK. Correlating self-directed learning abilities to lifelong learning orientation in Baccalaureate nursing students. Nurse Educ 2020;45(6):347–51.
8. Tekkol İA, Demirel M. An investigation of self-directed learning skills of undergraduate students. Front Psychol 2018;9:2324.
9. Kerr D, Ratcliff J, Tabb L, Walter R. Undergraduate nursing student perceptions of directed self-guidance in a learning laboratory: an educational strategy to enhance confidence and workplace readiness. Nurse Educ Pract 2020;42:102669.
10. Hill M, Peters M, Salvaggio M, Vinnedge J, Darden A. Implementation and evaluation of a self-directed learning activity

for first-year medical students. Med Educ Online 2020;25(1):1717780.
11. Jeong D, Presseau J, ElChamaa R, Naumann DN, Mascaro C, Luconi F, *et al*. Barriers and facilitators to self-directed learning in continuing professional development for physicians in Canada: a scoping review. Acad Med 2018;93(8):1245–54.
12. Ananthakrishnan N. Competency based undergraduate curriculum for the Indian medical graduate, the new MCI curricular document: positives and areas of concern. J Basic Clin Appl Health Sci 2018;1(1):34–42.
13. Yang C, Zhu Y, Jiang H, Qu B. Influencing factors of self-directed learning abilities of medical students of mainland China: a cross-sectional study. BMJ Open 2021;11(10):e051590.
14. Galvin E, Wiese A, Dahly D, O'Farrell J, Cotter J, Bennett D. Maintenance of professional competence in Ireland: a national survey of doctors' attitudes and experiences. BMJ Open 2020;10(12):e042183.
15. Leatemia LD, Susilo AP, van Berkel H. Self-directed learning readiness of Asian students: Student's perspective on a hybrid problem based learning curriculum. Int J Med Educ 2016;7:385–92.
16. State Council General Office of China. Guidelines on accelerating the innovative development of medical education of the State Council General Office, 2020. Available: http://www.gov.cn/zhengce/content/2020-09/23/content_5546373.htm
17. Uus O, Mettis K, Valjataga T. Self-directed learning: A case-study of school students' scientific knowledge construction outdoors. Cogent Educ 2022;9(1):2074342.
18. De Bruin ABH, Van Merriënboer JJG. Bridging cognitive load and self-regulated learning research: A complementary approach to contemporary issues in educational research. Learn Instr 2017;51:1–9.
19. Du Toit-Brits, C. Towards a transformative and holistic continuing self-directed learning theory. South Afr J High Educ 2018;32(4):51–65.
20. Schweder S, Raufelder D. Positive emotions, learning behavior and teacher support in self-directed learning during adolescence: Do age and gender matter? J Adolesc 2019;73:73–84.
21. Du Toit-Brits, C. Unleashing the power of self-directed learning: criteria for structuring self-directed learning within the learning environments of higher education institutions. Afr Educ Rev 2020;17(2):20–32.
22. Briggs S. 20 Steps towards more self-directed learning. (Online). Available at: www.opencolleges.edu.au/informed/features/29-steps-toward-more-selfdirected-learning/ [Acceded on: 5 Aug 2019].
23. Cadorin L, Bressan V, Palese A. Instruments evaluating the self-directed learning abilities among nursing students and nurses: a systematic review of psychometric properties. BMC Med Educ 2017;17(1):229.
24. Premkumar K, Vinod E, Sathishkumar S, Pulimood AB, Umaefulam V, Prasanna Samuel P, *et al*. Self-directed learning readiness of Indian medical students: a mixed method study. BMC Med Educ 2018;8(1):134.
25. Dost S, Hossain A, Shehab M, Abdelwahed A, Al-Nusair L. Perceptions of medical students towards online teaching during the COVID-19 pandemic: a national cross-sectional survey of 2,721 UK medical students. BMJ Open 2020;10:e042378.

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SYSTEMATIC REVIEW

SILVER NANO-PARTICULATE COATING ON IMPLANTABLE TITANIUM DEVICES: CAN WE TEACH AN OLD DOG A NEW TRICK? —A SYSTEMATIC REVIEW

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Implant materials are one of the most investigated topics in modern medicine. This review is focused only on addressing silver as coating substance on top of titanium based implantable devices, and focused on the last five years of silver coated titanium implants on human cell population. This study was conducted on Medline, PubMed, Science Direct, Scopus and Google Scholar. A time window of the previous five years was selected up to May of 2021. The study was done at HBS Dental College, Islamabad and the Higher Education Commission Library, Islamabad. The multi-location reviewing and gathering of articles assured lack of bias in the article selection. The search for articles was done using prescribed keywords and then the sieving of articles further using systemic review methodology via PRISMA flowchart. There was excessive evidence to suggest that the surface modification with silver is effective to eliminate surface microbes which might interfere in good quality healing of bone around the titanium implant material. Silver has great antimicrobial activity however it does not show simultaneously just as good compatibility. This study points towards evidence of establishing the 'sweet spot' between the most favourable cytotoxic concentrations and most effective antimicrobial concentrations.

Keywords: Silver, Ag, Nanoparticle, titanium, implantable devices, implant, dental, MTT assay

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INTRODUCTION

Although Titanium is a very good material to be used as an implant material in the dental prosthesis in restorative department, it is still understood that titanium itself has poor inherent antimicrobial properties. Therefore, to bridge this gap in Titanium's ability to ward off infections coating is done in a variety of metal oxides in order to give titanium a favourable antimicrobial profile. Use of Zinc, Copper, Cerium and Silver has always been a popular choice for antimicrobial activity.[1] The pleasantness of these compounds is determined by their ability to appear more tooth like or bone like. Zinc and silver in this matter win over the others by having closer tooth like colours such as silver or white and strong antimicrobial potentials.[2–5]

Silver has been used in medicine ever since middle ages and now with nano-technology we're teaching this old dog new tricks. When aimed at making silver an implantable material as a part of our silver coated implants, silver can be coated as an oxide or nitride.[6]

The main advantage of focusing on nano-particle is, the fact that they can provide a very high surface area in case of oxides that are capable of having antimicrobial activity on the contact surfaces.[7] At the same time these materials do not cause a lot of toxicity because the overall quantity is way below the lethal toxic dose.[8] They are used as delivery vehicles to intercellular deposition of growth factors. They behave like biological proteins and can pass through Lingard gates. Helping medicate regions of the body or the cell that are otherwise unapproachable by bigger molecules.[9] The use of silver as an antimicrobial material is as old as time itself, and yet its potential in functionality delivers its way forward with the number of researches published in the year 2021 alone is overwhelming. The number of publications crossing over 35,000 as per Google Scholar, and this review is written only halfway into 2021.

As the use of silver is one of the most popular one in the current biomedical world right now, the aim of this review is to study and quantify the degree of success that is achieved by its quoting on the titanium implants, be it orthodontic implant or prosthodontic implant. This review will highlight trends and upcoming advancements in the world of silver coatings in implantable devices.

MATERIAL AND METHODS

This study was conducted using databases of Medline, PubMed, Science Direct, Scopus, and Google Scholar. A time window of the previous five years was selected, leading up to May 2021, at HBS Dental College, Islamabad and the Higher Education Library, HEC, Islamabad. A multi-location reviewing and gathering of

articles assured the lack of bias in the article selection procedure. Search for articles was done with the prescribed keywords and then the sieving of articles was further done by the systemic review methodology via PRISMA flowchart. The keywords used were Ag, Silver, Nanoparticle, Titanium, Implantable devices, Dental implant, MTT assay. Studies with calcium compound and hydroxyapatite were excluded because they can cause increase cell adhesion in osteogenic cells and fibroblasts and were confounding to the study.

Inclusion criteria:

- *In vitro* studies
- Studies with cell studies
- Studies with cells with human origin
- Studies done after 2016
- Studies on titanium implant
- Studies with rat/mice cell assays
- Studies on commercially pure titanium implants
- Study conducted on titanium foils, sheets or rods
- Studies on titanium aluminium vanadium implants

Exclusion criteria

- Human trials due to lack of histological evidence
- Animal trials
- Studies with hydroxyapatite
- Studies with calcium compounds
- All in vivo studies
- Studies done prior to 2016

RESULTS

Thirteen (13) articles were selected from January 2016 to May 2021. The selection process of these articles was as shown in the PRISMA flowchart below. Calculation of biases was done via ROBINS tool.

The result of the articles selected is summarised in a tabulated form in this review. All studies were done *in vitro* settings and were non-clinical random sequencing and concealment of the test specimen was not required in these studies. The specimens were to hold identical properties and were tested against identical mediums. Personnel blinding or incomplete outcome reporting was also not prevalent. Only three of the following eleven studies were considered to have higher risk of bias because the authors do not initially opt to include the sample preparation methodology in their text. This being pointed out altogether, the risk of bias was very low in the *in vitro* studies.

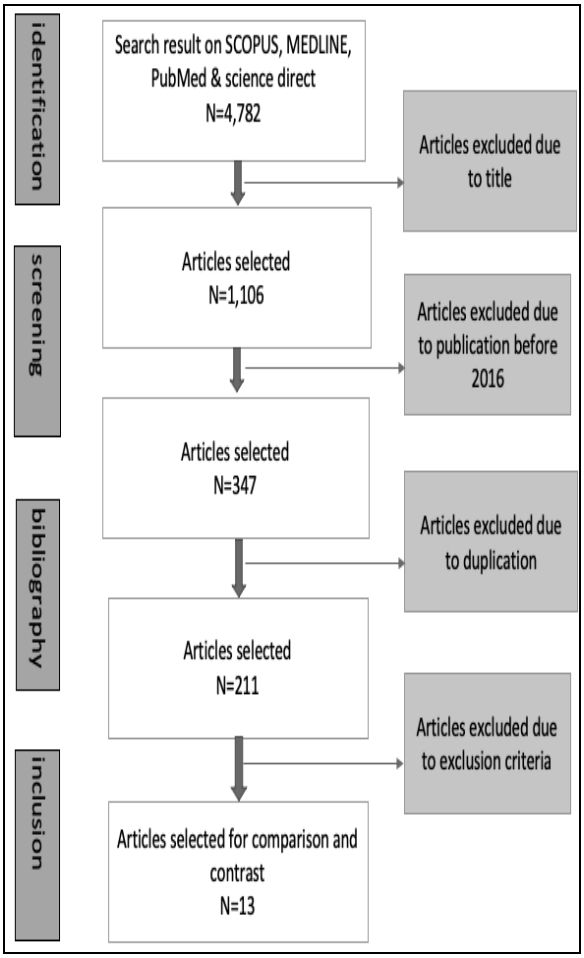

Figure-1: PRISMA flowchart applied to article selection process

Table-1: Evaluation of risk of biases based on ROBINS tool

Study	Random sequencing	Allocation blinding	Personal blinding	Outcome assessment blinding	Incomplete outcome assessment	Selective reporting
10	H	H	U	L	L	L
11	H	H	U	L	L	L
12	H	H	U	L	L	L
13	H	H	U	L	L	L
14	H	H	U	L	H	H
15	H	H	U	L	L	L
16	L	L	U	L	L	L
17	H	H	U	L	L	L
18	L	L	U	L	L	L
19	H	H	U	L	L	L
20	H	H	U	L	L	L
21	H	H	U	L	L	L
22	H	H	U	L	L	L

L=low risk, U=unclear risk, H=high risk

Table-2: Summary of results from selected articles

Study	Substrate	Particle size nm	Cells	Testing	Duration hours	Conclusion
Radtke *et al*[10]	Ti6Al4V	18–115	Human osteoblast-like MG63 cells	MTT assay	24, 72, 120	85% cell viability at 120 hrs
Zhang *et al*[11]	Medically pure Ti	10–40	Rat bone marrow derived mesenchymal cells	CellTiter-Blue® cell viability assay	24, 96, 168	Biocompatibility by suppressing intercellular ROS production
Yang *et al*[12]	Commercially pure Ti	1–10	Mice osteoblast	WST-1 cell proliferation assay kit	24, 72, 120, 168	Normal morphology of cells with full vitality
Kranz *et al*[13]	Ti6Al4V	25	Mice osteoblast	Florescent microscopy	48, 96	High initial biocompatibility
Marques *et al*[14]	Commercially pure Ti	<100	Adult human bone marrow cell	Florescent microscopy	3, 24, 144	Early attachment and proliferation
				MTT assay	3, 24, 144	Increased cell population progressively
Kaczmarek *et al*[15]	Commercially pure Ti	100–150	Human osteoblast	MTT assay	72, 96	Increased cell count
Guan *et al*[16]	Ti foils & rods	1–30	Mice osteoblast	Florescent microscopy	24	Normal cell morphology
Shivaram *et al*[17]	Commercially pure Ti	Unidentified	Human foetal osteoblast cells	MTT assay	96, 168	Increased cell density
Zeng *et al*[18]	Commercially pure Ti	20–30	Mice osteoblast	Cell counting kit-8	72, 120, 168	Proliferation increase by increasing nano particle concentration, new bone formation in proximity to 2% implant
Zhang *et al*[19]	Commercially pure Ti	20–50	Mice osteoblast	Florescent microscopy	12, 24, 120	Good vitality and cell differentiation
				MTT assay	96 168, 240	Favourable vitality and proliferation rate
Ulfah *et al*[20]	Ti6Al4V	134 diameters of nanotubular deposits	Human osteoblast	MTT assay	168, 336	cell viability 107=115% of control
Torres *et al*[21]	Ti6Al4V	52.5–95 diameters of nanotubular deposits	Human foetal osteoblasts	MTT assay	72, 168, 336	140–329% greater vitality than control
Sun *et al*[22]	Ti6Al4V	900	Mice osteoblast	MTT assay	24-168	100% cytocompatibility

DISCUSSION

The oral cavity is known to have a poly-microbial flora, which presents both pathological and probiotic microbial species.[23] Although antimicrobial properties in implants can be produced, but to choose between the perfect cytotoxicity is the real challenge.[24] Quite often, implant associated infections may be induced by Methicillin resistant Staphylococcus aureus.[25]

These infections can cause a great deal of delay, especially in orthodontic implant assisted treatments. This often occurs because children do not tend to their oral health who are prime candidate for orthodontic treatments.[26] In prosthodontic treatment, the implant surfaces are prone to infection because they are often given to individuals who are partially edentulous and are subject to old age or systemic disease.[27]

According to one of the earliest studies[28] conducted in 1998 a 0.3% concentration of silver in either sulfadiazine or nitrate form can lower the amount of Staphylococcus formed on the biofilm surface up to 3,000 times. The incubation period used for these experiments was 16 hours. The study showed that silver shows similar antimicrobial potency as levofloxacin or povidone-iodine.[28] Following years, numerous studies were done in order to evaluate and identify the antimicrobial properties of silver.[29,30] Soon after the idea of having nano-particulate materials that offer a greater surface area to volume ratio came about.[31]

Timorous quoted through a variety of methodologies, either it was sprayed over, electroplated, dip galvanised and quite recently anodised.[20,21,32,33] The anodization methodology, which is recently used has the advantage that it causes formation of nano-tubular structures which leads to an improvement in the hydrophilicity of the implant surface against the bone matter.[34,35] Hydrophilicity in implants is associated with reduction of implant rejection through the body's immune system. This increases its integration strength, reduces osteointegration time.[35,36] The improved intimacy of contact between bone and implant reduces the availability of pocket for infection formation as well.[37]

The studies that are being highlighted in this review are simply selected in order to ascertain if Nanoparticulate silver can be a resolve in choosing between silver's very established antimicrobial activity

versus its ability to be cytotoxic. Using silver in Nanoparticulate solution coming from control release methodology, such as that formed by anodization can lead to a perfect balance of antimicrobial activity, increased hydrophilicity, and increased osseointegration. Even though silver itself can be cytotoxic at certain doses, but the presence of silver nano-particles on titanium has caused increased cell growth with proper cell differentiation. Cell growth has been increased up to 170% in certain studies and over 340% in others.[21]

Interestingly enough, an unexpected output of this systematic review was that the silver nano-particles are more effective in sustaining cell differentiation and growth of cell count in human cells rather than that in mice cell populations.[21] The study shows that a controlled drug release methodology is the best way to reduce the amount of silver in circulation while giving it its maximum antimicrobial properties.[38]

CONCLUSION

According to these *in vitro* studies, it may be suggested that variable techniques of coating silver ions on top of any form of implantable titanium, commercially pure, medical grade titanium, or titanium vanadium aluminium alloy implants may serve as an effective methodology of improving cell integration. Silver ions have improved antimicrobial activity against the osteomyelitis causing organisms. Concentration, rate of release and times exposure are pivotal factor in determining the balance between title toxicity, an antimicrobial effects of silver.

REFERENCES

1. Kazemzadeh-Narbat M, Kindrachuk J, Duan K, Jenssen H, Hancock REW, Wang R. Antimicrobial peptides on calcium phosphate-coated titanium for the prevention of implant-associated infections. Biomaterials 2010;31(36):9519–26.
2. Fredj N, Burleigh TD. Transpassive dissolution of copper and rapid formation of brilliant colored copper oxide films. J Electrochem Soc 2011;158(4):C104.
3. Namba S, Hishiki Y. Color sensitization of zinc oxide with cyanine dyes1. J Phys Chem 1965;69(3):774–9.
4. Kim MI, Park KS, Park HG. Ultrafast colorimetric detection of nucleic acids based on the inhibition of the oxidase activity of cerium oxide nanoparticles. Chem Commun (Camb) 2014;50(67):9577–80.
5. Pasquet J, Chevalier Y, Couval E, Bouvier D, Noizet G, Morlière C, *et al.* Antimicrobial activity of zinc oxide particles on five micro-organisms of the Challenge Tests related to their physicochemical properties. Int J Pharm 2014;460(1–2):92–100.
6. Sim W, Barnard RT, Blaskovich MAT, Ziora ZM. Antimicrobial silver in medicinal and consumer applications: a patent review of the past decade (2007–2017). Antibiotics (Basil) 2018;7(4):93.
7. El-Sayed SM, Amer MA, Meaz TM, Deghiedy NM, El-Shershaby HA. Microstructure optimization of metal oxide nanoparticles and its antimicrobial activity. Measurement 2020;151:107191.
8. Sengul AB, Asmatulu E. Toxicity of metal and metal oxide nanoparticles: a review. Environ Chem Lett 202018(5):1659–83.
9. Sokolov AV, Limareva LV, Iliasov PV, Gribkova OV, Sustretov AS. Methods of encapsulation of biomacromolecules and living cells. Prospects of using metal-organic frameworks. Russ J Org Chem 2021;57(4):491–505.
10. Radtke A, Grodzicka M, Ehlert M, Jędrzejewski T, Wypij M, Golińska P. "To be microbiocidal and not to be cytotoxic at the same time..."–silver nanoparticles and their main role on the surface of titanium alloy implants, J Clin Med 2019;8(3):334.
11. Zhang H, Hatoko M, Yin D, Yang Y, Zeng Y, Komasa S, et al. Antibacterial activity and biocompatibility of nanoporous titanium doped with silver nanoparticles and coated with N-acetyl cysteine. J Hard Tissue Biol 2018;27(4):351–8.
12. Yang Y, Zhang Y, Hu, R, Huang Q, Wu K, Zhang L, *et al.* Antibacterial and cytocompatible AgNPs constructed with the assistance of Mefp-1 for orthopaedic implants, RSC Adv 2017;7(61):38434–43.
13. Kranz S, Guellmar A, Voelpel A, Lesser T, Tonndorf-Martini S, Schmidt J, *et al*. Bactericidal and biocompatible properties of plasma chemical oxidized titanium (TiOB®) with antimicrobial surface functionalization. Materials 2019;12(6):866.
14. Marques I da SV, Alfaro MF, Saito MT, da Cruz NC, Takoudis C, Landers R, *et al.* Biomimetic coatings enhance tribocorrosion behavior and cell responses of commercially pure titanium surfaces. Biointerphases 2016;11(3):031008.
15. Kaczmarek M, Jurczyk K, Koper JK, Paszel-Jaworska A, Romaniuk A, Lipińska N, *et al.* In vitro biocompatibility of anodized titanium with deposited silver nanodendrites, J Mater Sci 2016;51(11):5259–70.
16. Guan M, Chen Y, Wei Y, Song H, Gao C, Cheng H, *et al.* Long-lasting bactericidal activity through selective physical puncture and controlled ions release of polydopamine and silver nanoparticles-loaded TiO2 nanorods in vitro and in vivo. Int J Nanomedicine 2019;14:2903–14.
17. Shivaram A, Bose S, Bandyopadhyay A. Mechanical degradation of TiO2 nanotubes with and without nanoparticulate silver coating, J Mech Behav Biomed Mater 2016;59(1):508–18.
18. Zeng X, Xiong S, Zhuo S, Liu C, Miao J, Liu D, et al. Nanosilver/poly (DL-lactic-co-glycolic acid) on titanium implant surfaces for the enhancement of antibacterial properties and osteoinductivity, Int J Nanomedicine 2019;14:1849–63.
19. Zhang W, Wang S, Ge S, Chen J, Ji P. The relationship between substrate morphology and biological performances of nano-silver-loaded dopamine coatings on titanium surfaces, R Soc Open Sci 2018;5(4):172310.
20. Ulfah IM, Gibran K, Bachtiar BM, Ibadurrohman M, Slamet. Modification of Ti–6Al–4V alloys for dental implant and its capacity to grow human osteoblast ATCC. Biomed Eng Appl Basis Commun 2019;31(6):1950047.
21. Torres-Avila IP, Padilla-Martínez II, Pérez-Hernández N, Bañuelos-Hernández AE, Velázquez JC, Castrejón-Flores JL, *et al*. Surface modification of the Ti-6Al-4V alloy by anodic oxidation and its effect on osteoarticular cell proliferation. Coatings 2020;10(5):491.
22. Sun X, Gong H, Li D, Dong L, Zhao M, Wan R, Gu H. Ag^+ implantation induces mechanical properties, cell adhesion and antibacterial effects of TiN/Ag multilayers in vitro. Nanomedicine 2017;12(18):2257–68.
23. Jamkhande A, Kakade S, Gundawar AP. Probiotics and oral health: A review. South-Asian J Cranio-Maxillofac Dent Surg 2021;1(1):14–20.
24. Chen J, Hu G, Li T, Chen Y, Gao M, Li Q, *et al.* Fusion peptide engineered "statically-versatile" titanium implant simultaneously enhancing anti-infection, vascularization and osseointegration. Biomaterials 2021;264:120446.
25. Zhuang Y, Ren L, Zhang S, Wei X, Yang K, Dai K. Antibacterial effect of a copper-containing titanium alloy against implant-associated infection induced by methicillin-resistant Staphylococcus aureus. Acta Biomater 2021;119:472–84.
26. Sreenivasagan S, Subramanian AK, Rengalakshmi S. Prevalence and Cause of Mini-Implant Failure Encountered by Orthodontic Residents. J Long Term Eff Med Implants 2021;31(4):1–4.
27. Halpern LR, Adams DR. Medically Complex Dental Implant Patients: Controversies About Systemic Disease and Dental Implant Success/Survival. Dent Clin North Am 2021;65(1):1–19.

28. Akiyama H, Yamasaki O, Kanzaki H, Tada J, Arata J. Effects of sucrose and silver on Staphylococcus aureus biofilms. J Antimicrob Chemother 1998;42(5):629–34.
29. Zhang S, Du C, Wang Z, Han X, Zhang K, Liu L. Reduced cytotoxicity of silver ions to mammalian cells at high concentration due to the formation of silver chloride. Toxicol *In Vitro* 2013;27(2):739–44.
30. Li L, Bi Z, Hu Y, Sun L, Song Y, Chen S, *et al*. Silver nanoparticles and silver ions cause inflammatory response through induction of cell necrosis and the release of mitochondria in vivo and in vitro. Cell Biol Toxicol 2021;37(2):177–91.
31. Vidyadharan DM, Meethal BN, Jyothilakshmi VP, Swaminathan S. Highly transparent titania in mixed organic solvent with controlled surface area and porosity. Solar Energ 2021;213:43–52.
32. Ueda M, Yokota T, Honda M, Lim PN, Osaka N, Makita M, *et al*. Regulating size of silver nanoparticles on calcium carbonate via ultrasonic spray for effective antibacterial efficacy and sustained release. Mater Sci Eng C Mater Biol Appl 2021;125:112083.
33. Botelho CM, Fernandes MM, Souza JM, Dias N, Sousa AM, Teixeira JA, *et al*. New textile for personal protective equipment—plasma chitosan/silver nanoparticles nylon fabric. Fibers 2021;9(1):3.
34. Lin Y, Zhang L, Yang Y, Yang M, Hong Q, Chang K, *et al*. Loading gentamicin and Zn^{2+} on TiO_2 nanotubes to improve anticoagulation, endothelial cell growth, and antibacterial activities. Stem Cells Int 2021;2021:9993247.
35. Schuster AJ, de Abreu JLB, Pola NM, Witek L, Coelho PG, Faot F. Histomorphometric analysis of implant osseointegration using hydrophilic implants in diabetic rats. Clin Oral Investig 2021;25(10):5867–78.
36. Alipal J, Mohd Pu'ad NAS, Nayan NHM, Sahari N, Abdullah HZ, Idris MI, *et al*. An updated review on surface functionalisation of titanium and its alloys for implants applications. Mater Today Proc 2021;42:270–82.
37. Ständert V, Borcherding K, Bormann N, Schmidmaier G, Grunwald I, Wildemann B. Antibiotic-loaded amphora-shaped pores on a titanium implant surface enhance osteointegration and prevent infections. Bioact Mater 2021;6(8):2331–45.
38. Ghimire A, Song J. Anti-periprosthetic infection strategies: from implant surface topographical engineering to smart drug-releasing coatings. ACS Appl Mater Interfaces 2021;13(18)20921–37.

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