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CONTENTS

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EDITORIAL

UNITING MINDS AND SHAPING MEDICINE: THE CRUCIAL ROLE OF SCIENTIFIC CONFERENCES FOR PHYSIOLOGISTS

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Scientific conferences are essential to the advancement of physiology, serving as dynamic platforms for knowledge exchange, collaboration, and professional development. Far beyond formal gatherings, they foster interdisciplinary dialogue and expose attendees to cutting-edge research, emerging technologies, and innovative methodologies. Physiology, a foundational discipline bridging molecular biology and clinical practice, benefits immensely from these events, which attract diverse participants across medical and scientific domains. The Pakistan Physiological Society (PPS) exemplifies this impact through its biennial conferences, skill-development workshops, and prestigious awards that empower national researchers. Notable achievements, such as the launch of the Pakistan Journal of Physiology and the formation of the South Asian Association of Physiologists (SAAP), highlight the transformative potential of such gatherings. By nurturing intellectual growth and fostering regional and global collaboration, scientific conferences continue to shape the future of physiology and medicine—whether through groundbreaking discoveries or meaningful conversations among peers

Keywords: Scientific Conferences, Physiology Advancement, Interdisciplinary Collaboration, Professional Development

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Scientific conferences have long been a cornerstone of academic and professional development in the medical sciences. Far beyond mere formalities or travel opportunities, these gatherings serve as dynamic platforms for exchanging ideas, disseminating research, and cultivating collaborations. They are vibrant ecosystems where knowledge is shared, innovations are unveiled, and careers are shaped. For medical professionals—especially physiologists—conferences are not just beneficial; they are essential.[1]

Physiology, the study of how living organisms function, lies at the heart of medicine. It bridges the gap between molecular biology and clinical practice, offering insights into the mechanisms that sustain life.[2] The Nobel Prize in 'Physiology or Medicine', awarded annually by the Karolinska Institute, underscores the discipline's foundational role. Alfred Nobel's will explicitly named the category as 'Physiology or Medicine', reflecting the intrinsic link between the two.[3] A recent example is the 2024 Nobel Prize awarded to Victor Ambros and Gary Ruvkun for their discovery of microRNA and its role in gene regulation. Their work revealed a new layer of genetic control—impacting development, disease, and therapeutic strategies—and exemplifies how physiological research continues to revolutionize medicine.

Far from being an isolated field, physiology serves as the bedrock of numerous medical disciplines. It now encompasses molecular, cellular, organ-level, and whole-body studies, integrating knowledge from diverse domains to understand complex biological systems. Disciplines such as biochemistry, pharmacology, haematology, pathophysiology, psychophysiology, and emerging fields like systems biology and integrative medicine all trace their roots to physiological principles. This interdisciplinary nature makes physiology conferences uniquely diverse, attracting researchers and clinicians from a wide array of specialties. Such breadth fosters cross-disciplinary dialogue and sparks innovation.[2,4]

Scientific conferences offer a multitude of benefits for physiologists at all career stages. Attendees gain exposure to cutting-edge research, novel laboratory equipment, and advanced software tools that enhance research capabilities. Presentations and posters showcase ongoing projects from leading institutions, while recent conferences have spotlighted emerging themes such as wearable technologies, personalized medicine, and adaptive training protocols.[1]

These events also bring together a diverse mix of professionals—junior researchers, seasoned academics, clinicians, and industry leaders—creating fertile ground for mentorship, collaboration, and career advancement. Networking with peers from other institutions can inspire attendees to pursue opportunities at prestigious universities or research centers. Social evenings and gala dinners reveal the human side of science, strengthening bonds and fostering a sense of belonging within the academic community of physiologists.

Since its inception in 1987, the Pakistan Physiological Society (PPS) has consistently organized biennial conferences across various provinces of Pakistan on a rotational basis. These conferences adhere

to international standards, featuring keynote speakers, plenary sessions, original research presentations, and poster exhibitions. Pre-conference skill development workshops—particularly those aimed at faculty enhancement—have become a regular feature, with participation steadily increasing over the years.

To promote excellence, PPS has introduced several prestigious awards, including Best Paper by Young Faculty, Best Student Presenter, Best Student Poster, and Lifetime Achievement Awards. These initiatives have significantly empowered Pakistani physiologists and elevated the national profile of the discipline.

The Pakistan Journal of Physiology was conceptualized during the 9th Biennial Conference and officially launched at the 10th. Perhaps the most notable achievement of PPS conferences was the conceptualization and subsequent establishment of the South Asian Association of Physiologists (SAAP) following the 11th Biennial Conference in 2008. This milestone not only elevated the stature of Pakistani physiologists but also fostered regional collaboration among physiologists from seven South Asian countries.

Scientific conferences are indispensable for physiologists. They nurture intellectual growth, foster interdisciplinary collaboration, and catalyze innovation. As physiology continues to evolve and intersect with other fields, conferences will remain vital arenas for shaping the future of medicine. Whether through a Nobel-worthy discovery or a conversation over coffee, the impact of these gatherings is profound and enduring.

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ORIGINAL ARTICLE

SERUM MALONDIALDEHYDE AS A MARKER OF OXIDATIVE STRESS IN GESTATIONAL DIABETES MELLITUS: A COMPARATIVE STUDY

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Background: Oxidative stress is implicated in causation of many diseases including diabetes mellitus. An abnormally high level of oxidative stress may be involved in development of gestational diabetes mellitus (GDM). We aimed to compare degree of oxidative stress in healthy pregnant, and GDM women by measuring serum malondialdehyde (MDA) levels. **Methods:** This comparative cross-sectional study was conducted on age and gestational age matched 30 healthy pregnant women and 30 patients of GDM at Army Medical College and Pak Emirates Military Hospital, Rawalpindi over a period of one year. The diagnosis of GDM was made during second trimester after oral glucose tolerance test. The subjects with type-1 or type-2 diabetes, past history of gestational diabetes and systemic inflammatory disease were not included. Serum malondialdehyde levels of all subjects were measured with ELISA technique. Data were analysed on SPSS-22. Numerical data were expressed as Mean±SD and the comparison between two groups was done using independent samples *t*-test. The Pearson's correlation coefficient for association between numerical variables was assessed, and $p \leq 0.05$ was regarded statistically significant. **Results:** GDM group had significantly higher mean serum MDA level as compared to healthy pregnant women. Serum MDA had positive correlation with fasting plasma glucose and glycosylated haemoglobin. **Conclusion:** A significantly high MDA in GDM along with positive correlation with fasting glucose and glycosylated haemoglobin indicates the possible role of oxidative stress in GDM.

Keywords: Gestational diabetes mellitus, Hyperglycaemia, Malondialdehyde, Oxidative Stress

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INTRODUCTION

Gestational diabetes mellitus (GDM) is defined as the glucose intolerance that is first identified during second or third trimester of pregnancy and is not overt diabetes.[1] Its prevalence varies greatly in different areas with highest prevalence in South Asian countries.[2] In addition to geographic variance, its prevalence is also affected by criterion used for diagnosis. Highest prevalence of gestational diabetes is observed with International Association for Diabetes and Pregnancy Study Group (IADPSG) criteria of diagnosis and is equal to 34.9%.[3] Gestational diabetes mellitus is linked with adverse pregnancy outcome as well as development of complications in both mother and offspring. It increases the risk of premature labour, pre-eclampsia and premature operative delivery. Moreover, there are high chances of macrosomia, hypoglycaemia and hyperbilirubinemia in foetus in addition to risk of developing diabetes in future.[4] Women with gestational diabetes are also at risk of developing type-2 diabetes and cardiovascular problems.[5]

Pregnancy is associated with development of insulin resistance secondary to secretion of placental hormones. This insulin resistance is compensated by increased beta cell activity. It is suspected that beta cell dysfunction occurs during gestational diabetes due to which they are unable to regulate insulin secretion.[6] A number of factors like autoimmunity, gene mutation, inflammation and oxidative stress play a role in its causation. An abnormal inflammatory response especially occurs in obese women and may be a consequence of oxidative stress.[6] Oxidative stress occurs as a result of imbalance between pro-oxidant and antioxidant species.

The pro-oxidants are reactive oxygen species (ROS) like hydrogen peroxide, hydroxyl radical and superoxide anion. These ROS have damaging effects on various cellular components and can cause destruction of lipids, proteins and DNA, thus affecting their normal physiological functions.[7] During pregnancy, placenta produces a large number of reactive oxygen species. An increased number of lipid peroxidation markers like malondialdehyde, and 8-isoprostane are isolated from placenta of patients of gestational diabetes. An uncontrolled production of reactive oxygen species can lead to exhaustion of antioxidant defence mechanism and may cause cell injury and cell death.[8] The oxidative damage can involve peripheral tissues like pancreatic beta cells which are specifically susceptible to oxidative damage due to lack of antioxidant defence mechanism, thus resulting in increased apoptotic events and suppression of transcription factors involved in regeneration of beta cells. It also abolishes mitochondrial activity and ultimately causes decreased production of insulin. Oxidative stress also reduces the sensitivity of peripheral tissues to insulin by disrupting the process of

insulin signalling via decreasing tyrosine phosphorylation of insulin receptor substrate proteins as well as decreased expression of glucose transporters in muscle and adipose tissue.[9] Malondialdehyde (MDA) is a reactive, toxic aldehyde produced as a result of lipid peroxidation by ROS and is frequently used as a reliable marker of oxidative stress.[10]

Few previous studies show positive association between oxidative stress markers and development of GDM. However, studies elucidating the role of oxidative stress in gestational diabetes are scarce in our set up. To fill this gap, we compared the level of oxidative stress in normal pregnancy and in gestational diabetes by measuring serum levels of MDA. Identifying the association of oxidative stress and gestational diabetes may help us take steps in the direction of prevention of GDM development.

METHODOLOGY

This cross-sectional study was conducted at Army Medical College and Pak Emirates Military Hospital, Rawalpindi. The study was conducted over a period of one year from 1st Aug 2019 to 31 Jul 2020. Formal approval was obtained from ethical review board of the institution. The sample size was estimated using WHO calculator. Considering the estimated prevalence of GDM in Pakistan as 3.5%[11] and 95% confidence level, a sample size of 60 was calculated. Subjects were selected through non-probability convenient sampling after informed consent. The subjects were divided into two groups. Group I consisted of 30 healthy pregnant women at 24 weeks onwards gestation with normal glucose tolerance test (GTT). Group II consisted of 30 pregnant women at 24 weeks onwards gestation with diagnosis of gestational diabetes mellitus. The diagnosis of gestational diabetes was made after performing oral GTT on basis of IADPSG criterion approved by American Diabetes Association (ADA).[2] The women with type-1 or type-2 diabetes, previous history of gestational diabetes and systemic inflammatory disease were excluded from study. The baseline demographic data and relevant history and examination were recorded for all subjects.

Blood samples of subjects were collected after an 8 hour overnight fast. Oral GTT was performed. After determining concentration of haemoglobin and glycosylated haemoglobin (HbA1c), HbA1c/ haemoglobin ratio was expressed as percentage (% HbA1c). Serum malondialdehyde levels of the subjects were determined using Enzyme Linked Immunosorbent Assay (ELISA Kit Cat No. 10798-Glory Bios).

Data were analysed by using IBM SPSS-22. The quantitative variables were expressed as Mean±SD. For assessment of association of quantitative variables, Pearson's correlation was used and $p \leq 0.05$ was taken as significant.

RESULTS

Thirty healthy pregnant women and thirty patients of GDM were matched for age and gestational age. The groups did not show a significant difference regarding body mass index (BMI). The HbA1c levels of both groups were found to have significant differences (p=0.012). Serum MDA levels of GDM group were significantly higher as compared to healthy pregnant women. (Table-1). There was a significant positive correlation of serum MDA with HbA1c and fasting plasma glucose (FPG). (Table-2).

Table-1: FPG, HbA1c, serum MDA and BMI of the subjects

Variable	Control	GDM	*p*
FPG (mmol/L)	4.52±0.47	5.98±1.04	<0.001
HbA1c (%)	5.3±0.59	5.8±1.05	<0.05
MDA (ng/mL)	424.0±278.0	872.64±767.94	<0.01
BMI (Kg/m^2)	27.34±1.56	27.46±1.94	0.9

Table-2: Correlation of serum MDA with HbA1c, fasting plasma glucose

Parameter correlated	*r*	*p*
HbA1c	0.400	<0.001
Fasting plasma glucose	0.449	<0.001

*p is significant at ≤0.05

DISCUSSION

We conducted a study recruiting thirty healthy pregnant women and thirty women with GDM and compared serum MDA levels as marker of oxidative stress during 24–28 weeks of pregnancy. We found a significant increase in serum MDA in GDM group in addition to raised levels of HbA1c, fasting and postprandial plasma glucose levels. Serum MDA levels also correlated positively with HbA1c and fasting plasma glucose level.

Our results are in coherence with previously conducted similar studies. Qin Z *et al*[12] conducted a prospective study in China taking 130 GDM cases and 260 controls. They found a significant elevated mean level of serum MDA in GDM group as compared to healthy adults. They also found a positive correlation of MDA with fasting plasma glucose but a significant correlation was not found with HbA1c. The reason they didn't find a significant correlation with HbA1c may be because they measured HbA1c in second trimester and MDA in first trimester.

A study by Ayse Arsalan[13] also showed similar results. In that study, 40 GDM patients and 37 healthy controls were compared for assessment of degree of oxidative stress and antioxidant mechanisms by measuring serum MDA, glutathione peroxidase and catalase levels at 24–28 weeks of gestation. They observed a high mean serum MDA level along with low glutathione peroxidase level in GDM group.

Zhang C *et al*[14] carried a study on 93 patients of GDM and 82 healthy pregnant women. They found a

significantly increased serum MDA in GDM group compared to control group.

Contrary to above researches, a study[15] conducted on 200 subjects didn't show a significant difference regarding MDA between healthy pregnant women and those with GDM, although it revealed a high serum MDA in non-pregnant diabetic women compared to non-pregnant healthy women. A study[16] conducted on 51 pregnant women revealed no significant differences in serum MDA although it showed a significant difference in salivary MDA. Salivary MDA may be more sensitive than serum MDA to measure oxidative stress level as more free radicals are neutralized by plasma as compared to saliva.[16]

Pregnancy is a state of oxidative stress characterized by increased production of reactive oxygen species (ROS). This is the result of enhanced metabolism, increased consumption of oxygen and fatty acid utilization. ROS cause damage to biomolecules resulting in production of different by-products. One of the damaging effects of ROS is peroxidation of lipid molecules which results in formation of malondialdehyde.[8]

The damaging effects of this oxidative stress is counterbalanced by increased production of antioxidants. Excess production of glucose in GDM results in its auto-oxidation and increased production of reactive oxygen species. Depletion of antioxidant mechanisms in GDM further worsens the condition.[17] A previous study has shown increased oxidative stress shown as elevated levels of MDA and thiobarbituric acid reactive substances (TBARS) in GDM patients and decreased levels of antioxidants such as glutathione peroxidase and superoxide dismutase.[18] Another study did not reveal a significant difference in antioxidant levels in GDM despite increased levels of oxidative stress markers.[19]

Shang M *et al*[20] did a study on 68 pregnant women and assessed the levels of a number of oxidative stress markers as well as antioxidants in maternal, cord and placental blood. They also compared these levels in GDM patients diagnosed with different diagnostic criteria. They found increased levels of oxidative stress indicators like MDA, xanthine oxidase (XO) and 8-isoprostane, and decreased levels of antioxidants such as superoxide dismutase and total antioxidant capacity.

We found a positive correlation between MDA and HbA1c as well as fasting plasma glucose which is comparable to study of Shang *et al*[20]. This supports the hypothesis that hyperglycaemia and poor glycaemic control are associated with higher degree of oxidative stress.[21] Obesity as well as increased age results in increased oxidative stress.[22] Level of oxidative stress varies with the gestational age and is usually at peak in second trimester. In our study, GDM subjects were matched with controls regarding age, gestational age and BMI. The results of our study suggest the role of oxidative stress in hyperglycaemia and development of GDM. Measurement of oxidative stress markers like serum MDA may be helpful in early identification of gestational diabetes risk.

The limitations of our study include small sample size and one time measurement of biochemical markers. Moreover, it is a cross-sectional study measuring the oxidative stress markers at the time of diagnosis of GDM. Future prospective studies involving a variety of oxidative stress markers along with antioxidants are recommended to establish the link between oxidative stress and development of GDM.

CONCLUSION

A significantly high serum MDA level in GDM patients and a positive correlation between serum MDA and maternal glycaemic levels suggest role of oxidative stress in gestational diabetes. Identification of oxidative stress early in pregnancy may be helpful in better management of gestational diabetes.

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MK: Final draft and critical review

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ORIGINAL ARTICLE

COMBINED IMMUNE DEFICIENCY AND RENAL PHENOTYPE ASSOCIATED WITH *PLCE1* GENE VARIANT

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Background: Congenital Nephrotic Syndrome (CNS) is a paediatric kidney disease that is defined by massive protein loss in the urine, hypoalbuminemia, and hyperlipidemia. Mutations in *PLCE1* are associated with autosomal recessive form of nephrotic syndrome associated with elevated T-cells. **Methods:** A two-month-old female patient from a Pakistani family suffering from recurrent renal infections with fever and cough was investigated in this study. Laboratory tests including renal function test, lipid profile, lymphocyte subset analysis using flow cytometry, serum immunoglobulin level and blood complete picture were performed. After detailed clinical evaluation, whole blood samples were collected in EDTA tubes for genetic analysis. **Results:** Complete blood count (CBC) showed low haemoglobin levels and lymphocytosis. Flow cytometery revealed elevated CD4/CD8 T-cells. Low serum immunoglobulin levels were observed. Genetic analysis revealed a missense mutation [c.6790A>G; (p.Lys2264Glu)] in the gene *PLCE1*. **Conclusion:** The current study describes a novel homozygous genetic mutation in *PLCE1* gene. Clinical investigations revealed disease features partially fulfilling the criteria of inherited nephrotic syndrome.

Keywords: CRP, DNA Sequencing, Flow cytometery, Nephrotic Syndrome, *PLCE1* gene

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INTRODUCTION

Inherited Nephrotic Syndrome (INS) (OMIM 256300) is a rare paediatric disorder affecting glomerular function, characterized by massive proteinuria, hypoalbuminemia, and oedema that have impaired glomerular function.[1–3] Among children, the idiopathic type of nephrotic syndrome is more common, and most cases respond to corticosteroids; hence, it is termed steroid-sensitive nephrotic syndrome (SSNS).[4] Approximately 15–20% of these patients are steroid-resistant, referred to as steroid-resistant nephrotic syndrome (SRNS).[5,6] Among SRNS patients, 50% of the cases worsen in renal function and eventually develop end-stage renal failure (ESRF) within 15 years of diagnosis.[7]

Mutations in genes encoding podocyte-associated proteins are pivotal in certain forms of nephrotic syndrome, particularly the SRNS. Genetic mutations approximately 30% of SRNS patients are significant contributors to the disease pathogenesis.[7] Podocyte-related genes: Mutations in genes like nephrotic syndrome, type 1 (*NPHS1),* nephrotic syndrome, type 2 (*NPHS2)*, Wilms tumour gene 1 (*WT1)*, laminin subunit beta-2 (*LAMB2)*, and phospholipase C epsilon 1 (*PLCE1)* are commonly implicated. These genetic anomalies compromise the structure and functions of podocytes and immunological mechanisms. [8]

Studies have identified immune dysregulation in idiopathic nephrotic syndrome patients, including altered T-cell function and cytokine profiles.[9] The onset and progression of the nephrotic syndrome are linked to immune system dysregulation such as T-cell response, altered B-cell activity, and cytokine imbalance. This interplay between genetic predisposition and immune-mediated injury underpins the complexity of nephrotic syndrome, necessitating comprehensive diagnostic and therapeutic approaches. The convergence of podocyte gene mutations and immune system dysfunction highlights the multifactorial nature of this condition.[10]

The aetiology of childhood NS remains unclear; however, knowledge of disease pathogenesis continues to expand. Next Generation Sequencing (NGS) has yielded pathogenic variants in >80 genes that are enriched in the podocyte and have been associated with SRNS in 10–30% of cases.[11,12] Over the past decade, numerous genes associated with immune system have been discovered to play pivotal roles in the development of nephrotic syndrome. For example, *NPHS1* (Nephrin) and *NPHS2* (Podocin), primarily known as structural podocyte proteins, also mediate immune modulation within podocytes. *PLCE1* (Phospholipase C epsilon 1) contributes to podocyte differentiation and intracellular signalling pathways influencing cell growth and development. *CD2AP* (CD2-associated protein) is involved in T-cell activation and maintaining podocyte architecture, while *TNFRSF13B* (TACI) plays a regulatory role in B-cell function and humoral immunity.[13]

This study presents genetic analysis in a suspected nephrotic syndrome patient and reveals missense variant [c.6790A>G; (p.Lys2264Glu)] in *PLCE1* gene.

METHODOLOGY

The project was presented and received formal approval from ethical research board of the HBS Medical College, Islamabad. Written informed consent was obtained from all male and female participants before the start of the study. The mother of the patient voluntarily provided related clinical and family histories and consented to genetic analysis, permitted to publish clinical and family histories, and genetic findings. The study adhered to the principles of the Declaration of Helsinki and relevant national and institutional regulations regarding human genetic research.

A-2-months old female patient from a highly consanguine family was enrolled in this study. She was brought to emergency with complaints of fever, cough and a single episode of fit and, was admitted to the Department of Paediatrics, Aga Khan University Hospital, Karachi, Pakistan. Detailed clinical and family history was acquired form the mother of the patient. Complete blood count (CBC) was performed, and serum immunoglobulins were estimated. According to mother in two months this was second time she suffered from fever and acute cough. According to mother, family had a history of two early deaths of a male and a female child with similar clinical manifestations. To rule out the involvement of underlining aetiology related to immune system physician also ordered flow cytometry to evaluate lymphocyte subset levels. Complete blood count, Renal Function Test, Erythrocyte Sedimentation Rate, serum immunoglobulin levels, and using flow cytometry, lymphocyte subset analysis were performed in the Armed Forces Institute of Pathology, Rawalpindi, Pakistan.

Five mL whole blood samples from the patient and the parents were collected in EDTA tubes. Whole-exome sequencing (WES) was performed on genomic DNA using the SureSelect Human All Exon kit (Agilent) and sequenced on the Illumina HiSeq 2500 platform (Macrogen, Seoul, South Korea), generating 100 bp paired-end reads. Libraries were prepared with the Illumina Paired-End Sample Prep Kit VI. Reads were aligned to the GRCh38 reference genome using BWA-MEM algorithm.

RESULTS

A two-months-old female child weighing 5 Kg was enrolled from Attock City, Punjab, Pakistan. Family history revealed that two males (III-2 and III-3) and one female (III-4) patients had died (Figure-1A). The onset of the disease was at age 2 months to 1.5 years in all sufferers. At the time of admission our indexed patient had fever of 102 °F and acute cough.

Blood CBC revealed low haemoglobin levels (8.1 g/dL) and neutrophilia (42.9%). Low serum Immunoglobulin's were observed (IgA<0.15 g/L, IgM, 0.30 g/L and, IgG, 2.26 g/L). Details of Immunoglobulin with reference ranges are shown in Table-1. C-reactive proteins and renal function tests values were within the reference range.

Flow cytometery subset mediated lymphocyte subset analysis revealed increased absolute counts of CD3+ total T-Lymphocytes and CD19 total B-lymphocytes. CD56+ Natural Killer (NK) cells were moderately low. Both subsets of T-lymphocytes (CD4+ and CD8+) were elevated. The report suggested a case of Chronic Granulomatous Disease (CGD). (Table-2).

Genetic analysis revealed multiple homozygous and heterozygous variants. Two interesting variants were identified in *VISTA* and *PLCE1*. The interesting variants related to CGD were checked for their segregation in the family. A missense variant [c.6790A>G; (p.Lys2264Glu)] was identified in *PLCE1* gene (phospholipase C epsilon 1). The mutation was homozygous in the patient while heterozygous in the parents and one of the healthy siblings confirmed its segregation with the disease in the family (Figure-1).

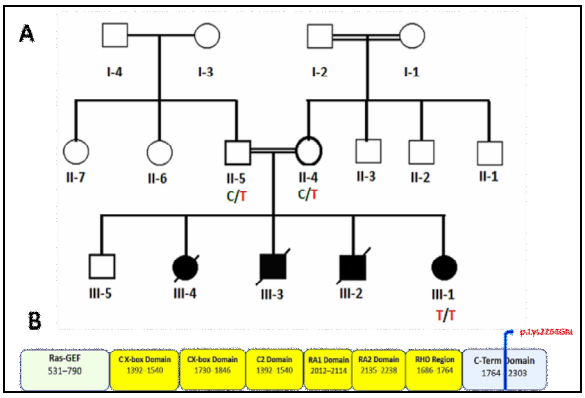

Figure-1: Family pedigree and protein structure

A. Squares represent males while circles represent females. Filled circle and squares represent patients. Double lines indicate consanguine marriages. Diagonal lines on circles and squares indicate deceased individual. 'C' below squares and circles (wild type) is abbreviation of cytosine, and mutated 'T' indicates thymine. The indexed female patient III-1 is homozygous for the mutated Thiamine. **B.** Protein structure of Phospholipase C epsilon 1 shows N-terminal RAs-GEF domain, CX-Box domain, CX domain, C2 domain, RA1 domain, RA2 domain, RHO domain, and a C-terminal domain along with their respective amino acids. Arrow head in C-terminal domain indicates point of mutation.

Table-1: CBC and serum immunoglobulin levels

Test	Results	Reference Range
Haemoglobin	8.1 g/dL	9.4–13 g/dL
Haematocrit	25%	28.0–42.0%
Red Blood Cells (RBC)	2.50×10^{12}/L	$3.1–4.3\times10^{12}$/L
White Blood Cells	20.5×10^{9}/L	$5–15\times10^{9}$/L
Lymphocytes	45.0%	67–80%
Eosinophils	0.4%	2–6%
Platelet Count	576×10^{9}/L	210–650
Serum Immunoglobulin Levels		
Immunoglobulin A (IgA)	<0.15 g/L	0.4–3.5 g/L
Immunoglobulin M (IgM)	0.30 g/L	0.5–3.0 g/L
Immunoglobulin G (IgG)	2.26 g/L	6.5–16 g/L

Table-2: Lymphocyte subset analysis

Test	Result	Reference Range
CD3+total T-Lymphocyte	4019	2800–3500
CD4+T-helper Lymphocytes	3535	800–1100
CD19+Total B-lymphocytes	2196	1000–1700

DISCUSSION

The *PLCE1* (OMIM, 608414) gene is localized on chromosome 10q23.33. It encodes the enzyme (Phospholipase C epsilon 1) a 230 kDa protein. The enzyme plays a crucial role in intracellular signalling by hydrolyzing phosphatidylinositol-4,5-bisphosphate (PIP_2) into two critical second messengers: inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG).[14,15] It is a multi-domain protein comprising of 6 important domains including a Ras Guanine Nucleotide Exchange Factor (Ras-GEF) domain, a Phosphoinositide Phospholipase CX-box domain, a Phosphoinositide Phospholipase C Y-box domain, a C2 domain, a C-terminus Ras-binding (RA) domains, and an intracellular RHO activation region.[16]

The protein *PLCE1* plays a crucial role signal transduction processes by hydrolyzing PIP2 intracellular signalling which lead various cellular responses, including cell proliferation and differentiation specially in nephrons. Through Ras Activation it activates MAP kinase pathway.[17,18] Patients with mutated *PLCE1* gene may suffer from Nephrotic Syndrome Type 3, cancers or high level of circulatory T-cells.[11,18–20]

We identified a missense mutation [c.6790A>G; (p.Lys2264Glu)] in RA2 (Ras-Associating 2) domain in C-terminal regulatory region of the protein. The C-terminal region is less characterized compared to its well-defined other protein domains. It may play a role in maintaining the structural integrity of the protein.[21,22]

Though we did not have any findings related to nephrotic syndrome, we observed elevated levels of circulatory T-cell (CD4+/CD8+) and hypoimmuno-globulenemia which is well documented in earlier studies.[17–23] As for as mutated *PLCE1* mediated nephrotic syndrome is concerned it is hypothesized that onset of disease may occur at a later age.

CONCLUSION

This study highlights a novel missense mutation [c.6790A>G; (p.Lys2264Glu)] in the C-terminal, Ras-Associating (RA2) domain of the *PLCE1* gene, a region not fully characterized yet. The immunological findings, including elevated circulatory T-cell populations and hypoimmunoglobulinemia, correlate with previously reported immune dysregulation linked to *PLCE1* mutations. It can be hypothesized that similar renal manifestations may emerge in our patient at a later stage of the disease. *PLCE1* variants may contribute to immune phenotypes independent of, or preceding, renal involvement. Further functional studies are warranted to clarify the role of the RA2 domain in T-cell regulation and to explore the potential age-related onset of nephrotic features in such cases.

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ORIGINAL ARTICLE

FREQUENCY AND PATTERN OF HYPOGLYCAEMIA IN LOW BIRTH WEIGHT BABIES DURING FIRST 48 HOURS OF LIFE

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Background: Hypoglycaemia is one of the most frequent metabolic problems in early neonatal period and is associated with an increased risk of morbidity and mortality. This study aims to determine the pattern and frequency of hypoglycaemia in low birth weight (LBW) babies during first 48 hours after birth. **Methods:** This cross-sectional study was conducted at Neonatology Unit, Ayub Teaching Hospital Abbottabad from 1st Feb to 30th Sep 2024. Babies with birth weight <2.5 Kg, preterm and term were included in the study. Blood sugar was checked and a value of <47 mg/dL (2.6 mmol/L) was labelled as hypoglycaemia. **Results:** There were 184 neonates in the study. Sixty-eight (36.9%) had at least one low glucose reading. A large group (55, 80.9%) of patients were asymptomatic compared to 13 (19.1%) symptomatic patients. Hypoglycaemia was more common in females (51, 75%) compared to males (17, 25%) neonates. Hypoglycaemia was seen in 56 (82.4%) preterm and 12 (17.6%) term neonates. Frequency of hypoglycaemia was highest (34, 18.4%) in 1–2 hours after birth followed by 6 hours after birth (17, 9.2%). **Conclusion:** Hypoglycaemia is a common finding in low birth weight babies being asymptomatic in majority, and warrants early glucose monitoring and management for better outcome.

Keywords: Hypoglycaemia, Low birth weight babies, Neonates, Preterm

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INTRODUCTION

Hypoglycaemia is one of the most common conditions faced by low birth weight (LBW) babies in first few hours of life and can be symptomatic or asymptomatic. Different studies use different values of blood glucose to define hypoglycaemia in neonates, some define hypoglycaemia as blood glucose measurement of <2.22 mmol/L (40 mg/dL) within 24 hours and <2.78 mmol/L (50 mg/dL) thereafter.[1] Symptoms of hypoglycaemia in neonates include abnormal cry, pallor jitteriness, tremulous, hypotonia, reluctant to feed, hyper-excitability and tachycardia.[2] It is due to sudden metabolic transition from foetal state to an independent extrauterine state that requires a coordinated and integrated change of hormones and enzymes. Risk of hypoglycaemia is higher in certain groups. The important contributing factors include prematurity, small for gestational age (SGA), large for gestational age (LGA), and maternal diabetes mellitus.[3] Premature or LBW babies do not have mature glycogenolytic and gluconeogenic mechanisms due to which they are more prone to hypoglycaemia.[1]

Maintenance of blood glucose level depends on glycogen stores, maturation of glycogenolytic and gluconeogenic pathways. In the term-babies this dynamic process is self-limiting because they have ability to maintain normal blood glucose even in fasting state by breakdown of endogenous glycogen in the liver and kidney, hepatic synthesis of glucose by gluconeogenesis and production of ketone bodies from fatty acid oxidation as alternative fuel for brain.[4] Low birth weight babies or premature babies may have abnormally low blood glucose because of inability to adapt the above mechanisms of blood glucose level maintenance.[4] Globally the frequency of hypoglycaemia in LBW neonates is reported to be 32.5%.[5] The hypoglycaemia was documented in 131 (37.4%) preterm babies in a study[6] conducted in Multan.

Glucose is an essential nutrient for the brain. Abnormally low blood glucose levels (hypoglycaemia) lead to adverse effects on nervous system and produce long term neurological problems. The level that causes the nervous system effect is variable and different studies consider different levels. Plasma glucose concentrations below 2–6 mmol/L were associated with reductions in Bayley motor and mental development scores at 18 months, even after adjustment for confounding factors known to influence development. The increased incidence of LBW because of increased incidence of prematurity which makes such studies very important for recommendation of causes, prevention and treatment within 48 hours of life to reduce neonatal mortality and morbidity.[3,7,8]

As incidence of low birth weight babies born in developing countries is high and increasing because of various risk factors like poor nutrition and improper antenatal check up and most of the deliveries are at home in unhygienic conditions.[9] This study was designed to gain an insight into the frequency of neonatal hypoglycaemia so that early recognition and timely management strategies are instituted for better outcomes and reduction in risk of neurological sequelae.

MATERIAL AND METHODS

This cross-sectional study was conducted at Neonatology Unit, Ayub Teaching Hospital, Abbottabad from 1st Feb to 30th Sep 2024 after approval

from Ethical Committee of the Hospital. WHO sample size calculator was used to calculate the sample size of 184 cases, with a 7% margin of error, 95% confidence level taking the expected incidence of hypoglycaemia as 37.4%.[6] Sampling technique was non-probability consecutive sampling. All term and preterm neonates with birth weight <2.5 Kg who presented in Nursery within first 48 hours of life were included in the study. Low birth weight babies born to diabetic mothers, and those with congenital anomalies were excluded from the study. Low birth weight babies with sepsis and hypoxic ischemic encephalopathy were also excluded from the study.

Demographic data were recorded on a specifically designed proforma. Complete physical examination including the anthropometry was done at the time of enrolment. The gestational age of each newborn was calculated by using obstetric notes and a new Ballard score. Blood was obtained via heel prick and serial monitoring of blood glucose was done at birth, 1–2 hours, 6 hours, 12–24 hours and 24–48 after birth on AccuCheck® glucometer, and value below 47 mg/dL (2.6 mmol/L) was considered hypoglycaemia. Babies who during observation developed poor feeding, jitteriness, irritability, lethargy, tremors, weak cry, apnoea, fits, vomiting, tachypnoea, and sweating were considered to have clinical signs of hypoglycaemia and were put on I/V fluids.

Data were entered and analysed on SPSS-22. For quantitative variables Mean±SD were calculated. Frequencies and percentages were measured for qualitative variables. Independent *t*-test was used for quantitative variables, Chi-square for qualitative variables, and $p \leq 0.05$ was considered significant.

RESULTS

There were 184 LBW babies in our study, 118 (64.1%) were preterm and 66 (35.9%) were term babies. A total of 83 (45.11%) patients were male and 101 (54.9%) were female. Sixty-eight (36.9%) patients were hypoglycaemic. Among the 68 hypoglycaemic LBW babies, 55 (80.9%) babies were asymptomatic and 13 (19.1%) were symptomatic. Hypoglycaemia was found in 82.4% of preterm and 17.6% of term neonates. Out of 68, hypoglycaemia was present in 10 neonates weighing <1 Kg, 22 neonates weighed 1–1.5 Kg, and 36 neonates weighed between 1.5 to <2.5 Kg. Hypoglycaemia was noted in 17 (25%) male neonates and 51 (75%) female neonates. The mean birth weight and gestational age was 1.74±0.25 Kg and 35.83±4.67 weeks respectively. (Table-1).

The mean blood glucose levels in study population were 3.12±0.41 mmol/L at birth (cord), 2.75±0.59 mmol/L at 1–2 hours, 2.81±0.45 mmol/L at 6 hours, 2.95±0.23 mmol/L at 12–24 hours and 3.13±0.53 mmol/L at 24–48 hours after birth. (Table-2).

There were statistically significant differences in blood glucose levels of LBW babies with variation of birth weight at 1–2 hours (p=0.03) and 6 hours (p=0.04) (Table-3).

Out of 68 infants, 8 cases had hypoglycaemia at birth (3 had blood glucose <1.94 mmol/L and 5 had blood glucose between 1.94–<2.6 mmol/L), 34 cases had hypoglycaemia at 1–2 hours (14 had blood glucose <1.94 mmol/L and 20 had blood glucose between 1.94–<2.6 mmol/L), 17 cases had hypoglycaemia at 6 hours (7 had blood glucose <1.94 mmol/L and 10 had blood glucose between 1.94–<2.6 mmol/L), 6 cases had hypoglycaemia at 12–24 hours (2 had blood glucose <1.94 mmol/L and 4 had blood glucose between 1.94–<2.6 mmol/L), and 4 cases had hypoglycaemia at 24–48 hours (1 had blood glucose <1.94 mmol/L and 3 had blood glucose between 1.94–<2.6 mmol/L). (Table-4).

Table-1: Patients' characteristics

Variables		Frequency	Percentage
Gender of total babies	Male	83	45.1
	Female	101	54.9
Presence of hypoglycaemia	Yes	68	36.9
	No	116	63.0
Type of hypoglycaemia (n=68)	Asymptomatic	55	80.9
	Symptomatic	13	19.1
Gestation of hypoglycaemic babies	Preterm	56	82.4
	Term (SGA)	12	17.6
Gender of hypoglycaemic babies	Male	17	25.00
	Female	51	75.00
Birth weight of hypoglycaemic babies	Up to 1 Kg	10	14.7
	>1–1.5 Kg	22	32.4
	>1.5–<2.5 Kg	36	52.9

Table-2: Blood glucose levels in study population at different sampling time (Mean±SD)

Age in hours	Blood glucose level (mmol/L)
At birth	3.12±0.41
1–2 hours	2.75±0.59
6 hours	2.81±0.45
12–24 hours	2. 95±0.23
24–48 hours	3.13±0.52

Table-3: Blood glucose (mmol/L) distribution according to birth weight (Mean±SD)

Sampling Time	Birth weight (Kg)		*p*
	<1.5	>1.5–<2.5	
At birth	4.07±0.21	3.83±0.28	0.42
1–2 hours	2.14±0.36	2.32±0.31	0.03*
6 hours	2.48±0.42	3.08±0.57	0.04*
12–24 hours	2.88±0.53	3.24±0.40	0.08
24–48 hours	3.38±0.41	3.93±0.63	0.14

Table-4: Frequency of hypoglycaemia at different sampling times [n (%)]

Sampling time	Blood glucose (mmol/L)		
	<1.94	1.94–<2.6	≥2.6
At birth	3 (1.6)	5 (2.7)	176 (95.6)
1–2 hours	14 (7.6)	20 (10.8)	150 (81.5)
6 hours	7 (3.8)	10 (5.4)	167 (90.7)
12–24 hours	2 (1.1)	4 (2.2)	178 (96.7)
24–48 hours	1 (0.5)	3 (1.6)	180 (97.8)

DISCUSSION

Neonates mount adaptive responses including mobilization of glucose and fatty acids from glycogen and triglyceride depots to meet the energy demands because nutrient supply from mother are discontinued after birth. These responses occur smoothly in term neonates but preterm and small for gestational age neonates tend to have low blood glucose because of low glycogen and fat levels, relatively high blood insulin levels and the fledgling gluconeogenic pathway, so they fail to mount an appropriate and adequate counter regulatory metabolic and endocrine response.[4,10] About one third of the patients in our study were hypoglycaemic. These finding are consistent with Hayat *et al*[6] who used cut-off value for hypoglycaemia as 2.22 mmol/L (40 mg/dL) in contrast to ours 2.6 mmol/L (47 mg/dL). In another study, 71.4% were found to be hypoglycaemic.[3] Khairuzzaman *et al*[4] reported 31.8% while George *et al*[5] reported 32.5%. Wang LY *et al*[11], reported 19.4% and Hosagasi NH *et al*[12], reported 17.8%. This variability of hypoglycaemia frequency may well be due to different definitions of hypoglycaemia, definitions used for classification of LBW babies, different policies of feeding and different degrees of neonatal sickness and the timing of testing of blood sugar levels done in these studies. Female were more affected than male in our study although the difference was not statistically significant. Similar results have been reported from Taiwan[13] (79 females *vs* 69 males).

The risk of hypoglycaemia is increased in premature, small for gestational age newborns. In our study, hypoglycaemia was more common in preterm compared to term babies. Zhou W *et al*[14] also reported more preterm babies as hypoglycaemic than term babies (55.6% *vs* 14.9%). Among the hypoglycaemic LBW babies, majority of babies was asymptomatic which is consistent with Khairuzzaman *et al*[4].

The mean blood glucose levels were significantly lower at 1–2 hours after birth and gradually increased to reach normal levels with increasing age. These finding are consistent with the findings of Yunarto *et al*[15]. Similar results were reported by Siddique *et al*[16], where hypoglycaemia was predominantly documented in first 24 hours and more commonly found in small for gestational age babies with no statistically significant differences.

CONCLUSION

Hypoglycaemia is well known complication of low birth weight babies and is asymptomatic in large majority. It warrants early glucose monitoring and use of pre-emptive measures for better outcome.

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ORIGINAL ARTICLE

DEPRESSION AND PLASMA OXYTOCIN LEVELS IN MOTHERS HAVING CEREBRAL PALSY CHILDREN

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Background: Children suffering from cerebral palsy (CP) experience many medical, social and psychological problems. Their family members, undertake a lot of difficulties emotionally, physically, socially, and economically. The study aimed to estimate the severity of depression in mothers looking after children suffering from severe CP and compared their depression scores with the scores of mothers having healthy children. Depression scores were correlated with the plasma oxytocin levels of the mothers. **Methods:** This cross-sectional comparative study included 21 mothers having children suffering from severe CP and 21 controls. All mothers were below 40 years of age. Depression was estimated using the Beck Depression Inventory (BDI). Plasma oxytocin was measured using Enzyme-Linked Immunosorbent Assay (ELISA). **Results:** A statistically significant difference was observed between the mean scores of BDI II between the two groups (p=0.011). By applying the Spearman correlation test, depression scores showed a negative significant correlation with the plasma oxytocin levels. **Conclusion:** Mothers having CP children are more prone to depression than mothers having healthy children. The significant negative correlation of plasma oxytocin levels with depression scores suggests its predictive role in depression and its use in adjunct therapy in treatment plans.

Keywords: BDI II, Cerebral Palsy, Depression, Oxytocin

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INTRODUCTION

Depression, the common mental illness, affects more than 322 million people around the globe.[1] During 2005 to 2015, the number of people living with depression increased by 18.4% making it the second leading global burden of disease.[1,2] Mothers having cerebral palsy (CP) children are prone to depression. CP is a non-communicable, commonest, disability of childhood caused by the brain hypoxia, has a global prevalence of 1.4–4 per 1,000 live births.[3] In Pakistan, District Swabi, KPK has a prevalence of CP at 1.22/1,000 live births.[4] Out of 160 cases in Faisalabad, presenting with abnormalities of posture, tone, and movement, 75% were suffering from CP.[5] There are increased chances of CP in cases with preterm delivery, intrauterine infection, congenital malformations or anomalies, birth defects, foetal growth restriction, multiple pregnancies, placental abnormalities, prolonged labour, and instrumental delivery.[6] The economic burden of children with CP exceeds far beyond the needs of healthy children adding to the physical and mental stress to their parents.[7] Mothers of these children are even under greater stress. Multiple studies were conducted to assess depression and effects of CP on the quality of life (QoL) of the mothers having children suffering from CP.[8,9] Higher depression and stress scores have been observed in mothers compared to fathers or parents of healthy controls.[3]

Oxytocin is a hormone secreted from hypothalamus and released from the posterior pituitary gland, and is strongly associated with social behaviour, affiliation, and stress.[10] It has a basal plasma level of 200–359 pg/mL in non-pregnant, non-lactating women.[11] Depressed women have lower plasma oxytocin levels compared to healthy women.[12] Multiple trials have shown a positive role of intranasal oxytocin as an adjunct treatment in depression.[13]

The present study aimed to investigate the effects of having CP children on maternal depression, and plasma oxytocin levels, and to compare it with healthy controls. It highlights the need of psychiatric support to improve the quality of life of the child, parents, and the family.

MATERIAL AND METHODS

This cross-sectional comparative study was conducted from Feb to Dec 2020. The Ethical Review Board of University of Health Sciences Lahore reviewed and approved the study (UHS/REG-19/ERC/1863), and the Institutional Review Board of Children Hospital/ Institute of Child Health Lahore also approved the study.

The study was conducted in Lahore, Pakistan. A total of 42 mothers aged up to 40 years were recruited from the Children Hospital, Lahore. Mothers were further divided into two groups of 21 each. Group I: mothers with age <40 years, having a child suffering from severe CP. Group II: mothers with healthy children. Gross Motor Functional Classification System (GMFCS) was used as a diagnostic tool and level V was taken as severe CP in children.[14] Sample size was calculated with WHO calculator, estimates were derived

based upon a previous study[15]. All pregnant or lactating mothers, mothers using steroid therapy, oral contraceptive pills or medication for depression were excluded. Women with any chronic medical condition were also excluded. The purposive sampling technique was used.

Well recognized self-reported measures with acceptable psychometric properties were utilized after consultation with the consultant psychiatrist. Depressive symptoms were assessed using BDI-II.[16,17] Demographic profile of subjects, past medical or drug history, and family history was collected on specially designed datasheets. History about antenatal visits, duration of pregnancy, mode of delivery, the delayed cry of the newborn, infections in neonate and family history of CP was also taken to rule out the cause of CP.

Serum bilirubin, total protein, alanine transaminase (ALT) and creatinine were measured on MicroLab-300 (Merck, New York). Modification of Diet in Renal Disease study equation (MDRD) was used to estimate the Glomerular Filtration Rate (GFR).[18,19] Random blood sugar (BSR) was measured on AccuCheck® glucometer. These investigations were carried out only to assess the liver and kidney functions to exclude any chronic disease.

Plasma oxytocin level was measured with ELISA. Venous blood samples were collected between 8 AM to 11 AM to avoid fluctuations due to circadian rhythm of the hormone.[20] Estimation of oxytocin was performed within 10 days of sampling on unextracted plasma sample with the commercially available ELISA kit (Elabscience Biotechnology Inc. USA).

Data were entered into Microsoft Excel and analysed using SPSS-25. Shapiro-Wilk test was applied to check the normality of data. Descriptive statistics were used to analyse the demographic data and other parameters. Mean±SD was given for the quantitative variables. Frequencies and percentages were given for qualitative variables. Student's *t*-test was applied to compare BSR between groups. Mann-Whitney test was applied to compare BMI, BDI scores and plasma oxytocin levels between the groups, Spearman correlation test was applied to find any association of the oxytocin levels with BDI scores, and $p \leq 0.05$ was taken as statistically significant.

RESULTS

This study included 42 women, 21 in each groups. No significant differences existed between the ages of mothers in the groups (p=0.103). All participants belonged to middle socioeconomic class. Among cases, only 2 children reported CP in their first-degree relatives. Twelve of the cases reported prolonged labour and 9 reported home delivery by midwives.

The differences in levels of BSR between the two groups were statistically significant (p=0.024). The BDI scores for depression among mothers taking care of CP children were significantly higher than in mothers taking care of healthy children (p=0.011). In mothers taking care of children suffering from severe CP the plasma oxytocin was lower than in mothers taking care of apparently healthy children, but this difference was not statistically significant. (Table-1).

Table-1: Comparison of study parameters in groups (Mean±SD)

Parameters	Group I (n=21)	Group II (n=21)	*p*
BDI score	7.57±5.33	4.62±5.12	0.011*
Plasma Oxytocin (pg/mL)	115.60±40.30	139.92±70.11	0.571

*Statistically significant

Among mothers having CP children 17 had minimal depression, 3 had mild depression and 1 had moderate depression. Out of 21 subjects in the control group II, 19 had minimal depression, 1 had mild and 1 had moderate depression. (Table-2). Depression scores showed a negative significant correlation with plasma oxytocin levels both in cases and controls (Table-3, Figure-1, 2).

Table-2: Depression in group I and II [n (%)]

Severity of Depression	Mothers with CP children (n=21)	Mothers with healthy children (n=21)
Minimal	17 (80.95)	19 (90.47)
Mild	3 (14.28)	1 (4.76)
Moderate	1 (4.76)	1 (4.76)
Severe	0 (0)	0 (0)

Table-3: Correlation of BDI score with plasma oxytocin levels in group I and II

	Group I (n=21)		Group II (n=21)		Total (n=42)	
Parameters	BDI	Plasma oxytocin	BDI	Plasma oxytocin	BDI	Plasma oxytocin
rho	1.00	-0.785	1.00	-0.486	1.00	-0.612
P	0.001*		0.026*		0.001*	

*Statistically significant

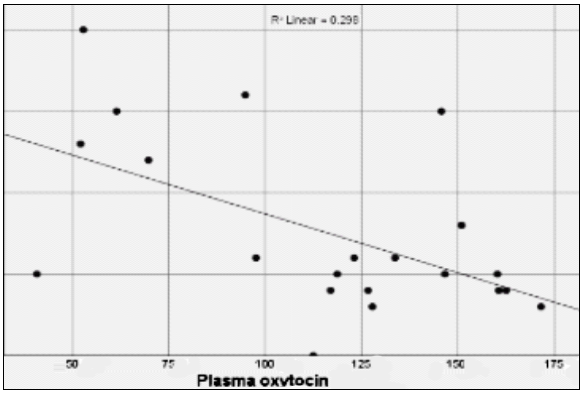

Figure-1: Scatter graph showing negative significant correlation between BDI scores and plasma oxytocin levels in mothers taking care of healthy children (n=21)

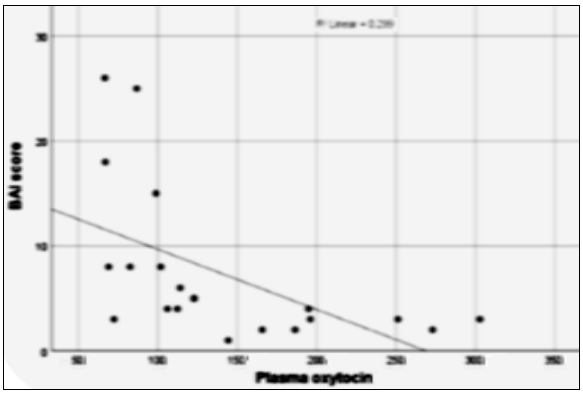

Figure-2: Scatter graph showing negative significant correlation between BDI scores and plasma oxytocin levels in mothers taking care of children suffering from CP (n=21)

DISCUSSION

This study included 21 mothers with healthy children and 21 mothers having children suffering from severe CP. The BDI scores were significantly higher in mothers taking care of CP children as compared to the mothers having apparently healthy children. A study[21] reported similar results to our study, the higher BDI II scores and low quality of life was observed in mothers having CP children. Previous studies[22,23] reported significantly higher levels of BDI scores in mothers having CP children than the control group. Mothers having CP children are depressed and severity can be categorized as mild, moderate and severe.[24] Results of our study are consistent with the above-mentioned studies. Studies suggest that CP children's mothers had a high prevalence of depression and anxiety that had a negative impact on children quality of life.[25,26] Studies conducted earlier suggest that factors like increase in age of mother as well as child, poor spousal support, lack of support from the family, unemployment and low education seemed to aggravate depression in these mothers. However, having another child with normal health and development, good family support and living in joint families served as protective factors. Presence of associated problems like epilepsy in the child, low intelligence, the severity of paralysis, motor disabilities, feeding issues and behavioural problems along with poor toilet training worsened the depression scores in mothers looking after the children suffering from CP.[27] Higher BDI scores were also observed by Altindag *et al*[15].

Physical disability in the child negatively affects the life of the mother. Maternal depression levels have a negative correlation with functional independence measurement and positive correlation with Gross Motor Function Classification System (GMFCS) scores in the CP child. Presence of CP child in a family is associated with maternal depression, musculoskeletal pain and negatively impacts the quality of life.[28] Increased psychological strain can be the triggering factor for the development of depression among mothers of CP children. Detailed history suggests that possible factors causing stress in mothers included in our study could be because of disabled child, behavioural problems or any other ailment associated with the child, limited leisure time, improper sleep, lack of family support, the social or economic burden. Anxiety and depression usually overlap each other in symptoms and treatment.

In our study plasma oxytocin levels showed a negative significant correlation with the BDI scores in both cases and controls. Literature shows a significant negative correlation between oxytocin and depression.[12,29]

Scarce literature is available to correlate plasma oxytocin levels in mothers having CP children. Our study correlates depression with the plasma oxytocin levels in mothers having CP children. It highlighted the need of psychological and social help for the mother taking care of CP children and emphasized on the need for the provision of proper medical facilities to the mother during pregnancy, during delivery and after birth. The study may help for future advancements about oxytocin and its use as a biomarker for prediction, prognosis, and treatment of depression.

CONCLUSION

Mothers taking care of CP children are more depressed as compared to the mothers taking care of healthy children. The significant negative correlation of plasma oxytocin with depression suggests its role as a predictor of depression. Further studies with large sample size are recommended. Awareness of the family and society, about the need for psychological help and need of social and family support to the mother having CP child is highly recommended.

LIMITATIONS

The study was a cross-sectional study which was conducted on a relatively smaller number of subjects, therefore it is hard to establish the exact cause-effect relationship.

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ORIGINAL ARTICLE

COMPARISON OF BUCCAL AND INTRAVENOUS MIDAZOLAM FOR THE TREATMENT OF ACUTE SEIZURES IN CHILDREN

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Background: Convulsive disorders often present as urgent neurological conditions requiring prompt intervention like IV midazolam. However, establishing an IV line especially in the ER can delay treatment institution. Therefore, rectal, buccal and intramuscular routes for administration are considered in such cases. Objective of this study was to compare the mean time for control of acute seizures by using buccal and intravenous midazolam in children. **Method:** This was a quasi experimental study carried over 6 months in the Paediatric Accident and Emergency Department, PAF Hospital, Mushaf, Sargodha. The study included a total of 60 children in actively fitting state. The subjects were divided into 2 groups of 30 in each, Group A having buccal midazolam and Group B having IV midazolam for control of seizure episode. Repeat dose was given after 5 minutes if seizures were not controlled. Time required to control seizures was noted for each patient. **Results:** Patients included in study had a mean age of 3.93±3.05 years, with 28 (46.6%) males and 32 (53.33%) female patients. Forty-seven (78.33%) had generalized seizures and 13 (21.67%) had partial seizures. Mean time for control of seizures was 198.33±133.19 seconds in Group A and 169.73±115.22 seconds in Group B. The difference was statistically insignificant with p=0.377. **Conclusion:** Buccal midazolam is an effective method for controlling acute seizures in children. It can be used as first line therapy instead of intravenous midazolam in pre-hospital and emergency settings.

Keywords: Acute seizures, Buccal, Children, Diazepam, Intravenous, Midazolam, Oral

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INTRODUCTION

In paediatrics, convulsive disorders often present as urgent neurological conditions requiring prompt intervention.[1] Although there are multiple aetiologies for seizures but prolonged seizure activity from any cause may lead to serious consequences in both patients, i.e., children and adults.[1,2] Despite the initial cause of seizures, if they do not stop spontaneously, they most often generate a positive vicious circle in which convulsions become self-perpetuating leading to status epileptics.[3] Timely control of seizures is important to prevent the patients from long term neurological complications.[2]

In the Accident & Emergency Department, the usual first line treatment for patients with seizures is IV midazolam or diazepam.[4,5] But most of the times establishing an IV line in a convulsing patient can be challenging and can delay treatment.[6,7] Therefore alternative routes for administration of midazolam like buccal, rectal and intramuscular routes are being practiced.[8,9] Despite various routes of administration of midazolam no consensus has yet been developed over the preferable route for administration of midazolam.[10–12]

In different studies, mean time for control of seizures has been reported as 225.6±23.4 seconds[13] after buccal midzolam, and 92.69±25.97 seconds[14] after IV midzolam. In another study mean time to control seizures by buccal midazolam group was 96±124.69 seconds with the lowest being 30 seconds and highest being 790 seconds and in IV diazepam group it was 83.40±124.27 seconds with lowest being 30 seconds and highest being 685 seconds. However, the difference in time to control seizures was not statistically significant (p=0.641).[15] Caregivers tend to prefer the buccal route over the rectal route, as it is considered safe and effective. If the buccal route is found to be more effective than intravenous administration, it could potentially become a standard approach for seizure control.[16] It offers the advantage of being easily and effectively used at home.[7]

Acute seizures are a frequently encountered emergency in paediatric care, often leading to serious consequences. Witnessing a child experience convulsions can cause significant anxiety for parents. It is urgent responsibility of healthcare professionals to manage and control seizures as quickly as possible.[2] This study aims to evaluate and compare different routes of drug administration for treating this emergency.

METHODOLOGY

This quasi-experimental study was conducted at the Paediatric Accident and Emergency Department, PAF Hospital Mushaf, Sargodha, from 28 Nov 2023 to 27 Nov 2024. Ethical approval was obtained from the hospital's ethical review committee, and informed consent was secured from the parents or guardians of all participants. A sample size of 60 was calculated using

the WHO sample size calculator for comparing two means: the mean time to control seizures with buccal midazolam (225.6 Sec)[13] and with IV midazolam (92.4 Sec)[14], 99% power, and 5% level of significance. Non-probability consecutive sampling was employed.

Children aged 6 months to 12 years presenting with acute seizures lasting more than 5 minutes were included in the study. Cases of prior administration of IV diazepam or other benzodiazepines within the past 24 hours, a history of narrow-angle acute glaucoma, known allergy to benzodiazepines, bradycardia (pulse <50/min) at presentation, and congenital anomalies like cleft palate were excluded. The patients were randomly assigned into two groups of 30 each.

Group A received buccal midazolam, while Group B received IV midazolam. Buccal midazolam was dosed according to age as 2.5 mg for ages 6–12 months, 5 mg for 1–4 years, 7.5 mg for 5–9 years, and 10 mg for children 10 years and older.[4] The drug was prepared as a solution in a syringe and administered with the child in the left lateral position, placing the syringe between the gums and cheek followed by gentle massage. The time to seizure cessation was recorded. A second dose was administered, up to a maximum of two additional doses, after 5 minutes if seizures persisted.

For Group B, IV midazolam was administered at a dose of 0.3 mg/Kg via an established IV line. Repeat doses were given after 5 minutes if needed.

The data were recorded on a structured proforma and entered and analysed using SPSS-20. Descriptive statistics (mean and standard deviation) were calculated for quantitative variables. Frequencies and percentages were calculated for qualitative variables. The mean seizure control times between the two groups were compared using the independent sample *t*-test. Data were stratified by age, gender, and seizure type, with post-stratification analysis performed using the Student's *t*-test, and $p<0.05$ as significant.

RESULTS

There were 28 (46.67%) males and 32 (53.33%) female patients. The mean age of patients was 3.93±3.05 years (Range: 5 months to 12 years). Mean time for controlling seizures was 184.03±124.31 seconds (Range: 30 to 500 Sec). Mean number of doses for controlling seizures was 1.30±0.56. Minimum dose was one and maximum dose was three.

Forty-seven (78.33%) seizures were generalized and 13 (21.67%) were partial. Mean time for seizures control was 198.33±133.19 Sec in Group A and 169.73±115.22 Sec in Group B. This difference was not statically significant (p=0.377). (Table-1).

Stratification was performed on the basis of age, gender and type of seizures. There was no significant effect of these effect modifiers on mean time to control seizures between the groups. (Table-2, 3, 4).

Table-1: Mean time for control of acute seizures between the groups

Groups	Time for control of acute seizures		*p*
	Mean	SD	
Group A	198.33	133.19	0.377
Group B	169.73	115.22	

Table-2: Association of age with mean time for control of acute seizures between the groups

Groups	Time for control of acute seizures		*p*
	Mean	SD	
0.5–3 years			
Group A	218.18	143.32	0.567
Group B	188.64	138.92	
4–12 years			
Group A	172.38	119.15	0.627
Group B	153.19	91.19	

Table-3: Association of gender with mean time for control of acute seizures between the groups

Groups	Time for control of acute seizures		*p*
	Mean	SD	
Male			
Group A	172.81	135.67	0.547
Group B	201.67	105.90	
Female			
Group A	227.50	128.92	0.082
Group B	148.44	119.13	

Table-4: Association of type of seizures with mean time for control of acute seizures between the groups

Groups	Time for control of acute seizures		*p*
	Mean	SD	
Generalized seizures			
Group A	201.17	121.32	0.08
Group B	146.12	86.08	
Partial seizures			
Group A	189.00	177.89	0.457
Group B	264.17	171.88	

DISCUSSION

The main focus of managing an acute episode of seizure is to control it at earliest. Efforts should be made to curtail the time of seizure so as to prevent long term squeal.[2,3] Trau SP *et al*[3] highlighted the prompt and successful acute seizure control to prevent neuronal injury. They also emphasized at providing patients with epilepsy, at home seizure abortive treatment, to decrease the time to initiate therapy.[3] Because of its feasibility and convenient route of administration, infrequent side effects and most significantly social acceptance, buccal midazolam could be the first choice of therapy for seizure control in patients with epilepsy at home and in circumstances in which there is a difficulty in gaining IV access, like less than 12 months old.[7,17]

The most important characteristics for any drug to be used as emergency treatment includes it safety, efficacy and decreasing seizure span.[12] Our study revealed that buccal midazolam is practical in controlling the acute seizure episode in kids with no side effects.

Previous studies had similar convincing results displaying that buccal midazolam is very effective in controlling seizures. Buccal midazolam reached a therapeutic level earlier as compared to intravenous route. Buccal midazolam can be used as an alternative to IV diazepam.[16]

Francesco Brigo *et al*[18] stated that Non-IV midazolam by any route is as effective as intravenous or rectal diazepam in terminating the seizures in children. In fact the time of arrival in Emergency to drug administration and convulsions control are shorter with non-IV midazolam than with IV or rectal diazepam. They also stated that buccal midazolam is easy to administer and socially more acceptable to patients and their parents for seizure control.

Studies conducted in Egypt to compare the efficacy, adverse effects and satisfaction after midazolam administration by buccal, intranasal or intramuscular route for the control of seizures in children at homes and in emergency settings also had interesting results. In their home group 67 patients received midazolam via buccal, 60 via intranasal and 69 via intramuscular route while in the A&E group 37 received buccal, 34 patients intranasal and 34 got intramuscular midazolam. Seizure cessation in 10 minutes was achieved in 94.2% and 85.3% after intramuscular midazolam in home and A&E groups respectively. The buccal route was effective in 91% in the home group and 78.4% in A&E group. The intranasal midazolam was successful in stopping seizures in 93.3% in home 88.2% in A&E group. They concluded that there was no statistical significant differences in efficacy between all groups (p=0.763 and p=0.509) among the home and A&E groups respectively. All of the above routes can be safely used in pre-hospital and emergency settings.[19,20]

McLntyre J *et al*[21] study included 177 patients having a total of 219 separate seizure episodes. The cut-off point for fit control was 10 minutes after the medicine administration. They concluded a success rate of 56% for buccal midazolam and 27% for rectal diazepam. Buccal midazolam had no associated adverse effects.[21]

Another clinical trail performed in Uganda by Mpimbaza A *et al*[22] found out that malaria was the most common cause of acute prolonged seizures (67.3%) in their children. Therapy failed in 71 (43%) of 165 patients who received rectal diazepam in comparison to 50 (30.3%) of 165 patients who received buccal midazolam. The only side effect noted in both groups was respiratory depression. The authors concluded that buccal midazolam is more effective and safer than rectal diazepam for treating seizures in children.[22]

Our study demonstrated that buccal midazolam is effective in terminating all types of seizures, regardless of their underlying aetiology. While the onset of action of IV diazepam is inherently faster due to its direct entry into the bloodstream, the overall initiation of treatment with buccal midazolam was quicker. This is largely because buccal administration can be performed immediately, without the need for IV access, which often delays treatment —especially in infants under 12 months, where establishing an intravenous line can be particularly challenging.

In contrast, buccal midazolam can be readily administered as a prepared solution into the buccal mucosa, allowing for faster overall seizure control. Our paediatric emergency team was well-prepared and efficiently administered buccal midazolam, which contributed to its effectiveness. As a result, buccal midazolam outperformed IV diazepam in terms of early seizure management.

However a few studies conducted on comparison of non-IV routes of midazolam, i.e., buccal, intramuscular, intranasal, and rectal midazolam, stated that the safety and efficacy of intramuscular midazolam is favourable as compare to buccal midazolam.[23,24] This part of research needs to be further investigated and elaborated as intramuscular absorption is slow in comparison to buccal route. The literature also lacks studies comparing buccal and IV midazolam. Most studies are conducted on buccal midazolam and rectal diazepam, appreciating the safety and efficacy of buccal midazolam. The findings of our research also emphasizes on effectiveness of buccal midazolam without any uncertainty.

Buccal midazolam at a dose of 0.2 mg/Kg has proven to be effective in terminating various types of seizures regardless of their aetiology. Given its ease of administration and rapid effectiveness, particularly in situations where IV access is challenging, buccal midazolam can be considered a first line treatment option for managing acute seizures in paediatric patients.

CONCLUSION

Buccal midazolam is as effective as intravenous midazolam in the management of acute convulsions in children. Due to its ease of administration, safety profile, and ability to be delivered without the need for IV access, it can be confidently used as a first line treatment option in both pre-hospital and emergency settings. It is particularly advantageous in young children and in scenarios where rapid IV access is not feasible.

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ORIGINAL ARTICLE

PATIENT-DRIVEN LIMITATIONS IN DENTAL IMPLANT THERAPY: A CROSS-SECTIONAL STUDY OF ITS BARRIERS

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Background: Implant dentistry offers a reliable, advanced alternative to traditional prosthetics for replacing missing teeth. The objective of this study was to document patient's related local and systemic causes that preclude dental implant prosthesis. **Methods:** This descriptive cross-sectional study was conducted over a period of 16 months in Prosthodontics Department, Peshawar Dental College, Peshawar, Pakistan. The study population consisted of patients seeking missing tooth/ teeth replacements. The data to identify factors hindering implant supported prosthesis was collected through a pre-structured proforma, interviews, clinical, and radiographic assessments. Data was analysed on SPSS-24 via post-stratification chi-square test. **Results:** The study involved 219 participants aged 19–65 years, with a mean age of 41.79±12.5 years. The implant supported prosthesis faced psycho-social obstacles (180, 82%) such as lack of information, time constraints, and procedure-related fear. Systemic causes included uncontrolled diabetes (26, 11.9%), active smoking (15, 6.8%), and age extremes (7, 3.2%), while local/oral factors included inadequate space and bruxism due to protracted tooth loss (11, 5%), and bruxism (13, 5.9%). **Conclusion:** Ignorance and cost are the major barriers to dental implant supported prosthesis selection, along with oral factors like bruxism and insufficient space, and systemic variables like diabetes and smoking. Public education regarding these issues is recommended.

Keywords: Complete denture, Fixed partial denture, Implant-supported fixed dental prostheses, Lifetime cumulative attachment loss, Removable partial denture

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INTRODUCTION

Implant dentistry presents a reliable and advanced alternative to traditional fixed prosthetics for replacing missing teeth. Over the past five decades, it has evolved into a predictable treatment option.[1] However, not all patients are eligible for implant placement due to various medical, psychosocial, and anatomical reasons.[2]

Psychosocial barriers such as cost, anxiety, lack of access, and insufficient perceived need often prevent patients from pursuing implant treatment. Oral health plays a vital role in determining implant success. Poor bone density and a history of periodontal disease can double the risk of implant failure.[3] Thorough systemic and oral health screening is crucial prior to treatment.[4]

Replacing lost teeth is essential for restoring function, speech, and aesthetics. The concept of osseointegration, introduced by Per-Ingvar Brånemark in the 1960s, underpins the success of implants by enabling titanium to fuse with bone. Osseointegration is affected by implant design, material properties, surgical techniques, and load conditions.[5]

Material science and biomechanics are integral to successful implant therapy. Ideal implant materials must be biocompatible and resistant to wear, corrosion, and fracture. Common materials include metals, ceramics, alloys, and polymers.[6] Key biomechanical considerations include implant length, diameter, crown-to-implant ratio, and the design of the occlusal surface to withstand forces and avoid complications.[7]

Achieving a passive fit of the prosthesis is critical to prevent biological and mechanical issues.[8] Stress distribution must be managed carefully, as poor fabrication steps, like errors in impressions or casting, can cause misfits.[8,9] Implant-protected occlusion (IPO) principles help minimize forces on implants through controlled occlusal design and positioning.[8]

Material choice for occlusal surfaces depends on existing dentition and location of the implant. Proper fixture length, placement, and use of night guards can prevent damage from parafunctional habits.[10] In some cases, tooth-implant supported prostheses (TISP) may be used to combine natural teeth and implants. The cantilevers should be minimized, stress breakers should be avoided, and occlusal loads should be oriented properly. However, TISPs should be avoided in patients with high caries risk or parafunctional habits.[11]

Patient awareness is vital in treatment acceptance.[12] Demographics such as age, gender, education, and number or location of missing teeth influence prosthesis choices. Dental implants offer excellent aesthetic and functional outcomes when planned properly.[13–17] Factors like bone quality, inter-arch distance, occlusion, cost, and treatment duration impact treatment planning.[14,18,19] The options like All-on-4 immediate loading are predictable solutions for

edentulous patients.[20] The objective of this study was to document and identify reasons that preclude dental implant prosthesis therapy.

METHODOLOGY

This was a descriptive cross-sectional study conducted in the Department of Prosthodontics, Peshawar Dental College and Hospital, for 16 months from Jun 2020 to Sep 2021. The sample size was calculated as 219 patients not opting for implant therapy, with an estimate of 17% in the population, with a 5% margin of error at 95% confidence interval. Consecutive sampling technique was used and included both genders, patients aged 19–65 years with at least one missing tooth.

The socio-economic status was categorized into 3 broad categories based on the modified Kuppuswamy's SES scale.[21] Patients with an overall monthly income of more than PKR 65,500 were categorised as High, 19,701–65,500 as Middle, and less than PKR 19,700 as Low SES. Exclusion criteria included age less than 19 or over 65 years, mentally handicapped patients, and patients not consenting.

Data collection was done through a pre-structured proforma which included Demographic data, psychosocial reasons, and local oral reasons. The proforma was divided into two parts: demographic data (name, age, gender, address, Out-Patient Department No., date of data collection, contact number, occupation, SES), and Psychosocial reasons (lack of awareness, high cost, fear of surgery, local oral reasons, aggressive periodontitis, poor quality and quantity of bone, inadequate space for implant, bruxism, conditions like uncontrolled diabetes, elderly, adolescence, survival after recent myocardial infarction, and immuno-suppression therapy).

The data was analysed on SPSS-24, quantitative variables were presented as Mean±SD, and qualitative variables as frequency and percentages. Chi-square test was applied to see association of various factors for preclusion of dental implants, and $p \leq 0.05$ was considered statistically significant.

RESULTS

Among 219 patients (mean age 41.79±12.5 years, range: 19–65 years), 74 (33.79%) were males and 145 (66.21%) were females. The main barriers to dental implants placement included lack of knowledge about dental implants (180, 82.19%), low SES (107, 48.86%), poor educational status (99, 45.20%) and high cost of the implant therapy (107, 48.85%). Low SES was seen in 107 (48.85%); middle SES in 53 (24.20%), and high SES was seen in 59 (26.94%) patients. Association of SES with gender was significant (p=0.04). (Table-1).

Table-1: Association of gender and SES among patients not opting for implant therapy [n (%)]

Socio Economic Status	Males	Females
Low	28 (37.83)	79 (54.48)
Middle	24 (32.43)	29 (20)
High	22 (29.72)	37 (25.51)
p	0.04	

There was significant association between education and time constraints for implant loading (p=0.001), with 10 (27.77%) postgraduates facing more immediate loading delays, i.e., 6–8 weeks, and 91 (49.72%) uneducated individuals delayed loading delays, i.e., 12–24 weeks. Majority (79, 54.85%) patients were females and unemployed (151, 68.94%). Lack of time was the factor responsible for preclusion in 36 (16.43%) patients, and in 28 (12.78%) fear of surgery was the factor for not opting for the dental implants. (Table-2).

Table-2: Association of educational status and time availability in patients not opting for implants [n (%)]

Educational Status	Immediate loading 6–8 weeks	Delayed loading 12–24 weeks
Uneducated	8 (22.22)	91 (49.72)
Primary	2 (5.55)	15 (8.19)
Secondary	7 (19.44)	48 (26.22)
Graduate	9 (25)	15 (8.19)
Postgraduate	10 (27.77)	14 (7.65)
p	0.001	

The fear of surgery (28, 12.78%) was significantly higher in females (25, 17.24%) than males (3, 4.05%), (p<0.01), and lack of time (36, 16.43%) was slightly and non-significantly higher in females (20, 13.80%) than males (16, 21.62%) that led to preclusion of implants. (Table-3).

Table-3: Factors for preclusion of dental implants [n (%)]

Factors	Males	Females	Total	*p*
Lack of time	16 (21.62)	20 (13.80)	36 (16.43)	0.139
Fear of surgery	3 (4.05)	25 (17.24)	28 (12.78)	0.006

Thirty-four (15.52%) patients who did not opt for implants were unemployed/unskilled, while 18 (8.21%) were students, 12 (5.47%) were professionals, and 4 (1.82%) were businessmen. (Table-4).

Table-4: Occupational status of patients not opting for dental implants

Occupational status	n (%)
Unemployed	34 (15.52)
Students	18 (8.21)
Professionals	12 (5.47)
Businessmen	4 (1.82)

Among the local oral factors limiting the placement of dental implants, the most commonly observed by gender was inadequate buccolingual ridge width (<6 mm) (47, 21.46%), followed by insufficient mesiodistal space (<7 mm) (23, 10.50%), bruxism (13, 5.93%), reduced interarch space (<7 mm) (11, 5.02%), presence of soft or hard tissue cysts (3, 1.36%), a

history of aggressive periodontitis (1, 0.45%), and Type IV bone quality (1, 0.45%). Thus, while these factors variably influenced implant eligibility, no significant gender-based differences were observed, indicating a minimal role of gender in the distribution of local anatomical limitations for implant placement. Diabetes and active smoking were the most common ones among the systemic factors precluding dental implants along with others. (Table-5).

Table-5: Systemic health reasons that precluded implant therapy

Systemic factors	n (%)
Uncontrolled diabetes	26 (11.87)
Active smoking	15 (6.84)
Elderly/Frail	7 (3.19)
Adolescence	5 (2.28)
Anticoagulation medication	3 (1.36)
Total	56 (25.57)

DISCUSSION

This cross-sectional study investigated patient-related barriers to dental implant therapy. Dental implants are increasingly regarded as the gold standard for tooth replacement due to their high survival rates, functional advantages, and positive impact on quality of life, with long-term success rates exceeding 90%.[22] Despite their clinical benefits, several patient-driven factors, psychosocial and clinical, limit their uptake and suitability.

Our findings showed that a higher proportion of female patients sought consultation compared to males, aligning with previous studies that also reported a slightly higher female participation rate in dental care settings.[23,24] This gender distribution may reflect cultural patterns in healthcare-seeking behaviour or greater aesthetic concern among female patients.

Educational and occupational status played a prominent role. The majority of patients were uneducated and unemployed. This is consistent with other studies indicating that low socioeconomic status is prevalent among those accessing public dental services.[6,18,25] A statistically significant association between gender and socioeconomic status was observed, supporting previous findings that women in such contexts are often more socioeconomically disadvantaged.[24]

The most frequently reported psychosocial barrier was a 'lack of awareness about dental implants', identified in 82.19% of participants. This proportion is notably higher than figures reported in earlier studies, which ranged from 50 to 70%.[11,24–26] This discrepancy underscores the persistent gap in patient education within the population studied. Following basic counselling, the 'cost of treatment' emerged as the next major deterrent (48.9%), comparable to other studies identifying financial burden as a critical barrier to implant acceptance.[26] 'Fear of surgery' was also prevalent and was significantly associated with gender (p=0.006), consistent with literature suggesting higher dental anxiety among female patients.[25] In contrast, 'lack of time'—although mentioned by some—did not show a significant association with gender (p=0.139), suggesting it may be a more generalized or logistical concern.[26]

Clinical barriers further influenced treatment feasibility. Local oral conditions such as 'inadequate buccolingual (21.46%), mesiodistal (10.50%), and inter-arch space (5.02%)', as well as 'bruxism (5.02%)', were common limiting factors. These findings align with earlier reports that highlight the anatomical constraints and parafunctional habits as significant predictors of implant candidacy.[24] Less frequent but noteworthy conditions included aggressive periodontitis, cysts, and poor bone quality, echoing similar clinical profiles reported in South Asian populations.[24]

Systemic health factors were also influential. Uncontrolled diabetes mellitus (11.87%), active smoking (6.84%), and frailty (3.19%) were the most reported medical conditions that contraindicated implant therapy. A strong association was found between smoking and gender (p=0.000), in agreement with prior data indicating higher smoking rates among males in the region.[24] Fewer participants reported anticoagulant use (1.36%) or adolescence (2.28%) as exclusionary factors. Notably, no patients had a history of chemotherapy or organ transplantation, likely reflecting either their low prevalence or patients being filtered out prior to referral for implant consultation.

The collective findings emphasize the need for enhanced patient education, cost-effective implant options, and comprehensive medical and dental screening. For example, the introduction of implant-retained mandibular overdentures, even supported by a single implant, could offer a viable and affordable alternative, especially in resource-limited settings.

This study is limited by its single-centre design, which may restrict generalizability. Future research should include multi-centre, large-scale studies to validate these results across broader demographic and geographic segments of the Pakistani population. Further studies incorporating the variable of governmental assistance may also give insight about its role in this matter. Both socioeconomic factors and systemic health play a critical role in determining candidacy for dental implant-supported prostheses.

CONCLUSION

Dental implant prosthesis is often not affixed to patients due to lack of knowledge, inferior level of education, low socioeconomic status, fear of surgery, lack of time for immediate as well as delayed loading, and high costs of implant therapy along with active smoking. To

address this issue, patients need to be educated about the benefits of dental implants, the consequences of tooth loss, and the importance of early prosthetic provision.

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ORIGINAL ARTICLE

EFFICACY OF SLOW INFUSION OF ADENOSINE VERSUS VERAPAMIL IN THE TREATMENT OF SUPRAVENTRICULAR TACHYCARDIA

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Background: Supraventricular tachycardia (SVT) is a critical cardiac emergency necessitating medical intervention. The comparative efficacy and safety profiles of adenosine and verapamil in Pakistani population have not been extensively studied. This study aims to compare the efficacy of slow infusion adenosine versus verapamil in the treatment of SVT in Pakistani patients. **Methods:** A prospective experimental study was conducted involving 100 patients with SVT at a tertiary care hospital in Pakistan. Patients were randomly assigned to receive either a slow infusion of adenosine (n=50, initial dose 6 mg followed by 12 mg if needed) or verapamil (n=50, 1 mg/min up to 20 mg) after the initial Valsalva manoeuvre. The primary outcome was successful termination of SVT. Secondary outcomes included haemodynamic changes and adverse effects. **Results:** The mean age was comparable between groups (adenosine: 52.02±12.19 years, verapamil: 51.98±13.90 years). Verapamil proved superior efficacy with a 100% conversion rate compared to 90% with adenosine (p=0.02). Both groups showed similar haemodynamic stability, with no significant differences in post-conversion systolic blood pressure, (verapamil: 123.70±22.35 mmHg *vs* adenosine: 122.14±15.79 mmHg, p=0.68) and diastolic blood pressures. The adenosine group reported higher rates of apprehension (62.9%) and ECG events (63.2%), while both groups showed comparable incidences of other side-effects. **Conclusion:** Verapamil showed higher conversion success compared to adenosine in treating SVT and maintained a favourable safety profile. Verapamil is an effective alternative to adenosine in the management of SVT.

Keywords: Adenosine, Pakistan, Supraventricular tachycardia, Verapamil

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INTRODUCTION

Supraventricular tachycardia (SVT) represents a significant cardiovascular challenge and is characterized by heart rates exceeding 150 beats/min, originating above the ventricles.[1,2] It affects approximately 2.25 per 1,000 persons of the general population and accounts for significant emergency department visits worldwide.[3] In Pakistan, SVT contributes substantially to cardiovascular-related emergency admissions.[4]

The pathophysiology of SVT encompasses several mechanisms, with atrioventricular nodal re-entrant tachycardia (AVNRT) being the most common, followed by atrioventricular re-entrant tachycardia (AVRT) and atrial tachycardia (AT).[1,5] These arrhythmias can cause significant patient distress, manifesting as palpitations, chest pain, dyspnoea, dizziness, and in severe cases, syncope.[3,6] The impact on patient's quality of life and healthcare resource utilization necessitates effective and efficient management strategies.

Current guidelines recommend a stepped approach to SVT management, beginning with Valsalva manoeuvre or carotid sinus massage.[7,8] When these first interventions fail, pharmacological management becomes necessary. Adenosine and verapamil represent two principal pharmacological options, each with distinct mechanisms of action and clinical considerations. Adenosine acts through rapid blockade of the AV node, while verapamil achieves its effect through calcium channel blockade, resulting in a slower but potentially more sustained response.[8]

While adenosine has traditionally been considered the first-line pharmacological intervention in many healthcare settings[1,2], several factors warrant a comparative evaluation with verapamil, particularly in the Pakistani healthcare context. These factors include: cost considerations in a resource-limited setting, availability and accessibility of medications, patient tolerability and acceptance, healthcare provider familiarity and experience, and infrastructure requirements for drug administration and monitoring.[6,7]

Limited data exists on comparing these medications in the Pakistani population. Genetic, cultural, and environmental factors affect drug responses, requiring local studies. Cost-effectiveness should also be evaluated alongside clinical efficacy.[8]

This study aims to compare the efficacy of slow infusion adenosine and verapamil in the treatment of SVT, and to evaluate the success rate of SVT termination with each drug. Secondary objectives include assessment of haemodynamic stability during and after drug administration, adverse effects, patient tolerability and comfort, and rate of SVT recurrence during the observation period.

METHODOLOGY

This prospective experimental study was conducted at Pakistan Ordinance Factory Hospital, Wah Cantt:, Pakistan from 1 Jun 2020 to 25 Aug 2021. The study protocol was approved by the institutional ethics committee Reference Letter No. 4119/HOD ER/Hosp.

Sample size was calculated by using WHO sample size calculator. A total of 100 patients presenting with SVT were enrolled in the study. Patients who are adults (age ≥18 years), had electrocardiogram-verified SVT with stable haemodynamics, and gave informed consent were included in the study. Patients who needed rapid cardioversion due to haemodynamic instability, had known hypersensitivity to study drugs, were pregnant or lactation, had history of asthma, significant heart block, and systolic blood pressure (SBP) <90 mmHg were not excluded.

Patients were randomly assigned in a 1:1 ratio to either the adenosine group (n=50) or the verapamil group (n=50) using computer-generated random numbers. Allocation concealment was maintained using sequentially numbered, opaque, sealed envelopes. All patients initially received a standardized Valsalva manoeuvre. If SVT persisted, medication was administered according to group allocation. The adenosine group received 6 mg slow IV bolus of adenosine and, if necessary, increased to 12 mg after 2 minutes followed by a quick saline flush. Verapamil group received slow infusion at 1 mg/min with a total dose of up to 20 mg IV, with continuous ECG monitoring.[9]

Demographic data, medical history including previous SVT episodes, vital signs at presentation, continuous ECG monitoring, blood pressure measurements at 5, 10, 15, and 30 minutes post-conversion, and any side-effects were recorded.

Successful termination of SVT, was defined as conversion to normal sinus rhythm. Haemodynamic parameters (blood pressure changes), adverse effects including patient's comfort and tolerability and need for additional interventions were noted.

Data was analyzed on SPSS-23. Continuous variables were presented as Mean±SD while categorical variables were presented as frequencies and percentages. Independent *t*-test was used for continuous variables and Chi-square test was used for categorical variables, and $p \leq 0.05$ was considered statistically significant.

RESULTS

The mean age was comparable between groups (verapamil: 51.98±13.90 years, adenosine: 52.02±12.19 years, p=0.98). Both groups showed similar age distribution across categories (<41 years, 41–60 years, and >60 years). The verapamil group comprised 26 (52%) males and 24 (48%) females, while the adenosine group had 20 (40%) males and 30 (60%) females, though this difference was not statistically significant (p=0.22). Prior diagnosis of SVT was reported in 32 (53.3%) patients in the verapamil group and 28 (46.7%) in the adenosine group (p=0.66).

Baseline clinical parameters were comparable between groups, with no significant differences in SBP (verapamil: 128.82±22.36 mmHg; adenosine: 127.88±19.66 mmHg; p=0.82), DBP (verapamil: 85.20±18.76 mmHg; adenosine: 82.76±13.69 mmHg; p=0.46), oxygen saturation (verapamil: 97.70±1.16%; adenosine: 97.86±1.78%; p=0.59), and initial heart rate (verapamil: 185.14±17.8 bpm, adenosine: 189.56±26.23 bpm, p=0.32). (Table-1).

Table-1: Pre-treatment patients' characteristics (Independent sample *t*-test) [n (%)]

Patient parameters	Verapamil	Adenosine	*p*
Age (Mean±SD)	51.98±13.89	52.02±12.19	0.98
Age n [%]			
<41 Years	11 (52.4)	10±47.6%	0.92
41–60 Years	25 (48.1)	27±51.9%	
>60 Years	14 (51.9)	13±48.1%	
Gender n [%]			
Male	26 (52.0)	20±40.0%	0.22
Female	24 (48.0)	30±60.0%	
Past SVT diagnosed			
Yes	32 (53.3)	28±46.7%	0.66
No	18 (45.0)	22±55.0%	
Systolic BP (mmHg, Mean±SD)	128.82±22.36	127.88±19.66	0.82
Diastolic BP (mmHg, Mean±SD)	85.20±18.76	82.76±13.69	0.46
Oxygen saturation (%)	97.70±1.16	97.86±1.78	0.59
HR at start (Beats/min)	185.14±17.8	189.56±26.23	0.32

The verapamil group demonstrated significantly higher success rates in SVT termination compared to the adenosine group (100% *vs* 90%, respectively; p=0.02). Both groups showed similar haemodynamic responses post-conversion, with no significant differences in SBP (verapamil: 123.70±22.35 mmHg, adenosine: 122.14±15.79 mmHg; p=0.68) or DBP (verapamil: 80.88±13.94 mmHg, adenosine: 78.44±15.21 mmHg; p=0.40) at the time of conversion. (Table-2).

Table-2: Immediate post-treatment vital signs (Independent sample *t*-test, Mean±SD)

Drugs Groups	Verapamil	Adenosine	*p*
Converted with initial treatment	50 (100%)	45 (90%)	0.02
Pre-conversion SBP mmHg	130.42±23.11	127.44±19.54	0.48
Pre-conversion DBP (mmHg)	84.12±21.77	82.34±12.97	0.62
SBP at conversion (mmHg)	123.70±22.348	122.14±15.79	0.68
DBP at conversion (mmHg)	80.88±13.94	78.44±15.21	0.40
Heart rate per minute	94.58±13.83	96.02±14.30	0.61

Systolic blood pressure measurements were recorded at 5, 10, 15, and 30 minutes post-conversion. Throughout the monitoring period, both groups kept stable haemodynamics with no statistically significant differences. At 5 minutes: SBP (verapamil:

120.94±24.69 mmHg; adenosine: 122.24±14.61 mmHg; *p*=0.75). At 10 minutes: SBP (verapamil: 119.34±17.89 mmHg; adenosine: 119.58±20.08 mmHg; *p*=0.95). At 15 minutes: SBP (verapamil: 119.90±16.50 mmHg; adenosine: 122.00±14.68 mmHg; *p*=0.50). At 30 minutes: SBP (verapamil: 119.46±15.04 mmHg; adenosine: 121.68±13.20 mmHg; *p*=0.43). (Table-3).

Table-3: Mean SBP changes during initial 30 min monitoring after conversion (Mean±SD)

BP measuring intervals	Verapamil	Adenosine	*p*
5 min	120.94±24.69	122.24±14.61	0.75
10 min	119.34±17.89	119.58±20.08	0.95
15 min	119.90±16.50	122.00±14.68	0.50
30 min	119.46±15.04	121.68±13.20	0.43

Appreciable differences in adverse effects between groups included. Apprehension was more common in the adenosine group (62.9% *vs* 37.1%, *p*=0.05), ECG events were more frequent in the adenosine group (63.2% *vs* 36.8%; *p*=0.20). Other side effects including nausea (53.7% *vs* 46.3%), light-headedness (55.9% *vs* 44.1%), musculoskeletal pain (52.8% *vs* 47.2%), chest tightness (55.6% *vs* 44.4%), dyspnoea (33.3% *vs* 66.7%), and headache (57.1% *vs* 42.9%) showed no statistically significant differences between groups. (Table-4).

Table-4: Association of problems with verapamil and adenosine group patients (Chi-square test) [n (%)]

Adverse effects	Verapamil	Adenosine	*p*
Past SVT diagnosed	32 (53.3)	28 (46.7)	0.41
Nausea	22 (53.7)	19 (46.3)	0.54
Apprehension	13 (37.1)	22 (62.9)	0.05
ECG changes	7 (36.8)	12 (63.2)	0.20
Light headedness	19 (55.9)	15 (44.1)	0.39
Musculoskeletal pain	19 (52.8)	17 (47.2)	0.67
Chest tightness	5 (55.6)	4 (44.4)	0.72
Dyspnoea	1 (33.3)	2 (66.7)	0.55
Headache	8 (57.1)	6 (42.9)	0.56

DISCUSSION

This experimental study, conducted in a tertiary care hospital in Pakistan, demonstrates the comparison between the effects of slow infusion of adenosine versus verapamil in terminating SVT. The 100% conversion rate observed with verapamil, compared to 90% with adenosine, suggests that verapamil may be a superior first-line agent for SVT management in this population.

These findings align with earlier studies that have also reported favourable efficacy with verapamil in certain SVT scenarios. Riaz *et al*[10] found comparable results in their randomized comparative trial, though with some variations in the overall conversion rates. The slower mechanism of action of verapamil, through calcium channel blockade as described by Antman *et al*[11] may provide a more sustained effect compared to the rapid but transient AV nodal blockade induced by adenosine.

Our study results are also consistent with Asghar *et al*[12] who reported similar efficacy between adenosine and verapamil in terminating SVT, though our investigation demonstrated superior conversion rates for verapamil. Verapamil selectively blocks calcium channels, prolonging the effective refractory period and slowing conduction through the AV node, thus terminating re-entrant tachycardia as explained by Godfraind[13].

While adenosine is often recommended as the first-line treatment for SVT due to its rapid onset and short half-life according to ACC/AHA Guidelines (2019), it is also associated with unpleasant side-effects such as chest pain, dyspnoea, and a sense of impending doom, which may contribute to patient apprehension.[14] Gupta *et al*[15] highlighted adenosine's multiple utilities but also acknowledged its significant side-effect profile. In our study, apprehension was significantly more common in the adenosine group (62.9%) compared to the verapamil group. ECG events were also more frequent in the adenosine group, though this difference did not reach statistical significance. These factors may influence patient tolerability and acceptance of adenosine, particularly in a setting where patient comfort is a priority. Feng and Liu's meta-analysis emphasized both the efficacy and safety considerations when selecting adenosine for SVT management.[16]

Both groups showed comparable haemodynamic stability, with no significant differences in post-conversion blood pressure measurements. This suggests that both drugs can be safely administered in haemodynamically stable patients with SVT, aligning with findings by Delaney *et al*[17]. Lim *et al*[9] also demonstrated that slow infusion of calcium channel blockers, including verapamil, was as effective as adenosine in treating SVT, with comparable safety profiles. However, continuous ECG and blood pressure monitoring are essential during and after drug administration to detect and manage any potential adverse effects. Marco and Cardinale[18] emphasized the need for vigilant monitoring following adenosine administration due to the risk of transient hypotension and bradycardia.

Shaker H *et al*[8] further support our findings by demonstrating the efficacy of verapamil in controlling SVT recurrence, suggesting its potential long-term benefits beyond immediate conversion. The higher cost and limited availability of adenosine in some healthcare settings may also favour the use of verapamil as a more practical and cost-effective alternative. The inclusion of verapamil in the WHO Essential Medicines List 2023[19] underscores its global importance as an accessible medication for cardiovascular conditions.

Further research is needed to evaluate the cost-effectiveness of verapamil versus adenosine in the Pakistani healthcare context, particularly considering the

findings of Ahmad *et al*[7] who emphasized the importance of systematic approaches to SVT management in resource-constrained settings. The availability of generic formulations of verapamil also contributes to its affordability and accessibility in resource-limited settings.

Our findings have important implications for clinical practice in Pakistan and similar settings. Given the higher efficacy, comparable safety, and potential cost-effectiveness of verapamil, it may be considered as a first-line agent for SVT management in haemodynamically stable patients. However, treatment decisions should be individualized based on patient characteristics, preferences, and the availability of resources.[6]

LIMITATIONS

The study was conducted at a single tertiary hospital with a small sample size, limiting generalizability and power to detect minor differences. It focused on stable patients and did not assess long-term outcomes or apply to unstable patients needing urgent cardioversion.

CONCLUSION

Slow infusion of verapamil showed higher conversion success compared to adenosine in treating SVT and supported a favourable safety profile. Verapamil can be an effective alternative to adenosine. Multi-centre large scale study is suggested to elaborate these findings.

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ORIGINAL ARTICLE

EFFECTS OF ALPHA LIPOIC ACID ON OXIDATIVE STRESS AND CONTRACTILE FUNCTIONS OF FAST MUSCLES IN TYPE 2 DIABETIC MALE SPRAGUE DAWLEY RATS

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Background: Metabolic derangements and oxidative stress due to Type 2 Diabetes Mellitus (T2DM) has deleterious effects on skeletal muscle contractile functions. This study aimed to see the effects of alpha lipoic acid (ALA) on oxidative stress and contractile functions of fast muscles in type 2 diabetic male Sprague Dawley rats. **Methods:** This quasi experimental study was conducted at Department of Physiology, CMH Multan Institute of Medical Sciences, Multan from Sep 2022 to Feb 2023. Ninety adult Sprague-Dawley rats were randomly divided into 3 equal groups (n=30). Group A (control group) Group B (Diabetic group) and Group C (ALA-treated group). Diabetes was induced in group B and C by injecting Streptozocin (35 mg/Kg body weight) intraperitoneally in the lower-right quadrant of the abdomen after 2 weeks. After 4 weeks, extensor digitorum longus (EDL) muscles were dissected and contractile functions assessed through iWorx data acquisition unit. Serum Malondialdehyde (MDA) and plasma glucose levels were estimated through cardiac blood sampling. **Results:** ALA group showed improvement in maximum fused tetanic tension, fatigue and recovery from fatigue protocol as compare to the diabetic group. No significant difference among all the groups was found in maximum tension and time to relax to peak twitch tension. Serum MDA levels were found significantly deceased in ALA group as compared to the diabetic group. **Conclusion:** ALA supplementation decreases oxidative stress which improves contractile force and delays fatigue in fast muscles of diabetic rats.

Keywords: Alpha lipoic acid, contractile function, malondialdehyde, muscle, type 2 diabetes mellitus

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INTRODUCTION

According to World Health Organization (WHO) approximately 462 million people are affected worldwide from Type 2 diabetes mellitus (T2DM) currently, and by 2035 this number may rise to 592 million.[1] Muscle fibres have been classified into two distinct categories: type I (slow twitch, oxidative) and type II (fast twitch, glycolytic).[2] Skeletal muscles utilize muscle glycogen and plasma glucose by oxidation for optimal adenosine triphosphate (ATP) generation for daily activities and during high intensity exercise.[3] In T2DM, decreased insulin sensitivity/resistance results in mitochondrial dysfunction with defective oxidative phosphorylation, decreased content and rate of glycogen synthesis, decreased glucose oxidation due to decreased pyruvate dehydrogenase (PDH) activity resulting in increased dependence on alternate sources of energy via accelerated lipid and protein catabolism. Consequently, there is increased plasma concentration of triglycerides and free fatty acids (FFAs)[4] which lead to formation of superoxide anion, hydroxyl radical and other reactive oxygen species (ROS) like hydrogen peroxide which causes cytotoxic damage to different proteins in mitochondria which affect fast muscle fibres contractile functions by decreasing its oxidative capacity.[5]

Alpha-lipoic acid (ALA) plays a crucial role in mitochondrial energy metabolism as a cofactor for α-ketoacid dehydrogenases and functions as a potent antioxidant. It scavenges ROS, regenerates intracellular antioxidants like glutathione and vitamins C and E, and enhances insulin sensitivity. ALA stimulates glucose uptake by translocating GLUT4 transporters and activating AMP-activated protein kinase, promoting fatty acid oxidation.

In T2DM, increased oxidative stress and impaired glucose metabolism lead to contractile dysfunction in skeletal muscles, particularly fast-twitch fibres. ALA mitigates these effects by reducing oxidative stress, restoring glycogen synthase activity, and improving glucose uptake, thereby enhancing muscle contractile function and delaying fatigue. Alpha-lipoic acid is naturally synthesized enzymatically in the mitochondrion from octanoic acid. In addition to *de novo* synthesis yeast, animal liver, kidney, and spinach, broccoli, potatoes are good plant sources of ALA.[6]

Many international studies have proven that ALA corrects metabolic derangements and oxidative stress in T2DM however no study has been conducted regarding its effects on contractile functions of skeletal muscles. This study aimed to see the effects of alpha lipoic acid on oxidative stress and contractile functions of fast muscles in T2DM male Sprague Dawley rats.

METHODOLOGY

This experimental control trial was carried out at Physiology and Anatomy Departments of CMH Multan Institute of Medical Sciences, Multan from Sep 2022 to Feb 2023 after formal approval from ERC/IRB of the Institution. Ninety Sprague Dawley rats (adult male) were recruited for the study.

Male Sprague-Dawley rats (82±6 days old) and 245±60 grams average body weight were included in the study. Three groups (each having 30 rats) were formed by random distribution. The control group was fed on low fat normal diet and free access to water. Diabetic and ALA groups were fed on fat rich/high fat diet (HFD) for 14 days. (Table-1).

Table-1: Composition of rat's feed

Control group		Diabetic and ALA groups	
Ingredients	Weight (g/Kg)	Ingredients	Weight (g/Kg)
Wheat	275	NPD	385
Dried skimmed milk	285	Casein	315
Mollasen	5	Lard	265
Salt	15	Cholesterol	30
Cooking oil (mL/Kg)	50	Vitamin/mineral	70
Raw meat	150	L-cystine	3
Vitamins	10	Yeast mixture	5
Wheat brawn	200	Sodium chloride	20

On 15th day, diabetes mellitus was induced by injecting Streptozocin (35 mg/Kg body weight) intraperitoneally in diabetic and ALA groups, while normal saline was injected in control group. On 21st day, development of T2DM in diabetic and ALA groups was confirmed (plasma glucose level >16.65 mmol/L) by measuring plasma glucose of all rats by tail vein sampling.[7]

Alpha lipoic acid (Thioctacid 600, AstaMedica, Germany) was injected intra-peritoneally (30 mg/Kg/day) for two weeks to ALA group, while normal saline was administered to other two groups.[8]

On 28th day, rats were euthanized by high dose of Ether. Samples of 3–5 mL blood was obtained and centrifuged for 15 minutes at 4,000 rpm at 4 °C. Serum was pipetted out and transferred into 1.5 mL labelled eppendorf tubes to assess biochemical parameters.

Serum malondialdehyde (MDA) levels were estimated using Rat MDA ELISA Kit, (Shanghai Crystal Day Biotech Co, Ltd.). Plasma glucose levels (PGLs) was estimated by Glucose kit (glucose oxidase method).

On 28th day, extensor digitorum longus muscle (EDL) was dissected and mounted on organ bath system of iWorx for animal physiology data acquisition unit containing 25 mL Krebs-Ringer bicarbonate buffer solution and supplied with 95% O_2 and 5% CO_2 continuously at fixed temperature of 30 °C.[9] Force transducer of iWorx was used for muscle stimulation with stimulation frequencies 5–110 Hz per second (with 3 minutes rest period between each stimulus) was used for measuring peak twitch tension (PTT), force-frequency relationship and relax time to 50% of the PTT. Maximum fused tetanic tension (MFTT) and recovery from fatigue was determined by stimulating muscle with optimum frequency for 1 minute with 5 seconds rest time in between.[10]

The Mean±SD was calculated using SPSS-23. ANOVA was applied to determine the statistically significant differences across the groups and followed by post hoc test, and $p \leq 0.05$ was considered significant.

RESULTS

Plasma glucose levels (PGLs) and body weight of all rats recorded on day one were within normal range. Diabetic and ALA rats groups successfully developed T2DM confirmed on 21st day of the study by measuring plasma glucose levels. As compared to control group, diabetic and ALA groups rats body weight was found to be increased due to fat rich high calorie diet. PGLs and BW of each rat was again measured at the end of study on 28th day and were found significantly high in the diabetic group, while the ALA group showed decrease levels compare to diabetic group (Table-2).

Serum MDA levels were 3.71±0.66 µmol/dL, 7.97±0.81 µmol/dL in diabetic group, and 4.45±0.72 µmol/dL in ALA group. Skeletal muscles parameters like TPTT (p>0.38), MTT (p>0.15), and relax time to 50% maximum twitch tension (p>0.29) were insignificant among all groups. Significant difference was found among the groups in fatigue protocols like MFTT (p<0.02), and tetanic tension measured after fatigue protocol (p<0.03), (Table-3).

Diabetic group was significantly different from control and ALA after application of post hoc Tukey's test, and control and ALA groups had non-significant differences (Table-4).

Table-2: **Body weight and plasma glucose levels in all groups at 1st, 21st, and 28th days (Mean±SD)**

Groups	Days	Control	Diabetic	ALA
Body weight (g)	1	215.55±5.04	248.62±6.47	251.54±5.32
	21	256.63±7.40	269.70±8.35	268.70±7.70
	28	265.71±7.15	278.70±7.61	269.86±8.34
Plasma glucose (mmol/L)	1	5.85±0.34	5.84±0.30	5.87±0.34
	21	5.83±0.31	23.13±0.40	22.90±0.41
	28	5.91±0.32	23.90±0.47	10.55±0.45

Table-3: Skeletal muscle contractile parameters comparison using one-way ANOVA on 28th day

Variables	Group I (Control)	Group II (Diabetic)	Group III (ALA)	p
PTT (N/g)	0.34±0.06	0.31±0.07	0.40±0.021	0.15
TPTT (mSec)	20.83±1.53	22.05±1.23	21.15±1.54	<0.38
Relax time to 50% PTT	21.2±3.50	22.8±4.30	21.9±4.00	<0.9
MFTT (N/g)	3.98±0.07	3.93±0.09	3.97 ± 0.06	**<0.02**
MFTT after fatigue protocol (N/g)	1.83±0.05	1.79±0.05	1.82±0.06	**<0.01**

Table-4: Comparison of groups using post hoc Tukey's test (*p*-values)

Contractile parameters	Control *vs* Diabetic	Control *vs* ALA	Diabetic *vs* ALA
MFTT	0.01	0.55	0.04
MFTT after fatigue protocol	0.00	0.48	0.03

DISCUSSION

This study demonstrates that ALA supplementation reduces oxidative stress markers and improves certain aspects of skeletal muscle contractile function in diabetic rats. The findings align with previous research highlighting ALA's antioxidant properties and its role in improving glucose metabolism and mitochondrial function.[6,11] The reduction in serum MDA levels observed in the ALA-treated group supports its role in mitigating oxidative stress, which is a key factor in T2DM-induced muscle dysfunction.[5] Furthermore, the improvement in maximum fused tetanic tension and recovery from fatigue suggests that ALA may contribute to better muscle endurance by restoring glycogen synthase activity and ATP availability.[4]

A major strength of this study is its integration of metabolic and functional assessments of skeletal muscle performance. Previous studies have established ALA's role in improving insulin sensitivity and reducing oxidative damage.[12,13] However, limited research has examined its direct impact on skeletal muscle contractility in diabetic models. This study adds to the existing body of knowledge by demonstrating that ALA may enhance muscle performance by reducing oxidative stress and improving glucose utilization in fast-twitch muscle fibres.

While the results support ALA's beneficial effects, claims regarding its ability to 'correct metabolic derangements' should be cautiously interpreted. The scope of this study was limited to biochemical markers of oxidative stress and muscle contractility, and it did not evaluate long-term metabolic adaptations or molecular mechanisms such as GLUT4 expression or mitochondrial function in detail. ALA supplementation reduced oxidative stress markers and improved muscle function in this model.

Malondialdehyde (MDA) is an end-product formed during increase lipid peroxidation which signifies cellular injury and oxidative stress. Serum MDA levels are used as biomarker of oxidative stress and were high in the diabetic group showing increase oxidative stress and damaging effects of reactive oxygen species (ROS). Serum MDA levels were decreased in the ALA group as compare to diabetic group signifying its antioxidant property.[14] Zhang T *et al*[15] documented the similar role of alpha-lipoic acid in protection against oxidative stress and apoptosis in rats with diabetic peripheral neuropathy by studying the mechanism of ALA through activated AMPK pathway.

The EDL muscle largely depends on glycogenolysis for ATP production because of abundant type II fast fibres.[16] There were no significant differences in MITT, TPTT and relaxation time to 50% MITT among the groups as these skeletal muscles contractile functions depends on available ATPs and calcium ions in sarcoplasm, release of Ca^{++} and transport back to sarcoplasmic reticulum via Ca^{++} pump. Adequate amount of ATPs are available in the diabetic muscle sarcoplasm for a single muscle twitch and the activity of Ca^{++} pump is also unaffected in early stages of T2DM.[17]

The MFTT and maximum muscle tension after fatigue protocol of the EDL muscle require large number of ATPs for tetanic contraction due its abundant fast fibres and is provided by already stored muscle glycogen. Glycogen synthase activity is halted in T2DM because of insulin resistance and oxidative stress causing significant reduction occurs in glycogen stores and ability to reuptake glucose.[18] Therefore, maximum tension generated and muscle tension following fatigue protocol in diabetic muscles is significantly less than controls and ALA group. ALA decrease oxidative stress by reducing ROS and improved insulin sensitivity leading to restoration of glycogen synthase activity which improves glycogen storage and increase glucose reuptake thus providing large amount of ATPs to produce maximum tension and improved contraction force after fatigue protocol comparable to the controls muscles.[19]

Hong OK *et al*[20] has extensively explored the role of ALA in preservation of skeletal muscle mass (gastrocnemius) in type 2 diabetic OLETF rats similar to our settings which further establish the role ALA on skeletal muscles of diabetic rats. However his work was only on muscle mass and weight and not entirely on contractile functions.

Our study explored the beneficial effects of ALA on contractile functions of fast skeletal muscles and oxidative stress in diabetic rats and can be exogenously used as an adjunct therapy in treating T2DM induced cases of contractile dysfunction of skeletal muscles.

LIMITATIONS OF STUDY

The mechanism of action of ALA on glucose transporters and contractile proteins in EDL muscles in male diabetic rats could be explored in depth by immunohistochemistry *in vivo*.

CONCLUSION

Alpha-lipoic acid supplementation aid in restoring muscle contractile performance in T2DM by effectively reducing oxidative stress and restores metabolic equilibrium leading to improved muscle function, increased maximum tension, and enhanced recovery from fatigue.

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ORIGINAL ARTICLE

METAL-CERAMIC PROSTHESIS PITFALLS: A CLINICAL CROSS-SECTIONAL STUDY AT A TERTIARY CARE HOSPITAL IN PAKISTAN

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Background: Tooth-supported Metal Ceramic Fixed Dental Prosthesis (MCFPDs) offers strength, aesthetics, and affordability, but often face minor complications. This study documents common complications of MCFPDs at a tertiary care hospital in Pakistan. **Methods:** This 12-month cross-sectional study included 150 patients with MCFDP complications. Data were collected through patient examination and records, and analyzed on SPSS-23 with Chi-square test, considering $p \leq 0.05$ as significant. **Results:** A total of 150 patients (63 males, 87 females) with a mean age of 38±8 years (Range: 20–60 years) were examined for complications in tooth-supported fixed-fixed metal-ceramic prostheses. Most belonged to high (52.7%) and mid (46%) socioeconomic status (SES); 1.3% were low-income. Education levels included primary (34.7%), secondary (40%), and higher (25.3%). Among 566 MCFDP units (323 retainers, 243 pontics), complications were more frequent in the maxillary arch (56%) and in anterior (32.7%) right posterior (32.7%) and left posterior regions (30.0%). Biological failures (78.7%), mainly secondary caries (35.3%) dominated. Failures occurred mostly in general practice (96.7%), and after 12 years (22%). **Conclusion:** The most prevalent fixed dental prosthesis (FDP) complication was secondary caries, followed by periodontal compromises. Dental practitioners must ensure effective case selection, diagnosis, and treatment plan.

Keywords: Biological Complications, FDP, Fixed-Fixed Metal Ceramic Fixed Dental Prosthesis, Frequency, MCFDP, Removable partial denture, RPD, Technical Complications

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INTRODUCTION

Metal-ceramic tooth-supported FDPs have been widely used since the mid-20th century for their durability, affordability and aesthetics.[1,2] Despite these advantages, they are associated with both biological and technical complications.[1] Most issues like Level-1 complications are minor and manageable, while Level-2 complications may require prosthesis removal or replacement.[1,3]

Tooth loss affects both functional and psychological health.[4] Treatment options include removable and fixed prostheses, implants, and overdentures, with patients' choices influenced by factors such as age, gender, education, and socioeconomic status (SES).[4,5]

Despite advances, conventional FDPs remain reliable and cost-effective. Clinical success relies on proper design, patient compliance, and regular follow-up.[6,7] Careful selection of pontics, retainers and connectors ensures stability and aesthetics.[8] Metal-ceramic prostheses, especially porcelain-fused-to-metal (PFM), remain common FDP option despite aesthetic limitations of some metal alloys.[9] Abutment tooth evaluation is critical, including assessment of crown height, crown-root ratio, and periodontal condition.[1]

Short clinical crowns may require interventions such as crown lengthening.[7] A ferrule of 1.5–2 mm enhances fracture resistance, often necessitating endodontic treatment.[10] Alternatives like telescopic retainers and non-rigid connectors are beneficial in cases with compromised abutments or longer spans.[11]

Common causes of FDP failure include caries, periodontal disease, de-cementation, and material fracture.[1,12] Accurate margin placement and proper cement selection are vital for long-term success. Composite resin cements are favoured for their bond strength and aesthetics.[13] Aesthetic issues such as shade mismatch or ceramic fracture can impact outcomes but are manageable with proper planning.[8,12,14] Reported survival rates for FDPs remain high over extended periods when complications are proactively addressed.[15]

This study aims to assess clinical complications of metal-ceramic prostheses to improve management and outcomes in a tertiary care setting.

METHODOLOGY

This 12-month (Sep 2020 to Aug 2021) cross-sectional study at Peshawar Dental College included 150 patients, using consecutive sampling, based on a 42% assumed MCFDP complication rate at 95% confidence interval. Socio-demographic data and prosthesis details usage duration and retention type were documented and clinical examination like abutment condition were undertaken to assess biological and technical complications of MCFDPs, categorized by type and severity (Level-1: manageable; Level-2: needing replacement).

Inclusion criteria were partially dentate patients aged 20–60 years with MCFDP complications, consenting to participate. Exclusion criteria were non-study site prostheses, implants, RPDs, edentulous uncooperative patients, systemic illness, or who were unable to undergo clinical examination.

Data were analyzed on SPSS-23. Descriptive stats with continuous data were expressed as Mean±SD/IQR and categorical as frequency and percentage. Chi-square test was applied and $p \leq 0.05$ was considered statistically significant.

RESULTS

A total of 150 patients, 63 (42%) males and 87 (58%) females were examined for common complications in tooth-supported fixed-fixed design MCFDPs. Eighteen (12%) patients from 20–30 years, 31 (20.7%) from 31–40 years, 50 (33.3%) from 41–50 years and 51 (34%) were from 51–60 years' age groups with a mean age of 38±8 years. Five (3.3%) failures occurred in specialist clinic (postgraduate) and 145 (96.7%) failures occurred in general clinics (undergraduate).

There were 566 units of MCFDPs, out of which 323 were retainers and 243 were pontics. The frequency of the number of units and complications recorded in each arch (maxillary and mandibular) as well as those recorded in both regions (posterior region and anterior region) are tabulated in (Table-1).

Table-1: Distribution of abutments/retainers, pontics, and sites of prosthetic complications

Parameter	Frequency (%)
Number of Abutments/Retainers	
2	134 (89.3)
3	14 (9.3)
5	1 (0.7)
8	1 (0.7)
Number of Pontics	
1	82 (54.7)
2	54 (36)
3	11 (7.3)
4	1 (0.7)
7	1 (0.7)
8	1 (0.7)
Number of Units	
3	79 (52.7)
4	54 (36.0)
5	6 (4.0)
6	8 (5.3)
7	1 (0.7)
12	1 (0.7)
16	1 (0.7)
Arch Type	
Maxillary	84 (56.0)
Mandibular	66 (44.0)
Location of the FPD within the Arch	
Right posterior	49 (32.7)
Left Posterior	48 (30.0)
Anterior	49 (32.7)
Anterior and Posterior	4 (2.7)

Majority (118, 78.7%) of the complications were biological, whereas 32 (21.3%) were technical. Secondary caries (53, 35.3%) was the most common complication followed by periodontal disease (36, 24%), pulp pathology (29, 19.3%), and de-cementation (17, 11.3%). The least common complication was unacceptable aesthetics (15, 10%). (Table-2).

Table-2: Biological and technical complications in relation to prosthesis duration of use [n (%)]

Complication Level	Biological Complications 118 (78.7%)		Technical Complications 32 (21.3%)	
	Secondary caries/ pulp pathology	Periodontal complication	Decementation	Unacceptable aesthetics
Level 1	53 (35.3)	21 (14.0)	11 (7.3)	12 (8.0)
Level 2	29 (19.3)	15 (10.0)	6 (4.0)	3 (2.0)
Missing	68 (45.3)	114 (76.0)	133 (88.7)	135 (90.0)

Most (145, 96.7%) of the patients reported failures at general dental clinics (undergraduate), while (5, 3.3%) at the specialist/private clinics (postgraduate). Failures were more common after 12 years (33, 22%), 11 years (12, 8.0%), 10 years (12, 8.0%), and 6 years (12, 8.0%). (Table-3).

Table-3: Duration of prosthesis used by the patients

Duration (Years)	Frequency (%)	Duration (Years)	Frequency (%)
2	2 (1.3)	**12**	33 (22.0)
3	2 (1.3)	**13**	9 (6.0)
4	3 (2.0)	**14**	2 (1.3)
5	4 (2.7)	**15**	9 (6.0)
6	12 (8.0)	**16**	6 (4.0)
7	7 (4.7)	**17**	1 (0.7)
8	10 (6.7)	**18**	9 (6.0)
9	8 (5.3)	**19**	1 (0.7)
10	12 (8.0)	**24**	5 (3.3)
11	12 (8.0)	**26**	3 (2.0)

DISCUSSION

This study evaluated complications associated with tooth-supported fixed-fixed designed MCFDPs in 150 patients, with a higher (58%) proportion of females. The majority were aged 41–60 years, with a mean age of 38 years. Similar age and gender trends were reported by Khan M *et al*[16], highlighting mid-life as a common period for prosthetic intervention. This implies that middle-aged adults are most likely affected by FDP-related complications due to cumulative oral health challenges.

Anterior and right posterior regions showed the highest complication frequencies. Similarly, a recent study by Pol *et al*[17] found 3-unit and maxillary FDPs to be more failure-prone due to biomechanical stress and aesthetic demands. Short-span maxillary FDPs in stress-bearing zones require enhanced planning and material selection.

Biological complications exceeded technical ones. The most frequent issues were secondary caries, followed by periodontal disease, and pulp pathology.

These findings are consistent with Khan *et al*[18] who identified carries and periodontal breakdown as major causes of FDP failure. Therefore, biological maintenance is key to long-term FDP survival, emphasizing oral hygiene and periodic monitoring.

Decementation and aesthetic concerns were the main technical complications in our study which was depicted in an international study[19]. This underscores that the clinician's technique and material choice significantly influence technical success.

Although failures occurred mostly after 10 years, especially in general dental clinics in comparison to specialist/postgraduate dental settings, this finding was not found to be popular in the recent literature, though certain aspects can be influenced by the dentistry undergraduate students' limited clinical experience.[20] It may be deduced that specialist-led care is not as critical factor as the others were for extending FDP lifespan.

CONCLUSION

Secondary caries, periodontal disease, de-cementation, and aesthetic concerns remain common complications in FDP, with a higher prevalence observed in females and the maxillary arch. It is recommended to prioritize thorough pre-operative assessment, proper case selection, and ongoing professional development to enhance treatment outcome.

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SWB: Data collection, Tabulation

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ORIGINAL ARTICLE

IMPACT OF SOCIO-DEMOGRAPHIC, LIFESTYLE AND NUTRITIONAL FACTORS ON HYPERGLYCAEMIA AMONG MALE UNIVERSITY EMPLOYEES

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Background: Hyperglycaemia poses significant health risks. This study investigates hyperglycaemia among male university employees due to their high-risk lifestyle factors and higher prevalence of diabetes in Pakistani males. **Methods:** The study was conducted among male university employees (teaching and support staff, n=243) at a university in Peshawar, Pakistan. Socioeconomic and lifestyle data were collected using validated questionnaires. Nutritional assessments included physical measurements and diet quality evaluation. Weight, height, and waist circumference (WC), were taken with standardized tools. Body composition parameters, including body mass index (BMI), total body fat (TBF), and visceral body fat (VBF), were assessed using validated techniques. Diet quality was evaluated through subjective measures. Fasting blood glucose (FBG) levels were determined with a glucometer. **Results:** Significant associations were observed between hyperglycaemia and anthropometric indicators, as well as body composition. Hyperglycaemic individuals had higher BMI (30.5 *vs* 24.9 Kg/m²), TBF (29.4% *vs* 22.2%), VBF (13.6% *vs* 8.3%), and WC (100.0 Cm *vs* 90.7 Cm), indicating central obesity. Hyperglycaemic participants also exhibited lower physical activity levels and poorer sleep quality. Linear regression analysis revealed significant predictors of FBG levels, with TBF (β=1.63, p<0.05) and BMI (β=1.80, p<0.05) positively associated, while higher consumption of the five food groups was negatively associated (β=-4.00, p<0.05). **Conclusion:** The findings underscore the importance of body composition, particularly BMI and TBF, in predicting hyperglycaemia. Promoting balanced diets and minimizing ultra-processed food consumption are essential strategies to mitigate hyperglycaemia risk among university employees.

Keywords: Body Mass Index, Diet, Hyperglycaemia, Life Style, Obesity, Sleep Quality

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INTRODUCTION

Glycaemic status, an essential measure of blood glucose concentration, serves as a key indicator of metabolic health. Maintaining optimal glycaemic levels is critical for physical and psychological well-being, as it significantly influences energy, mood, and overall health.[1,2]

Global projections indicate a rising prevalence of hyperglycaemia, increasing to an anticipated 10.9% (700 million) by 2045 from 9.3% (463 million) in 2019.[3] In Pakistan, the diabetes prevalence among adults reached 26.7% by 2022, with 33 million reported cases.[4] The disease burden is exacerbated by gender disparities and limited screening facilities, particularly in rural areas, necessitating urgent interventions to mitigate its impact on morbidity and mortality.[5]

Metabolic health is intricately linked to anthropometric and biochemical markers, such as blood glucose levels, reflecting the efficiency of energy metabolism pathways. Factors like age, lifestyle, dietary habits, and physiological conditions significantly influence glycaemic health.[6] Research demonstrates that balanced diets and regular physical activity correlate with healthier glycaemic profiles, while sedentary behaviours and poor dietary practices are associated with adverse outcomes.[7]

Obesity and hyperglycaemia are interrelated challenges that elevate the risks of cardiovascular diseases, diabetes, and certain cancers.[8] Overweight and obesity are the 5th leading causes of global mortality.[9] Sedentary job roles, common among university employees, exacerbate obesity and related metabolic disturbances. Dietary irregularities and low physical activity exacerbated these risks, highlighting the importance of targeted interventions.

This study investigates hyperglycaemia among male university employees due to their high-risk lifestyle factors and higher prevalence of diabetes in Pakistani males. The goal was to identify trends in hyperglycaemia across age groups and recommend healthier lifestyles for mitigating metabolic disorders.

MATERIAL AND METHODS

This cross-sectional study was carried out at the University of Agriculture, Peshawar, from Nov 2022 to Nov 2024. The initial sample size was 250, but due to

the minimal representation of female participants, they were excluded from the study. The final sample comprised 243 randomly selected male university employees. Participants included both teaching and support staff. Inclusion criteria were: university employees residing in Peshawar, free from any chronic diseases, (e.g., diabetes, hypertension, cardiovascular disease, kidney disease, or other metabolic disorders), and not on regular prescription, (i.e., medication taken consistently for ≥3 months). Exclusion criteria were: non-university employees, non-residents of Peshawar, individuals with chronic diseases, and those on regular prescription. Participants were selected from departmental records provided by the university registrar. Informed consent was obtained. Participation in the study was voluntary.

Socio-demographic, medicinal history, physical activity level, nutritional intake, and diet quality data were gathered using structured questionnaires. Anthropometric assessments and body composition evaluations were conducted using validated equipment. Weight and height were measured using a digital scale and stadiometer, respectively. Body mass index (BMI) (Kg/m²) was calculated and categorised per WHO 2001 guidelines as: Underweight (<18.5 Kg/m²), Normal (18.5–24.99 Kg/m²), Overweight (25–29.99 Kg/m²), and Obese (≥30 Kg/m²).[10] Waist circumference (WC) was measured with a tape, with cut-off values of ≥90 Cm for males indicating central obesity.[11]

Body composition, including total body fat (TBF) and visceral body fat (VBF), was assessed using an Omron BF-508 body composition monitor.[12] Fasting blood glucose (FBG) levels were measured using a glucometer via the finger-prick. The blood glucose levels were categorized into two groups: normal (below 100 mg/dL) and hyperglycaemia (above 100 mg/dL).[13]

Diet quality was assessed using the validated Diet Quality Index (DQI)[14] which evaluates intake across five food groups: vegetables, fruits, nuts and seeds, legumes, and animal foods. A maximum score of 5 indicates consumption of all groups. Additional scores included: non-communicable disease (NCD) Protect Score (0–9): Reflects adherence to global dietary recommendations (GDR) for non-communicable disease prevention, emphasizing protective food groups. Non-communicable disease (NCD) Risk Score (0–9): Measures intake of unhealthy food groups, with higher scores indicating greater consumption. Global Dietary Recommendations (GDR) Score (0–18): Combines protective and risky dietary factors, with scores ≥10 indicating adherence.

Physical activity was assessed using the International Physical Activity Questionnaire (IPAQ), with MET values categorized as low (<600 MET-min/week), moderate (600–1500 MET-min/week), or high (>1500 MET-min/week).[15] Sleep quality was evaluated using the Pittsburgh Sleep Quality Index (PSQI), with scores >5 indicating poor sleep quality.[16]

Data were analysed using SPSS-20 and Microsoft Excel. Mean±SD and frequencies and percentages were calculated for relevant variables. Independent *t*-tests assessed mean differences, and chi-square tests examined associations between categorical variables. Linear regression identified predictors of glycaemic status, with results presented as β-coefficients and 95% CI with $p \leq 0.05$ taken as statistically significant.

RESULTS

The mean age of total 243 participants was 38.5±6.6 years. Educational levels varied, with 118 (48.6%) holding university degrees. Majority (222, 91.4%) were married, with an average age at marriage as 26.1±5.3 years. Most respondents (214, 88.1%) were non-smokers, and 119 (49%) resided in rural areas. The reported average monthly income was PKR 63,400, with 131 (53.9%) living in joint families. Participants had an average family size of 11±4.9 members and an average of 4 children. Mean job duration was 12±7 years. (Table-1).

Hyperglycaemic individuals exhibited significantly higher weight, body mass index, waist circumference, body fat percentage, and visceral fat levels. The prevalence of overweight/obesity and central obesity among hyperglycaemic individuals was 52 (100%) and 41 (78.8%), respectively. The mean fasting blood glucose (FBG) level was 99.6±42.9 mg/dL. This group also reported lower physical activity levels (PAL-MET) and poorer sleep quality, as indicated by higher Pittsburgh Sleep Quality Index (PSQI) scores. The percentage of disturbed sleep (PSQI>5) was significantly higher (78.4%) in the hyperglycaemic group compared to normal group (35.9%), ($p<0.001$). (Table-2).

Table-1: Socio demographic characteristics of the university employees

Characteristics		Mean±SD/[n (%)]
Age (Years)		38.5±6.6
Education	Primary or below	44 (18.1)
	SSC/HSSC	81 (33.2)
	University	118 (48.6)
Job duration (Years)		12.0±7.0
Marital status	Married	222 (91.4)
Age at marriage (Years)		26.1±5.3
Residence	Rural	119 (49.0)
	Urban	124 (51.0)
Smoking status	Non-smokers	214 (88.1)
Monthly income in thousands (PKR)		63.4±61
House ownership	Own	200 (82.3)
Family type	Joint	131 (53.9)
Family size		11±4.9
No. of children in family		4±1.9

(PKR: Pakistani rupees, SSC: Secondary School Certificate, HSSC: Higher Secondary School Certificate)

Table-2: Physical health status by glycaemic status

Indicators	Glycaemic status Mean±SD/[n (%)]		*p*
	Normal	Hyperglycaemic	
Weight (Kg)	70.6±14.3	88.2±19.3	<0.0001
Height (Cm)	167.8±6.6	168.0±6.9	NS
BMI	24.9±4.5	30.5±7.0	<0.0001
Nutrition Status (based on BMI)			
Normal	124 (62.6)	0	<0.0001
Overweight/Obese	74 (37.4)	52 (100)	
Waist circumference (Cm)	90.7±11.4	100.0±11.5	<0.0001
Central Obesity Status (based on Waist circumference)			
Normal	108 (54.5)	11 (21.2)	<0.0001
Central obesity	90 (45.5)	41 (78.8)	
Total body fat	22.2± 7.2	29.4± 7.1	<0.0001
Visceral fat	8.3± 4.1	13.6± 6.0	<0.0001
Fasting blood glucose	78.8±6.5 192 (79)	177.6±30.0 51 (21)	<0.001
PAL-MET	1090.7±780.7	638.9± 405.2	<0.0001
PSQI-Score	4.7±2.1	5.4±1.1	<0.021
Physical Activity Level (based on MET)			
Low level activity<600	78 (40.6)	41 (80.4)	<0.001
Moderate activity 600–1500	62 (32.3)	8 (15.7)	
High level activity>3000	52 (27.1)	2 (3.9)	
Sleep Quality			
Normal (PSQI≤5)	123 (64.1)	11 (21.6)	<0.001
Disturbed Sleep (PSQI>5)	69 (35.9)	40 (78.4)	

Age, job duration, family size, body mass index, waist circumference, total body fat, visceral fat, and physical activity levels were positively related with FBG levels (p<0.05). Dietary factors, including adherence to all 5 food groups, NCD Protect scores, GDR, and fibre intake, demonstrated protective effects (p<0.05). Notably, the non-communicable disease NCD risk score showed a positive association with hyperglycaemia risk (p<0.05). (Table-3).

Table-3: Unadjusted determinants of fasting blood glucose (Unadjusted analysis)

Determinants	β-coefficients (95% CI)
Body mass index (BMI)	3.66 (2.82–4.50)***
Waist circumference	1.35 (0.94–1.77)*
Visceral fat	3.99 (3.05–4.94)***
Total body fat	2.69 (2.05–3.32)***
Age	1.42 (0.63–2.22)*
Job duration	1.19 (0.43–1.94)*
Family size	1.84 (0.77–2.91)**
All five food groups	-11.2 (-15.3– -7.12)***
NCD Protect score	-3.10 (-5.47– -0.72)*
NCD risk score	3.13 (1.56–4.70)***
GDR	-3.61 (-4.98– -2.24)***
Fiber	-0.47 (-0.80– -0.14)**
PAL	-0.01 (-0.02– -0.00)***

(*p<0.05, **p<0.01, ***p<0.001)

BMI (β=1.799, p<0.01) and total body fat (β=1.630, p<0.001) were positively associated with fasting blood glucose, while consumption of all five food groups (β=-7.657, p<0.001) had a protective effect. In contrast, the unadjusted analysis showed a broader range of factors associated with fasting blood glucose, including age, job duration, family size, waist circumference, visceral fat, and physical activity levels, as well as dietary factors like NCD Protect scores, GDR, and fibre intake. (Table-4).

Table-4: Adjusted determinants of fasting blood glucose (Adjusted Analysis)

Determinants	β-coefficients (95% CI)
Age	0.231(-0.52–0.98)
Family size	0.479 (-0.53–1.49)
Body mass index (BMI)	1.799 (0.64–2.95)**
Total body fat	1.630 (0.79–2.46)***
All five food groups	-7.657 (-11.65–-3.66)***
NCD risk score	0.550 (-0.93–2.03)
PAL	0.004 (-0.00–0.01)

(**p<0.01, ***p<0.001)

DISCUSSION

Our study confirms that socio-demographic factors like age, job duration, and large family size are strongly associated with elevated FBG levels, consistent with previous work.[17,18] Biological mechanisms underlying hyperglycaemia involve complex interactions between insulin resistance, pancreatic beta-cell dysfunction, and lifestyle factors. Adiposity, particularly central obesity, contributes to insulin resistance by promoting chronic inflammation and altering adipokine secretion leading to impaired glucose uptake in skeletal muscle and increased glucose production in the liver, ultimately resulting in hyperglycaemia.[19] University employees, given their sedentary lifestyles and unhealthy dietary habits, are particularly susceptible to glycaemic dysregulation.[20,21] Lower physical activity levels and suboptimal sleep quality exacerbate risk of hyperglycaemia.[22,23] Consistent with previous work[24], BMI, TBF, and VF were the significant predictors of elevated FBG. Excess adiposity is a primary driver of insulin resistance, a precursor to hyperglycaemia.[25] Effective management of these parameters is crucial for glycaemic control.

Recent studies have highlighted the role of dietary factors in modulating insulin sensitivity and glucose metabolism. Diets rich in fruits, vegetables, and whole grains, which are high in fibre and antioxidants, have been shown to enhance insulin sensitivity and reduce inflammation. Dietary adherence to all five food groups and higher fibre intake were inversely associated with fasting blood glucose levels, highlighting the protective role of a balanced diet. The fibre content in these diets can slow down glucose absorption, reducing the peak blood glucose levels and insulin demand.[26,27] These findings underscore the importance of dietary quality and lifestyle interventions in mitigating hyperglycaemia risk.

Our findings support the development of targeted interventions emphasizing weight management, dietary improvements, and physical activity. These strategies can effectively address hyperglycaemia and associated metabolic risks. The role of dietary diversity, particularly through adherence to GDR and NCD Protect scores, further underscores the need for comprehensive public health initiatives aimed at promoting healthier lifestyles.

CONCLUSION

The study highlights the significant influence of anthropometric factors, particularly BMI and total body fat, on fasting blood glucose levels. Dietary diversity and fibre intake serve as protective factors against hyperglycaemia. These findings emphasize the importance of dietary and lifestyle interventions in glycaemic regulation, suggesting that strategies focused on weight reduction, balanced nutrition, and increased physical activity can effectively reduce the burden of hyperglycaemia.

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ORIGINAL ARTICLE

COMPARISON OF ESTRADIOL LEVELS IN RAT MODEL OF CHRONIC ALTERNATING STRESS

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Background: Stress is a prevalent factor in our daily lives and is affecting the reproductive system too. The objective of this study was to compare the effect of chronic alternating stress on two generation of rats by evaluating the corticosterone and estradiol levels. **Methods:** One hundred and thirty-six healthy wistar albino rats 11 weeks old were used in this experimental case-control study. They were distributed into case and control parent groups after assaying for baseline parameters. Case parents were given three weeks of chronic alternating stress while the control parents were not given any stress. Following mating within respective groups the offspring were placed into 5 groups each. One group was assayed at 5 weeks without any stress, the other was given 3 weeks of stress and assayed at 8 weeks, the third group was given stress at the age of 5 and 11 weeks, one group was given stress only at 11 weeks of age. There were two control groups which were offsprings that did not receive any stress and were assayed at the ages of 8 weeks and 11 weeks. Blood sera were assayed for corticosterone and estradiol using Enzyme Linked Immunosorbent Assay Techinique. In the offspring generation behavioural tests were done and analysed. **Results:** The rats given early life stress had raised corticosterone levels and decreased estradiol levels shown by significant *p*-values. **Conclusion:** The early life stressed rats did not fare well as compared to rats given repeated stress.

Keyword: Chronic Alternating Stress, Corticosterone, Estradiol

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INTRODUCTION

The concept of stress was introduced by Hans Selye who implied that stress could weaken the organism and lead to the development of diesease.[1] Stress can make an organism prone to a lot of diseases like diabetes[2], heart diseases[3], obesity[4] and psychiatric and psychological diseases.[5] Crohn's disease and ulcerative colitis symptoms are exacerbated by stress.[6]

Stress is now thought to be linked to diseases in early childhood. If the parent had been exposed to stress be it mother or father this affects the fetus and results in the fetus being vulnerable to many psychological disorders like autism spectrum dieseases and other diseases later in life. Prenatal stress has been proved now to change the hypothalamic-pituitary-adrenal axis activity.[7]

The hypothalamo-pituitary-adrenal axis (HPA axis) has an important role to play in homeostasis which is the maintenance of a balanced internal environment and is important in immunity, metabolism and cardiovascular functions. A hypothalamic nucleus called the paraventricular nucleus releases corticotropin-releasing factor (CRF) which causes the secretion of adrenocorticotropic hormone (ACTH). This causes the secretion of corticosterone (CORT) from the cortex of the adrenal gland which also feeds back negatively to the brain.CORT in rats and cortisol in humans is a glucocorticoid hormone which is a stress hormone and is released in stressful situations and also in low glucose levels in the blood. It is an immunosuppressant and increases the blood glucose concentration. It has an important role to play in the metabolism of fat, carbohydrate and protein.[8]

Stress response depends on the HPA axis activation which inhibits the release of Gonadotropin Releasing Hormone (GnRH) from the hypothalamus. This inhibition is mediated by the releasing factor for ACTH called the CRF. The glucocorticoids released during stress decrease the secretion of Leuteinising Hormone (LH) from the pituitary thereby inhibiting the release of estrogen and progesterone from the ovary. This causes stress-induced hypothalamic amenorrhea and this has been seen in anxiety, depression, malnutrition and excessive chronic exercise. Studies on animal model also suggest that stress can affect the menstrual cycle in a manner which is insufficient to cause amenorrhea but can cause infertility.[9]

Genes can be altered when stress is given to parents. The physiology of the offspring may be altered. If the same stress is given to the offspring, they can either succumb to the stress or become resilient to stress. We could not use humans to find this out, so we designed a rat model. Our objective was to find the effect of chronic unpredictable stress on parents (P) and offsprings (F-1) of stressed and non-stressed rats by comparison of corticosterone and estradiol levels.

MATERIAL AND METHODS

This was an experimental case control study carried out on rats which spanned for a duration of 2 years. Ethical approval was given by Ethical Committee of Khyber Medical University (Reference No. DIR-KMU-EB/HS/000675) and from Peshawar Medical College ethical committee (Reference No=Prime/IRB/2023-207). Sample size was calculated with the help of resource equation and there were 136 rats in the parent generation while in offspring generation there were 156 rats in the offspring generation which were distributed via random sampling into various groups. In the parent generation we included healthy stress free 11 weeks old rats while unhealthy, stressed or pregnant rats were excluded. There were two groups of parents, the case parents to whom we gave stress and the control parents to whom we did not give any stress. The offspring of case parents and control parents were kept separate from each other and were analysed at the age of 5, 8, 11 and 14 weeks.

A total of 136 wistar albino rats aged 11 weeks were included in the parent generation and were all checked for anxiety by behavioural tests. The rats which were showing stressed behaviour on the behavioural tests were removed. The remaining rats were grouped into the control group and the case group. We subjected the case parents to three weeks of stress and then checked them with the help of behavioural tests for induction of stress. We removed those which were not stressed. The stressed parents were allowed to mate and their offsprings were called the case offsprings who were divided into 6 groups. To the control group of parents we did not give any stress and were allowed to mate at the same time as the case parents. Their offsprings were then divided into 6 groups. Both the groups of offsprings were given stress, some were given early life stress, some were given late life stress while some were given both. There were also offsprings who were not given any stress and served as controls. Blood sampling was done from both the parent groups and their offsprings and we took blood from 10 rats in each group and extracted blood via intracardiac puncture.[10] Blood sera were assayed for stress hormones using Enzyme Linked Immunosorbent Assay Techinique. In the offspring generation we recorded and analysed behavioural tests from 12 rats.

The stress protocol comprised of 3 stressors alternating with each other. On day 1 there was alteration of circadian rhythm when lights were turned on at night time and it was dark during the day time.[11] On day 2 there was immersion in cold water at 15 to 18 °C for 5 minutes with the height of water column of 15 Cm.[12] On day 3 we subjected them to restraint stress for 2 hours where they were kept in a cylindrical tube having ventilation holes but they could not move inside the tubes.[13] After 3 weeks of alternating stressors the rats were subjected to behavioural tests on 22nd day. Two behavioural tests were used, one was hole board test and the other was open field test.[14] The statistical analysis was done on SPSS-25. The data were not normally distributed. Kruskal Wallis test and Mann Whitney U test were used to find out differences between the groups, and $p \leq 0.05$ was taken as significant.

RESULTS

The offspring and parent groups are explained in Table-1. 'P1' stand for parent generation and 'F1' stands for first filial generation. 'A' stand for the case parents or the offsprings of case parents while 'B' stands for control parents or offsprings of control parents. In a rat study the offsprings of the same parents like the case parents are considered as the same and the point of assaying of an offspring can be generalised to all the offsprings of the same parents so the early life stressed offspring of the case parent is taken as representative of the early life stress given rats in F1A2 which was the group given stress two times except that the rat in F1A2 after early life stress was not assayed and was assayed after late life stress, at the age of 14 weeks.

Table-1: Description of various experimental groups

Abbreviation	Group description
P1B	Control group of parents to whom no stress was given
P1A	Case parent group to which stress was given
F1A	5 wks old rat who were the offspring of case parents and from whom preliminary parameters were collected
F1A1	Offspring of case parents given early life stress from 5 wks to 8 wks and were assayed at 8 wks
F1A2	Offsprings of case parents given stress twice once at 5 wks and the other at 11 wks and they were assayed at 14 wks age
F1A3	Offsprings of case parents given late life stress at 11 wks and were assayed at 14 wks
CtF1A1	Case parent offsprings not given stress and assayed at 8 wks
CtF1A2	Case parent offspring not given stress and assayed at 14 wks
F1B	5 wks old rat who were the offspring of control parents and from whom preliminary parameters were collected
F1B1	Offspring of control parents given early life stress from 5 wks to 8 wks and were assayed at 8 wks
F1B2	Offsprings of control parents given stress twice once at 5 wks and the other at 11 wks and they were assayed at 14 wks age
F1B3	Offsprings of control parents given late life stress at 11 wks and were assayed at 14 wks
CtF1B1	Control parent offsprings not given stress and assayed at 8 wks
CtF1B2	Control parent offsprings not given stress and assayed at 14 wks

The corticosterone levels were increased in the case parents P1A when compared with P1B ($p<0.01$) and to its offsprings. F1A1 had significantly raised corticosterone levels as compared to the F1A offspring who were assayed at 5 weeks before starting early life stress ($p=0.03$). F1B1 had significantly raised corticosterone levels as compared to F1B ($p=0.05$), CtF1B1 ($p=0.01$) and Ct F1B2 ($p<0.01$). The control group offsprings had decreased stress levels as shown by significantly decreased corticosterone levels as compared to the offspring given stress. Early life stressed offspring were more stressed as compared to the rest of the offsprings. (Table-2, 3).

Table-2: Corticosterone in groups showing significant *p* shown in bold in various groups

Groups	P1A	P1B	F1A	F1A1	F1A2	F1A3	CtF1A1	CtF1A2	F1B	F1B1	F1B2	F1B3	CtF1B1	CtF1B2
P1A		**<0.01***	**<0.01***	**0.03***	**0.05***	**0.02***	**<0.01***	**<0.01***	**<0.01***	0.32	0.49	0.08	**<0.01***	**<0.01***
P1B			0.59	**<0.01***	0.71	0.08	0.49	0.08	0.29	**<0.01***	0.08	**0.02***	0.07	0.19
F1A				**0.03***	0.88	0.14	0.82	0.13	0.73	**0.01***	0.11	**0.04***	**0.03***	0.11
F1A1					0.59	0.62	0.13	0.73	0.29	0.13	0.25	0.49	**0.01***	**<0.01***
F1A2						0.65	0.82	0.76	0.71	0.08	0.22	0.36	0.19	0.17
F1A3							0.23	0.88	0.41	0.17	0.32	0.36	**0.02***	**0.01***
CtF1A1								0.13	0.88	**0.04***	0.29	0.15	**0.03***	**0.05***
CtF1A2									0.49	0.08	0.33	0.25	**0.02***	**0.01***
F1B										**0.05***	0.29	0.17	**0.03***	0.07
F1B1											0.82	0.52	**0.01***	**0.00***
F1B2												0.571	0.820	**0.03***
F1B3													**0.04***	**0.02***
CtF1B1														0.59
CtF1B2														

*Significant

Table-3: Levels of Corticosterone in various experimental groups (Mean±SEM)

Groups	Corticosterone (ng/mL)
P1B	53.57±7.13
P1A	187.82±48.24
F1A	60.06±7.66
F1A1	93.16±12.82
F1A2	84.58±21.36
F1A3	89.67±15.93
Ct F1A1	71.33±16.81
Ct F1A2	83.25±13.90
F1B	80.11±16.18
F1B1	131.70±18.47
F1B2	122.57±23.16
F1B3	111.90±20.07
Ct F1B1	49.33±19.98
CtF1B2	40.20±10.08

Estradiol levels were significantly decreased in stressed parents P1A compared to control offspring CtF1B1 (p=0.01) CtF1B2 (p<0.01). F1A1 had significantly decreased estradiol levels compared to F1A (p=0.04), F1A2 (p=0.04), F1A3 (p=0.03) and Ct F1A2 (p=0.03). Thus late life stressed rat fared better than the early life stress given rat. The stress given offspring of control parents showed that F1B2 had the highest estradiol level followed by F1B3 and the early life stress given rat fared the worst. Offspring of control parents F1B1 which were early life stress given offspring had decreased estradiol levels compared to F1B2 (p=0.03), CtF1B1 (p=0.02), and CtF1B2 (p=0.01). The offsprings who were given stress at early and at late life seemed resilient than the rest (Table-4, 5).

Table-4: Table showing significant *p* in levels of estradiol in different groups

Groups	P1A	P1B	F1A	F1A1	F1A2	F1A3	CtF1A1	CtF1A2	F1B	F1B1	F1B2	F1B3	CtF1B1	CtF1B2
P1A		0.09	0.71	**0.05**	0.88	0.59	0.82	0.59	0.45	0.71	0.08	1.0	**0.01**	**<0.01**
P1B			**0.05**	**0.01**	0.09	0.29	**0.05**	0.49	**0.04**	0.22	0.82	0.15	0.94	0.32
F1A				**0.04**	0.65	0.45	0.94	0.29	0.54	0.36	**0.04**	1.0	**0.01**	**<0.01**
F1A1					**0.04**	**0.03**	0.08	**0.03**	0.17	**0.04**	**<0.01**	0.17	**<0.01**	**<0.01**
F1A2						0.49	0.76	0.54	0.23	0.36	**<0.01**	0.94	**<0.01**	**<0.01**
F1A3							0.55	0.76	0.19	0.82	0.17	0.76	**0.05**	**0.02**
CtF1A1								**<0.01**	0.71	0.45	0.11	0.88	**<0.01**	**<0.01**
CtF1A2									0.23	0.76	0.49	0.45	0.26	0.13
F1B										0.19	**0.019**	0.94	**0.01**	**<0.01**
F1B1											**0.03**	0.94	**0.02**	**0.01**
F1B2												0.76	0.36	0.23
F1B3													0.41	0.08
CtF1B1														0.41
CtF1B2														

*Significant

Table-5: Levels of Estradiol in pg/mL in various experimental groups (Mean±SEM)

Groups	Estradiol (pg/mL)
P1B	7.97±2.86
P1A	1.75±0.57
F1A	2.002±1.02
F1A1	0.71±0.35
F1A2	0.88±0.17
F1A3	3.06±1.35
Ct F1A1	2.29±1.01
Ct F1A2	13.66±9.40
F1B	1.72±1.13
F1B1	1.94±0.90
F1B2	4.49±1.21
F1B3	4.39±1.57
Ct F1B1	5.32±1.26
Ct F1B2	16.36±8.60

DISCUSSION

The lifestyle has dramatically changed in the past few decades and so has the incidence of various diseases. Epidemiological studies provide compelling evidence that stressful early life events such as famine or war may significantly influence the likelihood of developing diseases in future generations. Stress during pregnancy is closely linked to a higher likelihood of neurodevelopmental problems, such as attention deficit hyperactivity disorder[15] and schizophrenia[16]. Both the stress experienced by the mother and father may be transmitted to their kids either directly during pregnancy or via epigenetic modifications in the germ cells.[17]

Parents who are exposed to stressors during their reproductive years can have offsprings who are either resilient to stress or become more vulnerable to stress when they grow up. In this research project we compared the effect of stress across two generations of rats and we found different responses that were dependent on the age at exposure and the number of times the stress was given.

The stressed parents had increased CORT levels as compared to the control parents. The early life stressed offspring of case parents as well as the control parents had raised CORT levels. Our research project was akin to another research in which 6 to 7 week rats were given unpredictable stress and CORT levels showed a significant increase.[18] In a similar research by Zhang *et al*, CORT levels were raised in rats given chronic unpredictable mild stress.[19] However, Toth E *et al*, took two groups of rats, one group had young 30 days old rats and the other group had 60 days old rats and subjected them to chronic mild stress. They did not observe any difference in circadian CORT levels in the young rats and suggested that it was because of development of resilience to stress however the older rats did have increased corticosterone levels.[20] They used a different stress protocol for 4 weeks and used different behavioural tests like sucrose consumption test. Furthermore they took the blood sample in the middle of the stress protocol and after 2 weeks of the completion of the stress protocol. The age of sampling was younger than our rats so that may be a reason why corticosterone levels were not raised in their younger rats.

In a research by Henry *et al*, pregnant rats were given stress and when the litters were born they were subjected to stress alongside pups who were offspring of control rats to whom no stress was given. Prenatally stressed pups aged 3 and 21 days showed increased plasma CORT levels and altered HPA axis reactivity. In the adult control rats the CORT levels dropped after 2 hours while they did not do so in the rats given prenatal stress. This may be because they were exposed to high corticosterone levels of the mother who was given stress during pregnancy.[21] This corticosterone hormone can cross the placental tissue into the fetal circulation and can also reach the brain by crossing the blood brain barrier.[22] This research had similar findings to our research however they gave prenatal stress while we gave stress before mating. In our research the rats given stress at an early age were more stressed as evidenced by stressful behaviour and increased corticosterone levels as compared to the rats given late life stress.

The CORT levels were increased in the early life stressed rats but as the offspring rats were given the same stress again a second time in late life the CORT levels decreased which showed that the rats had developed some degree of resilience to the stress.

Estradiol levels were decreased in stressed parents P1A and in the offsprings given stress. Among the offsprings who were given stress F1B2 had the highest estradiol levels followed by F1B3 and F1A3. Thus, as the rat matured its estradiol levels increased and it helped the rat to cope with stress.

The hypothalamo-pituitary-adrenal (HPA) axis has an inverse relationship with the hypothalamo-pituitary-gonadal (HPG) axis. When one is activated it impacts the other axis. Stress activates the HPA axis which inhibits the HPG axis. Estrogen and Testosterone secretion is inhibited by activation of HPA axis due to stress. When an adult female rat is given stress the sympathetic axis is activated which releases noradrenaline into the ovary resulting in a noncyling ovary with the development of cysts.[23]

There was one human research which showed that stress decreased the estradiol levels in saliva on days that the patient perceived depressed mood in the same cycle.[24]

Stressors given experimentally such as isolation stress, keeping the rats blind folded or the odors of predators like fox urine increased the CORT levels while decreasing the gonadotropin levels, lowered luteal phase progesterone levels and reduced the occurrence of estrogen-dependent sexual behaviour.[24] Increasing evidence suggests that stress inhibits reproductive neuroendocrine function by hypothalamic mechanisms that reduce pulsatile GnRH release.[22] This could be thae reason why estradiol levels were decreased in the early life stress given rats.. The rats with decreased estradiol levels after stress showed increased occurance of stressful behaviour as compared to the rats with increased estradiol levels. It was also observed in this study that the rats given stress two times did not have reduced estradiol levels after the second stress exposure which made the rat resilient.

CONCLUSION

The early life stressed offsprings had increased corticosterone and reduced estradiol as compared to the offsprings given late life stress especially the offspring given both early and late life stress. Thus some stress within physiological levels is beneficial during the growing age so as to develop resilience in adulthood.

LIMITATIONS OF THE STUDY

Our study did not investigate the molecular mechanisms underlying the stress responses observed.

RECOMMENDATIONS

Future research should focus on elucidating the molecular mechanisms underlying the observed changes in this study. Understanding these mechanisms could provide critical insights into developing strategies for better stress management.

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ORIGINAL ARTICLE

EFFECT OF PREFORMED TAPERED NICKEL TITANIUM ORTHODONTIC ARCH-WIRES ON MANDIBULAR INTER-CANINE WIDTH TO PREDICT FUTURE LONG-TERM STABILITY

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Background: Maintaining the pre-treatment mandibular inter-canine dimension is crucial for stable post-treatment retention as minor changes can increase post-treatment relapse. This study aimed to identify changes in mandibular inter-canine width caused by active orthodontic treatment using tapered preformed NiTi mandibular arch-wires and predict future stability proportionally. **Methods:** A 6 months' study at School of Dentistry Islamabad involved 60 patients with tapered mandibular arch forms, fully erupted, non-anomalous, and permanent mandibular canines. The patients underwent non-extraction fixed orthodontic mechanical therapy treatment using conventional pre-adjusted edgewise brackets. The study involved detailed clinical examination, orthodontic study casts, orthopantomograms, and lateral cephalograms. The initial pre-treatment mandibular inter-canine width was recorded using digital vernier caliper. After levelling and alignment, the mandibular cast impressions were repeated, and mid-treatment inter-canine width was calculated. **Results:** Statistically significant differences were found between pre-treatment and post-treatment widths. The mean pre-treatment width was 26.81 mm, while the mid-treatment width increased to 27.26 mm after levelling and alignment. Statistically significant differences (p=0.001) were found between pre-treatment mandibular inter-canine width (T1) to during treatment mandibular inter-canine width when considering gender. When considering age-wise analysis, there were no statistically significant differences between pre-treatment mandibular inter-canine width (T1) to during treatment mandibular inter-canine width. **Conclusion:** The commercially available preformed tapered NiTi arch-wires alter the original mandibular inter-canine width in patients which have a natural tapered mandibular arch-form, increasing the risk of relapse, hence predicting future long-term instability.

Keywords: Arch-forms, Inter-canine width, Preformed arch-wires, Stability

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INTRODUCTION

Oral health is extremely imperative for overall physical health, as health is considered as supreme assets of human life.[1,2] A successful orthodontic treatment involves accurate diagnosis, a detailed treatment plan, careful planning, and a retention strategy.[3] A good clinician correlates the morphological arch-form of patients with preformed arch-wires, which helps achieve optimal aesthetics, functional occlusion, and stability.[4] Arch-wires, metal wires, were initially fabricated in gold alloy, but stainless steel became popular due to its high yield strength and corrosion resistance.[5] Cobalt Chromium alloy was introduced, but it became obsolete due to heat treatment and additional costs.[6] Many people can't undertake treatment due to affordability issue in low income nations as socioeconomic status plays a vital role and it is also a predictor of a broad range of outcomes over the course of person's life, including their psychological and physical health.[7,8]

Australian Arch-wire was developed, and Nickel Titanium arch-wires have been improved over time, becoming the first choice for initial alignment and levelling.[9] In the modern days, orthodontics has grasped incredible goals and success.[10] Effects of certain procedures has been constantly an issue of concern for both patients and clinician.[11] Most people undertake dental treatment to get rid from pain.[12–13]

Arch-form refers to the overall configuration of the dental arch, considering symmetry, roundness, elongation, and convexity.[14] Chuck was the first to classify arch-forms as tapered, square, and ovoid, and Ricketts correlated various factors like width, size, length, bracket position, and contact details.[15]

Research on the impact of anatomical arch-forms on inter-canine width during orthodontic treatment has been limited.[16] Studies have shown that inter-canine widths decrease with treatment in all types of malocclusion and decrease after treatment, suggesting that type of malocclusion doesn't significantly affect long-term stability.[17] There is ongoing debate on the

effects of extraction *vs* non-extraction therapy, with some researchers advocating extraction and others against it.[18] A randomized clinical trial found no significant differences between self-ligating bracket systems and conventional pre-adjusted edgewise twin brackets during the alignment and levelling phase of orthodontic therapy.[19] However, little is known about the changes brought about by the use of preformed commercially available NiTi arch-wires on the most prevalent arch-forms in Pakistani population.

The objective of this study was to detect the changes in the mandibular inter-canine width brought by the use of tapered preformed NiTi arch-wires during active orthodontic treatment on the tapered mandibular arch-forms and draw the predictability of future stability proportional to these changes.

MATERIAL AND METHODS

The study was conducted at Department of Orthodontics, School of Dentistry, Islamabad, focusing on patients undergoing fixed orthodontic therapy, from May to Oct 2024. Sample size was calculated using WHO sample size calculators with 5% level of significance, 80% Power of test, 2.68 Pooled standard deviation, 94.43 Test value of the population mean, 96.21 anticipated population mean.[19] Final sample size came out to be 60.

Sample was chosen from a pool of patients reporting to the Department, undergoing fixed orthodontic therapy with tapered mandibular arch-forms as determined by template method via non-probability consecutive sampling technique. Ethical permission was taken from the hospital and patients prior to the study.

Sample comprised patients undergoing fixed orthodontic therapy with conventional pre-adjusted Edgewise brackets (0.022*0.028 MBT slot). The arch-wire material used in the treatment patients was commercially available preformed NiTi (Industrial: Ortho-care tapered) with an arch-wire sequence range (NiTi round: 0.012±0.019*0.025 NiTi rectangular). Patients of both genders with permanent erupted mandibular canines and tapered mandibular arch-forms were included in the study. Patients having gross dental anomalies (congenitally missing, ectopic, transposed, impacted teeth, cleft cases, and cleft mandibularis cases) in the mandibular labial segments, mesially or distally tilted canines, non-extraction cases, crowding with less than 5 mm in the anterior segment mandibular segment (between mandibular 3 to 3), severe skeletal malocclusion (Class II div II, Class III skeletal bases), prosthetic crowns on mandibular 3's and cross bites were excluded from the study.

The study involved detailed clinical examination, orthodontic study casts, orthopantomograms, and lateral cephalograms. Data collection procedure was performed by measuring pre-treatment mandibular inter-canine width (T1) and during treatment mandibular inter-canine width, once levelling and alignment has been achieved (T2). Data collection tools were orthodontic study models, ortho-care arch-wire templates and vernier caliper.

Measurement of pre-treatment mandibular inter-canine width (T1) and during treatment mandibular inter-canine width, once levelling and alignment was achieved (T2) were checked twice by the same observer. The differences in intra observer readings were compared by Cronbach's alpha. The second observer was blinded towards the tested data, calculated pre-treatment mandibular inter-canine widths (T1), during treatment mandibular inter-canine width, once levelling and alignment had been achieved (T2) and transferred the readings on proforma. To reduce the method error, two observers independently calculated pre-treatment inter-canine width (T1) and mid treatment inter-canine width (T2), once levelling and alignment was achieved. The decision to calculate mid treatment inter-canine width (T2) was subjective to both observers when the observers mutually agreed that mandibular arch-form filled the clinical criterion of levelling and alignment, the study models for (T2) were poured in casts followed by measurement of mandibular ICW.

Data analysis was performed on SPSS-22. For each variable, the arithmetic mean and standard deviation was calculated. Intra and inter observer measurements were repeated to rule out errors/biases once data collection had been completed. The data collection procedure, i.e., measurement of inter-canine width at two intervals was standardized amongst observers. The comparison of measurements between inter-observer and intra-observer readings was done with sample *t*-tests, and $p<0.05$ was considered statistically significant.

RESULTS

Out of 60 patients, 38 were females while 22 were males. Mean±SD for comparison of inter-canine width among both observers are given in Table-1.

Statistically significant differences ($p=0.001$) were found between pre-treatment mandibular inter-canine width (T1) to during treatment mandibular inter-canine width when considering gender, once levelling and alignment was achieved (T2). (Table-2).

When considering age-wise analysis, there was no statistical significant difference ($p=0.129$ and 0.106) between pre-treatment mandibular inter-canine width (T1) to during treatment mandibular inter-canine width. (Table-3).

Table-1: Paired sample *t*-test for comparison of inter-canine width among both observers

Observer	Inter-canine width (T1)	Inter-canine width (T2)	*p*
Observer 1	26.80±1.30	27.26±1.29	0.000
Observer 2	26.83±1.33	27.22±1.31	0.000

Table-2: Independent sample *t*-test for comparison of inter-canine width among gender distribution

Gender	Observer 1		Observer 2	
	Inter-canine width (T1)	Inter-canine width (T2)	Inter-canine width (T1)	Inter-canine width (T2)
Male	22	22	22	22
Female	38	38	38	38
p	0.003	0.002	0.001	0.001

Table-3: Independent sample *t*-test for comparison of inter-canine width among different age groups

Observer	Inter-canine width	Age group (Yrs)		*p*
		11–17	18–27	
Observer 1	**Inter-canine width (T1)**	32	28	0.194
	Inter-canine width (T2)	32	28	0.129
Observer 2	**Inter-canine width (T1)**	32	28	0.119
	Inter-canine width (T2)	32	28	0.106

DISCUSSION

Past literature has focused on finding an ideal arch-form by analyzing healthy patients in normal occlusion and various patterns of malocclusion.[20] The goal of an ideal orthodontic treatment plan is to correct repositioning of teeth according to arch-form configuration for optimal aesthetics, function, and stability.[21]

We evaluated the mandibular inter-canine width change with the use of preformed NiTi arch-wires during active orthodontic treatment. Studies advocate the maintenance of pre-treatment mandibular inter-canine width, with special emphasis on the preservation of the mandibular inter-canine width because this tends to return, in most patients, to its original value after treatment.[22,23]

After 21 days, this study found a significant increase in mandibular inter-canine width with active fixed orthodontic mechanics during the initial levelling and alignment phase. This increase was consistent across all observers, regardless of age or gender. Gardner made similar conclusions that greatest inter-arch dimensional changes after orthodontic treatment were observed in the mandibular inter-canine width followed by a strong tendency of inter-canine dimension to return to its pre-treatment shape in the retention phase.[24]

Our study results also confine to another study which concluded that preformed arch-wires significantly increased the arch width dimensions during treatment as compared to the customized arch-wire system.[25] One more study concluded that greater the change in ICW dimension during active treatment, the greater the tendency for post-retention relapse.[26]

A meta-analysis by Burke concluded that mandibular inter-canine width tends to expand during treatment by 0.8 to 2.0 mm, regardless of mechanics.[27] Chances of cross infection among dental patients who are going long-term treatment are always there as well.[28]

In this study, statistically significant differences were found between pre-treatment mandibular inter-canine width (T1) to during treatment mandibular inter-canine width when considering gender, once levelling and alignment was achieved. This is similar to another study[23] where authors found that after orthodontic treatment, mandibular inter-canine width increased for class II div 2 malocclusion in males and females.[23] This is comparable to another study[29] where there were statistically significant differences.

When considering age-wise analysis, there were no statistically significant differences between pre-treatment mandibular inter-canine width to during treatment mandibular inter-canine width. This is in contrast to another study[30], where there were statistically significant differences between certain age-groups.

CONCLUSION

The commercially available preformed tapered NiTi arch-wires significantly increase the original pre-treatment mandibular inter-canine width in patients which have a natural tapered mandibular arch-form, hence predicting future long-term instability.

LIMITATIONS

Only Ortho-care preformed NiTi arch-wires were used whereas a number of commercially available preformed arch-wires can have their own implications. Small sample size and short duration were also the limitations of this study.

RECOMMENDATIONS

Our arch-wire composition was NiTi. Comparison of various arch-wire materials like SS, stranded coaxial, heat treated and their effects may provide the clinicians with more insight. In future ovoid and other arch-forms should be correlated along with other popular preformed arch-wires in the market. Future studies with other arch-form variables should be done to advocate the changes induced by preformed arch-wires, and a longer follow-up and longitudinal changes brought by the preformed arch-wires should be studied.

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ORIGINAL ARTICLE

EFFECT OF TAURINE AND β-ALANINE ON BLOOD GLUCOSE, SERUM INSULIN, AND INSULIN RESISTANCE IN TYPE 2 DIABETIC RATS

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Background: Taurine and β-alanine have similar structures and compete for the same transporter. The ability to retain taurine and carnosine is impaired in type 2 diabetes mellitus (T2DM). This study was carried out to compare the effect of taurine and β-alanine on blood sugar, serum insulin, and insulin resistance, in T2DM rats. **Methods:** This laboratory-based experimental study was conducted from Jul to Sep 2020. Ninety male Sprague Dawley rats were randomly divided into three groups, each comprising of 30 rats. All rats were fed on a taurine-free high-fat diet for 4 weeks. Rats were supplemented in drinking water, as follows: Diabetic control rats with 0.02% (w/v) taurine, diabetic β-alanine rats with 3% (w/v) β-alanine, and diabetic taurine rats with 3% (w/v) taurine. On the 14th day, a single intraperitoneal injection of low dose streptozotocin (STZ) (35 mg/Kg), was administered to all rats. On the 21st day, tail vein sampling was done to confirm the development of T2DM. On the 28th day, rats were terminally anaesthetized and intra-cardiac blood samples were used to estimate the blood sugar, serum insulin, and HOMA-IR. **Results:** Significant differences were found between the control and taurine groups, as well as the β-alanine and taurine groups. No significant differences were found in these parameters when control group was compared to β-alanine group. **Conclusion:** Taurine significantly improves glucose homeostasis in diabetic rats. Future studies should explore taurine in combination with insulin to assess potential dose-sparing effects.

Keywords: Beta-alanine, Diabetes mellitus, High-fat diet, Insulin resistance, Taurine

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INTRODUCTION

The incidence of type 2 diabetes mellitus (T2DM) continues to grow at an alarming rate. The most salient feature of T2DM is progressive damage to the β-cells of pancreatic islets, impaired insulin secretion, and hyperglycaemia. Sustained hyperglycaemia and hyperinsulinemia lead to β-cell failure.[1] The β-cell failure results primarily from glucolipotoxicity, which in turn is attributed to multiple biochemical effects, including oxidative stress (OS).[2]

Oxidative stress results when the cellular oxidative and antioxidant enzymes balance tips towards the former, and an excess of reactive oxygen species (ROS) is produced.[1] This initiates free-radical-associated oxidation of polyunsaturated fatty acids (PUFA) in lipid peroxidation (LPO) with increased synthesis of reactive carbonyl species (RCSs). The RCS reacts avidly with proteins via Michael addition (Figure-1) to generate diverse covalent adducts known as advanced lipoxidation end-products (ALEs).[3,4] The RCS also generates advanced glycation end-products (AGEs), and both ALEs and AGEs play a role in the pathogenesis and progression of diabetes.[2,4]

A vicious cycle ensues in which chronic hyperglycaemia and OS are mutually causative. Studies have reported 30% lower levels of antioxidant enzymes in the islets as compared to the liver, suggesting that the β-cells are especially prone to oxidative damage caused by ROS and that the protection of β-cells from such damage is dependent on the inhibition of glucolipotoxicity-induced ROS generation.[1]

There is a need to pursue novel interventions that can alleviate oxidative and carbonyl stresses to help hinder disease progression, offer better clinical prognosis, and have minimal toxic effects.[5] Previous animal and human studies have demonstrated the positive impact of carnosine (CAR) and taurine (TAU), on glucose homeostasis in diabetes.[1,2,5–7]

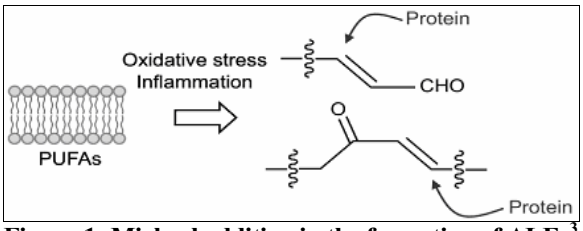

Figure-1: Michael addition in the formation of ALEs[3]

Taurine, a sulphur-containing amino acid, is richly distributed endogenously and has multi-target anti-oxidant effects that significantly alleviate glucolipo-toxicity induced OS and apoptosis in pancreatic islets.[1,8] TAU promotes insulin secretion by inhibiting the pancreatic ATP-sensitive K^+ channels, and thus, plays a pivotal role in insulin homeostasis.[8] CAR is an endogenously distributed dipeptide synthesized from β-alanine (BA) and histidine. The intracellular synthesis catalyzed by carnosine synthase (CARNS 1) is rapid, though greatly limited by BA levels.[6] CAR is a major endogenous carbonyl scavenger that quenches RCS via

intramolecular Michael addition and can mitigate aspects of metabolic disturbances in diabetes.[2]

TAU and BA share the same secondary active transport mechanism via the transmembrane TAU transporter (TAUT) found in target cells, including islets' β-cells and skeletal muscle cells.[6] The activity of the TAUT decreases in diabetes, which means that the antioxidant capacity of the cells decreases.[1] Moreover, significantly decreased TAU and CAR levels are found in diabetic animals[9,10] and humans[11,12], and the tissue levels of both TAU and CAR can be increased by oral supplementation.[8,12] This study was done to compare the effect of the two on glycaemic parameters in T2DM.

METHODOLOGY

This laboratory-based experimental study was conducted at the Physiology Department, Army Medical College, Rawalpindi, in collaboration with the National Institute of Health (NIH), Islamabad, from Jul to Sep 2020. Approval for research was obtained from the Ethical Review Committee of the College (ID/150). Ninety healthy male Sprague Dawley rats aged 60–90 days, weighing 250±50 grams, without pre-existing diabetes (plasma glucose levels >200 mg/dL)[12] as tested by tail vein sampling, were selected. Rats were housed in a well-ventilated room at 22±4 °C temperature and a 12-hour light/dark cycle.

Rats were randomly divided into 3 groups: I (Control, n=30), II (BA group, n=30), and III (TAU group, n=30). For 4 weeks, all 3 groups were fed with a TAU-free-high-fat diet.[13] For the same duration, the groups were supplemented in their respective drinking waters, as: Group I rats with 0.02% TAU to match the TAU content of standard rat chow, Group II rats with 3% BA to reduce plasma and tissue TAU content by 50%, due to competitive inhibition of TAU uptake by TAUT, and Group III rats with 3% TAU to equal the amount of BA documented to produce maximal TAU depletion.[14] Animals were allowed free access to diet and drinking water.

On the 14th day, a single intraperitoneal injection of streptozotocin (STZ), in a dose of 35 mg/Kg body weight was administered in the lower right quadrant of the rats' abdomen. On the 21st day, tail vein blood sampling was done to measure plasma glucose and insulin resistance (by HOMA-IR) to establish the development of T2DM with IR, according to the criteria of a cut-off value of plasma glucose level >200 mg/dL and HOMA-IR value of >3.9.[14,15] On the 28th day, after prior overnight fasting, rats were sacrificed by ether anaesthesia overdose. Intracardiac blood samples were collected in sodium fluoride tubes for plasma, and in gel separator tubes for serum. The samples were centrifuged at 4,000 rpm for 15 minutes to separate plasma and serum. After centrifugation, the plasma and serum were pipetted out, put into the polypropylene storage tubes, and stored at -80 °C for the assays of glucose and insulin, respectively. Plasma glucose was measured with glucose oxidase method, serum insulin was measured with Sandwich ELISA, and HOMA-IR was calculated from these values.

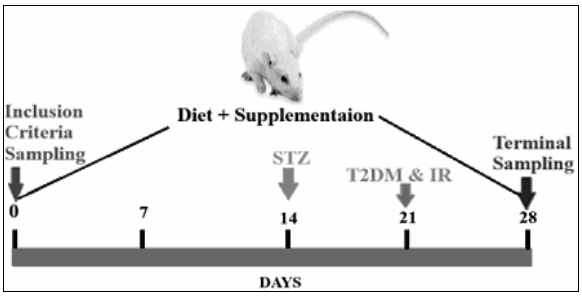

Figure-2: Experimental design

Data was analysed on SPSS-21 to calculate the Mean±SD of all variables. ANOVA was applied to determine the differences among groups. Post-hoc Tukey test was used for pair-wise comparison of groups, and $p \leq 0.05$ was considered statistically significant.

RESULTS

Table-1 shows the mean plasma glucose levels, serum insulin levels, and HOMA-IR values. ANOVA shows significant differences in all three glycaemic parameters among the groups ($p<0.001$).

Post hoc Tukey's test was applied to compare plasma glucose levels, serum insulin levels, and HOMA-IR between two groups to find which group significantly differed from the other. Significant differences were found in all three glycaemic parameters between the control and taurine groups. No significant differences were found in these parameters when β-alanine group was compared to controls. (Table-2).

Table-1: Comparison of plasma glucose, serum insulin, and HOMA-IR among control, β-alanine treated, and taurine treated groups, by ANOVA at the end of the study (Mean±SD)

Parameters	Control	β-alanine	Taurine	*p*
Fasting Plasma glucose (mg/dL)	282.23±9.77	282.80±10.24	122.03±7.51	<0.001
Serum insulin (µU/L)	3.61±1.15	3.41±1.66	5.48±0.92	<0.001
HOMA-IR	2.53±0.83	2.37±1.14	1.65±0.30	<0.001

Table-2: Comparison of plasma glucose, serum insulin, and HOMA-IR among control, β-alanine treated, and taurine treated groups, by Post Hoc Tukey test at the end of the study (*p*-values)

Parameter	Control and β-alanine	Control and taurine	β-alanine and taurine
Fasting Plasma glucose (mg/dL)	0.969	<0.001	<0.001
Serum insulin (µU/L)	0.818	<0.001	<0.001
HOMA-IR	0.757	<0.001	<0.01

DISCUSSION

The animal model used in this study closely replicates the natural history and metabolic characteristics of human T2DM. A high-fat diet induced insulin resistance (IR), while a low dose of STZ caused β-cell dysfunction. As compensatory hyperinsulinemia declined, it failed to counterbalance IR, leading to overt hyperglycaemia.[15]

Zhao *et al*[1] revealed that hyperglycaemia in T2DM primarily results from inadequate insulin secretion due to pancreatic β-cell loss, with individuals exhibiting a 30–63% reduction in β-cell volume compared to non-diabetic individuals. Their study showed that TAU supplementation alleviated hyperglycaemia in male rats fed a high-fat, high-glucose (HFHG) diet. This beneficial effect was attributed to TAU's antioxidant properties, specifically through activation of the nuclear factor erythroid 2 related factor 2/heme oxygenase 1 (Nrf2/HO-1) pathway. This pathway regulates cellular responses to OS by upregulating antioxidant gene expression and suppressing pro-inflammatory cytokines. Under glucolipotoxic OS induced by the HFHG diet, the protective effects of the Nrf2/HO-1 pathway were compromised, leading to severe pancreatic apoptosis. TAU supplementation mitigated this damage by enhancing antioxidant defences and suppressing OS. Compared to non-supplemented HFHG rats, TAU-supplemented HFHG rats exhibited increased Nrf2 and HO-1 activity, elevated superoxide dismutase levels, reduced malondialdehyde levels, and consequently, reduced pancreatic damage.[1] The animal model and TAU administration protocol used in their study were similar to ours; however, the 4-month duration of their study allowed for the detection of TAU's antioxidant effects through gene expression and protein synthesis.[1]

Murakami *et al*[16] reported that TAU had occasional but significant hypoglycaemic effects in STZ-injected C57BL/6J mice, accompanied by the upregulation of hepatic glucose transporter (GLUT-2) and UDP-glucose phosphorylase 2, suggesting that TAU improves hepatic glucose metabolism by promoting glucose uptake and glycogen synthesis. TAU's antioxidant effects were organ-specific, protecting the liver and kidney but not significantly protecting pancreatic β-cells or restoring insulin production.[16] The 3% dose of TAU, the same as in our study, was adequate to produce the initial hypoglycaemic effect in the C57BL/6J mice. Later, the OS-mediated extensive cytotoxic damage to the pancreatic tissue by the high dose of STZ (200 mg/Kg) may have overwhelmed the protective antioxidant effects of 3% TAU. Although the extent of β-cell destruction was not assessed in our study, the significant increase in serum insulin levels following TAU treatment suggests that a sufficient population of functional β-cells remained and responded to the insulinotropic effects of TAU.

Díaz-Rizzolo *et al*[7] investigated the preventive effects of a sardine-enriched diet on T2DM development, using the Finnish Diabetes Risk Score. Prediabetic subjects aged >65 years consumed a sardine-based diet (200 g/week) for a year. Sardine is a source of TAU (147 mg/100 g of serving). At the end of the study, there was a decrease in levels of HbA1c and glucose, compared to the pre-intervention values. This T2DM preventive effect was attributed to improved IR secondary to increased high-density lipoproteins and adiponectin, and decreased triglycerides.[7] In a randomized control trial[8], 8 weeks of TAU (1 g, three times per day) treatment in T2DM patients did not improve blood glucose, HbA1c, and lipid profile, despite improvement in serum insulin and HOMA-IR, and lowering of MDA and TNF levels, compared to the placebo group.[8] The discrepancy between the results could be due to factors such as the severity of T2DM, the dose of TAU, other medications, and the study duration.

Albrecht *et al*[17] reported an insulinogenic effect of CAR in BTBR ob/ob mice, a type 2 diabetes (T2D) model characterized by leptin deficiency, insulin resistance, and hyperglycaemia, which develops a phenotype similar to advanced human diabetic nephropathy (DN). Their study found a 2-fold increase in serum insulin levels, accompanied by elevated serum C-peptide levels, and a negative correlation with glycaemia. They suggested that either CAR activates the insulin-signalling cascade after internalization into the pancreas, or CAR is first hydrolyzed to beta-alanine (BA), which then opens voltage-activated L-type Ca^{++} channels, to promote insulin secretion. Although the BTBR ob/ob mouse model is useful for studying diabetic nephropathy, its leptin deficiency limits its similarity to human diabetes due to two key differences: leptin deficiency is not typical in humans with diabetes, and leptin administration can reverse the disease in these mice.[18]

Cripps *et al*[2] investigated the effects of CAR on INS-1 β-cells, isolated from CD-1 mice and exposed to glucolipotoxic conditions. The results showed a significant increase in ROS and impaired insulin secretion. However, treatment with 1 mM and 10 mM CAR reduced ROS levels and enhanced secretagogue-stimulated insulin secretion. Notably, the higher concentration of CAR (10 mM) resulted in a nearly two-fold increase in insulin secretion compared to the lower concentration (1 mM). While INS-1 cells are a reliable β-cell model, they are transformed cell lines[2] and may not fully replicate primary β-cell biology. Therefore, validating these results in whole animal models is crucial to ensure physiological relevance.

In a randomized controlled trial[19], CAR reduced glycaemia in 82 T2DM patients, probably by increasing insulin production from the pancreas and reducing OS. However, the patients received 2 other supplements (ALA and thiamine), hence, this effect cannot be assigned to CAR alone.[19]

CONCLUSION

Our findings substantiate previous studies of the beneficial effects of TAU on glucose homeostasis and support the supplementation of TAU as an adjunct therapy in T2DM. Future studies should investigate the optimal TAU dosing and duration, synergistic effects with existing diabetes treatments, and long-term efficacy and safety in diverse patient populations.

RECOMMENDATION

It is suggested that combined supplementation of insulin and taurine be investigated to assess whether combined therapy could be a more effective approach, that may also reduce the dose of insulin therapy in patients.

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AS: Analysis, interpretation, revision and drafting
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IR: Data analysis, interpretation
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ORIGINAL ARTICLE

BLOOD PRESSURE CHANGES IN HYPERTENSIVE PATIENTS: PRE- AND POST-OPERATIVE ASSESSMENT WITH LOCAL ANAESTHESIA DURING DENTAL PROCEDURES

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Background: Patients with hypertension undergoing dental treatments necessitate meticulous monitoring due to the possible cardiovascular implications of local anaesthetics including vasoconstrictors. The objective of this study was to evaluate and quantify changes in systolic and diastolic blood pressure within five minutes before and after administering a vasoconstrictor-containing local anaesthetic in hypertensive patients with stage 1 hypertension using standardized monitoring techniques. **Methods:** This quasi-experimental study examined the effects of local anaesthetic (2% lignocaine with 1:100,000 epinephrine) on blood pressure in hypertensive individuals receiving dental treatment. The study was conducted from Mar to Sep 2024 at School of Dentistry, Shaheed Zulfiqar Ali Bhutto Medical University, Islamabad, involved hypertensive patients (stage 1 hypertension, aged 30–60 years). Severe hypertension, co-morbidities, and being above the age of 60 were exclusion factors. A mercury sphygmomanometer was used to assess blood pressure five minutes before and after the administration of local anaesthetic. Data was analysed on SPSS-25, using paired *t*-tests to compare pre- and post-anaesthetic blood pressure readings. **Results:** The study analysed 62 individuals (mean age 49.83±7.12 years), with 36 (58%) men and 26 (42%) women. The mean pre-anaesthesia systolic blood pressure (SBP) was 135.57±3.98 mmHg, which decreased to 134.50±3.98 mmHg after anaesthesia, (p=0.000). Mean diastolic blood pressure (DBP) decreased from 85.85±4.15 to 84.57±4.03 mmHg (p=0.000). **Conclusion:** Study shows a clinically small but statistically significant drop in blood pressure in hypertensive individuals undergoing local anaesthesia during dental procedures.

Keywords: Hypertension, Local anaesthesia, Root canal therapy, Tooth extraction

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INTRODUCTION

Hypertension is defined as chronic high blood pressure caused by a complex interaction of hereditary and environmental variables. It is the main cause of cardiovascular disease and early deaths worldwide, highlighting its huge impact on global health.[1] According to the latest guidelines from the American Heart Association (AHA) and the American College of Cardiology (ACC), blood pressure is considered high if it routinely exceeds 130/80 mmHg, emphasizing the importance of close monitoring and management to avoid problems.[2] Hypertension affects almost 1.28 billion individuals worldwide, with a large proportion living in low and middle-income nations. These areas frequently confront obstacles such as restricted access to healthcare, low awareness, and ineffective hypertension management measures, aggravating the problem's prevalence and impact. Hypertension's broad prevalence raises the risk of heart attacks, strokes, renal disease, and other significant health issues, making it an important target of public health activities.[3]

Hypertension has developed as a major public health concern in Pakistan, owing to lifestyle changes, fast urbanization, and other variables such as eating choices, obesity, and stress. The prevalence of hypertension in the country has been alarmingly high. Data from comprehensive national research done between 2016 and 2017 revealed that the overall prevalence of hypertension has risen to 46.2%. This growth presents a substantial challenge for healthcare systems in terms of preventive strategies and treatment alternatives.[4] Regional variations in Pakistan highlight the problem's pervasive nature. For example, a survey done in central Punjab found that the prevalence of hypertension was 35.1%, revealing significant disparities between provinces and groups.[5] These regional disparities highlight the impact of factors such as socioeconomic position, healthcare infrastructure, and lifestyle choices. The rising prevalence of hypertension in Pakistan highlights the critical need for targeted health policies, public awareness initiatives, and increased access to medical care to reduce the burden of this condition and avoid long-term health implications.

Hypertensive patients are frequently seen at dental clinics and hospitals, where they pose special issues owing to the interaction of their condition with various dental procedures. Hypertension can complicate dental operations by raising the risk of excessive

bleeding and cardiovascular problems, particularly during invasive treatments or surgical interventions.[6] Dental procedure may become even more complicated when local anaesthesia (LA) is used on hypertensive individuals. To extend the duration of anaesthesia and lessen intra-operative bleeding, anaesthetics that contain vasoconstrictors, like epinephrine, are frequently used. But these vasoconstrictors can also raise blood pressure and heart rate, which can cause negative side-effects in people with poorly managed hypertension. Arrhythmias, hypertensive crises, and even cardiovascular events like myocardial infarction or stroke can be complications.[7] To reduce these risks, dental practitioners should alter the dose and use anaesthetics without vasoconstrictors whenever possible. Dental professionals must take patient's blood pressure before beginning any procedure to identify high-risk instances. If substantial problems arise, the patients should consult their primary care physician or cardiologist to build a complete treatment plan that is tailored to their specific medical needs.[8]

The purpose of this study was to look at the effect of local anaesthesia on blood pressure levels in hypertensive people during dental procedures. The primary objective was to examine blood pressure fluctuations before and after administering a vasoconstrictor-containing local anaesthetic to hypertensive patients. By identifying important changes and potential dangers, the study aims to improve safety measures, optimize management strategies, and prepare dental practitioners to provide care that promotes patient well-being while lowering hypertension risks.

METHODOLOGY

This study was carried out at the Oral and Maxillofacial Surgery (OMFS) and Operative Departments of the School of Dentistry, Shaheed Zulfiqar Ali Bhutto Medical University, Islamabad. The study period spanned from Mar to Sep 2024. The Raosoft sample size calculator was used to calculate the sample size, with a type I error of 5% and a 95% confidence interval. The study population comprised hypertensive patients visiting the Out-Patient Department of School of Dentistry, who were referred to the OMFS and Operative Dentistry Departments for further treatment.

Informed consent of the participants was taken, in addition to the ethical approval from School of Dentistry, Shaheed Zulfiqar Ali Bhutto Medical University, Islamabad. A convenience sampling method was used to select participants. Patients with stage-1 hypertension [Systolic BP (SBP) ≤140 and Diastolic BP (DBP) ≤90 mmHg], and aged 30–60 years were eligible. Exclusion criteria included patients with severe hypertension and co-morbidities such as heart illnesses, diabetes, cancer, and age above 60 years.

After written informed consent, a mercury sphygmomanometer was used for measuring blood pressure. A special performa was used for recording BP and demographic information. Blood pressure was measured 5 minutes before and after administering local anaesthesia (2% lidocaine with 1:100,000 epinephrine).

The statistical analysis was performed on SPSS-25. Descriptive statistics such as mean age and gender distribution were analysed. Paired sample *t*-test was used to compare pre- and post-administration blood pressure, with a statistical significance set at $p<0.05$.

RESULTS

The study included a total of 62 participants, with a mean age 49.83±7.12 years. There were 36 (58%) males and 26 (42%) females in the sample.

The mean SBP recorded five minutes before LA was 135.57±3.98 mmHg. Five minutes after administering LA, the SBP dropped marginally to 134.50±3.98 mmHg. Paired *t*-test analysis found a statistically significant difference in pre- and post-treatment SBP levels among stage-1 hypertensive patients ($p=0.000$).

The mean DBP five minutes before administering LA was 85.85±4.15 mmHg. Five minutes after delivery, the DBP decreased to 84.57±4.03 mmHg. Paired *t*-test analysis revealed that the change was statistically significant ($p=0.000$). (Table-1).

Table-1: Comparison of pre- and post-anaesthesia BP

Variables	Mean	SD	*p*
Systolic BP 5 min before LA	135.57	3.98	0.000
Systolic BP 5 min after LA	134.50	3.75	
Diastolic BP 5 min before LA	85.85	4.15	0.000
Diastolic BP 5 min after LA	84.57	4.03	

DISCUSSION

The aim of this study was to compare the changes in blood pressure in hypertensive individuals undergoing dental operations before and after local anaesthesia. Systolic and diastolic blood pressures were monitored before and after 5 minutes of administering 2% lignocaine with 1:100,000 epinephrine to hypertensive patients with stage-1 (SBP <140 and DBP ≤90). Several studies have assessed the effect of local anaesthesia or various local anaesthetic drugs, with or without vasoconstrictor, on hypertensive individuals.[9–12] This may be due to the action of adrenaline in the LA medication. Adrenaline in lower dose acts on more sensitive β_2 adrenergic receptors and causes vasodilatation in the skeletal muscles and some vital organs as a part of 'fight and flight' response. In high doses it stimulates α_1 adrenergic receptors causing widespread vasoconstriction combating shock status. This action overrides the action of low dose adrenaline as β_2 adrenergic receptors. In this study, the DBP and SBP did not increase following administration of LA with vasoconstrictor, but rather reduced, proving that

2% lignocaine does not produce a rise in BP in hypertensive patients.

A study conducted in India[13] reported results comparable to the current study on the effects of lignocaine and adrenaline on hypertensive patients undergoing tooth extraction. It discovered temporary elevations in systolic and diastolic blood pressure upon injection which corrected within 60 minutes. The alterations were not clinically significant, demonstrating that this LA formulation is safe for controlled hypertension individuals.[13] A research conducted in Iran[14] reported that providing 2% lignocaine with epinephrine had no significant effect on systolic or diastolic blood pressure in hypertensive patients having dental extractions.

Badar *et al* conducted a comprehensive evaluation of the cardiovascular effects of epinephrine in dental anaesthetics on hypertension individuals. According to them, using epinephrine-containing local anaesthetics resulted in minor changes in systolic and diastolic blood pressure that were not clinically significant.[15] Ilyas *et al* examined the impact of 2% lidocaine and 1:100,000 epinephrine on blood pressure levels in hypertensive individuals having their teeth extracted in Pakistan. They found that in pre-hypertensive, stage I and stage II hypertensive individuals, using three cartridges of this LA had no discernible effect on systolic or diastolic blood pressure.[9]

Several variables contribute to the stability of blood pressure shown in this study and similar investigations. First, the concentration of epinephrine (1:100,000) is low enough to cause regional vasoconstriction while without considerably raising systemic catecholamine levels. This localized impact diminishes systemic absorption and lowers the chance of blood pressure rises. Second, dental guidelines encourage regulated LA dosing and thorough monitoring of hypertensive patients to guarantee patient safety. These guidelines, which include preoperative assessments and stress reduction measures, are crucial in reducing any detrimental consequences.

While the majority of studies report outcomes that are similar to the current study, several studies have found temporary blood pressure rises after administering LA with epinephrine. A study[16] has shown that injecting local anaesthetic can be uncomfortable and may cause cardiovascular alterations such as hypertension or tachycardia. This emphasizes the need of proper local anaesthetic treatment, particularly in patients with cardiovascular disease, in minimizing deleterious consequences.[16] While epinephrine in local anaesthetics helps with haemostasis and prolongs the anaesthetic effect, another research found that it can also raise blood pressure. To avoid potential consequences from elevated blood pressure during dental treatments, individuals with hypertension were advised to use plain lidocaine without epinephrine.[17] These findings emphasize the haemodynamic stability associated with the administration of this LA in a controlled situation.

CONCLUSION

Delivering a vasoconstrictor-containing local anaesthetic to hypertensive dental patients resulted in a statistically significant but subjectively minor drop in blood pressure. Such anaesthetic procedures are safe when carefully controlled. Future interventional research with larger sample sizes and longer observation periods are needed to validate and build on these findings.

LIMITATIONS

This study employed a brief observation time (five minutes before and after anaesthesia), which may not have captured the entire range of BP fluctuations throughout the dental operation. More studies should look into extended monitoring periods and the effects of diverse anaesthetic formulations, particularly those with varied doses of vasoconstrictors. Investigating a bigger and more varied patient sample might improve the generalizability of the results.

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BA: Experimentation and study conduction
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MR: Critical review
EM: Manuscript writing
AM: Critical Review

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REVIEW ARTICLE

PANDORA BOX OF POSSIBILITIES AND LIMITATIONS: UNLEASHING THE POWER OF CHATGPT IN HEALTHCARE

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A lot of hype has been sparked by the words Chat, GPT (Generative Pretrained Transformer), and AI (artificial intelligence). The principle that nothing is truly free has perfectly aligned with the introduction of ChatGPT. Since its debut, so what is the penalty we are paying with the rising interchange of ideas with AI as the spontaneity and originality of human radiance are at risk? But it is important at the same time to adapt to the evolving world and smartly use these brilliant technologies to minimize and simplify workload with limitations. There are several uses of ChatGPT in every field including medical education, scientific research, public health, and medical assistance but with restricted ethical considerations. This comprehensive review represents the utilization of ChatGPT in the healthcare sector using data collected from the latest relevant articles, peer-reviewed research, and published reports.

Keyword: AI, ChatGPT, Dentistry, Healthcare system, Medical education, Research

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INTRODUCTION

What was once confined to science fiction is now a frequent element of our everyday lives, effortlessly merging without our conscious awareness. There has been a lot of hype sparked by the words Generative Pre-trained Transformer (GPT) and Artificial Intelligence (AI) since its release in the latter year 2022. After its launch people from all occupations are inquiring what it has for them. The story will not stop here, there will be a similar erratic cascade of artificial intelligence innovations that could be anticipated in the ensuing decade as this is just the beginning.[1]

Not long ago, although AI could read and write, it was incapable of comprehending the content, as it lacked the next degree of intellect that comes from comprehension. That succeeding level has finally been unlocked with the ChatGPT.[2] It is based on the GPT, a machine learning algorithm trained on vast amount of text data from the multiple internet resources.[3] ChatGPT aims to produce text that mimics real human conversation and can be applied to various processing tasks, including dialogue systems, language translation, and text summarization. It can be used to answer queries, write imaginative stories, and produce responses for chatbots. The text-generated tool has a quality close to that of real human language because it was trained using large volumes of internet-based text.[4] Due to GPT's ability to analyze sequential data, including natural language, ChatGPT is better able to comprehend the context of phrases and related text that came before it and produce coherent script as a result.

Interpretation is diverse on this topic while usage in certain aspects remains ambiguous in its application, accompanied by numerous unaddressed questions concerning privacy and ethical considerations. Nevertheless, these models pose concerns regarding deception, privacy, biases in training data, and misuse.[5,6] Given their ability to comprehend complicated concepts as well as responses to a variety of requests and queries (prompts), ChatGPT could potentially help in a variety of areas of medicine[7,8] (Figure-1). This manuscripts reviews how ChatGPT can potentially impact patient care, medical and dental diagnostics, medical research, and medical education, while also highlighting its limitations and ethical considerations.

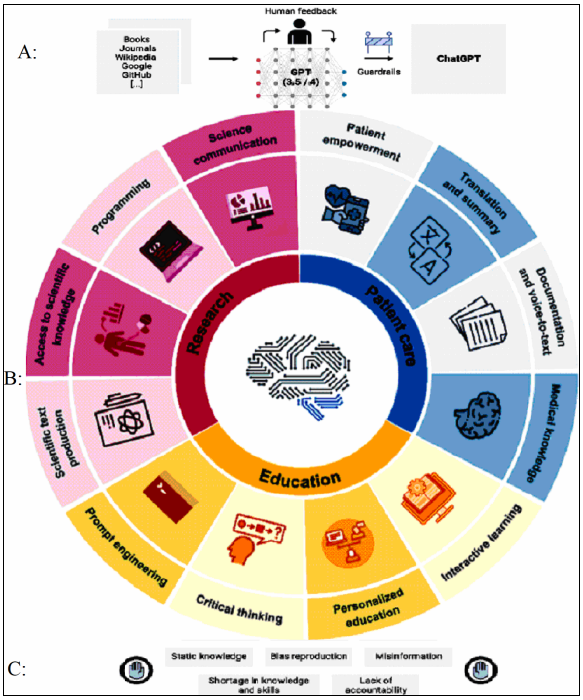

Figure 1: A: ChatGPT's architecture including training, iterations of reinforcement learning with human feedback, model selection, and the addition of guardrails for increased safety. B: Overview of patient treatment, research, and educational opportunities for large language models (LLMs) in medicine. C: Limitations of LLMs as they stand right now.[9]

METHODOLOGY

A thorough search of pertinent literature across many databases and sources was done using PubMed, Google Scholar, and specialized websites for peer-reviewed research, reports, and articles about the use of ChatGPT and related AI tools and their effects on healthcare quality. Studies and publications written in English, published in 2022 and 2023, using keywords, ChatGPT, AI, dentistry, medical education, research, and healthcare system. All the manuscripts concentrating on AI on healthcare quality met the inclusion criteria. Studies that did not assess the influence on healthcare outcomes, old literature, or did not focus on recent advances of AI in health systems were excluded.

DISCUSSION

Optimizing health administration and medical record keeping

Administrative documentation remains one of the most time-consuming tasks for clinicians. According to American Medical Association research, for every hour spent on patient visits, physicians devote roughly two additional hours to electronic medical records and desk work.[10] About 25% of clinicians' workdays is consumed by documentation and administrative responsibilities.[11]

AI-powered tools such as ChatGPT have the potential to alleviate this burden by generating structured clinical notes, patient summaries, and discharge instructions. This can optimize physicians time, reduce clerical workload, and minimize the risk of human error. For example, a large scale deployment of ambient AI scribes by the Permanente medical group in 2023 recorded over 2.5 million patient encounters in its first year, saving more than 15,000 hours of documentation time for physicians and contributing to lower burnout rates.[1,2]

More recently, Boston health AI launched Hami, describes as the worlds first AI-powered physicians assistant. Designed as an ambient scribing and clinical intelligence tool, Hami converts patient's provider conversations into structured SOAP notes and generates after visit summaries early developments across hospitals in Pakistan demonstrates how such innovations can enhance workflow efficiency while complying with global privacy standards like HIPAA and GDPR.[3]

Applications in public health and disease surveillance

ChatGPT can also be used by medical professionals and ordinary citizens to monitor global health statistics, providing real-time insights into potential outbreaks and facilitating early response efforts. ChatGPT can detect trends and abnormalities that may suggest the emergence of new diseases or the spread of an existing one by analyzing vast volumes of data from multiple sources such as news stories and public health databases. The model can also send automated alerts to public health officials, healthcare professionals, and the general population, allowing them to implement necessary disease-prevention measures.[12] Furthermore, by discovering patterns and connections in huge amounts of data, it has the potential to reveal new insights and breakthroughs in medicine.[13]

ChatGPT can also support health promotion by delivering multilingual educational content and reminders on areas such as nutrition, exercise, and lifestyle improvements. It can surely provide individualized guidance based on an individual's health profile and aspirations.[4]

Patient communication and remote monitoring

GPT-3 and its derivatives are capable of interpreting and creating text in several languages, facilitating communication between healthcare personnel and patients from various linguistic backgrounds.

ChatGPT can also be used to produce automatic responses to patients' questions about appointment scheduling and medication management as well as for patient triage, in which it asks patients about their symptoms and medical history, assisting in determining the severity and urgency of their condition. In near future, AI will most likely aid medical practice by assisting physicians in using technology to better clinical care and basic public health issues. Hopefully, human intelligence will be supplemented by technology rather than replaced.

Remote patient monitoring (RPM) is an increasingly popular technique to enhance patient outcomes while lowering healthcare expenditures. ChatGPT can remotely monitor patients by analysing data from wearables, sensors, and other monitoring equipment, allowing for real-time insights into a patient's health status. ChatGPT can analyze this data and notify healthcare practitioners if a patient's condition worsens or other problematic tendencies emerge. This allows healthcare providers to intervene sooner and prevent hospitalizations or other consequences.[12]

To eliminate possible hazards such as mistakes or misinterpretations that might hurt patients, the use of AI in healthcare communication must be controlled and supervised. To implement ChatGPT responsibly, one feasible technique may be to combine voice-to-text identification software with quick clinician editing of the letter and little human participation. This would enable the technology's potential applications to be investigated while reducing any possible risks.[14]

Assistance in medical and dental diagnosis

Diagnosis is the most fundamental and important part of medicine, acting as the cornerstone of patient care and the platform upon which effective treatment strategies are created a medical diagnosis is based on three factors: The patient's medical history, performing a physical

examination, and interpreting diagnostic testing and imaging investigations to accurately determine the underlying cause of a patient's symptoms or illness.[15] Keeping these three factors in mind there is no way that ChatGPT can diagnose medical or dental conditions all on its own but it can surely be used as a tool to assist but it can't replace a medical practitioner as they are trained to extract history from patients by their communication, interpersonal skills and by observing expression and doing cross-questioning.[16] ChatGPT's only job in medical or dental diagnosis is to assist in the generation of clinical notes, summaries, and other documentation, which would help optimize time and reduce the danger of human error. It may also be used to provide assistance and therapy recommendations based on the patient's symptoms and medical history.[17] We can't ignore the fact that with technological advancements patients no longer solely depend on physicians for medical information. Instead, patients are increasingly turning to search engines and, more recently, artificial intelligence chatbots as convenient and accessible sources of medical information. ChatGPT and other recently announced chatbots participate in conversational conversations and respond authoritatively to complex medical questions. Despite its potential, ChatGPT frequently delivers seemingly believable but inaccurate results, necessitating caution when evaluating its uses in medical practice and research.[18–20] These engines' dependability and correctness have not been evaluated, particularly in the context of open-ended medical queries that clinicians and patients are likely to ask. The most important factor of physician patient relationship of sympathy and kind words which give mental and psychological peace of mind to the patient is a big lacking factor with these artificial technologies.

Role in medical and dental education

ChatGPT's educational value is promising, and its implementation in medical education can provide an individualized learning experience to support the diverse learning needs of medical students. Assessments conducted in person are unlikely to be influenced directly by ChatGPT and do not necessitate any changes at this time. However, there is a danger of dishonesty in academic tasks undertaken by students off-campus, and medical educators must adopt suitable protocols to prevent such risks.[21] Most medical and dental institutions, particularly in developing countries, employ open-source online technologies such as Zoom and Webex to deliver exams remotely, allowing students to be watched on camera during the examinations. These platforms, however, did not allow candidates to limit internet access to assessment content during tests. This was somewhat countered by creating 'non-searchable' questions, which stopped students from quickly discovering solutions by searching the internet. With the availability of bots like ChatGPT, however, this method may be rendered ineffectual if remote evaluations are used without proctoring.[22]

While researching there was enough evidence of ChatGPT performance in medical education coming from different countries across the globe. Kung[22] and Gilson[23] in two separate studies, used the United States Medical Licensing Examination to assess ChatGPT's performance. Their findings indicated that ChatGPT might produce moderate accuracy and consequently a passing score. However, Fijačko discovered that ChatGPT failed the American Heart Association examinations.[24] According to Han, ChatGPT offered erroneous and insufficient information about cardiovascular illnesses.[25] Nisar and Aslam discovered that while ChatGPT could deliver relevant and correct replies, these answers lacked references and sources in the context of pharmacology education in Malaysia.[26] Wang used the Chinese National Medical Licensing Examination to testify about ChatGPT in China.[27] They reported that ChatGPT's performance was lower than the average score of medical students. Researchers in Korea[28], India [29], and Singapore [30] confirmed similar findings. According to the outcomes of these investigations, ChatGPT's performance in the sphere of medical education was not sufficient (Tabel-1).

Table-1: Ethical and legal questions regarding the use of ChatGPT-generated content including plagiarism, AI authorship, copyright issues of AI-generated content, and the detection of fake research and fraudulent papers

Plagiarism	How much help from AI is too much help?
AI authorship	Is AI eligible to appear as a co-author? If not how can its contributions be appropriately documented and acknowledged?
Copyright of AI-generated content	Does the AI generated content belong to the prompt creator, the AI tool, the tool creator, or the owners of the training data?
Fake research and fraudulent papers	How to detect AI-generated content effectively?

Contributions to medical research and scientific writing

The artificial intelligence language model ChatGPT may help medical researchers and scientists with writing, literature research, data summarization, structure, references, and title suggestions, and even developing an initial paper draft.

However, this is only the starting point for humans to further improve the text. ChatGPT can help to identify scholarly publications, summarize their results, and indicate areas of doubt, although the summary presented may lack critical examination of variations across research.

ChatGPT's key benefit is its capacity to swiftly analyse information and correlate evidence to make conclusions faster than humans, who have difficulties in

completely reading a wide range of literature and combining seemingly unconnected bits of information Nonetheless, due to ethical concerns, its use in scientific writing should be regulated. Authors may produce several versions of their material in only a few seconds using AI-powered content generation tools like ChatGPT, which might potentially help them overcome writer's block.[31] ChatGPT may be an effective tool for expanding on an existing text, refining information, and rewording content as needed. However, as medical research evolves, there is rising concern that ChatGPT may be abused to create articles devoid of clinical reasoning and critical thinking.[32] Certain scientific journals now require that authors of works containing ChatGPT-generated content list ChatGPT as an author. Nature, a renowned medical journal with highest impact factor, on the other hand, has rejected to recognize ChatGPT as an author since it cannot bear responsibility for anything published by it.[33] Whereas some other researchers suggest that the fact that AI cannot be a copyright holder does not preclude it from being registered as an author. If the writing was not done by a person, attributing it to a human author may not make sense.[34] Considering the constraints highlighted, the World Association of Medical Editors (WAME) has suggested that ChatGPT not be featured alongside the authors.[35]

An increasing number of researchers undertake plagiarism checks on articles and research manuscripts using relevant software applications such as iThenticate (Turnitin LLC).[36] However, plagiarism detection software primarily calculates the similarity index with published works available online and identifies the source.[37] Given ChatGPT's ability to generate fresh text, standard software systems used to detect plagiarism may not be reliable in recognizing ChatGPT results. Khalil further added that ChatGPT has the ability to create 50 essays based on several open-ended topics.[38] Turnitin was used to check half of the essays, yielding an average similarity score of 13.72%. The remaining half were tested using iThenticate, another plagiarism detection technology, which returned an 8.76% similarity score. The ChatGPT materials were deemed highly original with these scores.

A growing number of AI-based solutions, such as OpenAI's AI Text Classifier, Detect GPT, and GPT Zero, are newer software being developed to address this issue.[39] As language processing expands and becomes more sophisticated, such detection may become much more difficult in the future.[40] However, these tools are not always accurate and misclassification can happen. As a result, more study is needed to improve the precision of these technologies. When it comes to writing, however, ChatGPT has been demonstrated in generating incorrect or fake information.[41] Thus, the accuracy and reliability of ChatGPT have been called into doubt.[42] To overcome this situation researchers have emphasized the need to develop anti-plagiarism policies and educate students about academic integrity.[43]

One more important issue is the list of references, as most provided cited data by AI tool is fabricated. Once these references are chased in reality, they do not exist finally making the work unreliable!

Limitations and ethical considerations

ChatGPT's expertise is limited by its training data, which only extends up to 2021. This limitation stops it from providing real-time information, cross-referencing facts with recent events, or generating truly creative and fully unique responses. As a result, when confronted with post-2021 facts or modifications, this constraint may result in possibly incorrect or misleading replies.[44]

However, it has its own set of drawbacks and restrictions, use of ChatGPT for healthcare purposes may raise privacy problems, owing to the need for access to and analysis of patient data and medical history acquired from various internet sources, which may jeopardize patient privacy. This practice raises serious concerns regarding patient privacy and the handling of sensitive medical information.[45,46] Security flaws are also a legitimate source of worry, as a software system, it is vulnerable to hacking and other security risks. Misuse of ChatGPT has the potential to cause security breaches, resulting in unauthorized access, exchange, or theft of personal or sensitive information.

Another major limitation relates to accuracy and reliability. While ChatGPT can generate references and citations, studies show that most are fabricated or erroneous. For example in an evaluation of 115 references generated by ChatGPT, only 7% were authentic, while nearly half were fabricated and the rest contained significant errors. Such findings highlight the risk of relying on ChatGPT for scientific or clinical writing without rigorous human verification.

Furthermore, it may face difficulties in ethical and moral thinking. It has the potential to generate morally ambiguous or ethically dubious content that may not comply with recognized ethical norms, making it unsuitable for certain applications in the absence of sufficient human oversight.

The lack of emotional intelligence in ChatGPT is a key disadvantage that renders it unsuitable for conversations that need emotional sensitivity, empathy, or comprehension of complicated emotional states (Figure-2). When employing AI models like ChatGPT in emotionally charged or sensitive settings, users should exercise caution and consider the possibility of misinterpretation and insensitivity. ChatGPT, being a text-based AI language model, is unable to create visual material such as photos, videos, or graphs, restricting its application in multimedia content production and visual communication jobs.[47]

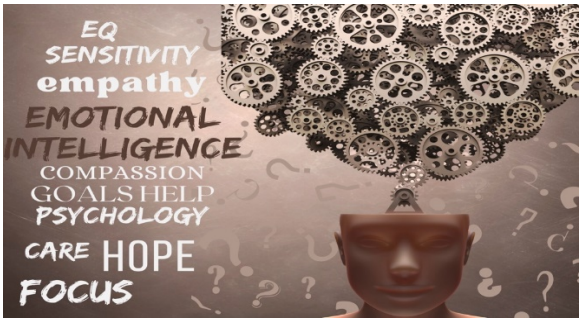

Figure-2: Lack of Emotional Intelligence in AI

This illustration highlights the essential components of emotional intelligence, the gears represent the intricacy and interdependence of these characteristics, highlighting the significant gap in AI's ability to comprehend and exhibit emotional intelligence.

CONCLUSION

ChatGPT's potential is analogous to opening a Pandora's box of ideas-vast promising, yet bounded by inherent restrictions and limitations. It's interpretation and impact often depend on how it is applied and understood. While AI-driven tools may appear rival to human intelligence, it is important to remember that at the helm of these technologies must remain the human minds, with its unique capacity for judgment, empathy and emotional awareness.

In healthcare, ChatGPT has already shown promise in easing administrative burdens, supporting diagnostics, enabling pandemic alerts and improving communication across linguistic barriers it also holds potential in medical education and research, though its impact in these areas remains ambiguous and requires further validation, implementation, and careful oversight. Ethical safeguards, policies and regulatory frameworks will be essential to ensure that these tools are integrated responsibly and do not compromise privacy accuracy or academic integrity.

Ultimately ChatGPT should not be seen as a replacement for human intelligence but rather as a supportive tool one that can augment healthcare professionals by streamlining workflows, reducing burnout, and expanding access to knowledge. If embraced responsibly, with well-defined ethical guidance and critical human oversight, ChatGPT may help reshape the future of medicine, education and research while preserving the irreplaceable role of human compassion at the core of healthcare.

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