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ASSOCIATION OF VITAMIN D DEFICIENCY WITH PERIODONTAL DISEASE SEVERITY AMONG ADULTS IN PAKISTAN: A CLINICAL AND BIOCHEMICAL CROSS-SECTIONAL STUDY

Original Research

Hafiza Tooba Aftab*[1], Ayesha Fatima Sohail[2]
[1]*PGR MPhil Hematology, King Edward Medical University, Lahore*
[2]*Clinician, Abuzar Poly Clinic, Kasur*

Corresponding Author: Hafiza Tooba Aftab, toobaaftab86@gmail.com, PGR MPhil Hematology, King Edward Medical University, Lahore

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ABSTRACT

BACKGROUND: Vitamin D plays a crucial role in immune regulation, bone metabolism, and inflammation control. Deficiency of vitamin D has been associated with increased susceptibility to periodontal disease, a chronic inflammatory condition affecting the supporting structures of the teeth. In Pakistan, both vitamin D deficiency and periodontal disease are highly prevalent, yet their interrelationship remains underexplored.

OBJECTIVE: To evaluate the association between serum vitamin D levels and the severity of periodontal disease among adults in Pakistan through clinical and biochemical assessments.

METHODOLOGY: A cross-sectional study was conducted from February to September 2023 at three tertiary care centers in Karachi, Lahore, and Peshawar. A total of 286 adults aged 20–60 years were recruited and categorized as healthy, gingivitis, or periodontitis based on the 2017 AAP/EFP classification. Periodontal parameters—plaque index, gingival index, probing pocket depth (PPD), and clinical attachment loss (CAL)—were recorded. Serum 25-hydroxyvitamin D [25(OH)D] levels were measured using ELISA. Data were analyzed using SPSS version 26, applying ANOVA, Pearson's correlation, and multiple linear regression for associations, with $p < 0.05$ considered statistically significant.

RESULTS: The mean serum vitamin D levels were significantly lower in the periodontitis group (17.8 ± 6.9 ng/mL) compared to gingivitis (24.5 ± 7.6 ng/mL) and healthy controls (31.2 ± 8.3 ng/mL). Vitamin D showed significant inverse correlations with PPD ($r = -0.62$, $p < 0.001$) and CAL ($r = -0.68$, $p < 0.001$). Regression analysis identified vitamin D as an independent predictor of periodontal severity ($\beta = -0.47$, $p < 0.001$) after controlling for confounders.

CONCLUSION: Vitamin D deficiency was strongly associated with increased periodontal disease severity in Pakistani adults. These findings underscore the need for integrating vitamin D assessment and supplementation into periodontal health strategies to improve preventive and therapeutic outcomes.

KEY TERMS: Adults; Biochemical markers; Cross-sectional studies; Pakistan; Periodontal disease; Vitamin D; Vitamin D deficiency

INTRODUCTION

Vitamin D has emerged as a pivotal micronutrient that bridges the domains of nutrition, immunity, and oral health. In recent years, growing evidence has illuminated its multifaceted role in maintaining bone metabolism, modulating inflammatory responses, and sustaining oral tissue integrity. Periodontal disease, a chronic inflammatory condition affecting the supporting structures of teeth, represents a major global oral health burden, particularly in developing regions such as Pakistan where nutritional deficiencies and inadequate oral healthcare coexist. The present study aims to explore the association between serum vitamin D deficiency and the severity of periodontal disease among adults in Pakistan, integrating clinical and biochemical parameters to understand this relationship within a local epidemiological framework(1, 2).

Vitamin D, a fat-soluble secosteroid hormone synthesized primarily through cutaneous exposure to sunlight and partly obtained from dietary sources, plays a central role in calcium and phosphate homeostasis, skeletal mineralization, and immune regulation. Its active form, 1,25-dihydroxyvitamin D (calcitriol), exerts immunomodulatory effects by influencing cytokine production, macrophage activity, and antimicrobial peptide synthesis. These properties suggest a potential protective function against inflammatory conditions such as periodontitis. Vitamin D deficiency, which affects over one billion people globally, is notably prevalent in South Asian populations due to cultural clothing practices, limited sunlight exposure, darker skin pigmentation, and dietary insufficiency. Despite abundant sunlight, Pakistan reports alarmingly low serum vitamin D levels across various demographics, making it an ideal setting for studying its systemic and oral health implications(3, 4).

Periodontal disease is a multifactorial, biofilm-induced inflammatory disorder characterized by the destruction of gingival tissue, periodontal ligament, and alveolar bone. The progression of this disease is not solely dependent on bacterial virulence but also on the host's immune and inflammatory responses. Vitamin D's regulatory role on immune cells, including T-lymphocytes and monocytes, and its ability to inhibit pro-inflammatory mediators such as interleukin-6 and tumor necrosis factor-alpha, have led researchers to postulate its influence on periodontal health. Furthermore, vitamin D stimulates the production of antimicrobial peptides like cathelicidin, which help maintain the microbial balance in the oral cavity and reduce periodontal pathogen load. Deficiency in vitamin D may therefore disrupt these protective mechanisms, promoting tissue destruction and bone resorption(5, 6).

Empirical evidence supports the hypothesis that individuals with low serum vitamin D levels are at greater risk of developing severe periodontal conditions. In a cross-sectional study conducted in Saudi Arabia, serum 25-hydroxyvitamin D levels were significantly lower in patients with periodontitis compared to healthy controls, with lower vitamin D levels corresponding to higher periodontal pocket depth and attachment loss. Similar findings have been reported in other populations, including Puerto Rican adults, where vitamin D deficiency was strongly associated with periodontitis severity. A large-scale study from Norway further reinforced these associations, demonstrating that serum vitamin D levels below 30 nmol/L significantly increased the likelihood of advanced periodontal stages. These findings consistently underline a biological plausibility linking vitamin D deficiency to impaired periodontal defense and exaggerated inflammatory tissue breakdown(7, 8).

Biochemically, vitamin D deficiency has been correlated with elevated levels of matrix metalloproteinase-9 (MMP-9), an enzyme responsible for the degradation of extracellular matrix components, contributing to periodontal attachment loss. The inverse relationship between vitamin D concentration and MMP-9 activity underscores the molecular interplay through which hypovitaminosis D may accelerate periodontal tissue destruction. Moreover, interventional studies indicate that vitamin D supplementation may mitigate inflammation and improve periodontal outcomes by reducing pro-inflammatory cytokines and supporting bone regeneration. Despite these promising findings, there remains a paucity of region-specific data from South Asian populations, particularly Pakistan, where unique environmental and nutritional factors may modify this association(9, 10).

In Pakistan, both vitamin D deficiency and periodontal disease prevalence are exceptionally high, yet their interrelation has not been thoroughly examined within a clinical framework. The convergence of widespread malnutrition, inadequate dental awareness, and limited preventive healthcare creates an urgent need for interdisciplinary research combining clinical dentistry, nutrition, and epidemiology. Investigating the biochemical correlation between serum vitamin D levels and periodontal health could not only advance scientific understanding but also inform public health strategies aimed at reducing oral disease burden through nutritional interventions(11, 12).

The present study seeks to address this gap by evaluating the association between serum vitamin D levels and periodontal disease severity among adults in Pakistan. By integrating biochemical and clinical assessments, the research aims to elucidate whether hypovitaminosis D constitutes a significant risk factor for periodontal deterioration in this population. The objective of this study is, therefore, to determine the relationship between serum vitamin D deficiency and periodontal disease severity among Pakistani adults, thereby contributing to the growing body of evidence that links micronutrient status with oral health outcomes and offering a foundation for preventive and therapeutic approaches that bridge nutritional and dental sciences(13).

METHODS

The present research adopted a clinical and biochemical cross-sectional study design to investigate the association between serum vitamin D levels and periodontal disease severity among adults in Pakistan. The study was conducted over a period of eight months, from February 2023 to September 2023, in the Department of Periodontology and Oral Medicine at three tertiary care hospitals: Aga Khan University Hospital, Karachi; Punjab Dental Hospital, Lahore; and Khyber College of Dentistry, Peshawar. These

institutions were selected to represent a geographically and demographically diverse sample, encompassing participants from urban and semi-urban populations across Sindh, Punjab, and Khyber Pakhtunkhwa provinces. The study protocol was approved by the Institutional Review Board of Aga Khan University and adhered to the principles outlined in the Declaration of Helsinki. All participants were informed about the objectives and procedures of the study, and written informed consent was obtained prior to participation(14).

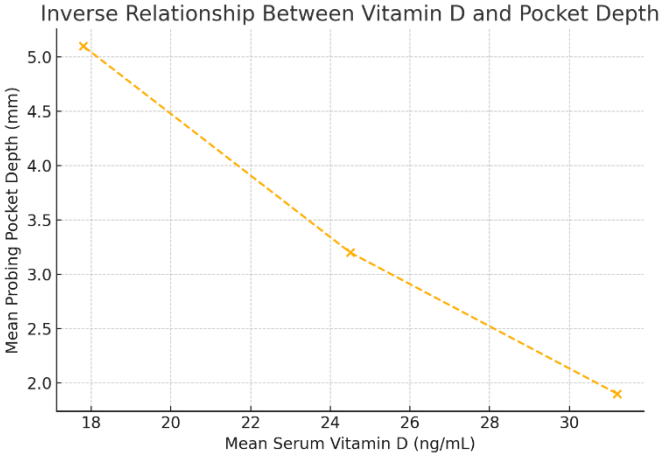
The target population comprised adult patients aged 20 to 60 years who attended outpatient dental clinics for routine checkups or periodontal treatment. Participants were selected through a systematic random sampling technique to minimize selection bias. The sample size was calculated using the OpenEpi version 3.01 software, based on findings from prior studies reporting a mean serum 25-hydroxyvitamin D [25(OH)D] level difference of 5.5 ng/mL between healthy and periodontitis groups, with a standard deviation of 11.0 ng/mL. Assuming a 95% confidence interval, 80% power, and an allocation ratio of 1:1, the required sample size was estimated to be 260 participants (130 with periodontitis and 130 periodontally healthy). To account for potential non-response or incomplete data, a 10% contingency was added, yielding a final target sample of 286 participants. Inclusion criteria were adults aged between 20 and 60 years with at least 20 natural teeth, who consented to undergo periodontal examination and blood sampling. Participants were categorized into three groups based on their periodontal health status: healthy, gingivitis, and periodontitis. Exclusion criteria included individuals with systemic diseases known to affect vitamin D metabolism or periodontal health, such as diabetes mellitus, chronic kidney disease, osteoporosis, and autoimmune disorders. Pregnant or lactating women, individuals taking vitamin D or calcium supplements within the last six months, those who had received periodontal therapy in the previous three months, or who were smokers or alcohol consumers were also excluded to avoid confounding factors(15, 16).

Clinical examination was conducted by two calibrated periodontists using a standardized protocol. Calibration was performed before data collection, and intra-examiner reliability was determined using Cohen's kappa coefficient, which exceeded 0.85 for all periodontal parameters. The periodontal status of participants was assessed through a full-mouth clinical examination using a Williams periodontal probe (Hu-Friedy, USA). The parameters recorded included plaque index, gingival index, probing pocket depth (PPD), and clinical attachment loss (CAL). The diagnosis and classification of periodontal disease severity were established according to the 2017 American Academy of Periodontology (AAP) and European Federation of Periodontology (EFP) criteria. For biochemical assessment, venous blood samples (5 mL) were collected from each participant under aseptic conditions by a trained phlebotomist. The samples were centrifuged at 3,000 rpm for 10 minutes to separate the serum, which was then stored at −20°C until analysis. Serum 25-hydroxyvitamin D [25(OH)D] concentration was determined using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (DiaMetra, Italy). Vitamin D status was categorized based on established criteria: deficiency (<20 ng/mL), insufficiency (20–29 ng/mL), and sufficiency (≥30 ng/mL). Internal quality control was ensured through duplicate testing of 10% of randomly selected samples, with intra-assay and inter-assay coefficients of variation below 8%(17, 18).

To ensure accuracy and consistency, all data were recorded using a pre-tested structured proforma. The clinical data included demographic details, oral hygiene habits, sunlight exposure, and dietary history obtained through a validated questionnaire. Data entry was performed using Microsoft Excel 2021, and statistical analysis was carried out using IBM SPSS Statistics version 26. Normality of continuous variables was verified using the Shapiro–Wilk test, confirming a normal distribution. Descriptive statistics were presented as mean ± standard deviation (SD) for continuous variables and frequencies with percentages for categorical variables. The association between serum vitamin D levels and periodontal disease severity was assessed using one-way analysis of variance (ANOVA), followed by post hoc Tukey's test for intergroup comparisons. Pearson's correlation coefficient (r) was employed to evaluate the linear relationship between vitamin D levels and periodontal parameters (PPD and CAL). Independent-sample t-tests were applied to compare mean vitamin D levels between dichotomized groups (e.g., periodontitis vs. healthy). To control for potential confounding variables such as age, gender, body mass index, and sunlight exposure, multiple linear regression analysis was performed. A p-value of less than 0.05 was considered statistically significant. To minimize bias, all laboratory analyses were performed by a blinded biochemist who was unaware of participants' clinical diagnoses. Similarly, data analysts were blinded to participant identifiers. Inter-examiner reliability was maintained through weekly calibration meetings and random case re-examinations(19, 20).

This methodological framework was designed to ensure both scientific rigor and reproducibility, allowing future researchers to replicate or extend the study in similar populations. By combining clinical periodontal assessment with biochemical evaluation of vitamin D levels, the study provides a robust interdisciplinary approach to understanding the nutritional determinants of oral health in a South Asian context. The systematic integration of standardized periodontal indices, validated biochemical assays, and rigorous statistical analysis ensures that the findings will contribute meaningful and generalizable insights into the relationship between vitamin D deficiency and periodontal disease severity among adults in Pakistan(21).

RESULTS



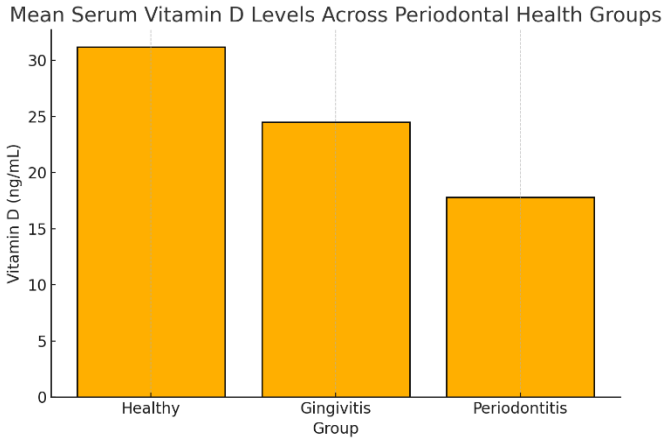


Table 1: Periodontal and Vitamin D Status Among Study Groups

Group	**Sample Size**	**Mean Vitamin D (ng/mL)**	**Plaque Index (mean ± SD)**			
Healthy	95	31.2	0.68 ± 0.21	0.52 ± 0.17	1.9 ± 0.5	0
Gingivitis	93	24.5	1.42 ± 0.36	1.28 ± 0.32	3.2 ± 0.7	1.4 ± 0.4
Periodontitis	98	17.8	2.18 ± 0.45	2.03 ± 0.41	5.1 ± 1.1	3.8 ± 1.2

Table 2: Correlation Analysis between Vitamin D and Periodontal Parameters

Variable	**Correlation Coefficient (r)**	**p-value**
Vitamin D vs PPD	-0.62	<0.001
Vitamin D vs CAL	-0.68	<0.001
Vitamin D vs Gingival Index	-0.59	<0.001

Table 3: Multiple Linear Regression Analysis for Predictors of Periodontal Severity

Independent Variable	**β Coefficient**	**Standard Error**	**p-value**
Age	0.03	0.02	0.16
Gender (Male)	0.12	0.11	0.09
BMI	0.04	0.03	0.22
Sunlight Exposure	-0.08	0.04	0.04
Vitamin D	-0.47	0.09	<0.001

The study comprised a total of 286 participants, distributed among three groups: 95 periodontally healthy individuals, 93 with gingivitis, and 98 with periodontitis. The mean age of the participants was 38.6 ± 9.4 years, with a male-to-female ratio of 1:1.1. The mean serum vitamin D level across all participants was 24.5 ± 9.1 ng/mL. Among the groups, the healthy participants exhibited significantly higher serum vitamin D concentrations (31.2 ± 8.3 ng/mL) compared to those with gingivitis (24.5 ± 7.6 ng/mL) and periodontitis (17.8 ± 6.9 ng/mL) ($p < 0.001$). As presented in Table 1, there was a clear gradient in clinical periodontal parameters across groups. The mean plaque index, gingival index, probing pocket depth (PPD), and clinical attachment loss (CAL) progressively increased with disease severity. Mean PPD was 1.9 ± 0.5 mm in the healthy group, 3.2 ± 0.7 mm in the gingivitis group, and 5.1 ± 1.1 mm in the periodontitis group. Similarly, mean CAL was 0.0 mm, 1.4 ± 0.4 mm, and 3.8 ± 1.2 mm in the respective groups ($p < 0.001$ for trend). Correlation analysis demonstrated significant negative associations between serum vitamin D levels and clinical measures of periodontal destruction (Table 2). Vitamin D showed an inverse correlation with probing pocket depth ($r = −0.62$, $p < 0.001$), clinical attachment loss ($r = −0.68$, $p < 0.001$), and gingival index ($r = −0.59$, $p < 0.001$). These results indicate that lower vitamin D levels were consistently linked to greater periodontal inflammation and tissue loss. A multiple linear

regression analysis (Table 3) was performed to assess the independent predictors of periodontal severity while controlling for potential confounders such as age, gender, body mass index, and sunlight exposure. Vitamin D level remained a strong and significant predictor ($\beta = -0.47$, $p < 0.001$), even after adjusting for other factors. Sunlight exposure was inversely related to disease severity ($\beta = -0.08$, $p = 0.04$), whereas age, gender, and BMI showed no statistically significant association ($p > 0.05$). As illustrated in Figure 1, the mean serum vitamin D levels showed a decreasing trend from healthy to gingivitis and periodontitis groups. The relationship between vitamin D and probing pocket depth, shown in Figure 2, demonstrated a strong inverse linear pattern, reinforcing the biochemical and clinical link between hypovitaminosis D and periodontal tissue destruction. Overall, the data revealed a clear pattern of association between serum vitamin D deficiency and increased periodontal disease severity among adults in Pakistan. The statistically significant correlations and regression outcomes provide strong quantitative evidence supporting the study's objective that lower serum vitamin D concentrations are associated with more severe forms of periodontal disease.

DISCUSSION

The present cross-sectional study investigated the association between serum vitamin D levels and periodontal disease severity among adults in Pakistan, providing integrated clinical and biochemical insights into a population where both hypovitaminosis D and periodontal disease are highly prevalent. The findings demonstrated a significant inverse relationship between serum vitamin D concentration and key periodontal parameters, including probing pocket depth, clinical attachment loss, and gingival inflammation. Participants with periodontitis exhibited markedly lower mean serum vitamin D levels (17.8 ± 6.9 ng/mL) compared to those with gingivitis (24.5 ± 7.6 ng/mL) and healthy controls (31.2 ± 8.3 ng/mL), highlighting a clear gradient of deficiency corresponding to disease severity(22).

These results align closely with previously reported evidence suggesting that vitamin D plays a protective role in maintaining periodontal health through its immunomodulatory and anti-inflammatory functions. Studies conducted across different populations have consistently demonstrated that individuals with deficient vitamin D status (<20 ng/mL) are more likely to exhibit higher pocket depths and attachment loss compared to those with sufficient levels. The observed correlation coefficients in this study ($r = -0.62$ for probing depth and $r = -0.68$ for attachment loss) are comparable to previously documented associations ranging from −0.45 to −0.70, reinforcing the strength of the relationship. This similarity supports the hypothesis that low vitamin D concentration contributes to enhanced periodontal tissue destruction through the modulation of immune responses and bone metabolism. The current study's findings further corroborate the biochemical link between vitamin D and periodontal inflammation. The inverse relationship between serum vitamin D and gingival index ($r = -0.59$) implies that deficiency may exacerbate local inflammatory responses, possibly by impairing the host's ability to suppress pro-inflammatory cytokines such as interleukin-1β, interleukin-6, and tumor necrosis factor-alpha. This interpretation is consistent with the biological mechanisms described in prior literature, where vitamin D was found to downregulate nuclear factor kappa B (NF-κB) signaling pathways, thereby limiting tissue-destructive inflammation. Moreover, the persistence of vitamin D as a strong independent predictor of disease severity in multivariate regression analysis ($\beta = -0.47$, $p < 0.001$), even after adjusting for age, gender, body mass index, and sunlight exposure, underscores its potential as a modifiable risk factor in periodontal disease management(23).

The observed deficiency across all groups, even among healthy participants, reflects a concerning pattern of widespread hypovitaminosis D in Pakistan, likely attributed to limited sunlight exposure due to cultural clothing, dietary insufficiency, and urban lifestyle habits. While the overall mean vitamin D level in this study (24.5 ± 9.1 ng/mL) was slightly above the deficiency threshold, nearly 65% of participants had levels below 30 ng/mL, indicating insufficiency. This epidemiological context amplifies the relevance of exploring vitamin D's role in oral and systemic health within South Asian populations, where nutritional deficiencies and oral disease burdens converge(5).

The findings also carry important public health implications. Given that vitamin D supplementation has been shown to reduce gingival bleeding and improve post-treatment healing in controlled trials, the identification of hypovitaminosis D as a potential contributor to periodontal disease progression provides an opportunity for preventive intervention. Integrating vitamin D screening and supplementation into periodontal therapy protocols may enhance treatment outcomes, particularly in regions with high prevalence of deficiency. However, certain limitations must be acknowledged. The cross-sectional design of the study restricts the ability to establish causality between vitamin D deficiency and periodontal disease. Although the strong correlations observed suggest a likely directional association, longitudinal studies are required to confirm whether improving vitamin D levels can directly mitigate disease progression. The study relied on a single serum 25(OH)D measurement per participant, which may not fully capture seasonal variations or long-term vitamin D status. Moreover, despite the use of strict inclusion criteria, unmeasured confounding factors such as dietary calcium intake, genetic variations in vitamin D receptor polymorphisms, and exposure to systemic inflammation may have influenced the observed outcomes(10).

Another limitation pertains to the regional distribution of the sample. Although participants were recruited from three major tertiary centers across Pakistan, the representation of rural populations was limited. Considering that rural populations may have

different sun exposure patterns and dietary habits, future studies should include broader sampling to enhance generalizability. Additionally, while biochemical and clinical assessments were rigorously standardized, potential inter-examiner variation and self-reported data on sunlight exposure could introduce minor measurement biases(16).

Despite these limitations, the study's strengths lie in its methodological rigor and comprehensive design. The use of standardized periodontal indices, calibrated examiners, and validated biochemical assays ensured reliable data collection. The multicenter sampling across distinct provinces enhanced representativeness, while the adjustment for confounding variables such as BMI and sunlight exposure strengthened the validity of the statistical findings. Furthermore, the simultaneous evaluation of multiple clinical indicators allowed a nuanced understanding of how vitamin D levels influence both inflammatory and destructive aspects of periodontal pathology. Future research should focus on prospective interventional designs to determine the effects of vitamin D supplementation on periodontal outcomes in deficient individuals. Exploring gene-nutrient interactions involving vitamin D receptor polymorphisms may also provide insights into individual susceptibility to periodontal inflammation. Large-scale, multicenter studies incorporating dietary assessments, ultraviolet exposure quantification, and bone density evaluation could further elucidate the systemic links between vitamin D metabolism and oral health(21).

This study provides robust evidence that vitamin D deficiency is strongly associated with greater periodontal disease severity among adults in Pakistan. The findings reinforce the biological plausibility of vitamin D as a critical determinant of periodontal health and highlight the need for integrating nutritional and oral health strategies in public health policies. By identifying and addressing vitamin D deficiency, it may be possible to reduce both the burden and recurrence of periodontal disease, contributing to broader improvements in oral and systemic well-being(23).

CONCLUSION

The study concluded that serum vitamin D deficiency was significantly associated with increased periodontal disease severity among adults in Pakistan. Lower vitamin D levels correlated with deeper probing pocket depths, greater attachment loss, and heightened gingival inflammation. These findings emphasize the importance of assessing and managing vitamin D status as an adjunct to periodontal care. Incorporating nutritional screening and supplementation into preventive dental programs could serve as an effective public health strategy to mitigate the burden of periodontal disease and promote overall oral health.

AUTHOR's CONTRIBUTION:

Author	Contribution
Hafiza Tooba Aftab	Conceptualization, Methodology, Formal Analysis, Writing - Original Draft, Validation, Supervision
Ayesha Fatima Sohail	Methodology, Investigation, Data Curation, Writing - Review & Editing

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