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Vitamin D Deficiency Trends During and After COVID-19 Pandemic Among Different Age Groups in Central Punjab with Special Focus on Okara and Sialkot, A retrospective Study

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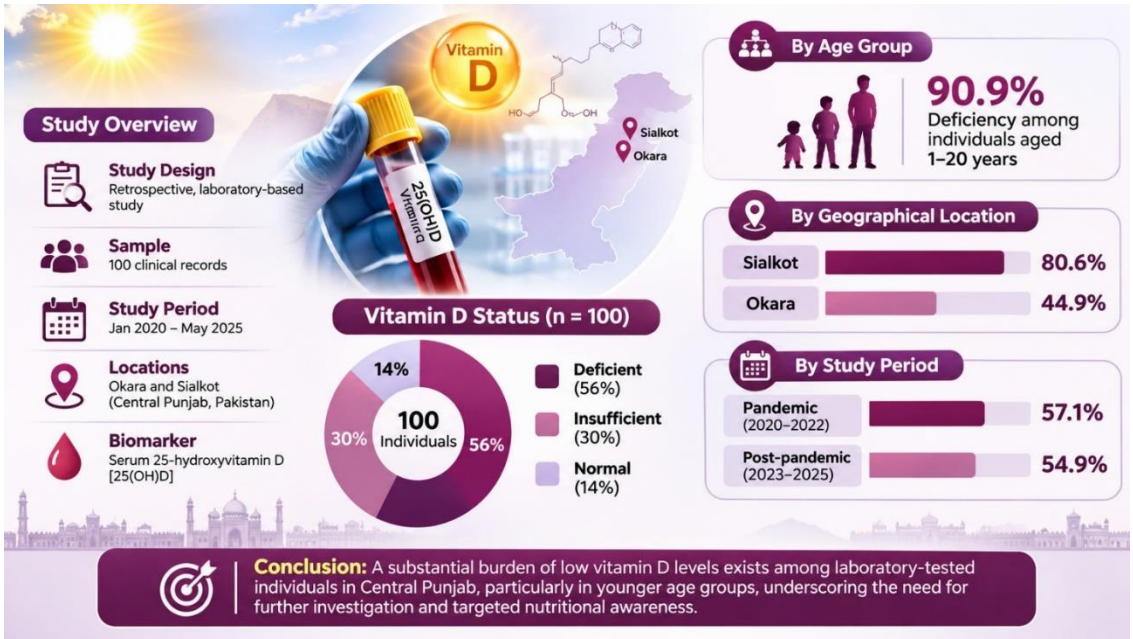
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Abstract

Vitamin D deficiency remains an important nutritional concern in Pakistan despite abundant sunlight. This retrospective laboratory-based study evaluated serum 25-hydroxyvitamin D [25(OH)D] levels in 100 clinical records from Okara and Sialkot, Central Punjab, between January 2020 and May 2025. The study compared vitamin D status across age groups, gender, geographical location, and pandemic (2020–2022) and post-pandemic (2023–2025) periods. Overall, 56% of individuals were vitamin D deficient, 30% were insufficient, and 14% had normal levels. Deficiency was highest among individuals aged 1–20 years (90.9%). The prevalence decreased slightly from 57.1% during the pandemic to 54.9% afterward. Sialkot showed a higher deficiency rate (80.6%) than Okara (44.9%). These findings indicate a substantial burden of low vitamin D levels among laboratory-tested individuals, particularly younger age groups, and support further investigation and targeted nutritional awareness.

Graphical Abstract



Introduction

Vitamin D is an essential fat-soluble nutrient involved in maintaining calcium and phosphorus balance, supporting skeletal development, and regulating several physiological processes. Its primary function is to facilitate the absorption of calcium and phosphorus, which are necessary for normal bone mineralization and skeletal strength. Vitamin D also participates in muscle function and various cellular processes. Inadequate vitamin D availability may contribute to impaired bone mineralization and conditions such as rickets in children and osteomalacia in adults. The importance of

vitamin D in human health has encouraged extensive research into its metabolism, nutritional sources, and distribution among different populations (Holick, 2007).

Unlike most vitamins, vitamin D can be produced naturally in the human body following exposure of the skin to ultraviolet B (UVB) radiation from sunlight. It is also obtained through dietary sources, including fatty fish, egg yolks, and fortified foods. Vitamin D exists mainly in two forms: vitamin D_2, or ergocalciferol, and vitamin D_3, or cholecalciferol. Following its synthesis or absorption, vitamin D undergoes hydroxylation in the liver to produce 25-hydroxyvitamin D [25(OH)D]. This metabolite represents the major circulating form and is commonly measured in clinical laboratories to assess vitamin D status. Further hydroxylation in the kidneys produces 1,25-dihydroxyvitamin D, also known as calcitriol, which is responsible for many biological effects of vitamin D (Holick et al., 2011).

Vitamin D deficiency has become a widespread nutritional and public health concern, affecting populations across different geographical regions and climatic conditions. Although limited sunlight exposure is an established contributor to reduced vitamin D synthesis, deficiency is also frequently observed in countries that receive considerable sunlight throughout the year. This indicates that environmental conditions alone do not determine vitamin D status. Dietary habits, clothing practices, time spent indoors, occupational activities, nutritional awareness, and access to fortified foods may also influence vitamin D availability. Previous research has described vitamin D deficiency as a widespread concern affecting populations in both developed and developing countries (Palacios & Gonzalez, 2014).

Pakistan experiences substantial sunlight throughout much of the year, yet vitamin D deficiency remains common among different population groups. This apparent contradiction suggests that sunlight availability does not necessarily translate into adequate individual exposure. Increasing urbanization, indoor employment, sedentary lifestyles, and limited consumption of vitamin D-rich foods may contribute to inadequate vitamin D levels. Cultural clothing practices and variations in outdoor activity may further influence vitamin D synthesis. Naeem (2010) described vitamin D deficiency in Pakistan as an overlooked public health problem, emphasizing the importance of greater awareness and preventive strategies.

The distribution of vitamin D deficiency may also vary according to age. Children and adolescents require adequate vitamin D during periods of rapid skeletal growth and development. Insufficient vitamin D during these stages may affect bone mineralization and overall skeletal health. Young adults may experience inadequate sunlight exposure because of educational commitments, office-based employment, and extended indoor activities. Middle-aged and older individuals may have different patterns of dietary intake, supplementation, and healthcare utilization. Consequently, examining vitamin D status across age categories is important for identifying groups that may require greater clinical attention.

The COVID-19 pandemic introduced substantial changes in daily activities and social behavior worldwide. During 2020–2022, restrictions on movement, temporary educational institution closures, remote working arrangements, and reduced recreational activities altered opportunities for outdoor exposure. Many individuals spent prolonged periods inside their homes, potentially reducing sunlight-dependent vitamin D synthesis. These changes raised concerns about whether pandemic-related lifestyle restrictions contributed to inadequate vitamin D levels, particularly among children, adolescents, and working-age adults.

Following the gradual relaxation of pandemic restrictions, educational institutions, workplaces, and public spaces resumed regular activities. The post-pandemic period provided an opportunity to examine whether changes in daily routines were accompanied by improvements in vitamin D status. However, differences between pandemic and post-pandemic vitamin D levels may also reflect dietary practices, supplementation, seasonal variation, or the characteristics of individuals undergoing

laboratory testing. Therefore, comparisons between these periods require careful interpretation, particularly when retrospective data are used.

Central Punjab provides a relevant setting for investigating vitamin D deficiency because its districts differ in occupational patterns, population characteristics, and everyday activities. Okara has a substantial agricultural economy, whereas Sialkot is widely recognized for its industrial and manufacturing activities. These differences may influence the amount of time individuals spend outdoors. Agricultural occupations can involve prolonged outdoor work, while many manufacturing and office-related activities take place indoors. Nevertheless, occupational exposure alone cannot explain regional differences without information about individual working conditions and sunlight exposure.

Laboratory-based studies provide useful information about vitamin D status through objective biochemical measurements. Serum 25(OH)D testing allows individuals to be classified according to predefined concentration ranges, making it possible to examine differences across demographic and geographical categories. Retrospective analysis of existing laboratory records is particularly useful for studying patterns across multiple years without requiring new specimen collection. However, such records generally represent individuals who underwent testing rather than a randomly selected community population.

Despite increasing attention to vitamin D deficiency in Pakistan, district-level comparisons covering different age categories and the pandemic and post-pandemic periods remain valuable. Understanding these patterns may assist healthcare professionals and researchers in identifying groups with a high frequency of low vitamin D concentrations and developing appropriate nutritional education strategies.

Therefore, the present study assessed serum 25(OH)D levels in 100 retrospective laboratory records from Okara and Sialkot, Central Punjab, collected between January 2020 and May 2025. The study examined the distribution of vitamin D deficiency according to age group, gender, geographical district, and study period. It also compared vitamin D status during the COVID-19 pandemic (2020–2022) and the subsequent post-pandemic period (2023–2025). The findings aim to contribute localized laboratory evidence regarding vitamin D deficiency and identify areas requiring further epidemiological investigation.

2. Materials and Methods

A retrospective, laboratory-based cross-sectional study was conducted using 100 clinical records collected between January 2020 and May 2025. The records represented individuals from Okara and Sialkot, Central Punjab, Pakistan. Data were obtained from previously available laboratory reports, including records associated with Al-Hameed Lab & Research Centre, Lahore.

The collected information included age, gender, testing date, geographical district, and serum 25(OH)D concentration. Participants were categorized into four age groups: Group A (1–20 years), Group B (21–40 years), Group C (41–60 years), and Group D (above 60 years).

Serum 25(OH)D concentrations were determined using chemiluminescence immunoassay (CLIA), as described in the laboratory methodology. Vitamin D status was classified according to the study's stated thresholds: deficiency below 20 ng/mL, insufficiency at 21–29 ng/mL, and normal status at or above 30 ng/mL.

The study period was divided into the pandemic phase (2020–2022) and post-pandemic phase (2023–2025). Statistical analysis was performed using SPSS version 25. Frequencies, percentages, chi-square tests, and independent-samples t-tests were reported. A p-value below 0.05 was considered statistically significant. Patient-identifying information was excluded from the analysis.

3. Results

Among the 100 laboratory records, 56% showed vitamin D deficiency, 30% showed insufficiency, and 14% had normal serum vitamin D concentrations. The

highest deficiency rate was observed in Group A (1–20 years), where 90.9% of individuals were deficient, followed by Group B (21–40 years), with 57.1% deficiency. The reported association between age and vitamin D status was statistically significant ($\chi^2 = 34.786$, $p < 0.001$).

During the pandemic period, 57.1% of individuals were vitamin D deficient, compared with 54.9% in the post-pandemic period. Mean serum vitamin D concentrations increased from 19.33 to 20.62 ng/mL, although the reported t-test indicated no statistically significant difference ($p = 0.385$). Regional analysis showed higher deficiency in Sialkot (80.6%) than in Okara (44.9%). The supplied analysis did not establish a statistically significant gender-based difference.

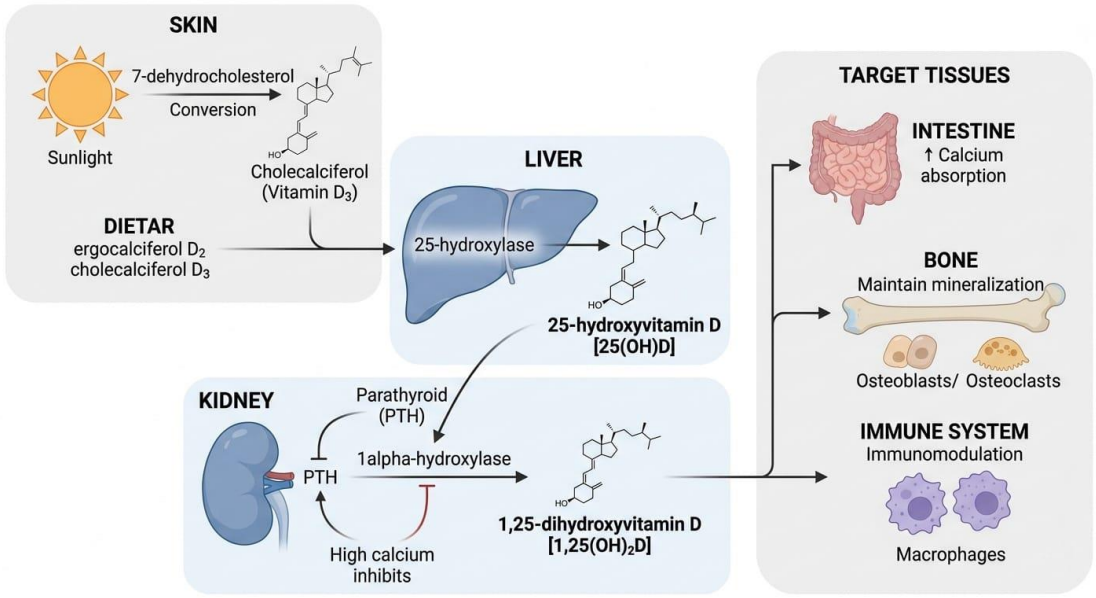
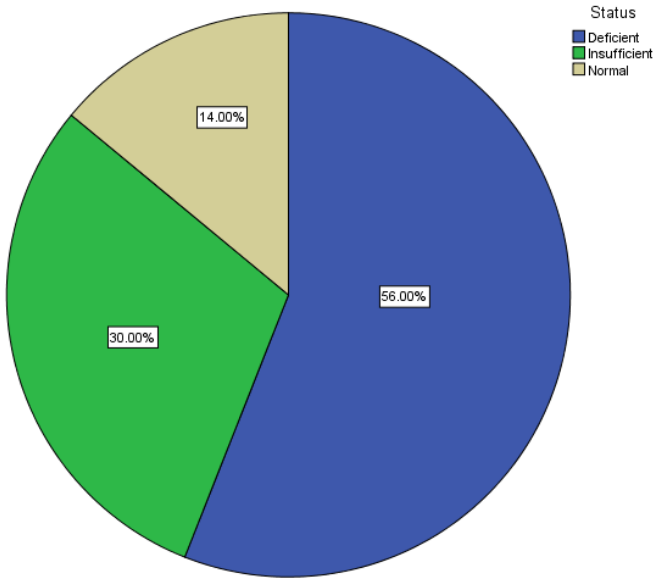


Table Age Group Classification and Sample Distribution

Group	Age Range (Years)	Frequency (n)	Percentage (%)	Rationale
Group A	1 – 20	11	11.0%	Children & adolescents — highest developmental vulnerability
Group B	21 – 40	63	63.0%	Young adults — largest working-age cohort; primary indoor workforce
Group C	41 – 60	16	16.0%	Middle-aged — transitional risk period; increasing comorbidities
Group D	Above 60	10	10.0%	Elderly — assessed for age-trend comparison

Frequency Distribution of Participants by Age Groups (N=100)

Age Category	Age Range (Years)	Frequency (n)	Percentage (%)
Group A	1 – 20	11	11.0
Group B	21 – 40	63	63.0
Group C	41 – 60	16	16.0
Group D	Above 60	10	10.0



4. Discussion

The present study examined the distribution of vitamin D deficiency among individuals from Okara and Sialkot, Central Punjab, using retrospective laboratory records collected between 2020 and 2025. The findings demonstrated that inadequate vitamin D levels were common among the tested individuals, with notable differences across age groups, geographical districts, and study periods. The overall prevalence of vitamin D deficiency was 56%, while another 30% of individuals had insufficient concentrations. Only 14% had vitamin D levels within the study's normal range. These findings indicate that most individuals included in the analysis had serum vitamin D concentrations below 30 ng/mL.

The high frequency of low vitamin D levels is particularly relevant because Pakistan receives substantial sunlight throughout the year. Despite favorable climatic conditions, vitamin D deficiency continues to be reported in different Pakistani populations. Naeem (2010) previously identified vitamin D deficiency as an important but insufficiently recognized public health concern. The present findings are consistent with this broader concern and suggest that the availability of sunlight alone does not ensure adequate vitamin D status. Factors such as indoor lifestyles, limited dietary sources, clothing practices, and individual health behaviors may contribute to low vitamin D concentrations. However, these factors were not directly measured in the

present study and should therefore be considered possible explanations rather than established causes.

Age-related variation was among the most important findings of this investigation. The highest proportion of vitamin D deficiency occurred among individuals aged 1–20 years, with 90.9% of the participants in this category classified as deficient. None of the 11 individuals in this group had vitamin D concentrations within the normal range. This observation is particularly concerning because childhood and adolescence are periods of active skeletal development, during which adequate vitamin D is essential for normal calcium absorption and bone mineralization. Persistent deficiency during these stages may increase the risk of impaired skeletal mineralization and other complications associated with inadequate vitamin D availability.

Several behavioral factors could potentially contribute to the high deficiency rate among younger individuals. Educational activities, increased screen time, reduced outdoor recreation, and changes in daily routines may limit exposure to sunlight. The COVID-19 pandemic may have further affected these patterns through school closures and restrictions on outdoor activities. Nevertheless, the available records did not contain individual information about sunlight exposure, dietary habits, or physical activity. Consequently, the observed deficiency among younger participants cannot be attributed directly to these factors. The relatively small number of individuals in this age category also limits the precision of the estimated deficiency rate.

The 21–40-year age group represented the largest proportion of the study population, accounting for 63% of all records. Within this category, 57.1% of individuals were vitamin D deficient, while 31.7% were insufficient. These findings indicate that inadequate vitamin D concentrations were also common among working-age adults. Individuals in this age range may spend considerable time in offices, educational institutions, commercial establishments, or other indoor environments. Such routines could reduce opportunities for sunlight exposure. The findings support the importance of nutritional awareness among young adults, although additional research is required to identify the specific determinants of deficiency in this population.

The supplied analysis also suggested that vitamin D status differed across age categories, with relatively lower deficiency proportions reported in older groups. A statistically significant association between age and vitamin D status was reported ($\chi^2 = 34.786$, $p < 0.001$). This pattern may reflect differences in lifestyle, nutritional practices, healthcare utilization, or supplementation. However, the study did not collect sufficient information to determine whether any of these factors contributed to the observed differences. Moreover, the reported age-group tables contain inconsistencies that require verification before firm conclusions can be drawn about the complete age-related trend.

The comparison between pandemic and post-pandemic periods provided another important observation. During 2020–2022, vitamin D deficiency was identified in 57.1% of laboratory records, compared with 54.9% during 2023–2025. The proportion of individuals with normal vitamin D concentrations increased from 10.2% to 17.6%. Similarly, the mean serum 25(OH)D concentration increased from 19.33 ng/mL during the pandemic to 20.62 ng/mL afterward. These descriptive findings indicate a modest improvement in vitamin D status following the pandemic period.

Despite these changes, the reported statistical analysis did not identify a significant difference between the two periods. The chi-square analysis was reported as $p = 0.124$, while the independent-samples t-test produced $p = 0.385$. Therefore, the observed differences should not be interpreted as conclusive evidence of improved vitamin D status after the pandemic. The small sample size, variation in patient characteristics, and absence of detailed lifestyle information may have influenced the results. Additionally, the retrospective cross-sectional design did not follow the same

individuals over time, preventing direct assessment of changes in individual vitamin D concentrations.

The slight improvement observed during the post-pandemic period may be consistent with the gradual resumption of educational, occupational, and recreational activities. However, increased outdoor activity was not directly measured, and other factors, including nutritional supplementation, dietary changes, and seasonal variation, could also influence serum vitamin D concentrations. The persistence of deficiency in more than half of the tested individuals during both periods suggests that inadequate vitamin D status remained common regardless of the changes associated with pandemic restrictions.

Geographical variation was another notable finding. The proportion of vitamin D-deficient individuals was 80.6% in Sialkot, compared with 44.9% in Okara. Similarly, normal vitamin D concentrations were reported in only 3.2% of individuals from Sialkot, compared with 18.8% from Okara. These findings demonstrate a substantial descriptive difference between the two districts within the analyzed laboratory records.

The contrasting occupational characteristics of Okara and Sialkot may provide one possible explanation for this variation. Okara has a considerable agricultural population, whereas Sialkot has a strong industrial and manufacturing sector. Individuals involved in outdoor agricultural activities may have greater opportunities for sunlight exposure than those working in enclosed industrial environments. However, occupation, working hours, clothing, and actual sunlight exposure were not recorded. Therefore, the regional difference cannot be attributed confidently to occupational or environmental factors. Differences in patient referral patterns, testing practices, and the characteristics of individuals seeking laboratory investigations may also have contributed.

The gender-based analysis did not establish a statistically significant association between gender and vitamin D status. This finding suggests that a clear gender-related difference was not demonstrated in the available sample. Nevertheless, the reported chi-square analysis contained very small expected cell frequencies, which limits the reliability of the statistical interpretation. As a result, the findings should not be considered evidence that gender has no relationship with vitamin D status in the wider population. A larger study using appropriately categorized vitamin D results would be necessary to evaluate this relationship more reliably.

From a clinical perspective, the findings highlight the importance of recognizing inadequate vitamin D concentrations among individuals undergoing laboratory investigations. Particular attention may be warranted for children, adolescents, and young adults, given the high proportions observed in these categories. Nutritional education, awareness of dietary vitamin D sources, and appropriate clinical assessment could help address potential deficiencies. Decisions regarding vitamin D testing and supplementation should be based on individual clinical indications and established healthcare guidance rather than the findings of this small laboratory-based sample alone.

The study also emphasizes the potential value of district-specific public health research. The difference observed between Okara and Sialkot suggests that vitamin D status may vary considerably between populations within the same province. Larger community-based investigations could help determine whether these differences persist after accounting for age, gender, socioeconomic characteristics, occupation, dietary intake, and sunlight exposure. Such evidence would provide a stronger basis for planning nutritional interventions and evaluating the potential benefits of food fortification programs.

An important strength of the present study was its use of laboratory-measured serum 25(OH)D concentrations rather than self-reported vitamin D status. The inclusion of records from both pandemic and post-pandemic periods allowed a

descriptive comparison across different time intervals. Furthermore, the examination of age, gender, and geographical location provided several perspectives on the distribution of vitamin D deficiency.

However, several limitations must be acknowledged. The study included only 100 records selected through non-probability sampling, limiting the generalizability of the findings. The retrospective design restricted access to information regarding dietary intake, supplementation, sunlight exposure, occupation, body mass index, and associated medical conditions. The study was also limited to laboratory-tested individuals from two districts, and the findings cannot be considered representative prevalence estimates for the entire population of Central Punjab. In addition, inconsistencies in some reported statistical tables require further verification.

Future research should involve larger, community-based samples and standardized laboratory procedures. Prospective studies incorporating dietary assessment, sunlight exposure, supplementation history, and seasonal variation would provide a more comprehensive understanding of the factors associated with vitamin D deficiency. Such investigations could also clarify whether the patterns observed during the pandemic and post-pandemic periods represent meaningful changes in population vitamin D status.

Overall, the present study identified a high frequency of vitamin D deficiency among laboratory-tested individuals from Okara and Sialkot, with the greatest observed burden among participants aged 1–20 years. Although a modest improvement was observed after the pandemic, the difference was not statistically significant. The findings reinforce the need for further research, appropriate clinical awareness, and evidence-based nutritional strategies to address vitamin D deficiency in Central Punjab.

5. Future Perspectives

Future research should focus on expanding the geographical and demographic scope of vitamin D deficiency investigations across Pakistan through larger, multicentre, and population-based studies. The present findings, particularly the high proportion of deficiency among individuals aged 1–20 years (90.9%) and the considerable regional difference between Sialkot (80.6%) and Okara (44.9%), highlight the importance of investigating population-specific nutritional, environmental, and socioeconomic determinants. Future investigations should incorporate participants from diverse urban, rural, agricultural, and industrial settings to establish more representative estimates of vitamin D status. Additionally, the integration of dietary habits, socioeconomic characteristics, occupational activities, supplementation history, and sunlight exposure into epidemiological assessments would provide a more comprehensive understanding of factors potentially associated with vitamin D deficiency. The importance of evaluating dietary and socioeconomic determinants of nutritional status among populations in Punjab has also been demonstrated in recent nutritional research (Riaz et al., 2026a).

Longitudinal and prospective studies are particularly important for understanding variations in serum 25-hydroxyvitamin D concentrations across different seasons and stages of life. The present study identified a modest increase in mean vitamin D concentrations during the post-pandemic period; however, this difference was not statistically significant. Therefore, future research should investigate whether these variations reflect changes in outdoor activities, dietary practices, supplementation, or seasonal influences. Particular attention should be directed towards children, adolescents, and young adults, considering the high deficiency proportion observed in the youngest age category. Further investigations could also examine the relationship between vitamin D status and other nutritional and physiological indicators, including calcium metabolism, bone mineral turnover, and growth-related biomarkers. Research examining dietary zinc supplementation and insulin-like growth factor-1 expression in adolescent athletes highlights the importance of investigating micronutrients alongside growth-related biochemical responses (Butt et al., 2026d).

Such approaches could provide a foundation for investigating the potential relationships between vitamin D concentrations, skeletal development, and other physiological indicators.

An important future direction involves the development and evaluation of vitamin D-fortified functional foods. The incorporation of vitamin D3 and calcium into commonly consumed food products may provide an alternative approach to improving micronutrient availability through regular dietary consumption. The development of calcium and vitamin D3-fortified chocolate spread demonstrates the potential of food product formulation and nutritional enrichment for delivering essential micronutrients through commercially acceptable food matrices (Zaidi & Randhawa, 2025). Future studies should evaluate the stability, bioavailability, nutritional composition, sensory acceptability, and storage characteristics of fortified products. The wider development of plant-based functional foods and innovative nutritional formulations may also provide opportunities for expanding the range of nutritionally enhanced products available to different population groups (Riaz et al., 2026b). In addition, research on sustainable hybrid protein formulations demonstrates opportunities for developing nutritionally valuable food products through innovative ingredient combinations and processing technologies (Butt et al., 2025b).

Future investigations should also explore personalized nutritional interventions based on individual biochemical profiles, dietary patterns, age, and physiological requirements. Rather than applying identical nutritional recommendations to all population groups, individualized approaches could help identify specific nutritional requirements and appropriate intervention strategies. The integration of nutritional biomarkers with personalized dietary assessment may provide opportunities for improving the effectiveness of micronutrient interventions. Research examining functional probiotic yogurt consumption in relation to metabolic health demonstrates the potential of functional nutritional approaches for addressing broader health requirements (Rashid et al., 2026). Similarly, emerging research on artificial intelligence-assisted nutrigenomic modelling provides a possible framework for integrating dietary information with molecular and metabolic characteristics to support personalized nutritional recommendations (Butt et al., 2026a).

Another promising research direction involves investigating interactions between vitamin D and other essential nutritional components involved in skeletal development, metabolic regulation, and physiological functioning. Future studies could examine vitamin D alongside calcium, phosphorus, magnesium, and other relevant nutritional indicators to determine whether combined nutritional interventions provide additional benefits compared with isolated micronutrient supplementation. Such investigations should incorporate appropriate clinical measurements and controlled intervention designs to establish meaningful physiological relationships. Research integrating nutritional biomarkers with musculoskeletal and rehabilitation outcomes also provides an opportunity to investigate nutritional status in relation to functional recovery and physical health (Zaidi et al., 2026).

Furthermore, future investigations should explore the integration of conventional biochemical assessment with advanced nutritional biomarkers and computational approaches. The combination of serum vitamin D measurements with other nutritional, metabolic, and bone-related indicators could support a more comprehensive assessment of individual nutritional status. Artificial intelligence and machine learning may provide opportunities to integrate demographic, biochemical, dietary, and environmental variables for identifying population groups at increased risk of vitamin D deficiency. Research integrating artificial intelligence with laboratory biomarkers, medical imaging, and molecular information provides a relevant methodological direction for developing more comprehensive diagnostic frameworks (Arif et al., 2026). Similarly, AI-guided precision fermentation research demonstrates the potential of computational technologies for nutritional optimization and

personalized functional food development (Butt et al., 2026b). Such predictive approaches would require adequately sized datasets, standardized laboratory measurements, and independent validation before their application in clinical or public health settings.

Finally, future research should emphasize the translation of epidemiological findings into practical nutritional and public health interventions. Community-based nutritional awareness programmes, targeted supplementation strategies, and the development of fortified food products could be evaluated through controlled intervention studies. Particular attention should be given to affordability, dietary compliance, consumer acceptability, and long-term sustainability when designing nutritional interventions for vulnerable populations. Combining longitudinal monitoring with nutritional assessment and laboratory-based measurements would help determine the effectiveness of these interventions. Such integrated research could contribute to more targeted nutritional policies and sustainable strategies for addressing vitamin D deficiency in Pakistan.

6. Integration with Other Sciences

Vitamin D deficiency represents a multidisciplinary nutritional and health concern that requires integration with food science, clinical biochemistry, human physiology, molecular nutrition, epidemiology, biotechnology, and computational sciences. The present investigation demonstrates the relevance of combining nutritional assessment with laboratory-based biochemical measurements to examine variations in vitamin D status across different age groups, geographical locations, and study periods. Integrating these scientific disciplines could provide a broader understanding of nutritional deficiencies and facilitate the development of evidence-based preventive, diagnostic, and nutritional approaches.

Integration with Food Science and Nutritional Technology: Food science and human nutrition provide important opportunities for addressing vitamin D deficiency through food fortification, functional food development, and nutritional product innovation. The development of calcium and vitamin D3-fortified chocolate spread demonstrates the practical application of food formulation technologies for incorporating essential micronutrients into familiar food products (Zaidi & Randhawa, 2025). Such approaches could be extended to other suitable food matrices, including dairy products, beverages, and commercially consumed foods. Future research should investigate micronutrient stability, bioavailability, sensory characteristics, and storage conditions to ensure that fortified products retain their nutritional and functional properties. Similarly, research on plant-based functional foods highlights the broader potential of food technology in developing nutritionally enhanced products for human consumption (Riaz et al., 2026b). Integrating these developments with population-based nutritional assessments could help identify appropriate food vehicles and target groups for vitamin D fortification initiatives.

Integration with Clinical Biochemistry and Human Physiology: The assessment of vitamin D status is closely connected with clinical biochemistry and human physiology because vitamin D is involved in calcium regulation, mineral metabolism, and skeletal development. Future investigations could integrate serum 25-hydroxyvitamin D measurements with additional biochemical and physiological indicators to provide a more comprehensive understanding of nutritional status. The assessment of nutritional biomarkers alongside musculoskeletal and rehabilitation outcomes represents an important interdisciplinary approach for connecting nutritional assessment with physiological performance (Zaidi et al., 2026). Similarly, research examining dietary zinc supplementation and IGF-1 expression in adolescent athletes demonstrates the relevance of investigating nutritional interventions through growth-related biochemical markers (Butt et al., 2026d). Such approaches could provide a foundation for future investigations examining relationships between vitamin D concentrations, skeletal health, and other physiological indicators.

Integration with Epidemiology and Public Health Sciences: Nutritional epidemiology provides an important framework for understanding variations in vitamin D status across populations. The geographical differences observed between Okara and Sialkot highlight the importance of considering socioeconomic characteristics, dietary practices, occupational patterns, and population demographics when investigating nutritional deficiencies. Research examining dietary habits, nutritional status, and socioeconomic factors among university students in Punjab demonstrates the relevance of integrating nutritional assessment with broader social and demographic variables (Riaz et al., 2026a). Applying similar multidisciplinary approaches to vitamin D investigations could help identify population groups requiring greater nutritional attention and support the development of targeted community-level interventions. Furthermore, collaboration between clinical laboratories, nutritionists, healthcare professionals, and public health researchers could improve the translation of biochemical findings into practical nutritional recommendations.

Integration with Molecular Nutrition and Nutrigenomics: Molecular nutrition provides an emerging scientific direction for understanding how dietary components interact with biological pathways and influence physiological responses. Future vitamin D investigations could integrate conventional nutritional assessment with molecular biomarkers and nutrigenomic approaches to examine individual variations in nutritional requirements. Research on phytochemical-rich functional diets and their relationship with epigenetic markers associated with obesity and insulin resistance demonstrates the potential of connecting nutritional interventions with molecular-level biological responses (Butt et al., 2026e). Similarly, AI-assisted nutrigenomic modelling of dietary bioactive compounds and gut microbiome interactions illustrates how nutritional information can be integrated with molecular and metabolic characteristics (Butt et al., 2026a). Applying comparable multidisciplinary frameworks to vitamin D research could help investigate molecular and metabolic characteristics associated with different nutritional profiles.

Integration with Artificial Intelligence and Computational Sciences: Emerging computational approaches provide additional opportunities for improving nutritional risk assessment and interpreting complex laboratory datasets. Artificial intelligence and machine learning could potentially be applied to integrate serum vitamin D concentrations with age, gender, geographical location, dietary information, socioeconomic characteristics, and other biochemical indicators. These approaches may facilitate the identification of complex patterns and the development of predictive models for nutritional risk classification. Research integrating artificial intelligence with laboratory biomarkers, medical imaging, and molecular information illustrates the potential of computational technologies in multidisciplinary healthcare assessment (Arif et al., 2026). Similarly, AI-guided precision fermentation demonstrates the potential of integrating computational technologies with food biotechnology for nutritional optimization (Butt et al., 2026b). Incorporating such approaches into future vitamin D research could support more comprehensive nutritional assessment, provided that adequate datasets and appropriate validation procedures are available.

Integration with Sports Nutrition and Exercise Physiology: Sports nutrition and exercise physiology provide another important area for multidisciplinary investigation because nutritional status is associated with skeletal health, physical performance, and physiological adaptation. Future studies could examine vitamin D status in relation to physical activity, musculoskeletal function, bone mineral turnover, and exercise-related physiological responses. Research examining nutritional biomarkers as predictors of rehabilitation outcomes provides an opportunity to connect nutritional assessment with functional recovery and musculoskeletal health (Zaidi et al., 2026). Furthermore, investigations into bone mineral turnover, hormonal stability, and lean mass preservation in endurance-trained adults demonstrate the relevance of integrating nutritional assessment with exercise physiology (Butt et al., 2026c). Such

interdisciplinary research could help establish a broader understanding of vitamin D status in physically active and sedentary populations.

Integration with Food Microbiology and Fermentation Biotechnology: Food microbiology and fermentation biotechnology offer additional opportunities for developing innovative functional food products. Fermentation technologies can influence the nutritional composition, functional characteristics, and quality of food products. Research involving Lactobacillus rhamnosus as a probiotic adjunct culture for tempeh development demonstrates the application of beneficial microorganisms in functional food production (Ahmed et al., 2024). Similarly, research on functional probiotic yogurt highlights the potential of fermented food products in nutritional and metabolic health applications (Rashid et al., 2026). Future investigations could explore the compatibility of vitamin D fortification with fermented food matrices, including the effects of processing, storage, and microbial activity on nutrient stability. Such research would require specific evaluation of vitamin D retention and bioavailability rather than assuming that fermentation directly improves vitamin D status.

Integration with Food Safety and Quality Assurance: The development of vitamin D-fortified and functionally enhanced foods requires integration with food safety, quality management, and analytical sciences. Fortified products must undergo appropriate evaluation to ensure nutritional accuracy, microbiological safety, physicochemical stability, and consistency throughout their shelf life. Research investigating the quality and safety attributes of food products, together with investigations into microbial safety and contamination risks in milk, demonstrates the importance of comprehensive food quality assessment in the development of safe nutritional products (Butt et al., 2024; Riaz et al., 2026c). Integrating these approaches into vitamin D fortification research could strengthen consumer protection and support the development of reliable fortified food products.

Integration with Clinical Rehabilitation and Musculoskeletal Sciences: Vitamin D research also has important connections with physiotherapy, orthopaedics, and rehabilitation sciences because nutritional biomarkers may provide additional information about musculoskeletal health and functional recovery. The integration of nutritional biomarkers with rehabilitation outcomes offers a multidisciplinary framework for investigating how nutritional status relates to physical functioning and recovery (Zaidi et al., 2026). Future research could combine serum vitamin D assessment with bone-related biochemical indicators, physical performance measurements, and musculoskeletal evaluations. Such an approach could extend nutritional investigations beyond the identification of biochemical deficiency towards a broader assessment of its potential clinical and functional implications.

Integration with Sustainable Food Systems and Nutritional Innovation: Sustainable food technology can contribute to the development of accessible and nutritionally enhanced food products through the efficient utilization of food ingredients and innovative processing strategies. Research on sustainable whey–corn hybrid protein powders and plant-based functional foods demonstrates the potential of developing nutritionally valuable products while considering functional properties and environmental sustainability (Butt et al., 2025b; Riaz et al., 2026b). Future vitamin D fortification research could explore suitable sustainable food matrices and assess their nutritional effectiveness, affordability, consumer acceptability, and commercial feasibility. Such integration could help connect nutritional interventions with broader food system development and accessibility objectives.

Collectively, integrating food science, clinical biochemistry, human physiology, molecular nutrition, epidemiology, biotechnology, sports sciences, and computational sciences could establish a more comprehensive framework for investigating vitamin D deficiency. Such collaboration would connect laboratory-based nutritional assessment with functional food development, population-level nutritional surveillance, and advanced analytical approaches. This multidisciplinary perspective

could contribute to the development of more targeted and sustainable strategies for improving nutritional status among vulnerable populations in Pakistan.

7. Conclusion

Vitamin D deficiency was common among the 100 laboratory records examined, affecting 56% of individuals, while an additional 30% had insufficient concentrations. The highest deficiency rate was observed among participants aged 1–20 years. Although vitamin D status showed a slight improvement during the post-pandemic period, the difference was not statistically significant. Sialkot demonstrated a higher proportion of deficiency than Okara. These findings highlight the importance of further population-based research, nutritional awareness, and appropriate preventive healthcare strategies, particularly for younger age groups.

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