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PAKISTAN BIOMEDICAL JOURNAL
https://www.pakistanbmj.com/journal/index.php/pbmj/index
ISSN (E): 2709-2798, (P): 2709-278X
Volume 9, Issue 01 (January 2026)

Agonists and Antagonists of Peptide Receptors: Therapeutic Approaches to Combat Cancer

OPEN ACCESS

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ARTICLE INFO

How to Cite:

Covenas, R. (2026). Agonists and Antagonists of Peptide Receptors: Therapeutic Approaches to Combat Cancer: Agonists and Antagonists of Peptide Receptors. Pakistan BioMedical Journal, 9(1), 01-02. https://doi.org/10.54393/pbmj.v9i1.1327

Despite the significant advances made in cancer diagnosis and treatment, much remains to be learned and explored. New strategies and lines of research must be pursued, in combination with those currently used (surgery, radiotherapy, chemotherapy, immunotherapy). Peptidergic systems are involved in many physiological functions and are also implicated in many human pathologies, including cancer. There is increasing research on these systems in relation to cancer development and progression, peptide receptor overexpression, biomarkers, prognosis, aggressiveness, relapse risk, and tumor size. Endogenous bioactive peptides exert oncogenic actions (e.g., cell proliferation and migration, anti-apoptotic action, angiogenesis) and antitumor effects (counteracting previous mechanisms). These bioactive peptides bind to peptide receptors that are overexpressed in cancer cells compared to normal cells. This is crucial for establishing more specific therapeutic strategies using antitumor peptides and oncogenic peptide antagonists, since these antagonists produce antitumor effects, including the apoptosis of tumor cells which is greater in tumor cells than in normal cells. Therefore, the co- administration of antitumor agonists and oncogenic peptide antagonists is a very promising anticancer line of research that needs to be developed. This treatment would not depend on either the clinical state or the biology of the tumor, since by overexpressing peptide receptors different tumors (e.g., glioma, lung cancer, hepatoblastoma, breast cancer), they could be treated with the same antitumor therapeutic procedure. What data currently exists to support what was written above and what peptide receptor agonists and antagonists act as antitumor agents? Fortunately, there is very promising data, some examples: 1) Neurokinin receptor antagonists: Aprepitant (Emend, neurokinin-1 receptor antagonist) exerts a broad antitumor action against numerous types of cancer (breast, pancreas, liver, lung, larynx, prostate, glioma, neuroblastoma, retinoblastoma, osteosarcoma, melanoma, thyroid, gastric, colon, leukemia) and the repurposing of this antiemetic used in clinical practice as an anticancer agent has been suggested [1]; 2) Angiotensin II receptor antagonists: Telmisartan and losartan (DuP-753, angiotensin II type 1 receptor antagonists) and PD-123,177, PD-123,319 ditrifluoroacetate, olodanrigan (EMA401) and A3E (angiotensin II type 2 receptor antagonists) exert an anticancer action against glioma and neuroblastoma [2]; 3) Neurotensin receptor antagonists: SR- 48692 and meclinertant (SR-48692) (neurotensin type 1 receptor antagonists) against glioma and ovarian cancer [2]; 4) Bradykinin receptor antagonists: SSR-240,612 (bradykinin type 1 receptor antagonist), Firazyr (HOE-140, bradykinin type 2 receptor antagonist), and BKM-570 (mainly a bradykinin type 2 receptor antagonist) against glioma [2]; 5) Vasopressin receptor antagonists: Tolvaptan (vasopressin type 2 receptor antagonist) against neuroblastoma [3]; 6) Neuropeptide Y receptor antagonists: BIBP- 3226 (neuropeptide type 1 receptor antagonist),

DOI: https://doi.org/10.54393/pbmj.v9i1.1327

BIIE-0246 (neuropeptide type 2 receptor antagonist) and L-152,804 (neuropeptide type 5 receptor antagonist) exert anticancer actions against neuroblastoma and breast, colon and prostate cancer [4]; 7) Galanin receptor antagonists: SNAP-37889 (HT-2157, galanin type 3 receptor antagonist) acts against leukemia [5], and 8) Glucagon-like peptide-1 receptor agonists, galanin type 2 receptor agonists (M89b) and angiotensin II type 2 receptor agonists also exert antitumor effects against pancreatic and colorectal cancer [6]. And there is something else, and it is very important: Somatostatin peptide analogs and gonadotropin-releasing hormone receptor agonists (triptorelin, lupron, zoladex (goserelin)) are currently used in clinical practice to fight lung, prostate, breast and neuroendocrine tumors [7]. These are some examples of the great antitumor potential of peptide receptor agonists and antagonists, alone or in combination with current therapies. In sum, peptide receptors are promising anticancer therapeutic targets and its exhaustive study will improve the diagnosis, management and treatment of tumors.

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PAKISTAN BIOMEDICAL JOURNAL
https://www.pakistanbmj.com/journal/index.php/pbmj/index
ISSN (E): 2709-2798, (P): 2709-278X
Volume 9, Issue 01 (January 2026)

Review Article

OPEN ACCESS

Innovative Therapeutic Paradigms in Diabetes Mellitus: Current Progress and Future Directions

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ARTICLE INFO

Keywords:
Diabetes Mellitus, Nanotechnology, Intestinal Microbiota, Insulin

How to Cite:
Sattar, A. (2026). Innovative Therapeutic Paradigms in Diabetes Mellitus: Current Progress and Future Directions: Novel Therapeutic Paradigms in Diabetes Management. Pakistan BioMedical Journal, 9(1), 03-10. https://doi.org/10.54393/pbmj.v9i1.1325

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Received Date: 10th November, 2025
Revised Date: 27th December, 2025
Acceptance Date: 23rd January, 2026
Published Date: 31st January, 2026

ABSTRACT

Diabetes mellitus is a chronic metabolic disease that is marked by inadequate glucose control that leads to eventual complications in the cardiovascular, renal, neurological, and ocular systems. The conventional approaches to treatment, such as insulin injections, oral hypoglycemic drugs, and structured lifestyle changes, continue to be the predominant approaches to treatment. However, the techniques are commonly limited so as to contain the symptoms and not achieve a long-lasting remission or to cure the causes of the disease. This has altered the field of science in the recent past, where creative approaches towards treatment have been given focus to change or even reverse the stages of disease. Stem cell interventions have the potential to cure pancreatic β-cells, and genetic therapy is meant to fix the genetic defects that lead to the disease. Immunotherapy transplantation is being undertaken to protect the β-cells in diabetes type 1 diabetes, and nanotechnology and smart insulin delivery systems can provide more precise glycemic control. In addition, the intestinal microbiota modulation has also become one of the promising adjunctive mechanisms. This review gives these new methodologies, the current status of these methodologies, and the possibilities of these methodologies in the management of diabetes.

INTRODUCTION

The term diabetes mellitus (DM) describes a set of chronic metabolic disorders, which are characterized by the constant high levels of blood glucose caused by impairments in the process of insulin secretion, its action, or both [1]. The disease is significant in terms of morbidity and mortality because it leads to damages of various organs, including the heart, kidneys, nerves, and eyes [2]. The world has been facing a social health crisis due to diabetes. According to the reference sources, over 540 million adults are already living with diabetes, and it is projected to reach 783 million people by the year 2045 (IDF, 2021). It is estimated that in 2021, 6.7 million deaths were related to diabetes in the global context, and it consumed an enormous sum of healthcare expenditure annually, estimated at USD 1 trillion (IDF, 2021).

Despite significant advances in innovative diabetes therapies, substantial research gaps remain in establishing their long-term safety, efficacy, and cost-effectiveness across diverse populations. Most emerging interventions, including stem cell therapy, gene editing, and nanotechnology-based systems, are supported primarily by early-phase trials with limited large-scale randomized evidence. Additionally, variability in treatment protocols and patient selection restricts generalizability and clinical translation. Future research should prioritize standardized methodologies, longitudinal studies, and equitable accessibility to ensure these therapies can be effectively integrated into routine diabetes care.

The rising prevalence rate and economic burden of this problem are signs of the urgent need to find effective

DOI: https://doi.org/10.54393/pbmj.v9i1.1325

treatments [3]. Diabetes mellitus is of two major types and of various other types [4] (Figure 1).

Figure 1: Types of Diabetes

Therefore, this review aims to synthesize recent advances in novel diabetes therapies and highlight key challenges and future directions for clinical translation.

Type 1 Diabetes Mellitus (T1DM)

T1DM is an autoimmune disorder in which the body's immune system assaults the β-cells of the pancreas, leading to a complete lack of insulin. It usually happens during childhood or adolescence, but more cases that happen in adults are increasingly becoming familiar. The long-term aspect of insulin therapy allows T1DM patients to live, and even with insulin treatment, these patients remain susceptible to such complications as diabetic ketoacidosis and long-term vascular damage [5].

Type 2 Diabetes Mellitus (T2DM)

T2DM is the most prevalent type, and more than 90% of all cases of diabetes are covered. It takes place due to muscle, fat, and liver insulin resistance along with progressive β-cell dysfunction [6]. Obesity, sedentary lifestyle, poor dietary decisions, and hereditary predisposition are the risk factors. T2DM is a disease that is normally not diagnosed for many years, and therefore, people are diagnosed when it is too late and when they have already developed complications [7].

Other Types

Other and less common forms of diabetes include gestational diabetes mellitus (GDM), which is a condition acquired during pregnancy; monogenic diabetes (including maturity-onset diabetes of the young, MODY); and secondary diabetes, caused by other ailments or by certain medications [8].

Weaknesses of Existing Interventions

However, these conventional approaches are predominantly symptomatic, focusing on glucose control rather than addressing the underlying pathogenic mechanisms. They are often limited by incomplete efficacy, the risk of hypoglycemia, treatment burdens, and an inability to halt the progressive decline in β-cell function or prevent long-term complications [9]. In T1DM, lifelong insulin replacement does not counteract the ongoing autoimmune destruction of β-cells. In T2DM, existing pharmacotherapy often fails to arrest the progressive β-cell failure and insulin resistance driving the disease [10]. These significant limitations highlight a clear unmet clinical need for interventions capable of modifying the disease course, restoring physiological regulation, and potentially inducing remission. The heterogeneity in pathogenesis is reflected in the disease classification. While T1DM and T2DM constitute the majority of cases, other forms such as gestational diabetes, monogenic diabetes (e.g., MODY), and secondary diabetes are increasingly recognized, each with distinct etiologies that may demand tailored therapeutic strategies.

Lifestyle Interventions (Diet, Exercise)

Together, there is strong evidence from clinical trials such as the Diabetes Prevention Program (DPP) that intensive lifestyle change prevents the occurrence of type 2 diabetes by 4560 percent in people at high risk [11]. These advantages are mediated by a group of physiologically related processes (Figure 2).

Figure 2: Mechanistic pathways linking lifestyle interventions to improved metabolic outcomes in diabetes

The important pathways involve enabling skeletal muscle and liver insulin sensitivity through AMPK activation and GLUT4 translocation, systemic inflammation reduction, and 2 -cell functionality through the reduction of glucolipotoxicity [12]. The schematic outlines the main biological processes of how diet adjustment (caloric restriction, high fiber, low glycemic load) and physical activity produce their positive influence. They are: (1) Enhanced Insulin Sensitivity: Stimulation of AMPK in muscle and liver, increasing glucose uptake and decreasing hepatic gluconeogenesis; (2) -Cell Preservation: Reduction of glucolipotoxicity, reducing metabolic stress on pancreatic -cells; (3) Palliative of Inflammation: Suppression of adipose tissue production of pro-inflammatory cytokines (e.g., TNF- a, IL-6); and (4 All these combined routes lead to a better glycemic regulation, weight loss, and cardiovascular risk.

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Stem Cell Therapy

In either type 1 diabetes (T1D) or type 2 diabetes (T2D), stem cell therapy is expected to replace or regenerate insulin-generating pancreatic 8-cells that have been destroyed in diabetes or are dysfunctional. The pluripotent stem cells (PSCs), such as embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), can be induced into glucose-responsive 6 -like cells, and this presents a potentially inexhaustible source of cell source to be used in transplantation [10]. According to the most recent clinical trials, like those of ViaCyte, the safety and initial effectiveness of encapsulated stem-cell-derived pancreatic progenitors in people with T1D have been noted. A decisive trade-off exists in the selection of the source of stem cells in terms of efficacy, safety, ethical issues, and stage of development.

Gene Therapy

The goal of gene therapy is to fix the genetic or functional defects of diabetes. The expression of the β-cell protective genes (e.g., PDX1, MafA) in T1D or the regulation of genes related to insulin sensitivity (e.g., GCKR, PPARG) in T2D might be used as strategies, and CRISPR/Cas9 can provide precise editing abilities [13]. Whereas preclinical models promise, there is a complex array of translational issues that prevent clinical implementation. The barriers to delivery are paramount; there is still difficulty in attaining efficient, targeted, and sustained delivery to the pancreatic or hepatic tissues. Viral vectors (e.g., AAV) are associated with immunogenicity, whereas non-viral approaches are characterized by low efficacy. It does not have long-term safety, and there is the risk of off-target edits, immune response to edited cells or delivery vectors, and oncogenesis as a result of potentially insertional mutagenesis [14]. Moreover, there is an extensive and dynamic regulatory route through which gene therapies have to pass, which is a potential obstacle. The clinical trial design is complex, long-lasting, and expensive due to the requirements by agencies such as the FDA and EMA to provide exhaustive preclinical information on whether the vectors are distributed, genotoxic, and long-term follow-up plans [15]. These bottlenecks on the delivery, safety, and regulatory side need to be overcome before gene therapy can be translated from a great idea to a clinical therapy that will help diabetics.

Immunotherapy

In other cases, type 1 diabetes requires immunotherapy. The etiology of T1DM is autoimmune destruction of β-cells; immunotherapy is an option for the future. The monoclonal antibodies are among the strategies and are applicable to monitor the immunological responses, vaccines against the diabetogenic antigens, and regulatory T-cell (Treg) therapy [16]. In the United States, recently, a monoclonal antibody, teplizumab, which targets T cell receptor CD3, has been approved to delay the onset of T1DM in those at high risk [17]. Other clinical trials are investigating other agents that either preserve the remaining β-cell or rebuild immune tolerance. The major challenges are the achievement of long-term remission and the minimization of adverse effects such as immunosuppression [18].

Nanotechnology in Diabetes

Nanotechnology offers transformative platforms for diabetes care, primarily through smart insulin delivery systems (e.g., glucose-responsive nanoparticles, hydrogels, microneedle patches) and advanced nanobiosensors for continuous glucose monitoring. These systems promise autonomous, physiologically responsive glycemic control, potentially reducing hypoglycemic risk and treatment burden [19]. However, translating these promising preclinical concepts into reliable, widely available therapies faces several formidable translational challenges.

Digital Health and Artificial Intelligence

Artificial intelligence (AI) has become an essential tool of diabetes care within a selected period of time [20]. Machine learning will be capable of calculating the probability of getting diabetes, the most effective dose of the insulin therapy, and the tailored treatment recommendations. The AI-based continuous glucose monitors (CGMs) and mobile health apps that allow monitoring glucose levels, diet, and exercise in real-time are now in existence. The so-called artificial pancreas (AI-controlled closed-loop insulin pumps) is an example, which is implemented to optimize insulin and better glycemic control based on the reduction in the incidence of hypoglycemia. Besides treatment, AI-based systems are also providing the prospect of the early detection of diabetic complications such as retinopathy due to retinal imaging [21].

Gut Microbiome and Probiotics

The gut microbiome has emerged as a major regulator role in host metabolism and glucose homeostasis [22]. Dysbiosis or changes in the composition of microbes have been linked to insulin resistance and T2DM. The experimental therapy is supposed to restore the microbial balance, which is based on probiotics, prebiotics, dietary fibers, and fecal microbial transplantation (FMT) [23]. Animal research and small clinical trials suggest that insulin sensitivity and glycemic control can be improved with the help of certain bacterial strains, such as Akkermansia muciniphila and Lactobacillus species. In spite of the promising nature of the field, it requires larger randomized controlled trials in order to establish efficacy

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and safety [12].

Transplant and Regeneration Medicine

Whereas the provision of the cellular raw material is done by the stem cell research field, the regenerative medicine and transplantation field is dedicated to the challenging engineering aspect of delivering and maintaining the cells in vivo. Allogeneic islet transplantation (the Edmonton protocol) is the historical standard that demonstrates that cell replacement can be used to restore insulin independence, but that has to be accompanied by life-long immunosuppression, and there is a shortage of donors [24]. Long-term graft survival without the use of immunosuppression is hence the primary aim of the central pursuit. This has triggered the promotions in immunoisolation techniques based on semi-permeable biomaterials. The existing encapsulation techniques strive to allow the two-way diffusion of oxygen, nutrients, insulin, and glucose and protect the graft against immune cells and antibodies [25]. The optimal response to fibrotic overgrowth (the foreign body response) is by optimizing biomaterial properties, which include the porosity, surface chemistry, and mechanical strength of the polyethersulfone or synthetic hydrogels, which starve encapsulated islets of oxygen [24]. There are new strategies of macroencapsulation (implantable, retrievable chambers) and microencapsulation (coating individual islands or clusters). One of the brightest examples is the current clinical translation of stem-cell-derived pancreatic endoderm cells in a framework of macroencapsulation devices that seek to offer a controlled environment of cell growth and functionality [26]. Combining angiogenic factors and oxygen-capable materials in these structures is one of the future directions in order to improve the graft vascularization and viability, which is the main limitation to long-term graft survival of a functional bioartificial pancreas.

Literature Search Strategy

This narrative review aimed to explore the innovative therapeutic paradigms of diabetes in detail. A systematic search of the literature in the databases of PubMed, Scopus, and Web of Science was carried out, and articles published from January 2019 to December 2024 were selected. The keywords were the following: diabetes mellitus, novel therapy, stem cell, regenerative medicine, immunotherapy, gene therapy, nanotechnology, artificial intelligence, digital health, and microbiome. The inclusion criteria gave priority to original research articles, clinical trials, systematic reviews, and meta-analyses, which were published in English. We filtered out studies not in English, and those where conventional therapies alone were studied without new mechanisms, and those that had been published before 2019, with exceptions being seminal works. Relevant information on mechanisms, efficacy, safety, and obstacles was identified and systematically synthesized thematically to give a critical picture of the current state of the field and the future.

Challenges and Limitations of Diabetes Management

High manufacturing costs and complex logistics for cell/gene therapies and advanced devices currently restrict them to affluent healthcare systems, exacerbating global health inequities [27] (Table 1).

Table 1: The challenges and limitations of novel therapeutic approaches for diabetes management

Specific Challenge	Explanation	References
Economic Constraint	Cell- and gene-based therapies are expensive to develop, manufacture, and deliver. Infrastructure, regulation, and quality control further increase costs, limiting accessibility.	[28]
Accessibility In Low-Income Countries	Many low- and middle-income countries lack specialized facilities, regulatory systems, and cold-chain logistics necessary for novel therapies. Access is often restricted to wealthy or urban populations.	[29]
Ethical Concerns – Gene Therapy	Gene editing raises moral concerns about heritable modifications, consent, and long-term effects. Risk of off-target mutations and misuse.	[30]
Ethical Concerns – Stem Cells	Use of embryonic stem cells raises ethical debates over embryo destruction, donor consent, and tumor formation risk.	[31]
Clinical Trial Limitations	High variability in stem cell types, dosages, and delivery routes makes it difficult to generalize findings across populations.	[32]
Insufficient Long-Term Data	Many trials show short-term benefits but lack evidence on durability, immune response, and tumorigenicity over extended periods.	[33]
Immunological Risks	In stem cell and transplant therapies, immune reactions and graft rejection remain significant barriers despite encapsulation technologies.	[34]
Regulatory Barriers	Different countries have varying regulations for gene editing, stem cell use, and nanomedicine, delaying translation to clinical practice.	[35]
Technological Challenges	Large-scale production and consistent differentiation of β-cells remain technically demanding.	[36]
Cost-Effectiveness	Novel interventions are not yet cost-effective compared to standard insulin therapy and oral drugs.	[37]

DOI: https://doi.org/10.54393/pbmj.v9i1.1325

Limited Evidence Base	More large-scale, long-term RCTs are required to confirm benefits, optimize protocols, and assess sustainability.	[38]

Thus, the study of scalable, automatable manufacturing (e.g., the scale-up of production with stem cells in bioreactors) and cheap biomaterials is not only a technical but also an ethical necessity. Second, the attainment of sustained efficacy and chronic safety is an essential validation gap of the majority of approaches. In the case of regenerative and immunotherapies, it is necessary to show long-term glycemic control and immune tolerance compared to temporary studies. At the same time, to obtain regulatory approval and popular acceptance, the creation of effective, standardized safety surveillance systems of such risks as tumorigenicity (stem cells), off-target effects (gene editing), and algorithmic bias (AI) is an initial step [39]. Lastly, hybrid regulatory approaches of combination products (e.g., cell device) and AI-as-a-medical-device should be developed. Research, industry, and regulators must work together to specify endpoints and evidence standards of such new therapeutic classes, or promising innovations may remain stagnant in development, developmental dead ends [40]. These three issues that are prioritized, namely, affordability, lasting safety/efficacy, and regulatory clarity, will play a crucial role in determining which new paradigms can shift into the realm of clinical reality on a broad scale.

DISCUSSION

Immunotherapy aims to disrupt the autoimmune pathogenesis of type 1 diabetes (T1D), representing a paradigm shift from managing hyperglycemia to modifying the underlying disease. The recent FDA approval of teplizumab, an anti-CD3 monoclonal antibody, to delay the onset of clinical T1D in high-risk individuals marks a significant milestone, validating the concept of immune intervention [15]. However, its modest efficacy and transient effects underscore that monotherapy is unlikely to induce lasting remission, highlighting the critical need for combination strategies and precision patient selection. The therapeutic landscape extends beyond a single agent. Current investigational approaches include: 1) Antigen-specific therapies (e.g., GAD65, proinsulin peptides) to induce immune tolerance; 2) Cytokine-targeting agents (e.g., anti-IL-21, anti-IL-6) to modulate the inflammatory milieu; and 3) Cellular therapies, notably the expansion and infusion of regulatory T cells (Tregs) to restore immune balance [40]. Combining these modalities—for instance, an anti-CD3 agent to reset the immune system followed by an antigen-specific vaccine to establish lasting tolerance—is a key focus of next-generation clinical trials. Success in this field is heavily dependent on identifying the optimal therapeutic window and stratifying patients using predictive biomarkers. Intervention is most effective during the pre-symptomatic stage or at clinical onset when a substantial reservoir of functional β-cells remains. Biomarkers such as autoantibody titers, T-cell phenotype assays, and genetic risk scores are being refined to identify individuals most likely to respond to specific immunotherapies and to monitor treatment efficacy [41]. The overarching challenge remains achieving durable immune tolerance without causing generalized immunosuppression, a goal that requires continued innovation in both therapeutic agents and the biomarkers used to guide their deployment. However, its widespread application is restricted because of its high price, inaccessibility in low-income regions, and unpredictability regarding the regulation. Moreover, the ethics in gene editing and embryonic stem cell has its opportunities as well as challenges that transcend science and extend to society [42]. The current trends in the curative approaches to diabetes give rise to the potential and the challenge of the conventional glucose-lowering systems [43]. However, this has not yielded consistent results due to the heterogeneity of the type of stem cells, dosage, route of administration, and patient selection, and the long-term durability and standardization of the process remain a doubtful issue. All of these combined prove the fact that safety is accepted, but the problems of immune response and tumorigenicity remain a concern that has yet to be resolved. Generally, the literature suggests a paradigm shift towards disease modification of glucose as opposed to symptomatic regulation of glucose [44]. To date, the most promising evidence is stem cell and immunotherapy therapies, but they should be validated in large, long-term randomized trials. Future research should center on early intervention, combination treatment methods, and alternative medicine, and the problem of cost, availability, and ethical matters. When such new strategies overcome these hurdles, it is then and only then that they can be translated into clinical practice and potentially revolutionize care provision in diabetes especially in patients whose β-cells have been severely destroyed as the case is, in patients with long-lived type 2 diabetes and in diabetes in preclinical phase or early-stage type 1 diabetes where some of the β-cells continue to survive. The most likely scenario is that the management of the future will require the two synergies: the immune modulation of the existing β-cells, and the regenerative solutions relying on the replacement of the already lost ones.

The primary limitations of these emerging therapies include high manufacturing costs, limited accessibility in low- and middle-income countries, insufficient long-term safety and efficacy data, and ethical concerns surrounding gene editing and stem cell use. Additionally, variability in

DOI: https://doi.org/10.54393/pbmj.v9i1.1325

treatment protocols and patient response hinders broad clinical application. Future efforts should prioritize scalable, cost-effective manufacturing processes, conduct large-scale long-term clinical trials, and develop clear regulatory pathways. Research should also focus on personalized combination therapies, early intervention strategies, and equitable global access to ensure these innovations benefit diverse patient populations.

CONCLUSION

Diabetes mellitus remains a major global health challenge, with conventional therapies limited to symptom control rather than cure. Emerging treatments such as stem cell therapy, immunotherapy, nanotechnology, and AI-driven personalized medicine offer a paradigm shift by targeting the root causes of the disease. Overcoming challenges related to cost, regulation, and long-term validation will be essential to translate these innovations into clinical practice.

Authors' Contribution

Conceptualization: AS
Methodology: AS
Formal analysis: AS
Writing and drafting: AS
Review and editing: AS

All authors approved the final manuscript and take responsibility for the integrity of the work.

Conflicts of Interest

The authors declare no conflict of interest.

Source of Funding

The author received no financial support for the research, authorship and/or publication of this article.

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DOI: https://doi.org/10.54393/pbmj.v9i1.1321

PAKISTAN BIOMEDICAL JOURNAL
https://www.pakistanbmj.com/journal/index.php/pbmj/index
ISSN (E): 2709-2798, (P): 2709-278X
Volume 9, Issue 01 (January 2026)

Review Article

OPEN ACCESS

Insights into Narcissistic Personality Disorder: A Narrative Review with Cultural and Biological Insights from Pakistan

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ARTICLE INFO

Keywords:
Narcissistic Personality Disorder, Pakistan, Genetics, Treatment, Diagnosis, Polygenic

How to Cite:
Syed, M., Shahzad, Z., Rajis, C., Lodhi, U. F., & Zulfiqar, S. (2026). Insights into Narcissistic Personality Disorder: A Narrative Review with Cultural and Biological Insights from Pakistan: Narcissistic Personality Disorder: A Narrative Review with Cultural and Biological Insight. Pakistan BioMedical Journal, 9(1), 11-19. https://doi.org/10.54393/pbmj.v9i1.1321

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Received Date: 20th November, 2025
Revised Date: 21st January, 2026
Acceptance Date: 26th January, 2026
Published Date: 31st January, 2026

ABSTRACT

Narcissistic Personality Disorder (NPD) is a heterogeneous and complicated personality disorder that is marked by grandiosity, admiration, and a lack of empathy. The purpose of this narrative review was to conduct a synthesis of literature on the etiological, diagnostic, genetic, and cultural aspects of NPD in the Pakistani sociocultural setting. The PubMed, PsycINFO, Scopus, and Google Scholar databases were used to conduct a narrative literature review. A search was conducted on peer-reviewed articles that were published from 2019 to 2024 with the keyword's narcissistic personality disorder, genetics, epigenetics, diagnosis, culture, and Pakistan. The relevant studies were applied to such topics as etiology, diagnosis, and cultural influences. The cultural values where collectivism is the dominant trait in Pakistan can affect the manifestation and perception of narcissistic features, which, in most cases, do not lead to the expression of overt grandiosity as in individualistic cultures, and that, consequently, complicates the clinical diagnosis. Despite the implicated role of dopaminergic and serotonergic pathways in the causes of NPD, such as DRD4, 5-HTTLPR, and COMT, no concrete genetic biomarkers have been defined. The emergent data point to the influence of the epigenetic processes, according to which the early-life adversity, trauma, and sociocultural factors regulate the expression of genes without changes in the sequences. It is important to have an insight into how genetic vulnerability and cultural situation relate to help diagnose and intervene appropriately in the treatment of NPD in Pakistan. It may enhance psychosocial outcomes and therapeutic interventions in the framework of culturally sensitive assessment models and biologically informed research.

INTRODUCTION

Narcissistic Personality Disorder (NPD) is a heterogeneous and complicated psychiatric disorder that is marked by widespread grandiosity, the need for admiration, and poor empathy for others [1]. NPD is a serious problem for both the clinician, researchers, and victims because it is a great challenge to the functioning of the individual in interpersonal relationships, emotional stability, and social behaviours as defined in the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5)(Cluster B personality disorders) [2]. The narcissism concept is a Greek myth where Narcissus is absorbed by his libidinous reflection, meaning that it is a pathological process of self-absorption and self-idealization [3]. In clinical terms, patients with NPD are often characterized by their over-importance and continuous fantasies about unlimited success or power, a feeling of exceptionalism, and a necessity to be validated and adored by others [4]. These external manifestations often conceal some underlying weaknesses of the psyche, such as poor self-esteem, feelings of inadequacy, and the inability to develop a true emotional bond. As much as people with NPD might come out as confident or socially competent at first, long-term interaction usually brings out emotional insecurity, a manipulative relationship style, and relationship problems that make it difficult to engage with them during therapy and maintain relationships with them [5]. The prevalence

DOI: https://doi.org/10.54393/pbmj.v9i1.1321

of NPD through epidemiological data, mainly based on population-based surveys and clinical investigations in Western nations, estimates the prevalence to range between 6.2 and 7.7 in males and between 4.8 and 6.2 in females. Twin and family studies, which are also extensively done in Western populations, suggest a strong heritable component with heritability estimates of about 24% in community samples, and up to 77% in clinical samples, with little support for shared environmental effects or sex-specific effects [6]. Despite these results, NPD diagnosis remains largely dependent on the clinical interview and standardized psychometric measures, which are prone to the self-reporting bias and are possibly determined by cultural norms and expectations [7]. Modern etiological theories define NPD as the result of a multifaceted interaction of genetic predisposition and environmental factors. It is believed that genetic vulnerability is a contributory factor to narcissistic personality traits as a subset of the larger heritable personality dimensions [8]. Negative early life experiences, such as childhood neglect, abuse, lack of parental validation, or overpraise, can also disrupt normative self-development and interpersonal functioning in genetically susceptible individuals and lead to a greater expression of narcissism [9]. These characteristics may be further enforced by cultural settings that contribute to the importance of individual success, competition, and social status. Psychiatric genetics, such as genome-wide association studies (GWAS), candidate gene studies, and molecular genetic studies, have found a number of genetic variants that may be linked to narcissism, although none of the results have been confirmed to be definitive genetic biomarkers of NPD [10].

Empirical studies of NPD are not well studied and represented in the literature in Pakistan. Previous research has also mainly concentrated on predicting the extent of prevalence of personality disorders as a general term, and not NPD as such. The lack of diagnostic instruments that have been validated in a specific cultural context, mental health stigma, and lack of access to psychiatric care are significant obstacles to proper diagnosis and treatment. Moreover, the impact of cultural norms that promote collectivism, family respect, and social rank could also affect the expression of the manifestations of narcissism and related interpretations, which can lead to a higher chance of under recognition or misclassification in clinical environments. The article by the author is a narrative review of the literature available on the topic of narcissistic personality disorder with specific emphasis on the etiological, diagnostic, genetic, and sociocultural aspects of the issue in the Pakistani context.

The present article was carried out in the form of a narrative literature review to generalize the available evidence regarding the biological, genetic, diagnostic, and sociocultural aspects of narcissistic personality disorder (NPD), focusing specifically on the findings that could be used in the context of Pakistan and other collectivistic societies. The narrative approach has been chosen to enable the incorporation of divergent study designs and theoretical outlooks that cannot be accommodated in a quantitative meta-analysis. The literature search was conducted in several electronic databases, such as PubMed, Scopus, PsycINFO, and Google Scholar, to cover the comprehensive psychiatric, psychological, genetic, and sociocultural studies. The predefined search keys and phrases were used as China searches were taken, either alone or in combination, such as narcissistic personality disorder, narcissism, genetic factors, epigenetics, neurobiology, diagnosis, cultural factors, South Asia, and Pakistan. There were a set period of publication (20192024) and a clear inclusion/exclusion criterion. Manual screening of reference lists of the relevant articles was also performed to find some more relevant studies. Scholarly articles had to meet the inclusion criteria of being peer-reviewed and focused on at least one of the areas of interest: etiology, genetic or neurobiological correlates, diagnostic models, cultural factors, or psychosocial and functional consequences of NPD. Since there were few literature sources on the specifics of the region, a study carried out outside of Pakistan was incorporated, where the results were conceptually applicable or offered comparative data on collectivistic versus individualistic cultures. Articles that described subclinical narcissism but not personality pathology were not considered. Opinion pieces, abstracts of conferences, and other unpublished material were not taken into consideration. The overall parts of the articles selected and included were done based on their relevance in terms of the themes covered, methodological clarity, and their contribution to conceptual knowledge, and not on quantitative limits. The literature reviewed consisted of different study designs, including cross-sectional surveys, twin and family studies, neuroimaging studies, and organizational or educational population-based studies. The case-based discussions mentioned in this review only mention already published empirical studies and not illustrative or hypothetical cases. The method of data extraction was qualitative and concentrated on the nature of the studies, population context, evaluation practices, and main conclusions. Formal meta-analysis was not done because results were not homogeneous based on study designs, outcome measures, and population. Rather, the synthesis of

DOI: https://doi.org/10.54393/pbmj.v9i1.1321

findings was done in a narrative manner and grouped into thematic sections to bring out the convergent evidence, differences in context, and gaps in the literature. This methodological review allows the systematic and integrative coverage of the narcissistic personality disorder, besides considering the drawbacks of heterogeneity of the studies and the lack of large-scale empirical studies conducted in Pakistan.

Pathophysiology

Historically, the concept of narcissistic personality disorder (NPD) has been defined as a maladaptive personality characteristic, which is mainly defined by dysfunctional interactions. Nevertheless, during the last decades, the research interest has been more and more focused on the biological and neurobiological processes, which could be involved in the development and support of the disorder. The existing data indicate that the pathophysiology of NPD is multifactorial with a complex interplay between neurobiological changes, genetic susceptibility, and environmental factors. The neuroimaging has given an understanding of the structural and functional brain variation with NPD. Changes have been observed in brain areas that are involved in emotion regulation, social cognition, empathy, as well as self-referent processing [11, 12]. These areas create neural networks that are interrelated and facilitate the ability to be affective and to understand the other person, which often fail in narcissistic pathology. Findings of structural neuroimaging studies, which are mainly carried out in young male clinical samples, reveal that there are declines in white matter microstructural integrity in major neural networks, indicative of impaired connections, which could be the basis of impairment in emotional processing and interpersonal functioning [13]. Besides neurobiological evidence, new models show that gene-environment interactions determine narcissistic traits. Existing environmental stressors, especially in the sensitive developmental stages, might affect the expression of genes via epigenetic processes affecting neurodevelopment, personality formation, and susceptibility to psychopathology. These processes could be the reason behind the fluctuation and wavering of narcissistic personalities in individuals over time. In combination, the evidence available suggests that NPD pathophysiology involves a combination of interacting neurophysiological, hereditary, and environmental factors, as summarized conceptually in figure 1.

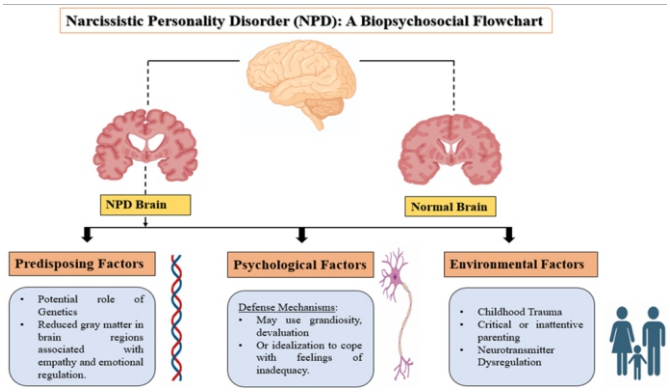

Figure 1: Schematic Representation of Biological, Psychological, and Environmental Factors Involved in the Development of Narcissistic Personality Disorder

Although there has been increased knowledge in neuroimaging, molecular genetics, and epigenetics, the biological basis of NPD has not been fully comprehended yet. The majority of the results are correlational, and causal mechanisms have not been well defined. This has resulted in the fact that targeted biological interventions have not been able to be developed and, as a result, there is a need to conduct further integrative and longitudinal studies to help understand the etiological processes that may be involved in such a complex and debilitating disorder.

Narcissistic Personality Disorder in Pakistan

The Pakistani society is largely collectivistic and highly emphasizes family pride, status in the society, and interdependence. As an individual, one is usually highly related to the family and the social role, and the social demands tend to favor group unity rather than the self-expression of an individual. In this cultural background, narcissism can manifest itself in different ways compared to individualistic societies. The grandiosity and the desirability of being admired may be formed in terms of any form of success that raises family prestige, but not individual distinction, and in such ways, the practices may be viewed as culturally normal as opposed to psychopathology. This means that narcissistic personality disorder can be misunderstood or misdiagnosed either in clinical or social practice [14]. Pakistan has socially constructed and maintained gender roles. The males are supposed to exhibit authority, dominance, and leadership, which can intersect with narcissistic aspects of entitlement and control, in addition to being socially acceptable instead of pathological [14]. Conversely, the narcissistic qualities in females can be more secretive, and adoration can be gained with the help of a physical appearance, societal status, or even home and family success. Mental health is also socially stigmatized, thus making diagnosis and treatment more difficult, especially for women who may not seek treatment because of the

DOI: https://doi.org/10.54393/pbmj.v9i1.1321

possibility of being ostracized by society [15]. Despite the slow increase in mental health awareness in Pakistan, significant infrastructural impediments to accessing mental care continue to exist, such as stigma, lack of resources, and misunderstandings about mental illness [16]. In a family system, narcissistic individuals are usually linked to dysfunctional relationship behaviors such as emotional neglect, interpersonal conflict, manipulation, and emotional detachment. Being often observed by partners and family members, the psychological distress manifests in emotional damage, confusion, and relationship instability that can lead to the disruption of the family over the long run. The emerging, albeit scant, evidence of the social and relational effects of narcissistic traits is presented through the empirical studies carried out in Pakistan. A cross-sectional study carried out in Multan had a negative correlation between narcissistic traits and healthy family functioning, where the levels of narcissism were greater among men than it was among women. The authors proposed that gender differences could be partially supported by culturally supported positions of authority, and women were more negatively impacted in family contacts, which could be because of higher emotional commitments and relational sensitivity [17]. Model 1: Narcissistic tendencies, forgiveness, and empathy predictors of social connectedness among university students and (B) Model 2: The influence of narcissistic personality disorder on cognitive organizational cynicism, mediated by psychological capital in selected hospitals [18, 19], as shown in figure 2.

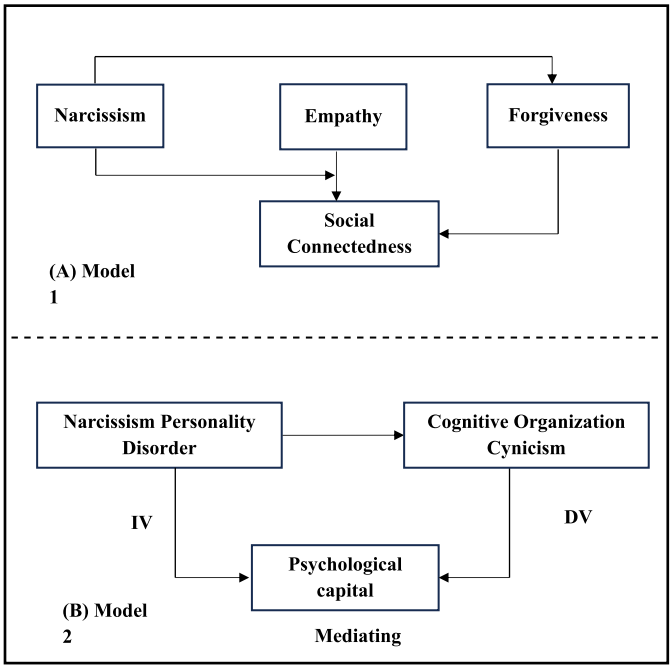

Figure 2: Published Empirical Studies from Pakistan

Some published empirical studies that were carried out within academic and occupational settings have also investigated the narcissistic traits among Pakistani populations further. In the study that was conducted on 280 university students of the University of Lahore, descriptive statistics, correlational analyses, moderated regression, and independent sample t-tests were used to analyze the relationships between narcissistic tendencies, empathy, forgiveness, and social cohesion. The results stated that narcissistic personality trait had a negative relationship with social relationships, and empathy and forgiveness had a positive relationship with social connectedness, where gender and family structure acted as moderating variables [18]. The other quantitative research conducted on the relationship between narcissistic traits and organizational cynicism in Pakistani nurses, and the study determined psychological capital (PsyCap) as a mediating variable. The study employed correlational and mediation analyses and revealed a significant correlation between narcissism and organizational cynicism, though with the reduction of PsyCap, which was higher. Cronbach and Kaiser-Meyer-Olkin values were acceptable, and thus indicated that organizational interventions to increase psychological capital can be used in the reduction of maladaptive narcissistic behaviors in a professional context [19, 20]. Other studies have analyzed narcissism in students and employees in terms of aggression, self-esteem, use of social media, and unethical pro-organizational behavior. Research on college and university students has shown positive correlations between narcissism and aggression, and significant gender variations on certain dimensions of narcissism. The studies of the social media usage process have shown that a greater involvement in the use of social media platforms such as Facebook and Instagram could be related to a greater level of narcissism among younger and more educated users, and the sociocultural context, individual personality, and digital surroundings interact to a complex degree [21]. Moderating effect of organizational change on the connection between narcissism and unethical pro-organizational behavior (UPB), and its resultant effect on guilt and shame [22], as shown in figure 3.

DOI: https://doi.org/10.54393/pbmj.v9i1.1321

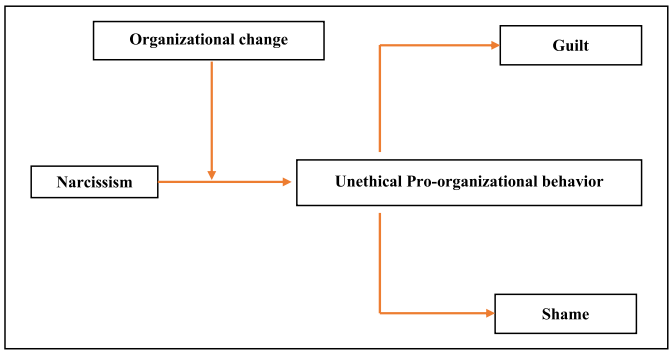

Figure 3: The Moderating Effect of Organizational Change Between Narcissism and UPB

Occupational research employing a three-wave design involving 287 employees has further demonstrated that narcissistic traits are associated with unethical pro-organizational behavior, particularly during periods of organizational change. These findings align with trait activation theory, suggesting that situational cues may amplify narcissistic tendencies while attenuating feelings of guilt and shame, thereby increasing the likelihood of unethical actions [22]. Conceptual frameworks derived from selected published studies are illustrated in Figures 2 and 3; these figures represent descriptive schematics based on existing literature and do not depict original empirical data.

Genetic Underpinnings

Twin and family studies provide uniform support for a genetic factor in narcissistic personality disorder (NPD). According to a meta-analysis of twin and family-based studies, which were mostly carried out in Western countries, it was determined that the contribution of heredity to the development of narcissistic personality disorders is about 0.51, which is proportional to moderate or significant genetic influence on the condition [23, 24]. These results imply that genetic predisposition is an important factor in the formation of narcissistic character, and environmental factors are also important. Candidates' genes of dopaminergic and serotonergic neurotransmission have been of primary interest in molecular genetic research since it has been proven to play a role in the processing of rewards, affect regulation, and social behavior. It was also reported that narcissistic traits are correlated with polymorphisms in various genes, including SLC6A3 and SLC6A4, including the 5-HTTLPR variant of the serotonin transporter gene [24, 25]. Other reports have put forth the possible associations with other genes like COMT, DRD4, and other serotonergic variants, but again, results have been mixed, and no one genetic marker has been shown to be related definitively with NPD [26]. Altogether, existing data indicate a polygenic and multifactorial genetic architecture in the form of a non-deterministic genetic factor [26].

Neurobiological Correlates

The development of neuroimaging, especially functional magnetic resonance imaging (fMRI), has also made a significant contribution to the comprehension of the neurobiological correlates of narcissistic personality disorder. The functional studies always indicate a change in the activity in parts of the brain that are related to reward processing, self-referential thinking, and empathic functioning. To illustrate, those with NPD have exhibited decreased ventral striatum activation when performing social reward or acceptance regarding activities, which indicates a distortion in reward sensitivity in the interpersonal dimensions [27]. Conversely, greater medial prefrontal cortex activation has been found in self-referential or self-enhancing tasks, which are used to indicate an increased focus on self and self-evaluation processing [28]. Neuroimaging and structural neuropathology findings also corroborate these results by showing a loss of gray matter volume in the fronto-paralimbic systems, such as the anterior insula, medial and dorsolateral prefrontal cortices, and the medial cingulate cortex [29]. These are the areas that play a pivotal role in emotional consciousness, empathy, and social cognition. Endogenous changes in the anterior insula, especially, have been linked to the lack of empathic processing, which is one of the key clinical signs of NPD. Though these neurobiological results contribute to the disorder, they can only be considered as correlates and not as causal processes.

Diagnosis of Narcissistic Personality Disorder

Narcissistic personality disorder diagnosis depends mainly on a clinical evaluation with the help of standardized diagnostic criteria provided in the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5). The major diagnostic characteristics are the presence of pervasive grandiosity, the major need for admiration, and lack of empathy, with impaired functional levels or interpersonal distress in the social, occupational, or relational spheres. Even though biological correlates, represented by neuroimaging and genetic findings, have been observed to correlate with the narcissistic characteristics, they are not yet brought into the mainstream of the diagnostic practice [30-32]. Diagnosis is, thus, still based on clinical interviews, behavior observation, and validated psychometrics. Since cultural norms affect the way people express their personality, especially in collectivistic cultures like Pakistan, one should be cautious when it comes to the sociocultural context, as one may be over- or underdiagnosed. Biological results are not to be considered diagnostic measures but

DOI: https://doi.org/10.54393/pbmj.v9i1.1321

rather, secondary data, which supports the necessity to adopt culturally sensitive and integrative systems of evaluation, as shown in table 1.

Table 1: Potential Treatment Approaches For Narcissistic Personality Disorder

S. No.	Treatment Approach	Description	Potential Benefits	Limitations	References
1	Psychotherapy	Individual therapy focusing on self-awareness, emotional regulation, and interpersonal skills	Improve self-esteem, relationships, and social functioning	May be a lengthy process, and NPD individuals might be resistant to change	[30]
2	Schema Therapy	Identifies and modifies maladaptive cognitive patterns	Address core beliefs and emotional needs underlying NPD behaviors	Limited research on effectiveness specifically for NPD	[11]
3	Family Therapy	Involves family members in the therapeutic process	Improve communication and family dynamics, but requires all parties to be willing to participate	May not be suitable for all families	[23]
4	Medication	Not a primary treatment, but might be used for co-occurring conditions like depression or anxiety.	Help manage symptoms, but doesn't address the core issues of NPD	Does not target core personality pathology	[31]

Clinical pathway for narcissistic personality disorder: From DSM-5 diagnostic criteria to multi-modal treatment planning and long-term management, as shown in figure 4.

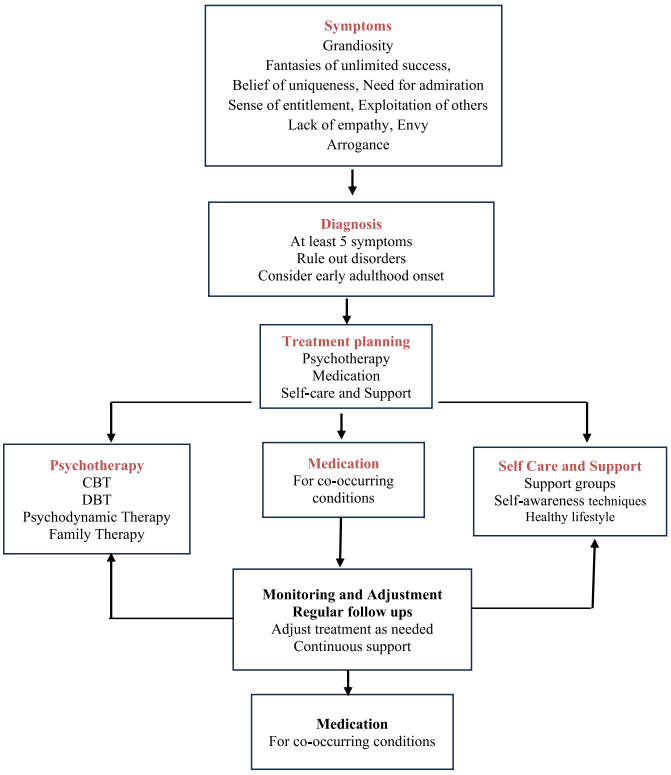

Figure 4: Clinical Pathway for Narcissistic Personality Disorder: From DSM-5 Diagnostic Criteria to Multi-Modal Treatment Planning and Long-Term Management

Role of Genetic Testing

Genetic testing has been suggested as a research-based method to comprehend the complex interrelation between hereditary vulnerability and environmental factors that cause narcissistic personality disorder (NPD). Modern tools, including genome-wide association studies (GWAS), can be used to identify genetic variants that may be related to narcissistic behavior by comparing genomic data of people with the disorder to that of control groups. Such methods have increased our understanding of the polygenic nature of personality traits and associated psychopathology, but the results are preliminary and generalized [33]. Genetic testing is currently not utilized in clinical practice as a diagnostic test in NPD. Since people who have a family history of narcissistic behaviors might be more susceptible, genetic results are not adequate to determine the onset or severity of the disorder. Rather, these data might be added to general risk profiling in their interpretation together with developmental history, psychosocial stressor and clinical examination [34]. Genetic counseling can be helpful as a supportive intervention because genetic risk, which is likely to occur, can be comprehended in a biopsychosocial context instead of being perceived as a deterministic fact. Ethics are still at the forefront in discussing the issue of genetic testing in personality disorders. The matters of privacy, data security, stigmatization, possible discrimination, and the psychological factor of risk disclosure should be considered. Proper management of data and informed consent, as well as careful interpretation of findings, is necessary in order to reduce harm and safeguard autonomy at the individual level. Genetic testing must therefore be seen as a research tool and not a clinical application at the moment. Genetic research could be useful in future progression of NPD research, especially when combined with psychological assessment and neurobiological results; however, at this point, its contribution to everyday diagnosis and treatment planning is limited [35].

DOI: https://doi.org/10.54393/pbmj.v9i1.1321

Future Prospects for NPD Research and Treatment

Future research on narcissistic personality disorder (NPD) holds substantial potential for advancing both theoretical understanding and clinical practice. Longitudinal study designs are particularly important, as they can clarify developmental trajectories and identify early-life factors that contribute to the emergence and persistence of narcissistic pathology across the lifespan. Such studies may help distinguish transient narcissistic traits from clinically significant disorders. Another critical direction involves the investigation of gene–environment interactions. Examining how genetic susceptibility interacts with childhood experiences, trauma, parenting styles, and sociocultural stressors may enable the development of more targeted and preventive intervention strategies. This approach is especially relevant in culturally diverse settings, where environmental influences may modify genetic risk expression. The identification of reliable biological markers represents an important objective for future research. Advances in neuroimaging techniques and molecular genetics may facilitate earlier identification of individuals at risk and improve prediction of treatment response. In this context, precision medicine and pharmacogenomics offer promising avenues for tailoring therapeutic interventions according to individual biological profiles, potentially enhancing treatment efficacy while minimizing adverse effects. In addition, interventions targeting neuroplasticity may support improvements in emotional regulation, self-awareness, and interpersonal functioning among individuals with NPD. The increasing use of social media platforms also warrants further investigation, as digital environments may reinforce or amplify narcissistic behaviors. Finally, family- and community-based interventions, along with interdisciplinary collaboration across genetics, neuroscience, psychology, and social sciences, are essential for refining diagnostic tools and developing culturally sensitive, person-centered treatment models. Collectively, these strategies may contribute to improved clinical outcomes and enhanced quality of life for individuals affected by NPD and those around them.

Study Limitations

This is a narrative review that has a number of limitations. The process of study selection was not systematic, and thus, it can add selection bias. A good deal of the biological and genetic evidence is obtained in Western populations, which restricts the generalizability of culture. The literature on Pakistani research is limited, and most of it evaluates non-clinical samples of narcissistic qualities, instead of diagnosed NPD. The difference in study designs and measurement instruments also limited direct comparison of the results.

CONCLUSION

The review adopts a multidisciplinary approach to narcissistic personality disorder by integrating genetics, neuroscience, psychology, and social research. It highlights the combined role of genetic and environmental factors, including epigenetic mechanisms, in NPD development. Emphasis is placed on a multimodal diagnostic and treatment framework involving psychological assessment, clinical observation, and emerging technologies such as pharmacogenomics. Despite advancements, key research gaps remain in gene–environment interactions, biomarker identification, long-term outcomes, and ethical considerations of personalized genetic testing.

Authors' Contribution

Conceptualization: MS
Methodology: MS, ZS, CR, UFL, SZ
Formal analysis: MS, ZS, UFL
Writing and Drafting: MS, ZS, CR, UFL
Review and Editing: MS, ZS, CR, UFL, SZ

All authors approved the final manuscript and take responsibility for the integrity of the work.

Conflicts of Interest

The authors declare no conflict of interest.

Source of Funding

The author received no financial support for the research, authorship and/or publication of this article.

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PAKISTAN BIOMEDICAL JOURNAL
https://www.pakistanbmj.com/journal/index.php/pbmj/index
ISSN (E): 2709-2798, (P): 2709-278X
Volume 9, Issue 01 (January 2026)

Original Article

Association Between Gestational Diabetes Mellitus and Maternal Bone Metabolism: A Cross-Sectional Study

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ARTICLE INFO

Keywords:
Osteocalcin, Gestational Diabetes Mellitus, Bone-Specific Alkaline Phosphatase, Biomarkers

How to Cite:
Hafiz, W. U., Asif, M., Manzoor, M., Umar, I., Ahmad, W., Asim, F., Mushtaq, M. N., Ayub, A., Affan, M., & Khan, Z. M. (2026). Association Between Gestational Diabetes Mellitus and Maternal Bone Metabolism: A Cross-Sectional Study: Gestational Diabetes Mellitus and Maternal Bone Metabolism. Pakistan BioMedical Journal, 9(1), 20-25. https://doi.org/10.54393/pbmj.v9i1.1323

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Received Date: 9th December, 2025
Revised Date: 10th January, 2026
Acceptance Date: 26th January, 2026
Published Date: 31st January, 2026

ABSTRACT

Gestational diabetes mellitus (GDM) is a prevalent metabolic condition complicated by pregnancy that relates to poor maternal and infant outcomes. The connection between glucose intolerance and a shift in the bone metabolism in pregnant women is a developing field whose applicability of bone turnover measurements in GDM is yet to be determined. **Objectives:** To determine the relationship between GDM and maternal bone turnover indices and GDM predictors. **Methods:** The groups of pregnant women with GDM and those without diabetes were 120 and 60, respectively, in the second trimester of this cross-sectional study. Serum osteocalcin, a cross-linked C-telopeptide of type I collagen (CTX), and bone-specific alkaline phosphatase (B-ALP) were measured. It comprised a series of clinical, biochemical, and obstetric data, such as the body mass index (BMI) and insulin resistance, which was determined using the homeostasis model assessment (HOMA-IR). ROC curve analysis and logistic regression analysis were carried out. **Results:** GDM women were significantly older women with much higher BMI and HOMA-IR compared to controls ($p<0.001$). B-ALP level of the GDM group was substantially low ($p<0.05$), and CTX did not change. The results of the logistic regression analysis identified the independent predictors of GDM as osteocalcin, BMI, and HOMA-IR (OR: 0.565; 0.442-0.722; OR: 1.309; 1.062-1.614; OR: 2.289; 1.090-4.805). The discriminative power (AUC = 0.905; $p<0.001$) was found to be powerful. **Conclusions:** GDM is also related to bone metabolism, osteocalcin, BMI, and HOMA-IR are independent predictors.

INTRODUCTION

Approximately 7–14% of pregnant women worldwide develop GDM, which is characterized by glucose intolerance during pregnancy [1]. The prevalence of GDM in China and Asia is reported to be 14.8% and 14%, respectively [2, 3]. GDM upsurges the risk of obstetric complications such as preterm delivery, macrosomia, neonatal metabolic disturbances, and cesarean birth [4]. Insulin resistance and dysfunction of pancreatic β-cells are central to the pathogenesis of GDM, though recent work has increasingly implicated osteocalcin and other bone-derived hormones in regulating energy metabolism and glucose homeostasis [5]. Although once regarded

DOI: https://doi.org/10.54393/pbmj.v9i1.1323

primarily for its structural role, recent studies have revealed that the skeleton also exerts essential regulatory effects on metabolic functions [6]. Osteocalcin, a non-collagenous protein secreted predominantly by osteoblasts in bone, has been increasingly recognized not just for its structural role in mineralization, but also for important endocrine functions in energy metabolism. In its fully γ-carboxylated form, osteocalcin binds to hydroxyapatite in bone; however, an undercarboxylated fraction (ucOC) released during bone resorption appears to be the hormonally active form that impacts glucose and lipid homeostasis [7]. The ucOC enhances beta cell proliferation, enhances insulin secretion, and develops insulin sensitivity in adipose tissue and muscles, and thus lessens fat mass as proved in animal studies. Also, insulin resistance develops in osteocalcin-deficient mice; osteocalcin administration reverses these metabolic derangements [8]. Despite strong preclinical evidence, several gaps remain unexplored. For example, the human studies do not differentiate between ucOC vs carboxylated fraction of osteocalcin. Furthermore, the studies omit concurrent measurement of other turnover makers like B-ALP, CTX, and HOMA-IR [9, 10]. Notwithstanding, these markers are often used to measure changing processes in bone growth and bone resorption.

Limited human evidence exists regarding the relationship between gestational diabetes mellitus (GDM) and maternal bone metabolism, particularly the role of bone turnover markers such as osteocalcin, CTX, and B-ALP in predicting metabolic dysfunction during pregnancy. Most previous studies have either focused on general glucose regulation or failed to assess multiple bone biomarkers alongside insulin resistance parameters, leaving uncertainty about their combined clinical relevance. This gap creates a challenge for early identification of metabolic alterations that may influence maternal and fetal outcomes. Therefore, this study aimed to examine the association between GDM and selected bone turnover markers in second-trimester pregnant women to better understand their potential as indicators of metabolic risk.

METHODS

The study was a cross-sectional one conducted in the University of Lahore Teaching Hospital (ULTH) in the period between January and December 2023. Ethical approval was received by the Institutional Research Ethics Committee (IREC) of the Department of Pharmacy, University of Lahore (No. IREC-2023-30H), and informed written consent was taken from all the subjects as per the institutional ethics. The research was done in accordance with the Declaration of Helsinki. The sample size was calculated by Open Epi with a 95 percent level of confidence, the power was 80 percent, and the anticipated differences in osteocalcin levels as reported in the literature were 108; 120 women were recruited to cover dropouts. The study sample was recruited sequentially in the normal antenatal care, with 60 having GDM and 60 having normal glucose tolerance (frequency-matched on ages and gestational age). The inclusion criteria were uncomplicated singleton pregnancies of 2428 weeks of gestation with ultrasound (Aplio 300 system, Canon Medical, Japan) transvaginal (PVT-375BT) and abdominal (PVT-475BT) transducer (16 MHz). A 75g OGTT: fasting glucose >95mg/dL, 1h>180mg/dl, or 2h 153mg/dl was used to diagnose GDM based on IADPSG criteria. Women who had pre-gestational diabetes, thyroid conditions, chronic kidney or liver disease, or women taking drugs that influence the bone or glucose metabolism were excluded. Maternal age, BMI, blood pressure, and obstetric history were taken. Plasma glucose, insulin, lipid profile, and bone turnover markers. Venous blood plasma samples were collected by means of fasting. Enzymatic measurements were performed to determine glucose and lipids (Roche Diagnostics, Germany), insulin through chemiluminescent immunoassay (Abbott Laboratories, IL, USA), and HOMA-IR as [fasting insulin (μIU/mL) × fasting glucose (mg/dL)]/ 405. Measurement of bone markers was done via ELISA: osteocalcin (Immunodiagnostic Systems, UK), B-ALP (MicroVue BAP EIA, Quidel, USA), and CTX (Serum CrossLaps ELISA, IDS, UK). IBM SPSS version 26.0 was used to conduct statistical analysis. Continuous data are mean and SD, and categorical data are in the form of frequencies and percentages. The difference between groups was measured using the 2x2 test, and the Pearson correlation coefficient was employed to establish the independent predictors of GDM. Significance was set at $p<0.05$.

RESULTS

Continuous variables, including maternal age, BMI, fasting glucose, osteocalcin, B-ALP, CTX, and HOMA-IR, were summarized using descriptive statistics. The measured values were within the range of values anticipated in adult female and generally consistent across the subjects. These variables did not miss any data. Out of the 120 participants, there were 60 who were diagnosed with GDM, and 60 who were non-GDM controls, so it was possible to compare the two similar groups of 60 each. About 61.7 per cent of women gave birth via cesarean section. The proportion of neonatal hypoglycemia was found in 52/60 (87.5) infants born to mothers with GDM and 3/60 (5) non-GDM, which is associated with a corrected calculation (Table 1).

DOI: https://doi.org/10.54393/pbmj.v9i1.1323

Table 1: Descriptive Statistics of Maternal and Biochemical Parameters Among Study Participants(n=120)

Parameters	Values
Age	29.3 ± 3.7 Years
BMI	26.8 ± 2.9 kg/m^2
Fasting Glucose	91.7 ± 12.1mg/dL
Osteocalcin	11.9 ± 3.0 ng/mL
B-ALP	22.9 ± 4.6 U/L
HOMA-IR	2.3 ± 0.8
CTX	0.5 ± 0.1 pg/mL

An association was found between GDM and delivery mode. Women with GDM were more likely to deliver their babies by cesarean(61.7%%)than women without GDM(38.3%)(χ^2(1)= 5.08, p=0.024). Thus, there was a strong correlation between GDM and neonatal hypoglycemia. Among babies born to women with GDM, hypoglycemia was reported in 87.5%, whereas only 12.5% of babies in the non-GDM group were affected (χ^2 (1) = 9.22, p=0.002). Results suggest that maternal glycemic control may be associated with the baby's metabolic health(Table 2).

Table 2: Descriptive Statistics of Cesarean Delivery and Neonatal Hypoglycemia Among Women with Gestational Diabetes Mellitus

Variables	Category	Frequency (%)
GDM	Yes	60 (50 %)
	No	60 (50 %)
Cesarean delivery	Yes	74 (61.7 %)
	No	46 (38.3 %)
Neonatal Hypoglycemia	Yes	105 (87.5 %)
	No	15 (12.5 %)

The association between osteocalcin and key glycemic indicators was assessed using Pearson correlation coefficients. Lower osteocalcin levels were correlated with higher fasting blood glucose, and a significant negative correlation was observed between osteocalcin and insulin resistance (HOMA-IR). These findings suggest that reduced osteocalcin may be linked to adverse glycemic regulation in pregnant women(Table 3).

Table 3: Pearson Correlation of Osteocalcin with Fasting Glucose and HOMA-IR in Pregnant Women

Variables	Fasting Glucose Levels	HOMA-IR
Osteocalcin	r = 0.474**	r = 0.396**
	p<0.001	p<0.001

**Pearson correlation coefficient. p<0.001 (2-tailed) indicates statistical significance.

To compare biochemical markers and maternal characteristics between women with and without GDM, an independent sample t-test was used. Maternal age showed no significant difference between the two groups (p=0.544). However, women diagnosed with GDM demonstrated increased insulin resistance and poorer glucose control, reflected by higher BMI (p<0.001), fasting glucose (p<0.001), and HOMA-IR (p<0.001) relative to the control group. Differences were also observed in serum bone turnover markers. Osteocalcin levels were significantly lower in women with GDM compared with those without GDM (10.07 vs. 13.91 ng/mL, p<0.001). Bone-specific alkaline phosphatase (B-ALP) levels were modestly but significantly decreased in the GDM group (p=0.025), while CTX levels showed no significant difference between the groups(p=0.131)(Table 4).

Table 4: Descriptive Statistics of Maternal Age, BMI, Fasting Glucose, and Osteocalcin in Women with and without Gestational Diabetes Mellitus

GDM	Age	BMI	Fasting Glucose	Osteocalcin
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
No	29.15 ± 3.709	25.60 ± 2.292	83.04 ± 8.120	13.906 ± 1.2
Yes	29.57 ± 3.825	28.06 ± 3.055	100.40 ± 8.996	10.073 ± 0.9

*SD means Standard deviation

Binary logistic regression analysis was performed to evaluate the relationship between clinical and biochemical variables and GDM. BMI, osteocalcin, B-ALP, CTX, and HOMA-IR were the predictor variables in the model. The general model was statistically significant, 5 (5) = 77.79, p<0.001, which showed that the variables were useful in distinguishing between women with and without GDM. Cox and Snell R 2 and Nagelkerke R 2 indicated that the model explained 47.7% and 63.6% of the variance in GDM status, respectively. The Hosmer-Lemeshow goodness-of-fit test (2 = 14.70, p=0.065) showed that the model fitted the data adequately. The results indicated that a higher BMI(p=0.011, OR = 1.31), lower osteocalcin levels (p<0.001, OR = 0.57), and higher HOMA-IR (p=0.029, OR = 2.29) were independent predictors of GDM. On the other hand, there were no strong associations found between B-ALP and CTX in the final model. These results signify that insulin resistance, osteocalcin, and BMI are significant and independent variables that are connected with the threat of developing GDM. The logistic regression analysis produced a receiver operating characteristic (ROC) curve that illustrated excellent discrimination power in women with and without GDM. The curve was steeply rising towards the upper-left part, indicating a high sensitivity and specificity at different cutoff points. Generally, the model performed well with an AUC of 0.911. The Area Under the Curve Is 0.911, Indicating Excellent Predictive Performance(Figure 1).

DOI: https://doi.org/10.54393/pbmj.v9i1.1323

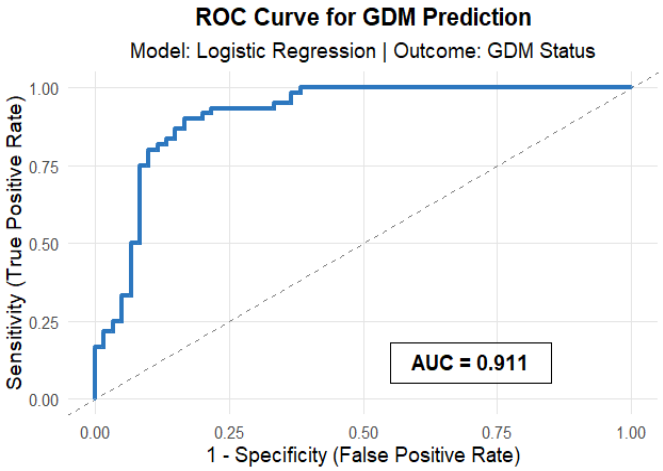

Figure 1: Receiver Operating Characteristic (ROC) Curve for the Logistic Regression Model Predicting Gestational Diabetes Mellitus Status

DISCUSSION

The effect of GDM on biomarkers of bone turnover, like osteocalcin, CTX, and B-ALP, was investigated in the current study. The findings revealed a significant alteration in these biomarkers compared to women without GDM. Thus, it suggests that there exists a potential association between impaired bone metabolism and glycemic control. During normal pregnancy, a steady decline in insulin activity is logical as it increases serum glucose level, thus ensuring a healthy supply of glucose to the fetus, especially during the second and third trimesters [11]. Thus, such a form of insulin resistance is considered natural and healthy for the fetus. During GDM, conversely, the pancreatic beta cells endue insufficient compensation towards serum glucose, which leads to maternal hyperglycemia [1, 12]. It is well known that bone-derived biomolecules are stimulated during the process of osteogenesis, and these molecules are involved in energy metabolism, consequently extending the role of bone beyond structural support. By the way, osteocalcin, a non-collagenous protein secreted by osteoblasts, has been reported to stimulate β-cell proliferation, improve insulin sensitivity, and increase insulin secretion in peripheral tissues [13]. Therefore, any physiological change in bone turnover, as seen during pregnancy, may affect glucose homeostasis and osteocalcin levels [14, 15]. The findings of the current study endorse the previous observations, as glucose tolerance and insulin resistance were obvious in the GDM patients [14]. Nevertheless, the physiological implications of this finding are complex enough, as osteocalcin exists in both uncarboxylated (ucOC) and carboxylated form, with metabolic regulation predominantly driven by the uncarboxylated isoform of it [16, 17]. The inability to analyze these isoforms separately is a recognized limitation of this study. Moreover, the serum level of a biomarker of osteoblast activity, e.g., B-ALP, was found to be lower compared to that of healthy pregnant women. While glucose level is not regulated by B-ALP levels, a reduced level of it may suggest compromised bone mineral density in GDM patients, which is potentially linked to its low level of circulatory level [18]. Studies have shown that dysfunctional insulin metabolism in type 2 diabetes is associated with lower bone formation [19], and equally, a comparable mechanism may also be relevant in the context of GDM. The lack of significant variation in the level of CTX suggests that variability in glucose level during GDM may primarily influence osteoblast function, with minimal effects on osteoclast, and so warrants further investigations on this aspect. Overall, our findings align with previous observations where Gong *et al.* reported a persistent reduced level of osteocalcin in women with GDM after delivery, and these women were found to be at higher risk of abnormal glucose metabolism [11]. Similarly, Hwang *et al.* have found that pregnant women with higher osteocalcin concentrations demonstrated greater insulin sensitivity [20]. Collectively, the evidence underscores a bone-pancreas interaction, with done-derived factors contributing to the control of glucose regulation and energy metabolism. Assessment of bone turnover markers alongside metabolic parameters may help identify women at higher risk of GDM, characterized by insulin resistance, elevated BMI, and low osteocalcin levels.

Although the relationship between gestational diabetes mellitus and bone turnover is illuminated, due to the cross-sectional study design, it is impossible to draw conclusions based on the causal or time relationship. Moreover, the inability to differentiate uncarboxylated and carboxylated osteocalcin, which play different functions in metabolism, and the failure to directly determine the bone mineral density are significant drawbacks of the methods. Longitudinal and mechanistic research on bone-derived factors and glucose metabolism in and after pregnancy requires the inclusion of analyses of osteocalcin isoforms and follow-ups in the postpartum period to enhance understanding of the relation between bone-derived factors and glucose metabolism.

CONCLUSIONS

The research revealed that there is a close relationship between GDM and bone turnover markers. The findings of the research showed that GDM is associated with lower bone turnover marker levels, particularly osteocalcin. Osteocalcin, as well as BMI and HOMA-IR, had independent relationships with GDM, and the role of the bone metabolism biomarker in affected women is possible. Although the study demonstrates the association, it does not allow making causal or predictive inferences because the study is cross-sectional. More studies should be

DOI: https://doi.org/10.54393/pbmj.v9i1.1323

conducted to affirm these results, as well as to investigate the biological processes that are involved, e.g. the interaction between the bone and pancreas.

Authors Contribution

Conceptualization: WUH, MA, MM, WA, FA, AA
Methodology: MA, IA, WA, FA, MNM,
Formal analysis: WUH, MM, WA, ZMK
Writing and Drafting: MM, IU, WA, FA, AA, MA
Review and Editing: WUH, MA, MM, IU, WA, FA, MNM, AA, MA, ZMK

All authors approved the final manuscript and take responsibility for the integrity of the work.

Conflicts of Interest

The authors declare no conflict of interest.

Source of Funding

The author received no financial support for the research, authorship and/or publication of this article.

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DOI: https://doi.org/10.54393/pbmj.v9i1.1323

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DOI: https://doi.org/10.54393/pbmj.v9i1.1333

PAKISTAN BIOMEDICAL JOURNAL

https://www.pakistanbmj.com/journal/index.php/pbmj/index

ISSN (E): 2709-2798, (P): 2709-278X

Volume 9, Issue 01 (January 2026)

Original Article

OPEN ACCESS

Association of *CYP19A1* Gene Polymorphism with Male Infertility in Khyber Pakhtunkhwa Population, Pakistan

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ARTICLE INFO

Keywords:
CYP19A1 Gene, Steroidogenesis, Male Infertility, Testosterone, Genetic Variations, Hormonal Regulations

How to Cite:
Alamgeer, M., Irfan, M., Ahmad, I., Khan, M. F., Rehman, F. U., Khan, S., & Muhammad, H. (2026). Association of CYP19A1 Gene Polymorphism with Male Infertility in Khyber Pakhtunkhwa Population, Pakistan: CYP19A1 Gene Polymorphism and Male Infertility in Khyber Pakhtunkhwa. Pakistan BioMedical Journal, 9(1), 26-32. https://doi.org/10.54393/pbmj.v9i1.1333

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Received Date: 4th December, 2025
Revised Date: 15th January, 2026
Acceptance Date: 19th January, 2026
Published Date: 31st January, 2026

ABSTRACT

Male infertility is a multifactorial disease that is controlled by genetic, hormonal, and semen factors. In this research, the authors examined the relationship that exists between the *CYP19A1* gene polymorphisms, semen parameters, and hormone profiles in male infertility. **Objectives:** To determine the Polymorphism *CYP19A1* Gene and Male Infertility among the Khyber Pakhtunkhwa Population, Pakistan. **Methods:** 186 men were recruited into the study, consisting of 106 infertile men and 80 healthy controls. Sanger sequencing was done on three *CYP19A1* SNPs (rs17703883, rs726546, and rs10046). ELISA was used to determine serum hormonal levels of luteinizing hormone (LH), follicle-stimulating hormone (FSH), prolactin, and testosterone, and semen parameters, such as sperm count, morphology, and motility. **Results:** Genotype and allele frequency analysis showed a significant relationship between male infertility and the polymorphisms of the following: rs 17703883 and rs 726546, and not the polymorphism of rs10046 ($p>0.05$). Nonetheless, none of the SNPs that were researched were significantly correlated with serum testosterone levels. **Conclusions:** These observations indicate that the same *CYP19A1* gene (rs17703883 and rs726546) might have an implication on male infertility among this population, but they do not seem to be the cause of lowered serum testosterone. More research with an increased sample size and functional studies needs to be done to elucidate the functions of these variants. Additional studies on the genetic role of oestrogen metabolism in the occurrence of male infertility need larger samples and other functional variants of *CYP19A1*.

INTRODUCTION

Fertility refers to the ability to conceive and bear children, whereas infertility, as defined by the World Health Organization as the failure to conceive after 12 months of unprotected sexual contact [1, 2]. Infertility affects approximately 8-12% of couples worldwide, corresponding to nearly 80 million couples, with a higher burden in low and middle-income countries [3, 4]. In industrialized nations, infertility affects approximately 10-15% of reproductive-age couples due to abnormalities in gamete production, fertilization, and inadequate embryo development [5]. In Pakistan, infertility prevalence has been reported to be as high as 21.9%, affecting more than one-fifth of the married population [6, 7]. Male infertility is commonly associated with sperm abnormalities, low sperm count, impaired motility, morphological defects, and azoospermia [8]. Various factors such as genetic abnormalities, lifestyle factors, infections, endocrine disorders, and testicular pathologies contribute to male Infertility [9, 10].

DOI: https://doi.org/10.54393/pbmj.v9i1.1333

Spermatogenesis is regulated by the hypothalamic pituitary gonadal axis, in which gonadotropin-releasing hormone stimulates the secretion of LH and FSH. LH acts on Leydig cells to promote testosterone synthesis, while FSH supports Sertoli cell function and spermatogenesis [11, 12]. Leydig cells (LCs), located between seminiferous tubules, are responsible for testosterone production through Steroidogenesis, a process that converts cholesterol into biologically active steroid hormones. Testosterone and estrogen play an essential role in male reproductive development and adult spermatogenesis [13]. Aromatase, encoded by the *CYP19A1* gene, exists in multiple transcriptional variants and has been linked to altering enzymatic activity due to certain genetic polymorphisms [14, 15]. The *CYP19A1* gene encodes aromatase, a member of the Cytochrome P450 family, which catalyzes the conversion of androgens such as testosterone and androstenedione into estrogens [16]. Mutations can inhibit or stimulate the expression of the enzyme aromatase, and this enzyme is expressed in both sexes' gonads. The *CYP19A1* gene is located on Chromosome 15q21.2 and consists of 18 exons [17].

Male infertility is a multifactorial condition with growing evidence suggesting that genetic variations, particularly in steroidogenesis-related genes such as CYP19A1, may influence reproductive function; however, data from the Khyber Pakhtunkhwa population remain scarce. Most existing studies focus on other ethnic groups, creating a research gap in understanding the role of *CYP19A1* polymorphisms within this genetically diverse region. Therefore, the present study aimed to investigate the association between selected *CYP19A1* gene polymorphisms (rs17703883, rs726546, and rs10046) and male infertility, as well as their relationship with hormonal profiles and semen parameters. Addressing this gap may improve the understanding of genetic susceptibility to male infertility and support population-specific diagnostic strategies.

METHODS

A case-control study was carried out from 5 September 2023 to 5 September 2024 at an infertility centre at Imperial Polyclinic, Peshawar, Pakistan. The study included 106 infertile males (cases) and 80 matched healthy fertile males (controls). All participants were residents of the Khyber Pakhtunkhwa province. While the province is ethnically diverse, recruitment was not stratified by specific ethnic subgroups. This potential source of population stratification is considered a limitation of the study design. Cases were recruited consecutively from men presenting for infertility evaluation at the clinic during the study period who met the inclusion criteria. Controls were recruited via convenience sampling from men accompanying patients to general outpatient departments, after verification of their fertility status. Demographic and clinical data were collected through interviews using a semi-structured questionnaire. Ethical approval was obtained from the institutional review board of Khyber Medical University, Peshawar (Ref. No. KMU/IBMS/IRBE/7th meeting/2023/209-4). All participants were provided with informed consent, which was written. Interviews using a semi-structured questionnaire were used to gather demographic and clinical data. Semen samples were collected through masturbation after a period of abstinence in accordance with conventional guidelines. Physical parameters were also examined; volume, viscosity, and pH of samples were found after proper dilution with the help of the counting chamber under light microscopic study. The assessment of the sperm motility and morphology was based on the standard laboratory procedures. The fructose test was used to determine the levels of seminal fructose to measure the functionality of the seminal vesicles. The G + Power software (version 3.1.9.7) was used to calculate a priori sample size. The calculation was done based on finding a relationship between an SNP and male infertility through a chi-square test of independence. Using an assumed odds ratio (OR) of 2.5, a minor allele frequency (MAF) of 0.15 in the control group, alpha (0.05) error probability, and power (1-β) of 0.80, the required sample size was 88 participants per group. This requirement is met by our study sample of 106 cases and 80 controls, which would have sufficient statistical power to perform the main genetic association test. The DNA sequences of genes were retrieved through the NCBI database, and the primers of each SNP were written with the Primer 3 plus programme. The phenol-chloroform method of DNA extraction was used. Amplification of extracted genomic DNA was done using polymerase chain reaction (PCR). A TCY48 thermocycler was used in PCR amplification. The Sanger sequencing was done to determine specific single nucleotide polymorphism (SNPs) of the *CYP19A1* gene. The commercial ELISA and chemiluminescence kits were used to measure testosterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH in the serum, according to the protocols of the manufacturers. DNA sequences of genes were obtained from the NCBI database, and primers of each SNP were designed using the Primer 3 plus program (https://www.primer3plus.com)(Table 1).

DOI: https://doi.org/10.54393/pbmj.v9i1.1333

Table 1: Primers for gene amplification

SNP	Primer	Length	Tm	GC	ANY	Product Size
rs17703883, rs726546	AAAGAGCTGACAGTCTTGCT (F)	20	57.1 C	45.0	17	531 bp
	AGAGATGGGGAGTCAGGCAA (R)	20	60.3 C	55.0	0.0	
rs10046 SNP	AGCCATCCTCGTTACACTTCTG (F)	22	60.1 C	50.0	0.0	402 bp
	TCTCCCTCAAACTCTTGGCC (R)	20	59.3 C	55.0	0.0	

Statistical analyses were performed using SPSS version 25. Genotype and allele frequencies were calculated, and the Hardy-Weinberg equilibrium was assessed in the control group. Associations between SNPs and male infertility were evaluated using the chi-square test and odds ratios with 95% confidence intervals. The distribution of continuous variables was examined using the Shapiro–Wilk test, and equality of variances was assessed using Levene's test. For all continuous variables compared (e.g., age, weight, hormone levels), the assumptions of normality and homogeneity of variances were satisfied ($p>0.05$ for both Shapiro–Wilk and Levene's tests), allowing for the use of parametric tests. Comparisons between cases and controls were therefore performed using independent-samples t-tests. Comparisons of continuous variables were performed using independent-samples t-tests when statistical assumptions were satisfied. Associations between genetic polymorphism and hormone concentrations were analyzed using Pearson's correlation coefficient. Statistical significance was defined as a two-sided p-value of less than *0.05*.

RESULTS

This study was performed on 106 infertile men and 80 healthy controls with known demographic data in KPK province of Pakistan. The results obtained in this study concluded that out of 106 infertile males, 77 (72.64%) had primary infertility and 29 (27.35%) had secondary infertility. Various factors were studied include age, weight, hormonal profiles, different semen parameters, and single-nucleotide polymorphism, to know the association of these factors with infertility. Characteristics of the study population were compared by case-control status. The Mean ages of infertile males were 31.87 ± 6.07 years (Table 2).

Table 2: Age Distribution of Infertility Type

Sr. No.	Age (Years)	Primary Infertility, n (%)	Secondary Infertility, n (%)	Total, n (%)
1	21-30	43 (40.56%)	10 (9.43%)	53 (50%)
2	31-40	30 (28.30%)	15 (14.15%)	45 (42.45%)
3	41-50	4 (3.77%)	4 (3.77%)	8 (7.54%)
Total	21-50	77 (72.64%)	29 (27.35%)	106 (100%)

Furthermore, the study examined infertility distribution across different body weights. The mean weight of the participant was 72.4 ± 8.47kg (Table 3).

Table 3: Weight Distribution of Infertility Types

Sr. No.	Age (Years)	Primary Infertility, n (%)	Secondary Infertility, n (%)	Total, n (%)
1	60-80	68 (64.15%)	24 (22.64%)	92 (86.79%)
2	81-100	9 (8.49%)	3 (2.83%)	12 (11.32%)
3	101-118	0 (0%)	2 (1.88%)	2 (1.88%)
Total	60-118	77 (72.64%)	29 (27.35%)	106 (100%)

The study revealed and analyzed infertile patients with sperm pathologies. This study found that 39% asthenospermic, 27% azoospermic, 18% normal, 13% teratospermic, and 3% oligospermic. Out of 106 infertile men, 27 had azoospermia, 13 had teratospermia, 3 had oligospermia, and 39 had asthenospermia (Figure 1).

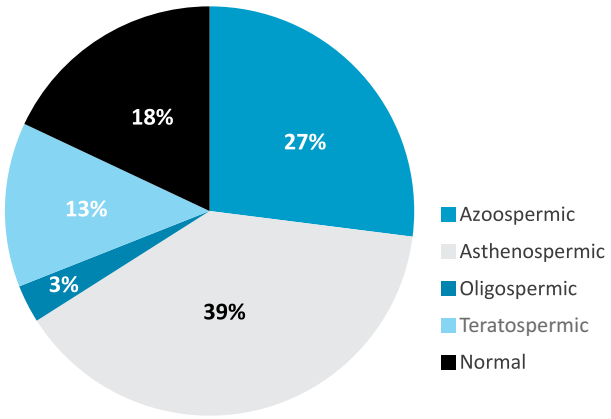

Figure 1: The Percentage of Infertile Patients Having Different Sperm Abnormalities

Chromatogram results were analyzed by Finch TV and Bio-Edit, and Well-defined peaks indicate a successful sequencing reaction. SNPs 726546 showing a C>T variation with peaks for both C and T, and rs17703883 displaying an A>G variation with distinct peaks for both alleles A and G. The presence of peaks for alleles at each SNP Position Suggests genetic variation within the sample. The study examined the genotypes and allelic frequencies for the selected SNPs, rs17703883, rs726546, and rs10046. The analysis showed the AG and AG+GG genotypes and the G allele were more frequent in cases than in controls. Similarly, rs726546 demonstrated the TC and TC+TT genotypes, and the T allele was significantly enriched among infertile men. In contrast, rs10046 showed no significant differences in genotype or allele distributions between cases and controls (Table 4).

DOI: https://doi.org/10.54393/pbmj.v9i1.1333

Table 4: Shows the Frequencies of Genotypes and Alleles in Three Selected Snaps of the *CYP19A1* Gene

Sr. No.	SNP Name	Genotype	Cases	Controls	OR (95%)	p-value
1	SNP rs17703883	AG	27 (25.47%)	8 (10%)	3.0721 (1.3105 - 7.2018)	0.009
		GG	1 (0.94%)	1 (1.25%)	0.9103 (0.0559 - 14.8264)	0.947
		AG+GG	28 (26.41%)	9 (11.25%)	2.8319 (1.2511 - 6.4102)	0.012
		Alleles				
		A	183 (86.32%)	150 (93.75%)	1	–
		G	29 (13.67%)	10 (6.25%)	2.3770 (1.1223 - 5.0347)	0.023
		AG	27 (25.47%)	8 (10%)	3.0721 (1.3105 - 7.2018)	0.009
2	SNP rs726546	CC	92 (86.8%)	79 (98.75%)	1	–
		TC	13 (12.26%)	0 (0%)	23.2054 (1.3577 - 396.6202)	0.029
		TT	1 (0.94%)	1 (.25%)	0.8587 (0.0528 - 13.9541)	0.914
		TC+TT	14 (13.20%)	1 (1.25%)	12.0217 (1.5462 - 93.4722)	0.017
		Alleles				
		C	197 (92.92%)	158 (98.75%)	1	–
		T	15 (7.8%)	2 (1.25%)	6.0152 (1.3554 - 26.6957)	0.018
3	SNP rs10046	AA	103 (97%)	77 (96.3%)	1	–
		AG	2 (1.9%)	2 (2.5%)	0.7476 (0.1030 - 5.4260)	0.773
		GG	1 (0.94%)	1 (1.25%)	0.7476 (0.0460 - 12.1414)	0.837
		AG+GG	3 (2.83%)	3 (3.75%)	0.7476 (0.1469 - 3.8054)	0.726
		Alleles				
		A	207 (97.64%)	155 (96.87%)	1	–
		G	4 (1.89%)	4 (5%)	0.7488 (0.1844 - 3.0410)	0.685

The relationships between the three *CYP19A1* SNPs (rs17703883, rs726546, and rs10046) and serum testosterone levels were analysed using Pearson's correlation, as specified in the methods. Testosterone levels were treated as a continuous variable, and genotypes were coded additively (e.g., 0, 1, 2 for the number of minor alleles). The analysis revealed no significant linear correlations: for rs17703883 (r = -0.045, p=0.649), for rs726546 (r = -0.087, p=0.374), and for rs10046 (r=0.019, p=0.847). These results indicate no statistically significant association between any of the studied SNPs and serum testosterone levels in the study population (Table 5).

Table 5: Associations of SNPs with Testosterone Level

Sr. No.	SNP	Allele (n)	Mean testosterone level (ng/mL)	p-value
1	rs17703883	G 27 (25.2%)	2.230-6.420	0.406
		A 78 (72.9%)	1.140-7.850	
2	rs726546	T 14 (13.2%)	1.950-5.550	0.710
		A 93 (87.7%)	1.140-7.850	

The study displays a detailed chromatogram obtained from sequencing, which includes (a) the rs726546 and (b) the rs17703883 SNPs. Each segment of the chromatogram provides insight into the nucleotide composition and quality of the sequencing data for these specific genetic regions (Figure 2).

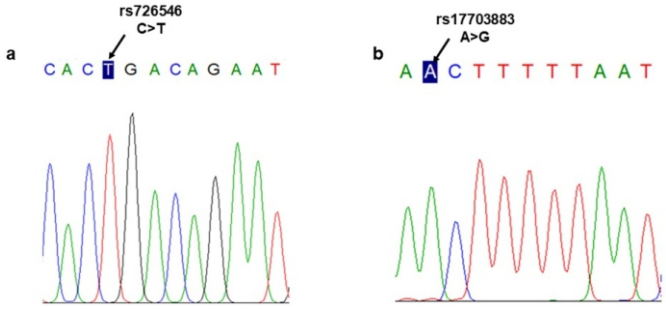

Figure 2: A Detailed Chromatogram Obtained from Sequencing

DISCUSSION

The genetic factor of male infertility is very high, and the single-nucleotide polymorphisms (SNPs) have an important role in the spermatogenic failure and the reproductive dysfunction. The current research assessed the relationship between polymorphisms of the *CYP19A1* gene (rs17703883, rs726546, and rs10046) and male infertility in one of the least studied ethnic groups, Khyber Pakhtunkhwa (KPK), Pakistan, and offers innovative data on the genetic foundation of infertility in the group. The *CYP19A1* gene codes for the aromatase enzyme, a cytochrome P450 family enzyme, which converts androgens (testosterone) into estrogens (estradiol and estrone). Despite being regarded as female hormones, estrogens are synthesized in the male reproductive tissue through aromatization and have been found to play major roles in male fertility. Estrogens have now been identified as obligatory regulators of male fertility and participating in

DOI: https://doi.org/10.54393/pbmj.v9i1.1333

spermatogenesis, sperm maturation as well as regulation of the hypothalamic-pituitary-gonadal axis [18, 19]. *CYP19A1* genetic variations can alter the aromatase activity, disrupt the androgen-estrogen balance, and, thus, cause hampered sperm production and functionality. Genetic studies were conducted in this paper where the mean age of a participant was 31.87 ± 6.07 years, and the mean weight was 72.4 ± 8.47kg. Past literature has indicated the correlation between *CYP19A1* polymorphisms and the absence of normal spermatogenesis, defective semen metrics, and disturbed metabolism [20]. The fact that genetic variations in *CYP19A1* are observed to correlate with what is hypothesized in this study is that the variation in *CYP19A1* could influence the production of estrogen in the local area of the testes, and could not be due to a complete deficiency of the hormone. Infertile men might also have a high rate of asthenospermia and azoospermia in this study, which can be partially attributed to extreme cases of genetic influence. According to the functional research, destabilized estrogen activity in the testis, the result of alterations in aromatase encoded by the *CYP19A1* gene, is associated with impairments in spermatogenesis and abnormalities in sperm maturation and motility. Male aromatase-deficient mice were developed progressive spermatogenic arrest and infertility as a result of suppressed estrogen production during germ cells development [21], and the *CYP19A1* knockout rabbits had reduced numbers of sperm, impaired spermatogenesis, and lower levels of sperm motility with defective flagellar structure [22]. These hereditary effects can be independent of the sperm count, and this is why the role of molecular mechanisms in infertility in males is also important. As the current research showed no statistically significant relationship between serum testosterone and the SNP polymorphisms: rs17703883, rs726546, and rs10046, it can be assumed that the SNP polymorphisms do not affect the levels of circulating testosterone in our population (Table 4). This lack of association is aligned with other past genetic studies that have been conducted to determine *CYP19A1* polymorphisms with respect to hormones. In a study that examined whole gene sequencing of aromatase gene variants in a large cohort of males, it was also found that whilst some *CYP19A1* haplotypes were linked with the variations in estradiol and luteinizing hormone, these haplotypes did not have a significant impact on the levels of circulating testosterone, complex interactions between aromatase variants and steroid hormone-regulating activities instead of a direct effect on testosterone concentration itself [23]. On the same note, some studies have found no apparent or consistent relationship between the prevalent *CYP19A1* SNP and blood levels of sex hormones. The research conducted on 19 polymorphisms of *CYP19A1* in women in China discovered the association with estrogen metabolites, but no distinct association of the other genetic polymorphisms assessed in the study, such as testosterone levels [24]. The association between some *CYP19A1* haplotypes and SNPs with serum testosterone and other sex hormones in some sub-groups has also been observed in another study in postmenopausal Japanese women, and demonstrates that the effect that aromatase gene variation has on hormones may be population- and context-specific. Research findings are opposite to the past findings [25]. The polymorphism rs10046 has been commonly researched on various occasions. Even though other researchers explain its correlation to estrogen-related conditions or disease phenotype (e.g., cardiovascular risks in postmenopausal women or hormone-related cancers), they seem to be context- and tissue-specific and are not always represented in systemic concentrations of testosterone [26]. In addition, meta-analysis of *CYP19A1* variants has provided substantial risk factors to the disease like Alzheimer's under certain genetic models, but also did not indicate a direct relationship to the varied testosterone levels in males [27]. Although *CYP19A1* polymorphisms other than those studied in the current study (e.g., rs749292 or rs2414096) have been linked to hormone levels and sperm parameters in specific subgroups of the population (e.g., obese male), they do not apply to the SNPs under study. The total genetic effect on hormone levels of *CYP19A1* variants is likely a result of interactions between genes and the environment, rather than a single SNP effect. Also, polymorphism in *CYP19A1*, like rs749292 and rs2414096 were found to be associated with the change in reproductive hormone profile, which included reduced circulating testosterone and increased estrogen levels in obese males with idiopathic infertility, indicating that certain *CYP19A1* variants may modulate hormone homeostasis in specific physiological contexts [28]. These investigations indicate that although rs17703883, rs726546, and rs10046 did not significantly correlate with testosterone in the current sample, other *CYP19A1* gene variants are capable of relating to the alteration of sex steroid levels in various groups and in different clinical conditions. The lack of correlation in the existing data can also indicate the multifaceted aspect of testosterone regulation by various enzymes and receptors, as well as aromatase, 5alpha-reductase, luteinizing hormone receptor pathways, and androgen binding proteins, which have proven to play a vital role in testosterone metabolism and reproductive endocrinology. Overall, the absence of correlation between the rs17703883, rs726546, and rs10046 with the testosterone level in this study is consistent with the previous studies, indicating that the genetic variations of *CYP19A1* do not directly predict the level of systemic testosterone in adults.

DOI: https://doi.org/10.54393/pbmj.v9i1.1333

Instead, the consequences of these polymorphisms can be delicate, tissue-specific, or controlled by an estrogen biosynthesis pathway that needs further functional studies. The general analysis failed to present strong evidence of the direct correlation that exists between the *CYP19A1* polymorphism and male infertility. This implies that these variants might not significantly contribute to infertility vulnerability in the population under study, and it might be other genetic, environmental, or epigenetic influences.

This study is limited by its modest sample size, potential population stratification within the ethnically diverse Khyber Pakhtunkhwa region, and the use of convenience sampling for controls, which may affect generalizability. The lack of association between the studied SNPs and serum testosterone suggests that other *CYP19A1* variants, gene-gene interactions, or local testicular estrogen dynamics may be more relevant. Future studies should employ larger, multi-center cohorts with ethnic stratification, include functional assays to assess aromatase activity, and explore a broader panel of *CYP19A1* variants alongside environmental and epigenetic factors to fully elucidate the genetic architecture of male infertility.

CONCLUSIONS

This study found no statistically significant association between the *CYP19A1* gene polymorphisms rs17703883, rs726546, and rs10046 and male infertility in the Khyber Pakhtunkhwa population. Additionally, none of the studied SNPs showed a significant relationship with serum testosterone levels. Further investigations involving larger sample sizes and additional functional variants of *CYP19A1* are required to clarify the potential genetic contribution of estrogen metabolism to male infertility.

Authors Contribution

Conceptualization: MA, HM
Methodology: MFK, FUR, SK
Formal analysis: MI, IA,
Writing and Drafting: MA, MFK, HM
Review and Editing: MA, MI, IA, MFK, FUR, SK, HM

All authors approved the final manuscript and take responsibility for the integrity of the work.

Conflicts of Interest

The authors declare no conflict of interest.

Source of Funding

The author received no financial support for the research, authorship and/or publication of this article.

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DOI: https://doi.org/10.54393/pbmj.v9i1.1326

PAKISTAN BIOMEDICAL JOURNAL
https://www.pakistanbmj.com/journal/index.php/pbmj/index
ISSN (E): 2709-2798, (P): 2709-278X
Volume 9, Issue 01 (January 2026)

Original Article

OPEN ACCESS

Clinical Learning Experiences of Nursing Students at Medical Teaching Institute, Mardan College of Nursing and Mardan Medical Complex: A Qualitative Study in Khyber Pakhtunkhwa, Pakistan

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ARTICLE INFO

Keywords:
Nursing Education, Clinical Learning, BS Nursing, Learning Experiences, Clinical Learning Environment, Nursing Student, Clinical Staff, Staff Nurses

How to Cite:
Khan, S., Muhammad, D., Ali, S. B., Khan, H., Naz, N., Khanum, S., & Aslam, Z. (2026). Clinical Learning Experiences of Nursing Students at Medical Teaching Institute, Mardan College of Nursing and Mardan Medical Complex: A Qualitative Study in Khyber Pakhtunkhwa, Pakistan: Clinical Learning Experiences of Nursing Students: A Qualitative Study in Khyber Pakhtunkhwa. Pakistan BioMedical Journal, 9(1), 33-39. https://doi.org/10.54393/pbmj.v9i1.1326

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Received Date: 14th December, 2025
Revised Date: 25th January, 2026
Acceptance Date: 29th January, 2026
Published Date: 31st January, 2026

ABSTRACT

Clinical and practical learning is an important element of teaching in the profession of nursing, as it allows for the transformation of classroom theory into practical clinical situations. This integration of theory into practice could be affected by many challenges in the clinical learning environment that students encounter. Students' perceptions regarding difficulties faced in the clinical setting could be of help in assessing and addressing these challenges. **Objectives:** To explore the nursing students' opinions on their clinical learning experiences. **Methods:** A qualitative descriptive study design using a phenomenological approach was used to explore the opinions of the students of bachelor nursing program on their clinical learning experiences. Thematic analysis was done. The sample included three males and three female students. **Results:** Five themes emerged from the data, which included: anxiety towards clinical learning environment, barriers to clinical learning, inappropriate teaching practices and learning context, ineffective coordination in the learning environment, and inefficient clinical faculty and preceptor role. **Conclusions:** The study results indicated nursing students' overall dissatisfaction with their clinical learning experiences. The findings advocated for bringing improvement in the clinical learning environment and correcting the highlighted deficiencies in the best possible way. The nursing faculty, clinical staff, and administration should play their role in this regard.

INTRODUCTION

Clinical training is an integral element of nursing education as it enables the integration of theoretical knowledge acquired in the classroom into practical clinical situations [1]. The effective transformation of theory into practice is influenced by several factors, particularly the clinical learning environment [2]. The clinical learning environment comprises a combination of physical, psychological, emotional, and organizational factors that affect students' learning [3]. A learning environment that is conducive to clinical education and centered on students' learning needs is essential for ensuring quality nursing education [4]. Clinical learning experiences include all

DOI: https://doi.org/10.54393/pbmj.v9i1.1326

activities undertaken to integrate theory into practice, provide effective student coaching, and deliver constructive feedback related to clinical training with the aim of improving learning outcomes [5, 6]. Favorable clinical learning experiences facilitate the transition of students from theoretical learners to competent individuals capable of managing practical clinical situations. In contrast, insufficient or inappropriate learning experiences may leave students feeling unprepared and lacking confidence [7]. Evidence suggests that positive clinical learning experiences are associated with active student involvement in clinical activities and adequate supervision by clinical staff [8]. Negative clinical learning experiences include a lack of faculty support, limited challenging learning opportunities, and poor communication among students, clinical staff, and nursing faculty [5]. Similarly, an insufficient number of teachers and preceptors required to guide students' learning can negatively influence clinical learning [1]. Additional factors that may impede students' clinical learning include work overload, role ambiguity, and inadequate knowledge and expertise among faculty, clinical teachers, and preceptors [9]. Baraz et al. reported that inadequately qualified nursing faculty and the absence of supportive learning environments adversely affect clinical learning [10]. Lawal et al. further identified factors such as preceptorship, preceptor-to-student ratios, cooperation from clinical staff, and the quality of pre-clinical conferences as key influences on clinical learning [11]. Evidence indicates that students consistently perceive a need for improvement in their practical preparation. Available data also highlight varying levels of anxiety and confusion among students, particularly at the beginning of clinical training [12]. At times, students in clinical settings may perform routine care activities rather than engage in meaningful learning. However, the consequences of these challenges on students' clinical learning have not been sufficiently explored [13]. Understanding students' perspectives on their clinical learning experiences is therefore crucial, as these experiences are fundamental for integrating theoretical concepts into practice. Students' satisfaction with clinical learning experiences is considered a vital factor in improving and tailoring clinical education to meet learners' needs [14].

Despite this importance, literature exploring nursing students' views on clinical learning experiences remains limited in Pakistan. While some studies have addressed challenges faced by nursing students, most have focused primarily on the clinical environment. Qualitative evidence exploring students' perspectives on their clinical learning experiences is scarce. Therefore, this study aims to explore nursing students' opinions regarding clinical learning experiences at Mardan College of Nursing and Mardan Medical Complex (MMC), Mardan.

METHODS

A qualitative descriptive study design employing a phenomenological approach was used to explore nursing students' opinions regarding their clinical learning experiences. The study was conducted at the Medical Teaching Institution (MTI), Mardan College of Nursing, Bacha Khan Medical College (BKMC), and Mardan Medical Complex (MMC), Khyber Pakhtunkhwa, Pakistan, over four months from 20 April 2025 to 17 August 2025. Ethical approval was obtained from the Institutional Review Board of MTI Mardan College of Nursing (Ref No: 690/BKMC). Participants were selected using a purposive sampling technique. Inclusion criteria required participants to be enrolled in the Bachelor of Science in Nursing (BSN) program at MTI Mardan College of Nursing and to have completed at least one clinical rotation. Students not currently engaged in clinical rotations or those enrolled in other nursing programs (e.g., diploma courses), were excluded. Sample size was determined based on data saturation, assessed through concurrent data collection and analysis. After the fifth and sixth interviews, repetition of codes and categories was observed with no emergence of new themes, indicating informational redundancy. Consequently, six interviews were conducted. Data were collected using semi-structured interviews guided by the study objectives and relevant literature. The interview guide was reviewed by two senior nursing education experts to ensure clarity and relevance. Written informed consent was obtained before audio-recorded interviews, which were transcribed verbatim within two days of completion.

Data analysis followed Braun and Clarke's thematic analysis approach [15]. Transcripts were read repeatedly during open coding to identify and label relevant statements. Axial coding involved grouping similar codes into categories to develop themes and sub-themes. Selective coding was then used to refine relationships among categories. Redundant codes were removed, enabling refinement of themes and improved consistency of interpretation.

RESULTS

Among the total 6 participants, 3 (50%) were males, and 3 (50%) were females. Majority of them were in the age range of 22-24 years, where 67% were enrolled in the Generic BSN and 33% in Post RN BSN. Regarding year of study, the majority of them (67%) were studying in the Generic BSN 4th year. Majority of the participants (67%) had 3 years of clinical exposure (Table 1).

DOI: https://doi.org/10.54393/pbmj.v9i1.1326

Table 1: Demographic Profile of the Participants (n=6)

Participants	1	2	3	4	5	6
Gender	Male	Female	Female	Male	Male	Female
Age (In Years)	23	27	22	23	22	25
Educational Status	Generic BSN (Enrolled)	Post RN BSN (Enrolled)	Generic BSN (Enrolled)	Generic BSN (Enrolled)	Post RN BSN (Enrolled)	Generic BSN (Enrolled)
Present Year of Studying	4th Year	2nd Year	4th Year	4th Year	4th Year	1st Year
Duration of Clinical Exposure	3 Years	1.5 Years	3 Years	3 Years	3 Years	7 Months

Analysis of the students' data on their opinions on clinical learning experiences resulted in the emergence of five predominant themes. These included: anxiety towards the clinical learning environment, barriers to clinical learning, inappropriate teaching practices and learning context, ineffective coordination in the learning environment, and inefficient clinical faculty and preceptor roles.

Theme 1: Anxiety Towards the Clinical Learning Environment

The study participants expressed feelings of worry and anxiety in the clinical learning environment. Participants' anxiety was mainly related to apprehension of being in a new environment and a changed routine, poor professional self-concept, and stress associated with work overload.

1.1. Apprehension of Being in a New Environment and a Changed Routine

Participants reported anxiety when entering the clinical environment for the first time, mainly due to unfamiliarity with ward routines, procedures, and expectations. Changes in routine across different clinical units were also identified as a source of anxiety. One participant explained, "Because of the different routine in each unit, we faced difficulties in the beginning; nonetheless, it got easy after 2 to 3 days" (Participant 2). Participants suggested that proper orientation before clinical rotations could help reduce anxiety.

1.2. Poor Professional Self-Concept

Another major source of anxiety reported by participants was poor professional self-concept. Students felt that their knowledge and opinions were often undervalued during discussions or clinical procedures, which negatively affected their motivation and self-esteem. Participants also associated poor professional self-concept with their nursing uniform, which they believed contributed to low self-esteem. One student stated, "Doctors and other people associate our nursing uniform with diploma nursing and thus think that we don't know anything. One day, I was in a scrub suit in CCU, while my colleagues were wearing white uniforms. The behavior of the consultant was completely different from me, and he involved me more in learning. If our uniform is changed, that will enhance our self-esteem and motivation" (Participant 1).

1.3. Stress of Work Overload

Participants reported that anxiety was also caused by work overload in the clinical setting. Students felt pressured to perform duties of clinical staff, which limited opportunities to achieve their learning objectives. One participant stated, "We used to go there with a set of learning objectives to achieve, but they kept us restricted to learning bed making and IV cannulation and involved us in their own duties" (Participant 1).

Theme 2: Barriers to Clinical Learning

This theme highlights certain hindrances faced by the students during their clinical learning. These learning barriers include: uncooperative clinical staff, inadequate clinical instruction and evaluation, and poorly equipped learning infrastructure.

2.1. Uncooperative Clinical Staff

Students expressed the concern that the clinical staff restricted their learning by involving them in performing only the basic learning skills like vital signs, bed making, etc., and didn't allow them to advance to skills as per their learning objectives. Another barrier expressed by the participants was the perception of non-acceptance of nursing students' learning needs in the clinical setting. A student mentioned: "We used to select a patient there for case discussion and application of nursing process, or to prepare a teaching plan for the patient, or to perform a physical assessment; they did not allow us to do all that by saying that you are nursing students and it's not your job" (Participant 1).

2.2. Inadequate Clinical Instruction and Evaluation

Participants expressed dissatisfaction with clinical teaching and evaluation, which acted as a learning barrier in the clinical area. Ineffective clinical supervision was the main concern. One of the students highlighted: "We do not get the supervision in clinical the way we need it, and we perform the skills without supervision. Neither the teachers are there to supervise us nor the clinical staff" (Participant 3).

2.3. Poorly Equipped Learning Infrastructure

The participants expressed their concern over poorly equipped wards and skills as a learning barrier. A student stated: "Proper learning facilities and equipment should be made available both in the skills lab and in the hospital to facilitate our clinical learning. On the hospital side, only CCU and ICU have sufficient equipment; the rest of the wards have insufficient equipment. Like we are taught

DOI: https://doi.org/10.54393/pbmj.v9i1.1326

about infusion pumps in the classroom, but that is not available in each ward, infusion pumps are not there, even in CCU and ICU, only syringe pumps are there. Only by operating an infusion pump can we practically learn about it, and for that, it should be available in the wards" (Participant 1).

Theme 3: Inappropriate Teaching Practices and Learning Context

This theme describes the inappropriateness of teaching practices and the learning environment as perceived by the students. The participants stressed the need for effective clinical teaching and the provision of an overall supportive learning environment.

3.1. The Need for Appropriate Teaching Practices

The participants raised the concern of not properly transforming theory into practice. A student verbalized: "We are unable to integrate theory into practice, and in this way, we face many problems that lead to negative feelings among students"(Participant 4).

3.2. Provision of a Supportive Learning Environment

Participants highlighted that the clinical environment was overall unsupportive for clinical learning and pointed out certain concerns in this regard. One of the students explained the ingredients of a supportive clinical environment as: "The staff should guide the students on each point during their clinical duty. Moreover, patients should also be available for students' learning (Participant-3). Yet another student argued: "In a supportive clinical learning environment, the clinical staff should have proper communication with students, and they should be cooperative with students. They should be problem solver to the students. Similarly, the faculty should be cooperative"(Participant 5).

Theme 4: Ineffective Coordination in the Learning Environment

This theme concerns the lack of proper communication and coordination for students' learning to be effective. This ineffective coordination is elaborated with reference to interpersonal relations of students with faculty, clinical staff, and seniors, an ill-matched clinical roster, and recognition and understanding among health professionals towards BS Nursing.

4.1. Interpersonal Relations with Faculty, Clinical Staff, and Seniors

Students realized the need for good interpersonal relations with the clinical staff for proper clinical learning. One student stated: "There should be a good relationship between the students and clinical staff because if you have a good understanding with staff, then they support you in different learning occasions"(Participant 5).

4.2. Recognition and Understanding among Clinical Staff Regarding BS Nursing

The students stressed proper communication among faculty, the clinical staff, hospital administration, and students, as one of the participants emphasized: "There should be proper communication from the college side to the hospital authorities regarding our clinical learning". He added: "There should be coordination between clinical staff and faculty"(Participant 4).

4.3. Ill-Matched Clinical Roster

Congruence between the theoretical content taught and practical learning experiences ensures the effective integration of theory into practice. This is required when a clinical rotation is being planned. However, participants in the current study were discontented with this process and hence, pointed out the need for correspondence between the theoretical content and the clinical rotation. A student suggested: "Students should be assigned to the concerned ward related to the theory content taught in the classroom simultaneously"(Participant 5).

Theme 5: Inefficient Clinical Faculty and Preceptor Role

Participants opined that the role of clinical faculty and the preceptor was inefficient. The clinical faculty has the primary role of planning and administering the clinical teaching & learning activities and supervising students for clinical practice. A preceptor has a crucial role in guiding, facilitating, supervising, and monitoring the students in accomplishing their clinical learning outcomes. However, participants expressed their dissatisfaction with both faculty and preceptors' supervision in the mentioned context. One of the participants stated, "I would mainly talk about supervision. As a teacher can't be with each student all day, how can he supervise each student individually? They hand over our supervision to staff nurses in the ward, but the nurses expect us to do whatever they want. We do not get the supervision in clinical the way we need it, and we perform the skills without supervision. Neither the teachers nor the clinical staff are there to supervise us" (Participant-3).

DISCUSSION

Nursing faculty and clinical nursing staff have the primary responsibility to provide students with opportunities for appropriate clinical practice and support within the clinical environment to facilitate optimal learningStudents in the present study reported feelings of worry and anxiety in the clinical setting, mainly due to, fear of being novice, low self-esteem, and difficulties in time management resulting from excessive assignments and the expectation to perform clinical staff duties. Existing evidence supports that novice nurses commonly experience insecurity, confusion, and stress during role transition [16, 17]. Similarly, instruction in effective time management skills has been shown to reduce students' stress levels [18], a need also emphasized by participants in this study. One participant highlighted the concern that students were

DOI: https://doi.org/10.54393/pbmj.v9i1.1326

being restricted to only basic nursing skills and that the clinical nursing staff involved them mostly in their own duties. Mismatched expectations regarding nursing students' roles and responsibilities can lead to communication gaps, which negatively affect relationships between students and staff nurses [19]. Supportive clinical staff are therefore, essential in facilitating students' practical learning experiences [20]. Clinical instruction is a multidimensional process that includes diverse teaching and learning activities. However, participants expressed concerns related to inadequate supervision, lack of graded clinical evaluation, and an insufficient number of clinical faculty. Similar findings were reported in a study conducted in Iran, where students expressed dissatisfaction with the quality of clinical teaching provided by faculty members [21]. Participants also highlighted the absence of a formal evaluation of clinical learning as a significant concern. Evaluation plays a crucial role in maintaining professional standards and ensuring that students acquire the competencies required for safe clinical practice [22]. Notably, the university with which our college is affiliated, has recently initiated graded evaluation of clinical and practical learning in the end-of-semester examinations, a practice that was previously absent. Effective learning further requires the availability of adequate resources and facilities, particularly those supporting practical training [23]. These challenges extend beyond the local context and require urgent attention from nursing faculty to initiate meaningful improvements. Evidence from existing literature has demonstrated inadequate coordination and collaboration between nurses and doctors and emphasized the importance of fostering positive professional relationships [24]. Participants also expressed dissatisfaction with the supervision provided by both faculty members and preceptors. The preceptor's role is critical in the clinical preparation of undergraduate nursing students; however, evidence consistently indicates insufficient preparation of clinical nurses for this role [25].

This study has several limitations. Findings from a single institution may limit transferability, and the small sample size, despite achieving data saturation, remains a constraint. Additionally, the possibility of social desirability bias cannot be excluded. Future research should include multiple institutions and adopt mixed-methods designs to enhance generalizability. Intervention-based studies are recommended to develop and test models aimed at improving the clinical learning environment. Preparing a pool of well-trained clinical preceptors is therefore essential for the effective clinical supervision and optimal practical preparation of the future nursing workforce.

CONCLUSIONS

The study findings indicate that nursing students were largely dissatisfied with their clinical learning experiences. Students reported significant anxiety in clinical settings due to their novice status, changes in routine, poor professional self-concept, and stress related to academic assignments and ward responsibilities. Several barriers to effective clinical learning were identified, including limited cooperation from clinical staff, inadequate clinical instruction and evaluation, and insufficiently equipped skills laboratories and hospital wards. Participants emphasized the need for appropriate teaching strategies and a supportive clinical learning environment. Concerns were also raised regarding weak coordination between academic and clinical settings. Students highlighted the importance of professional interpersonal relationships with faculty, clinical staff, and senior healthcare professionals, as well as better awareness of BS Nursing students' learning needs and structured ward rotations aligned with learning objectives. Furthermore, the role of clinical preceptors was perceived as ineffective, despite being critical to clinical learning. Addressing these issues may help develop a confident and clinically competent nursing workforce.

Authors' Contribution

Conceptualization: SK[1], DM, NN, SK[2]
Methodology: SK[1], SBA, HK, NN, ZA
Formal analysis: SK[1], DM, SK[2]
Writing and Drafting: SK[1], SBA
Review and Editing: SK[1], DM, SBA, HK, NN, SK[2], ZA

All authors approved the final manuscript and take responsibility for the integrity of the work.

Conflicts of Interest

The authors declare no conflict of interest.

Source of Funding

The author received no financial support for the research, authorship and/or publication of this article.

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DOI: https://doi.org/10.54393/pbmj.v9i1.1328

PAKISTAN BIOMEDICAL JOURNAL
https://www.pakistanbmj.com/journal/index.php/pbmj/index
ISSN (E): 2709-2798, (P): 2709-278X
Volume 9, Issue 01 (January 2026)

Original Article

OPEN ACCESS

Effectiveness of a Self-Stretching Exercise Program on Restless Leg Symptoms in Patients with Type 2 Diabetes Mellitus: A Pre-Post Intervention Analysis

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ARTICLE INFO

Keywords:
Restless Legs Syndrome, Type-2 Diabetes Mellitus, Self-Stretching, Restless Legs Syndrome-Diagnostic Index, Non-Pharmacological Intervention

How to Cite:
Mehmood, N., Iqbal, M., Zia, M., Asif, M., & Ahmed, W. (2026). Effectiveness of a Self-Stretching Exercise Program on Restless Leg Symptoms in Patients with Type 2 Diabetes Mellitus: A Pre-Post Intervention Analysis: Restless Leg Symptoms in Patients with T2DM: A Pre-Post Intervention Analysis. Pakistan BioMedical Journal, 9(1), 40-45. https://doi.org/10.54393/pbmj.v9i1.1328

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Received Date: 12th December, 2025
Revised Date: 26th January, 2026
Acceptance Date: 28th January, 2026
Published Date: 31st January, 2026

ABSTRACT

Restless legs syndrome (RLS) symptoms, characterized by an urge to move the legs, unpleasant sensations, worsening during rest, and predominance at night, are prevalent sleep-disrupting complaints. **Objectives:** To evaluate the effectiveness of a home-based self-stretching exercise program in reducing RLS symptoms among adults with Type 2 Diabetes Mellitus. **Methods:** This experimental study recruited two hundred (200) adults aged 40–59 years with a confirmed diagnosis of T2DM (HbA1c >6.5%) via convenience sampling at District Headquarter Hospital, Lodhran, and Bahawal Victoria Hospital, Bahawalpur. Following baseline assessment, participants received standardized instruction and hands-on training in a daily lower-limb self-stretching program to perform at home for four weeks. Restless legs symptoms were quantified using the RLS Diagnostic Index (RLS-DI). Pre-and post-changes in RLS-DI Sum Score and Grand Total Score were examined with paired-samples t-tests; item-level frequency shifts were analyzed via chi-square tests (α=0.05). **Results:** The mean RLS-DI Sum Score decreased significantly from 7.39 ± 5.60 to -0.79 ± 6.25 (mean paired difference 8.17, 95% CI 7.06–9.28, t=14.56, p<0.001). The Grand Total Score fell from 3.31 ± 6.83 to -2.27 ± 6.74 (mean difference 5.57, 95% CI 4.38–6.76, t=9.20, p<0.001). Item-level analyses revealed significant reductions in frequency of urge to move (χ^2=17.395, p=0.002), unpleasant sensations (χ^2=13.671, p=0.008), worsening during rest (χ^2=12.009, p=0.017), and sleep disturbance (χ^2=22.665, p=0.001). **Conclusions:** A 4-week self-stretching program was associated with substantial reductions in restless leg syndrome symptoms among adults with T2DM. These findings position self-stretching as a feasible, low-cost, non-pharmacological strategy for managing RLS symptoms in routine outpatient diabetes settings.

INTRODUCTION

Restless legs syndrome (RLS) is a sensorimotor disorder of sleep, which is characterized by an urge to move legs frequently accompanied by unpleasant sensations. The typical symptoms, which generally develop or deteriorate during rest, improve with activity, and exhibit circadian patterns in the evening or at night, cause difficulty in the initiation and maintenance of sleep, as well as in the quality of sleep [1, 2]. Since symptoms often appear when a person is idle and are alleviated temporarily through activity, RLS may have a significant impact on bedtime habits, increase the duration between waking up and going to bed, and disrupt sleep through the night, thereby worsening daytime performance and reducing health-related quality of life [2, 3]. The modern clinical decision-making focuses on the diagnosis based on the symptom and follows the consensus criteria and the exclusion of common mimics, e.g., nocturnal leg cramps, positional pain, pain of peripheral neuropathy, and habitual movement of legs,

DOI: https://doi.org/10.54393/pbmj.v9i1.1328

which are not provoked by a sensory desire [1, 4]. There is growing observational data to indicate that RLS is more prevalent in diabetes patients as compared to non-diabetic groups. Recent meta-analysis (observational studies) has indicated that about a quarter of diabetic individuals might experience the symptoms of RLS and that diabetes is related to higher risks of RLS than non-diabetic controls [5]. Research on RLS among patients in various clinical environments indicates a significant prevalence of RLS among patients with type 2 diabetes and its linkages with diabetes complications, especially neuropathy [6-10]. Mechanistically, suggested connections between T2DM and RLS are dysregulated iron management and dopaminergic signaling, microvascular pathophysiology, oxidative damage, and changes in sensory pathways associated with diabetic peripheral neuropathy, which can symptomatically overlap with RLS or serve as an RLS precipitant [6, 8]. This medical overlap highlights the importance of thorough differential diagnosis in clinical settings with diabetes because leg pain can be classified as neuropathy, whereas a contributing pathology of RLS is under-appreciated [1, 8]. The evidence-based management of RLS currently involves treating underlying conditions (e.g., iron deficiency), checking the medications that could worsen the symptoms, and applying pharmacologic treatment in cases when the symptoms are clinically prominent [11]. Nonetheless, pharmacologic treatment can be challenging due to adverse events, polypharmacy implications in the elderly with T2DM, and the potential risk of the development of dopaminergic augmentation when using specific medications with time. In line with this, recent evidence-based practices are placing a more accurate focus on personalized care and regard non-pharmacologic approaches as significant elements of treatment, particularly in groups with a strong risk of drug risks or access limitations [11, 12]. Biologically plausible interventions to RLS include exercise-based and body-based interventions since acute movement removes symptoms and potentially affects peripheral afferent signaling, muscle tone, microcirculation, and arousal related to stress. Symptomatic population clinical trials (such as the patients on hemodialysis with a high rate of secondary RLS) show that exercise and specific stretching exercises could decrease the severity of RLS symptoms and enhance sleep-related outcomes [13, 14]. Meanwhile, lifestyle-based interventions like yoga have demonstrated potential in randomized controlled trials, and decreases in RLS symptoms and their accompanying sleep and mood outcomes, in favor of the larger theory that low-cost movement interventions can be therapeutically useful [15, 16]. While evidence remains heterogeneous across populations, these findings provide a rationale to evaluate feasible self-stretching programs that can be delivered in routine outpatient settings and practiced safely at home. In recent Pakistani rehabilitation studies, structured exercise and stretching protocols have been delivered successfully in supervised and home-based formats, supporting the feasibility of self-directed programs in local outpatient settings [17, 18]. Despite increasing global literature, local data from Pakistan, particularly South Punjab, remain limited regarding the burden of RLS symptoms in adults with T2DM and the responsiveness of RLS symptoms to practical, clinic-to-home interventions. Additionally, given the common coexistence of neuropathic symptoms, sleep fragmentation, and EDS in diabetes, there is a need for interventions that are simple, scalable, and acceptable to patients, while being measurable through validated symptom and sleepiness indices [4, 18]. The study hypothesized that regular self-stretching would reduce RLS symptom burden and potentially improve related daytime functioning, offering a low-risk adjunct to comprehensive diabetes care.

The study lacks a control group, objective outcome measures, long-term follow-up data, and assessment of key confounders like iron status, limiting causal inference and generalizability. The study cannot prove causation, subjective data, the benefits' sustainability is unknown, and real-world feasibility is unclear. Therefore, the present study evaluated the effectiveness of a structured self-stretching exercise program on restless leg symptom severity in adults with T2DM, using standardized symptom scoring and paired pre-post comparisons.

METHODS

This study used an experimental pre-post intervention design to evaluate changes in restless leg symptoms after a home-based self-stretching exercise program among adults with type 2 diabetes mellitus (T2DM). Data collection was conducted for three months from April 2025 to June 2025 at District Headquarter Hospital, Lodhran, and BVH, Bahawalpur. Approval of the study was obtained from the Ethical Review Committee of the Department of Physical Therapy, The Islamia University of Bahawalpur; reference No. 2026-A/DPT. Participation was voluntary, and written informed consent was obtained before enrollment. Participant confidentiality was maintained by anonymizing data and restricting access to study records to the research team. Baseline assessment and 4-week follow-up assessment were performed using the same procedures at the participating sites. Participants (n=200) were recruited using a convenience, non-probability sampling technique from patients attending the participating hospitals during the study period. Adults aged 40-59 years of either sex with

DOI: https://doi.org/10.54393/pbmj.v9i1.1328

a diagnosis of type 2 diabetes mellitus (T2DM) and HbA1c > 6.5% who were willing to participate were eligible for inclusion. Exclusion criteria were type 1 diabetes mellitus, nephropathy, heart disease, history of trauma, musculoskeletal conditions that could limit safe stretching performance or confound lower-limb symptoms, and diabetic neuropathy. Restless leg symptoms were assessed using the Restless Legs Syndrome Diagnostic Index (RLS-DI) [19]. The RLS-DI captured core symptom domains, and summary scores were derived according to the scoring approach used in the study dataset. Participants received instructions and demonstrations in a standardized, home-based self-stretching exercise program. The program was delivered by qualified physiotherapy staff and prescribed for four weeks. Instruction was provided in a gender-appropriate manner (female participants by female staff and male participants by male staff). Participants were advised to perform the prescribed stretches at least five times a week and at least two times a day at home throughout the intervention period as per the schedule and guidance provided. After ethical approval and permission from the Ethical Committee of the Department, eligible patients were approached in the outpatient setting. Baseline data were collected using the RLS-DI. Participants were then trained in the self-stretching program and followed for four weeks. At the end of week 4, the RLS-DI was re-administered using the same data collection procedures.

Data were analyzed using SPSS (version 23.0). Continuous variable, i.e., RLS-DI total score, was summarized using mean and standard deviation. Pre-post changes in continuous RLS-DI total scores were evaluated using paired-samples tests (paired t-test where applicable). Categorical symptom responses and categorized ESS outcomes were examined using cross-tabulations, and associations were evaluated using chi-square tests. Statistical significance was set at $p<0.05$.

RESULTS

A total of 200 participants consisted of 97 (48.5%) males and 103 (51.5%) females with a mean age of 50.76 (SD 5.537). All participants completed both baseline (pre-intervention) and follow-up (post-intervention) assessments. Both RLS-DI summary measures improved significantly at follow-up. For the Sum Score, the mean decreased from 7.39 ± 5.60 to −0.79 ± 6.25, corresponding to a mean pre-post difference of 8.17 points (95% CI: 7.06 to 9.28), t=14.56, p<0.001. For the Grand Total score, the mean decreased from 3.31 ± 6.83 to −2.27 ± 6.74, corresponding to a mean difference of 5.57 points (95% CI: 4.38 to 6.76), t=9.20, p<0.001 (Table 1).

Table 1: Pre-Post Changes in RLS-DI Summary Scores (Paired-Samples t-Tests)

Outcomes	Pre	Post	MD (Post)	t-test	p-value
	Mean ± SD	Mean ± SD			
Sum Score (RLS-DI)	7.390 ± 5.602	-0.790 ± 6.249	8.170	14.556	<0.001
Grand Total (RLS-DI)	3.310 ± 6.825	-2.270 ± 6.737	5.570	9.201	<0.001

Item-level analyses demonstrated marked shifts from higher-frequency symptoms ('occurs regularly') toward lower-frequency categories ('occurs occasionally' and 'not present') for multiple core domains. Statistically significant distribution changes were observed for urge to move the legs (p=0.002), unpleasant sensations (p=0.008), worsening at rest (p=0.017), and sleep disturbances (p=0.001). Relief with movement (p=0.180) and evening/night predominance (p=0.092) did not reach significance at the 0.05 level (Table 2).

Table 2: Pre and Post-Distribution of RLS-DI Symptom-Frequency Items and Chi-Square Tests

Item	Pre-Not Present, n (%)	Pre-Occasionally, n (%)	Pre-Regularly, n (%)	Post Not Present, n (%)	Post Occasionally, n (%)	Post Regularly, n (%)	χ^2	p-value
Urge to Move Legs	13 (6.5%)	45 (22.5%)	142 (71.0%)	70 (35.0%)	106 (53.0%)	24 (12.0%)	17.395	0.002
Unpleasant Sensations	9 (4.5%)	69 (34.5%)	122 (61.0%)	75 (37.5%)	101 (50.5%)	24 (12.0%)	13.671	0.008
Worsen at Rest	18 (9.0%)	71 (35.5%)	111 (55.5%)	72 (36.0%)	99 (49.5%)	29 (14.5%)	12.009	0.017
Relief with Movement	55 (27.5%)	83 (41.5%)	62 (31.0%)	59 (29.5%)	86 (43.0%)	55 (27.5%)	6.272	0.180
Evening/Night Increase	30 (15.0%)	50 (25.0%)	120 (60.0%)	92 (46.0%)	82 (41.0%)	26 (13.0%)	7.986	0.092
Sleep Disturbances	25 (12.5%)	50 (25.0%)	125 (62.5%)	73 (36.5%)	102 (51.0%)	24 (12.0%)	22.665	0.001

Using Total Score categorization, RLS prevalence decreased from 19.0% (38/200) at baseline to 1.0% (2/200) at follow-up. Using Grand Total categorization, prevalence decreased from 18.0% (36/200) to 1.5% (3/200). Despite these large descriptive reductions, chi-square tests were not statistically significant for either 2×2 table, likely due to very low post-intervention RLS counts and small expected cell frequencies (Table 3).

Table 3: Pre and post Categorical RLS Status and Chi-Square Tests

Categorization	Pre RLS, n (%)	Post RLS, n (%)	No→ No	No→ RLS	RLS→ No	RLS→ RLS	χ^2	p-value
Total Score	38 (19.0)	2 (1.0)	160	0	36	2	0.474	0.491
Grand Total	36 (18.0)	1 (1.5)	162	0	35	1	0.485	0.486

DOI: https://doi.org/10.54393/pbmj.v9i1.1328

DISCUSSION

In this hospital-based sample of adults with type-2 diabetes mellitus (T2DM), a structured self-stretching program was followed by a marked reduction in Restless Legs Syndrome Diagnostic Index (RLS-DI) symptom burden. Paired-sample analyses showed highly significant pre-to-post improvements in the RLS-DI sum score and the total score, with large-to-moderate within-subject effect sizes. Symptom-pattern crosstabs further suggested clinically meaningful shifts: the proportion reporting "occurs regularly" for key sensory-motor features (urge to move the legs and unpleasant sensations) fell substantially, while the proportion reporting no symptoms increased. Sleep-related impact improved as well, with a notable rise in the proportion reporting no sleep disturbance after the program. Non-pharmacological management is frequently recommended as a first step for many patients with mild-to-moderate RLS symptoms, especially when comorbidities, medication burden, or access barriers make pharmacotherapy less attractive. Contemporary reviews of conservative and rehabilitative approaches describe exercise, stretching, massage-type modalities, and movement-based strategies as plausible symptom-relieving options, although study designs and intervention doses vary considerably [20]. In the broader RLS literature, exercise-based programs have repeatedly been associated with symptom reduction, sleep improvement, and better daytime functioning in selected populations. For example, a meta-analysis of exercise training trials in hemodialysis populations reported consistent improvements in RLS symptom severity and related outcomes [14]. Although our population differed (T2DM rather than end-stage kidney disease), both groups share risk pathways that may amplify sensory symptoms (e.g., metabolic-vascular dysfunction, neuropathic features, and sleep disruption), potentially making movement-based strategies relevant. Evidence specific to diabetes is also converging on the idea that RLS is not rare in T2DM and that addressing RLS may have measurable patient-centered benefits. Observational work has documented the presence of RLS in T2DM cohorts and linked it with reduced sleep quality and quality of life [21]. Notably, even pharmacologic treatment studies in T2DM have shown that symptom improvement is accompanied by better sleep indices, and in some reports, modest improvements in glycemic control, suggesting that reducing nocturnal symptoms may help relieve sleep-related metabolic stress [22]. Within this context, our findings support the plausibility that a low-cost, home-based stretching program can meaningfully reduce symptom burden in a T2DM sample, at least in the short term. The pathophysiology of RLS is multifactorial. Contemporary synthesis work highlights contributions from iron handling and central dopaminergic signaling, as well as interactions with sleep-wake regulation and peripheral sensory inputs [23]. In people with T2DM, additional contributors, such as microvascular changes and diabetic neuropathy, may add sensory symptoms that overlap with RLS complaints or increase their salience. Because neuropathic pain, cramps, positional discomfort, and peripheral neuropathy can mimic RLS, careful clinical characterization is essential, and diabetes-focused guidance has emphasized structured assessment to differentiate true RLS from diabetic neuropathy and related mimics [24]. Supportive evidence for stretching-centered programs comes from interventional studies in clinical populations where RLS is common. For instance, a stretching exercise program in hemodialysis patients reduced RLS severity compared with usual care [25], and intradialytic stretching training has also been associated with improved RLS symptoms and sleep quality [26]. While mechanisms may differ across populations, these studies strengthen the biological and behavioral plausibility of stretching as a symptom-modulating strategy. The largest practical improvements in our crosstab analyses were observed in domains most closely tied to discomfort during rest, urge to move the legs, unpleasant sensations, and worsening at rest. These domains are also the ones most likely to be influenced by changes in muscle tension, peripheral sensory input, and immobility-related discomfort. Sleep disturbance showed a clear improvement, consistent with the idea that reducing evening symptoms decreases sleep fragmentation and improves perceived restrictiveness. In contrast, domains reflecting the defining pattern of RLS (relief with movement and evening/night predominance) showed comparatively smaller shifts and non-significant χ^2 results. This is not necessarily inconsistent with clinical improvement: for participants who continued to have residual symptoms, those symptoms may still have retained the classic RLS pattern (movement-responsive and worse in the evening), even if their overall intensity or frequency was reduced. From a pragmatic standpoint, the observed improvement suggests that structured self-stretching can be considered a low-risk adjunct within T2DM clinics, particularly where access to specialist sleep services or long-term medication monitoring is limited. Finally, while iron therapy and pharmacologic options remain important for selected patients, conservative approaches can be positioned as first-line or complementary strategies alongside medical evaluation, particularly for individuals with mild symptoms or those reluctant to use long-term dopaminergic agents.

This study used a pre-post design without a parallel control group; therefore, causal inference is limited, and

DOI: https://doi.org/10.54393/pbmj.v9i1.1328

improvements could partly reflect regression to the mean, expectancy effects, or concurrent changes in lifestyle and medication. Outcomes were based on self-reported diagnostic index scoring rather than clinician-confirmed diagnosis or objective sleep measures (e.g., polysomnography or actigraphy). The study did not include biochemical characterization (e.g., ferritin or transferrin saturation), standardized assessment of neuropathy severity, or systematic screening for sleep apnea, each of which could confound symptom reporting. Adherence to the home program was not objectively monitored, and longer-term durability of improvement was not assessed. Future work should evaluate self-stretching using randomized controlled designs with appropriate comparators (e.g., education-only, sham stretching, or standard care) and longer follow-up to test durability. Stratification by neuropathy status, iron indices, obesity, and sleep apnea risk could clarify which subgroups benefit most. Incorporating validated severity scales (e.g., IRLS), objective sleep endpoints, and glycemic outcomes (HbA1c) would help clarify whether symptom improvement translates into measurable metabolic benefit. Finally, implementation-focused studies in resource-limited settings should test pragmatic delivery methods (printed protocols, mobile reminders, brief physiotherapist coaching) and quantify adherence, feasibility, and cost-effectiveness.

CONCLUSIONS

A self-administered stretching exercise program was associated with substantial reductions in restless leg syndrome symptoms among adults with type 2 diabetes mellitus. The pattern of improvement was most pronounced for rest-related sensory-motor symptoms and for perceived sleep disruption. These findings support the feasibility and potential clinical value of incorporating structured self-stretching as a conservative management option for restless leg syndrome symptoms in diabetes type 2 diabetes care settings.

Authors' Contribution

Conceptualization: NM
Methodology: MI, MZ
Formal analysis: NM
Writing and Drafting: NM, MI, MZ, MA, WA
Review and Editing: NM, MI, MZ, MA, WA

All authors approved the final manuscript and take responsibility for the integrity of the work.

Conflicts of Interest

The authors declare no conflict of interest.

Source of Funding

The author received no financial support for the research, authorship and/or publication of this article.

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DOI: https://doi.org/10.54393/pbmj.v9i1.1344

PAKISTAN BIOMEDICAL JOURNAL
https://www.pakistanbmj.com/journal/index.php/pbmj/index
ISSN (E): 2709-2798, (P): 2709-278X
Volume 9, Issue 01 (January 2026)

Original Article

OPEN ACCESS

Association of Screen Time with Posture and Gross Motor Development in Adolescents

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ARTICLE INFO

Keywords:
Screen time, Posture Motor Coordination, Adolescents, Musculoskeletal Disorders, Ergonomics

How to Cite:
Faryad, A., Jabbar, M., Touqeer, S., Ahmed, T., Chisti, K. F., Faryad, I., & Faryad, I. (2026). Association of Screen Time with Posture and Gross Motor Development in Adolescents: Screen Time with Posture and Gross Motor Development . Pakistan BioMedical Journal, 9(1), 46-51. https://doi.org/10.54393/pbmj.v9i1.1344

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Received Date: 26th November, 2025
Revised Date: 10th January, 2026
Acceptance Date: 19th January, 2026
Published Date: 31st January, 2026

ABSTRACT

Overuse of digital devices (e.g., smartphones) often causes significant negative effects on muscle development and joint health among adolescents, including poor posture and problems with fine motor skills. There is an increasing amount of evidence to support these concerns, due to the high reliance upon devices for learning, communicating with friends and family, and being entertained. **Objective:** This study aimed to determine the association of screen time with posture and gross motor development in adolescents. **Methods:** A cross-sectional study was conducted with 184 adolescents (ages 10-19) from different schools/colleges in Lahore. Participants completed self-assessments on daily computer use, motor coordination, and posture-related behaviors using the Adolescent Postural Habits Questionnaire (APHQ) and the Developmental Coordination Disorder Questionnaire (DCDQ). **Results:** The study found that 59.5% of participants reported low back pain, and 47.8% spent more than 4 hours daily on smartphones for social media. A significant positive correlation was found between daily screen time and poor postural habits ($r=0.42$, $p<0.001$). Furthermore, screen time showed a significant negative correlation with motor coordination scores ($r= -0.38$, $p<0.001$), indicating that higher screen time was associated with poorer coordination. **Conclusions:** This research supports the conclusion that adolescents who use screens more frequently have a decreased ability to control their body position as compared to adolescents who use screens less frequently. The results further indicate that an educational intervention that focuses on teaching correct posture and increasing physical activity will combat the adverse effects associated with excessive screen time on the health and overall well-being of adolescents.

INTRODUCTION

With the modern digital era, the use of electronic appliances is rapidly growing, including smartphones, laptops, tablets, and televisions, which are changing the everyday lives of adolescents. Screen time, which is the time devoted to electronic screens to engage in different activities, such as education, socialization, and revitalization, has increased over the past years [1]. Adolescence is a sensitive stage of intense growth and neuro-motor development that is related to musculoskeletal alterations and elevated chances of postural and functional change in the outcome of overexposure to screens [2]. The study investigates the connection between screen time and musculoskeletal conditions in terms of posture, motor coordination, and musculoskeletal pain among adolescents. Global research has found that most adolescents (greater than two-thirds) complain of neck or shoulder pain attributed to screen time [3]. In Lahore, Pakistan, where the study was conducted, lower back pain among adolescents was reported to be 59%, with a large proportion of them spending more than

DOI: https://doi.org/10.54393/pbmj.v9i1.1344

four hours a day in front of screens [4]. These tendencies point to the increased anxiety over the problem of posture, such as the enhancement of lumbar lordosis and the decrease of motor coordination that is caused by extensive screen exposure. Additionally, research has revealed that high screen time in adolescents is highly linked with impaired gross motor skills and distorted spinal orientation [5, 6]. Specifically, screen-related sedentary behaviors are associated with musculoskeletal disorders, such as the so-called text neck, tech back conditions, and are associated with biomechanical alterations in the cervical and lumbar spine [7, 8]. The negative impact of screen time also has a spillover to cognitive and psychosocial health because excessive screen time on digital media is linked with anxiety, depression, and disturbed sleep patterns in adolescents [9].

This research aims to assess the relationship between screen time, posture-related behaviors, and motor coordination in adolescents in Lahore, Pakistan, as limited evidence exists regarding the prevalence of musculoskeletal pain, postural deviations, and motor coordination issues linked to screen exposure in this population. The significance of this study lies in its potential to raise awareness about the harmful effects of excessive screen time on adolescent musculoskeletal health and contribute to the development of targeted interventions. The rationale is to provide evidence-based data that can inform preventive physiotherapy and public health strategies to mitigate the potential musculoskeletal and developmental risks associated with increasing digital device usage in this vulnerable population. Therefore, this study aimed to assess the association between screen time, postural habits, and gross motor coordination in adolescents in Lahore, Pakistan.

METHODS

The study was a cross-sectional observational study that was implemented in Lahore, Pakistan. A purposive technique of sampling was used to enroll a total of 184 adolescents. The a priori calculation of the sample size pertained to the formula of correlation studies. According to a pilot study, it was assumed that there would be a moderate correlation (r = 0.25) between screen time and motor coordination. The required minimum sample size of 123 was determined with the desired power of 80 percent and alpha error of 0.05. The sample size was raised to 184 in order to take into consideration the possibility of dropouts and missing data. The sample population was selected among different schools and colleges in Lahore in March 2024 and June 2024. The instrument involved in the data collection was the written informed consent of all the participants, which was received in accordance with the ethical principles of the Declaration of Helsinki. They were adolescents aged 10 to 19 years old, who had 2-4 hours of daily use of electronic devices (smartphones, tablets, and/or computers) and attended schools in Lahore. The exclusion criteria were adolescents with neurological, orthopedic, or congenital conditions (ex: cerebral palsy, scoliosis, spina bifida) [10]; any history of spinal surgery; a history of neck, back, or shoulder pain [5]; the use of assistive walking devices or braces; visual or vestibular balance disorders; physiotherapy or medical management due to postural or motor difficulty. Three questionnaires that were known to be valid were used to evaluate the study variables. Measures of device usage were done using the Screen Time Questionnaire (STQ) to establish the type and amount of time used on the device per day. The assessment of postural habits was made with the help of the Adolescent Postural Habits Questionnaire (APHQ) [11]. The Gross motor development was measured with the help of the Developmental Coordination Disorder Questionnaire (DCDQ) [12]. The internal consistency of chosen questionnaire sample was acceptable, with Cronbach's alpha of 0.79 for the APHQ and 0.82 for the DCDQ. The a priori calculation of the sample size pertained to the formula of correlation studies. The pilot study led to an assumption of an expected moderate correlation based on screen time and motor coordination (r=0.25). The required minimum sample size of 123 was determined with the desired power of 80 percent and alpha error of 0.05. The sample size was raised to 184 in order to take into consideration the possibility of dropouts and missing data. Cronbach's alpha was used to establish the internal consistency of the questionnaires in current sample. The values were reasonable, and the Cronbach alpha of the APHQ and DCDQ is 0.79 and 0.82, respectively.

Data were analyzed using SPSS version 29.0. Descriptive statistics (mean, standard deviation, frequency, and percentage) were computed for demographic and questionnaire variables. The Shapiro-Wilk test was used to check the normality of continuous data. As screen time data were not normally distributed, the association between screen time (categorized into groups) and postural/motor coordination scores was analyzed using Spearman's rank correlation coefficient. Chi-square tests were applied for associations between categorical variables. A p-value ≤ 0.050 was considered statistically significant, and 95% confidence intervals were calculated for key correlation coefficients.

RESULTS

The adolescents who were involved in the study were 184 in number, and their average age was 14.72 ± 2.35 years, with a range of 10-19 years. The sample was relatively evenly balanced in gender, as 101 (54.9) out of the participants

DOI: https://doi.org/10.54393/pbmj.v9i1.1344

were female and 83 (45.1) were male. A stratification of the academic level of the participants was made as follows: 81 (44.0%) middle academic level, 76 (41.3%) high academic level, and 27 (14.7%) college level. One hundred and fifty-nine (86.4) were right-handed, and 25 (13.6) were left-handed. On the nature of the digital devices, the highest percentage of 105 (57.1%) participants indicated the use of smartphones, then 38 (20.7) participants indicated the use of laptops/PCs, 26 (14.1) participants indicated the use of tablets, and 15 (8.2) indicated the use of televisions. On weekdays, 66 (35.9) of the participants had 3-4 hours of TV or video watching, and 44 (23.9) participants had more than 4 hours of watching. A large number of respondents also used smartphones to chat or do social media, with 88 (47.8) spending over 4 hours a day doing so. On weekends, 87 (47.3) of the participants of the study indicated that they spent more than 4 hours on computer/laptop-based schoolwork (Table 1).

Table 1: Gender of Participants, Dominant Hand, Types of Digital Devices Used, and Screen Time Habits on Weekdays (n=184)

Valid	Frequency (%)
Gender of Participants	
Female	101 (54.9%)
Male	83 (45.1%)
Dominant Hand	
Left	25 (13.6%)
Right	159 (86.4%)
Types of Digital Devices Used	
Laptop/PC	38 (20.7%)
Smartphone	105 (57.1%)
Tablet	26 (14.1%)
Television	15 (8.2%)
Screen Time Habits on Weekdays	
1–2 hours	30 (16.3%)
3–4 hours	66 (35.9%)
<1 hr	37 (20.1%)
>4 hrs	44 (23.9%)
None	7 (3.8%)
Total	184 (100%)

Posture-related behaviors while using devices were also assessed. Participants reported moderate support for their back while sitting, with a mean score of 3.31 ± 0.85. However, many participants exhibited forward trunk lean while using their smartphones, with a mean score of 3.16 ± 0.97. Sitting cross-legged while using devices was less common, with a mean score of 2.93 ± 1.05 (Table 2).

Table 2: Posture-related Behaviours

Posture Behavior	Mean ± SD
Back Well Supported on Chair Backrest	3.31 ± 0.85
Body Tilted Forward While Using Phone	3.16 ± 0.97
Sitting Cross-Legged While Using Devices	2.93 ± 1.05

A significant association was observed between screen time and the study's outcome measures. These findings suggest that excessive screen use negatively impacts both posture and motor skills (Table 3).

Table 3: Correlation between Screen Time, Postural Habits, and Motor Coordination

Variables	Statistic	Screen Time (hours /day)	APHQ Score (Posture)	DCDQ Score (Motor Coordination)
Screen Time (hours/day)	Spearman's rho	1.00	0.42**	-0.38**
	p-value	—	<0.001	<0.001
	95% CI	—	0.29 to 0.54	-0.50 to -0.25
APHQ Score (Posture)	Spearman's rho	0.42**	1.00	-0.31**
	p-value	<0.001	—	<0.001
	95% CI	0.29 to 0.54	—	-0.44 to -0.17
DCDQ Score (Motor Coordination)	Spearman's rho	-0.38**	-0.31**	1.00
	p-value	<0.001	<0.001	—
	95% CI	-0.50 to -0.25	-0.44 to -0.17	—

The correlation between the time spent at the daily screen and the choice of musculoskeletal and motor coordination parameters in adolescents. The more the screen time, the more it is positively associated with poor posture, like forward trunk lean and increased lumbar lordosis, and the more it is associated with a higher likelihood of having a postural problem like back pain. Moreover, it has been established that excessive screen exposure has a negative correlation with motor coordination, especially hand-eye coordination and bilateral coordination, thus leading to poor performance in activities involving coordinated movements (Table 4).

Table 4: Gender of Participants, Dominant Hand, Types of Digital Devices Used, and Screen Time Habits on Weekdays (n=184)

Variables	Screen Time (hours/ day)	Posture (e.g., lumbar lordosis, forward trunk lean)	Motor Coordination (e.g., hand-eye coordination, bilateral coordination)
Screen Time	—	Positive association with poor posture (increased lumbar lordosis, forward trunk lean)	Negative association with motor coordination (difficulty with hand-eye coordination, bilateral coordination)
Postural Issues (e.g., back pain)	Increased with screen time	Significant correlation with forward trunk leans and increased lumbar curve.	—
Hand-eye Coordination	Negative correlation with excessive screen time	—	Decreased performance with high screen use
Bilateral Coordination	Negative correlation with excessive screen time	—	Increased difficulty with tasks requiring bilateral coordination

DOI: https://doi.org/10.54393/pbmj.v9i1.1344

DISCUSSION

We establish a high correlation between the duration of screen time and postural and coordination problems among adolescents in the city of Lahore. These results are in line with previous studies that have indicated that protracted time spent on screens may deteriorate postural and coordination development in adolescents [8]. As observed, adolescents who spent more than 4 hours in front of a screen per day reported more neck and back pain than those who did not spend excessively in front of a screen. Hence, lack of physical activity affects the health and development of an adolescent negatively. The existing literature results suggest that sitting position on or operating equipment is a key risk aspect that promotes the development of musculoskeletal pain. In our research, 59.5% of the respondents had the perception that protracted sitting contributed largely to their low back pain. The study conducted by Banadaki *et al.* established that the number of painful and uncomfortable experiences was significant in response to the use of static postures (particularly extreme forward head position or slouching) [13]. Almutairi *et al.* also discovered that there is a high relationship between the use of poor postures by adolescents when using electronic devices and the occurrence of musculoskeletal disorders [14]. Although there were considerable respondents who indicated that they were very likely to have poor posture because of the use of devices, about 59% of them mentioned that their posture on the device had changed since then [15]. These findings are in line with Zhang *et al.* results, where they found that prolonged screen time (primarily from devices) resulted in spinal misalignments in the cervical and lumbar spines [8]. Together, it appears that initiating timely interventions may result in the reestablishment of correct postural habits and reduce the potential for long-term musculoskeletal dysfunction. Digital device overuse diminishes adolescents' motor coordination development as determined by Developmental Coordination Disorder Questionnaire (DCDQ) assessment results [12]. Adolescents who utilize devices for four or more hours per day experience greater difficulties completing two separate movement types (i.e., eye-hand coordination) and multiple coordinated movements simultaneously bilateral coordination). The data collected herein agrees with the study of Parra-Fernandez *et al.* [3]. In their study, adolescent mobile phone dependence exhibited an inverse relationship to adolescents' musculoskeletal and motor coordination capabilities. Balanced sedentary behaviors among adolescents and enabling them to participate in physical activity/coordination exercise is in agreement with the research study by Fan *et al.* [16]. Of the participating adolescents, a significant percentage indicated difficulty completing activities involving catching or throwing a ball accurately (mean = 2.96) due to the lack of motor coordination seen in many adolescents who are regularly sedentary [5]. Therefore, adolescents must engage in an adequate amount of balanced physical activity to ensure their continued development of motor coordination. In contrast with Priftis and Panagiotakos, previous assumptions regarding the impact of screen time on posture development may not be as great as previously thought. Their study indicated that sedentary behavior, regardless of whether it was through screen use or other non-screen-related activities such as reading and studying, has a greater impact on musculoskeletal health in adolescents than screens alone [10]. They further claim that sedentary lifestyles in general are a contributing factor to postural problems as well as to some degree motor problems, not just the result of screen time only. Their conclusions challenge the perception that eliminating screens alone would cure postural and motor issues, while they suggest broader-based interventions that would improve overall physical activity and reduce sedentary behavior associated with different types of contexts, such as non-screen-based activities [17]. While excessive screen time may be associated with negative effects on posture and coordination, recent studies have shown that motor skills may be enhanced through screen-based activities if there is physical involvement. As shown in the work of Fan *et al.* adolescents who participated in interactive screen-based activities, such as video games that require physical activity (e.g., active gaming systems), possessed increased motor coordination and balance than those who did not. This study demonstrates that not all screens adversely affect motor skill development, and that the type of screen-based activity (i.e., passive vs. active) has a strong influence on the development of motor skills [16]. Thus, the results of this research support the use of screen-based physical activities as an intervention for the harmful effects of passive screen time experience [18]. Current results indicate a significant finding that shows a greater number of Female (54.9%) than Male (45.1%) participants; this may reflect a difference regarding levels of screen time and musculoskeletal health based on gender. Research indicates females, including young females, are more likely than males to develop postural problems related to sedentary behavior. However, additional research is needed to fully understand the differences in genders related to screen time exposure and their effect on postural alignment and motor coordination issues in female participants [19]. The implications of this study on the physiotherapy profession are considerable; the results indicate that excessive screen time can lead to posture-related and motor-coordination-related issues in children and adolescents and that these issues can be addressed through early detection and intervention [20].

DOI: https://doi.org/10.54393/pbmj.v9i1.1344

This research supports the use of physiotherapists as providers of individualized programs of corrective exercise that will help adolescents improve their postural control and motor coordination through corrective exercise. In addition, as noted by Priftis & Panagiotakos [10], incorporating ergonomic education and awareness programmes into school curricula could reduce the negatives produced by excessive screen time on children and adolescents.

This research has a few limitations that are worth taking into consideration when interpreting the research. The cross-sectional design restricts the opportunity to determine the causal relationship between screen time, postural problems, and motor coordination issues. Also, the research was done in one city, and its sample size was too small to be able to be generalized to the wider population. The studies that are intended to be conducted in the future must be larger, have a multi-centric nature, and be based on longitudinal designs to gain a better insight into the causal relationships, as well as consider the impact of various kinds of screen-based activities and intervention strategies to decrease the prevalence of sedentary behavior and enhance the musculoskeletal health and motor coordination within adolescents.

CONCLUSIONS

This study concludes that excessive screen time is significantly associated with poorer postural habits and reduced gross motor coordination in adolescents. The high prevalence of musculoskeletal discomfort, particularly low back pain, among heavy screen users underscores a significant public health concern. These findings highlight an urgent need for targeted interventions, including physiotherapy and ergonomic education, to mitigate the long-term health risks associated with increasing digital device use in this population.

Authors' Contribution

Conceptualization: AF, MJ
Methodology: KTC
Formal analysis: ST, TA, IF, IF
Writing and Drafting: AF, TA
Review and Editing: AF, MJ, ST, TA, KTC, IF, IF

All authors approved the final manuscript and take responsibility for the integrity of the work.

Conflicts of Interest

The authors declare no conflict of interest.

Source of Funding

The author received no financial support for the research, authorship and/or publication of this article.

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DOI: https://doi.org/10.54393/pbmj.v9i1.1335

PAKISTAN BIOMEDICAL JOURNAL
https://www.pakistanbmj.com/journal/index.php/pbmj/index
ISSN (E): 2709-2798, (P): 2709-278X
Volume 9, Issue 01 (January 2026)

Case Report

OPEN ACCESS

Cervicovaginal Agenesis: A Case Report of a Rare Congenital Anomaly with Delayed Clinical Recognition

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ARTICLE INFO

Keywords:
Premenarchial, Hematometra, Cervical Agenesis, Uterovaginal Anastomosis, Case Report

How to Cite:
Ali, N., Osama, M., Attique, M., Asghar, R., & Aman, A. (2026). Cervicovaginal Agenesis: A Case Report of a Rare Congenital Anomaly with Delayed Clinical Recognition : A Case Report of Cervicovaginal Agenesis . Pakistan BioMedical Journal, 9(1), 52-56. https://doi.org/10.54393/pbmj.v9i1.1335

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Received Date: 19th January, 2025
Revised Date: 4th July, 2025
Acceptance Date: 12th July, 2025
Published Date: 31st January, 2026

ABSTRACT

Rare congenital Mullerian anomalies include cervical agenesis. It has been found in 39% of cervical agenesis instances, which makes its association with vaginal agenesis which occurs even less frequently much rarer. For this ailment, a hysterectomy was the standard of care. However, due to improvements in assisted reproductive techniques and surgical advancements, conservative surgery can now be considered the first-line therapy Patients with primary amenorrhea should be actively evaluated for underlying causes. cervicovaginal agenesis is uncommon and has a variety of presentations. In a successful case of cervicovaginal agenesis treated with cervicovaginoplasty, a 14-year-old adolescent girl who had been experiencing cyclical pelvic discomfort and primary amenorrhea for five months came to the Women and Children Hospital Dera Ismail Khan's outpatient department. The patient's menstrual periods returned after surgery, and her cyclical abdominal pain decreased. Patients with primary amenorrhea should be actively evaluated for underlying causes. cervicovaginal agenesis is uncommon and has a variety of presentations. This might cause misdiagnosis and treatment delays. it is important to establish early diagnosis and proper management of these surgically amenable lesions to preserve normal physiology and fertility and prevent complications.

INTRODUCTION

Cervical agenesis is a rare congenital Mullerian anomaly occurs in one in every 80,000 to 100,000 births. It has been documented in 39% cases of cervical agenesis, making its relationship with vaginal agenesis, which is even more uncommon in occurrence [1]. Cervical agenesis is categorized as Ib by the American Fertility Society (AFS), which has historically been frequently utilized due to its simplicity and convenience of usage [2]. Due to the blockage of menstrual flow from the uterus, patients with cervical agenesis typically appear with amenorrhea and cyclic pelvic discomfort in early adolescence, around the time of menarche. Preventing significant endometriosis requires early identification and treatment since it can cause irreparable harm to reproductive potential and requiring major surgery like a hysterectomy or adnexectomy [3]. Two cases of cervicovaginal agenesis reported with the normal uterus, which managed them with a conservative surgical approach. They created neovagina and connected it to the uterine cavity, but after six months, there was no passage between the uterus and neovagina. Despite recurrent reconstructive surgeries, the patient presented with pelvic infection and sepsis, for which the hysterectomy was done [4]. Considering these harmful consequences of uterine conserving surgery, in the case of cervicovaginal agenesis, there are some important issues for both the patient and surgeon before choosing the best

DOI: https://doi.org/10.54393/pbmj.v9i1.1335

surgical decision; first, the patient and her parents should be completely informed about regular follow-up visits, appropriate hygiene, regular use of vaginal dilators after surgery. Second, in conservative surgery after the creation of the neovagina in the case of cervical agenesis or cervical malformation, insertion of the stent between the lower uterine segment and neovagina may prevent cervical stenosis [5]. This work has been reported with respect to the SCARE 2023 criteria [6]. Ethical approval was obtained under reference number 134/GJMS/JC.

Cervicovaginal agenesis is a rare Müllerian anomaly that often presents with primary amenorrhea and cyclic pelvic pain, leading to delayed diagnosis and mismanagement, particularly in resource-limited settings. Due to its low incidence and variable clinical presentation, there is limited standardized guidance regarding optimal timing, surgical technique, and long-term outcomes of conservative management. Most available literature consists of isolated case reports, with insufficient long-term follow-up data on fertility preservation and postoperative complications. Therefore, this case report aims to highlight early diagnostic considerations, describe a successful conservative surgical approach, and contribute to the existing evidence on functional and anatomical outcomes in cervicovaginal agenesis.

Case Presentation

A 14-year-old young girl with primary amenorrhea and severe cyclical pelvic pain for 5 months presented to the Outpatient department at Women and Children hospital Dera ismail khan, she took multiple treatments for abdominal pain at private setup but was not resolved. she was referred for gynaecological examination. On physical examination, her breast development was tanner stage 2, axillary and pubic hairs stage 3. She had no known history of medications, allergies, adverse reactions or her family disease. Transabdominal ultrasonography revealed a fluid collection of 172ccin intrauterine cavity. These findings were consistent with hematometra. The patient was admitted to hospital for examination under anaesthesia, external genitalia was normal, a blind-ending vagina 1.5 cm from the introitus. Dissection was given in loose areolar tissue between urethra and rectum, there was no connection between uterus and vagina, the cervical opening was not visualized. So vaginoplasty with abdominopelvic approach was planned. laparotomy followed by hysterotomy was done. Hegar dilator 7 was introduced from above to create a space followed by the insertion of foley's catheter to guide the cervical opening, inflated and secured from below. A Mold was kept in the space created to prevent from stenosis. Which was changed after 72 hrs. Her post-operative period went unremarkable. She had no pain or discomfort. She was stable and discharged home on postoperative day five. The patient successfully underwent cervicovaginoplasty through a combined abdominopelvic approach leading to the creation of a neovagina and continuity was established between the uterine cavity and the neovaginal tract using a Foley catheter as a temporary stent to maintain patency. The immediate postoperative period was uneventful. The patient remained stable, experienced no pain or complications, and was discharged on the fifth postoperative day. Vaginal molds were introduced postoperatively to maintain the tract and prevent restenosis. At her first follow-up one month after surgery, the Foley catheter had been removed, and she reported spontaneous menstruation 15 days' post-discharge. Clinical examination revealed a neovaginal length of 5–6 cm, with a palpable cervical dimple and adequate epithelialization at the neocervical junction. Ultrasound confirmed absence of hematometra and free flow of menstrual blood. The patient was followed up for six months. She continued mold use consistently and was monitored regularly over a six-month follow-up period. Menstrual cycles remained regular with no recurrence of pelvic pain or signs of vaginal stenosis. Vaginal patency and cervical continuity were maintained, and no further intervention was required. No complications such as restenosis, infection, or sexual dysfunction (although the patient had not yet initiated sexual activity) were reported. The patient and her guardians were counselled on the importance of continued annual follow-up to monitor vaginal and cervical status and future fertility potential. The experience and recommendation while dealing such case are, the patient should continue using vaginal molds for at least 3–6 months postoperatively to maintain the vaginal tract and prevent stenosis. She should be advised to monitor her menstrual flow and report any symptoms of dysmenorrhea, reduced flow, or amenorrhea, which may indicate re-stenosis. Every 3–6 months follow ups for the first year and then annually to assess: Vaginal Caliber and epithelialization status. Cervical patency using ultrasound (to ensure no hematometra formation). Hormonal Evaluation: Breast and pubic hair development at Tanner stage 2-3 suggests possible incomplete puberty or hormonal imbalance. Serum FSH, LH, estradiol, and AMH may be checked to assess ovarian function.

DOI: https://doi.org/10.54393/pbmj.v9i1.1335

DISCUSSION

Because of the variation in presentation, the therapy strategy for cases of hematometra caused by cervical atresia or agenesis is debatable Compared to patients with full agenesis, those with fibrous cord/fragmentation may have a greater chance of repair [1]. This condition often manifests in adolescent girls with a 46, XX karyotype, normal ovarian function, normal developed secondary sexual characteristics, and primary amenorrhea [2]. It is crucial to comprehend and define the precise anatomy of the Mullerian abnormality before considering genital tract reconstruction or surgical repair. Examining the current instance revealed an outflow restriction at the cervix level conservative surgical methods [4]. Since a stent was unavailable, a self-made Mold was used to put between the lower uterine segment and the neovagina after the neovagina was created in cases of cervical agenesis or cervical malformation. This may help avoid cervical stenosis. It's unclear how long the stent should be left in place, although some research suggests leaving it in place for at least six months to allow the catheter's surrounding tissue to fully epithelialize [5]. To keep the newly formed vaginal canal open and stop the tissues from settling inward, vaginal molds are helpful. They also guarantee that the vaginal canal is the proper size and form and aid in its shaping. According to studies, individuals who utilize vaginal molds following surgery had a greater success rate for vaginal dilatation and a lower risk of restenosis. It is crucial to remember that vaginal molds are not always required, and the choice to use them should be evaluated individually. The extent of the cervical agenesis, the size of the newly formed vaginal canal, and the patient's adherence to the mold-wearing protocol are all factors the surgeon should consider while doing surgery on this patient [7]. One surgical strategy that presents several difficulties is the restoration of utero-cervix/neocervix-vagina/neovagina continuity in individuals with cervical deformity. After menarche, and preferably after starting a sexual relationship, is the optimal age. Making a neovagina is a rare procedure that calls for surgical expertise and a multidisciplinary team. Rebuilding a neocervix is an even more difficult and uncommon procedure [8]. It is clear that using two approaches at the same time comes with challenges and possible consequences. Some writers contend that in order to prevent problems such ascending infection and the necessity for reintervention because of stenosis, the first line of treatment should be traditional care (hysterectomy) [9]. However, taking into account the preservation of fertility, the new surgical methods might be employed as the first resort [10]. There are significant risks of recurrence and complications associated with early uterovaginal anastomosis operations that involve cervical drilling or catheterization. Numerous papers report that uterovaginal anastomosis by laparotomy or laparoscopy has been effective in cases with cervix agenesis [11]. Regretfully, if conservative measures are unsuccessful, a hysterectomy may be required. Even in the ill-reputed types of congenital cervical agenesis, cervicoplasty with mucosal lining allows for the development of a patent cervical canal [8]. When doing vaginoplasty in situations of congenital cervical and vaginal agenesis, the abdominopelvic approach necessitates meticulous planning and risk assessment. The creation of a vaginal canal that is sufficiently long and wide, the preservation of the urethral and rectal sphincters, the maintenance of bladder and bowel function, the reduction of infection and hematoma formation risk, and the achievement of a cosmetically acceptable result are all important surgical factors. Hematoma development, vaginal stenosis, infection, persistent pelvic discomfort, urethral and rectal fistulas, and sexual dysfunction are possible side effects. Before beginning, the surgeon should thoroughly examine the patient's anatomy and medical history and have expertise doing this kind of surgery [12]. Congenital anomalies of the female reproductive tract, particularly cervicovaginal agenesis, pose a significant diagnostic and surgical challenge due to their rarity and variable clinical presentation. The complexity of such malformations often leads to delayed recognition, particularly in settings where adolescent menstrual irregularities are overlooked or misinterpreted. Acién and Acién provide a comprehensive overview of complex genital tract anomalies, emphasizing that early diagnosis is critical for planning optimal surgical intervention and for psychological well-being [13]. However, as seen in many cases, including the one discussed in this report, diagnosis is frequently delayed until adolescence or early adulthood when symptoms such as primary amenorrhea and cyclic pelvic pain manifest. Carranza-Mamane *et al.* stress the importance of a systematic approach to the diagnosis of congenital reproductive anomalies, recommending the use of imaging modalities such as MRI for precise anatomical delineation [14]. This is particularly relevant in cervicovaginal agenesis, where detailed imaging aids in differentiating among types of Müllerian anomalies and informs surgical planning. Leitao *et al.* describe a successful uterovaginal anastomosis for cervicovaginal agenesis, reinforcing that timely intervention using appropriate surgical techniques can result in restoration of menstrual function and preservation of reproductive potential [15]. Similar approaches have been supported by Folch *et al.* who categorize Müllerian agenesis and its subtypes, emphasizing individualized treatment strategies depending on anatomical and functional considerations [16]. In resource-limited settings, Adeyemi *et al.* highlight the utility of non-surgical approaches such as vaginal

DOI: https://doi.org/10.54393/pbmj.v9i1.1335

dilation in selected cases, particularly for vaginal agenesis. While this may not be applicable in cases with a functional uterus and cervical agenesis, it underscores the need for context-specific treatment modalities and counselling [17]. Liu *et al.* document the use of modified laparoscopic uterovaginal anastomosis techniques in cervical agenesis, demonstrating the advancements in minimally invasive procedures that improve patient recovery and reduce long-term complications [18]. Their report supports the growing body of literature advocating for laparoscopy as the preferred approach in experienced centers. Dreisler *et al.* explore long-term outcomes of vaginal reconstruction using bowel segments in cases where conventional techniques are unfeasible. While this technique is more commonly used in vaginal agenesis without a functional uterus, it serves as an alternative in complex cases requiring neovaginal construction, with satisfactory functional outcomes [19]. Mungan *et al.* emphasize the importance of long-term follow-up in patients undergoing surgical correction of vaginal agenesis. Complications such as stenosis, fistula formation, and sexual dysfunction may arise if postoperative care and patient compliance with dilation protocols are inadequate [20].

This study is limited by its single-case design and relatively short follow-up duration of six months, which restricts the assessment of long-term reproductive outcomes, fertility potential, and risk of restenosis. Additionally, advanced imaging modalities such as MRI were not utilized for detailed preoperative anatomical mapping. Future studies should focus on multicenter case series with longer follow-up periods to evaluate reproductive outcomes, quality of life, and surgical success rates. The development of standardized management protocols and long-term registries for congenital cervical anomalies would further strengthen clinical decision-making and improve patient outcomes.

CONCLUSIONS

The underlying causes of primary amenorrhea should be carefully assessed in patients. Cervicovaginal agenesis is a rare condition with a range of manifestations, despite vaginal anomalies like TVS. Misdiagnosis and treatment delays might result from this. For these surgically treatable lesions to maintain normal physiology and fertility and avoid consequences, early identification and appropriate care are crucial. For teenagers in particular, psychosocial support is a critical component of treatment. Two well-known side effects of the illness include infertility and restenosis.

Authors' Contribution

Conceptualization: NA, MA, RA
Methodology: NA, MA, RA
Formal analysis: MO, MA, RA
Writing, and Drafting: NA, MO, AA
Review and Editing: NA, MO, MA, RA, AA

All authors approved the final manuscript and take responsibilities the integrity of the work.

Conflicts of Interest

The authors declare no conflict of interest.

Source of Funding

The author received no financial support for the research, authorship and/or publication of this article.

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