Correlation of Vitamin D Deficiency with Levels of Calcium and Phosphate in the Adult Population of Khairpur Mir's.

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ABSTRACT:

Objective: To determine the correlation between vitamin D level and serum calcium and phosphate in the adult population of Khairpur Mir's, and to identify independent predictors of vitamin D deficiency.

Methodology: This cross-sectional study enrolled 210 asymptomatic adults aged 20-39 years from Khairpur Mir's. Serum vitamin D, calcium, and phosphate were measured biochemically, and demographic and lifestyle data were recorded. Vitamin D deficiency was defined as <20 ng/mL. Associations were assessed using chi-square and independent-samples t-tests; independent predictors of deficiency were identified using univariate and multivariable binary logistic regression; and the vitamin D-calcium and vitamin D-phosphate relationships were quantified using Pearson's and Spearman's correlation coefficients.

Results: Mean serum vitamin D was 19.54±8.00 ng/mL; 53.3% of participants were deficient. Mean calcium was 8.75±0.98 mg/dL (36.7% deficient) and mean phosphate was 3.31±0.73 mg/dL (32.4% deficient). Vitamin D correlated moderately with calcium ($r=0.451$, $p<0.001$) but only weakly and non-significantly with phosphate ($r=0.125$, $p=0.071$). On multivariable logistic regression, employment (adjusted OR=4.52, 95% CI 2.41-8.48, $p<0.001$) and inadequate sun exposure (adjusted OR=4.41, 95% CI 2.32-8.40, $p<0.001$) were independent predictors of vitamin D deficiency; age, sex, education, and dietary intake were not.

Conclusion: Vitamin D deficiency is common among adults in Khairpur Mir's and correlates significantly with serum calcium but not phosphate, consistent with their differential regulation. Employment and inadequate sun exposure, rather than demographic factors, independently predict deficiency, supporting targeted, exposure-based screening and supplementation strategies for this and similarly under-studied districts.

Keywords: Vitamin D deficiency; Calcium; Phosphate; Correlation; Khairpur Mir's; Pakistan.

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Introduction:

Unlike other vitamins, the body synthesizes most of its vitamin D needs. The conversion of a cholesterol precursor into previtamin D3 in the skin occurs in response to UVB exposure; previtamin D3 is later hydroxylated in the liver to give 25-hydroxyvitamin D and then, in the kidney, to 1,25-dihydroxyvitamin D. 1,25-dihydroxyvitamin D is active, acting through the vitamin D receptor to regulate intestinal calcium and phosphate absorption, renal handling of calcium and phosphate, and bone mineralization.[1] But calcium and phosphate are not just innocent bystanders, downstream targets of this system. Vitamin D and the bone-derived hormone, FGF23, are regulated by a three-way relationship where each regulates the production of the other and each regulates renal handling of phosphate.[2,3] The end result is that calcium and phosphate do not necessarily reflect vitamin D status. In vitamin D deficiency, absorption of calcium from the gut falls to 10%-15%, and phosphate absorption falls to 50%-60%, leading to secondary hyperparathyroidism, bone loss and ultimately osteomalacia over time.[4]

Despite receiving more sunlight than most regions globally, South Asia and the Middle East report some of the highest adult prevalence rates of vitamin D deficiency, averaging around 50%.[5] This paradox is particularly evident in Pakistan. A 2024 meta-analysis of 37 studies involving over 19,000 participants estimated the national prevalence at 78%[6], and recent investigations among young adults have confirmed similarly high rates alongside early cardiovascular changes.[7] In Sindh, a study from Jamshoro demonstrated that housewives and indoor workers were significantly more deficient than outdoor workers.[8] Moreover, Pakistan's adult burden of deficiency exceeds that of most neighboring countries in the region.[9]

This distinction reflects the differing mechanisms of calcium and phosphate mobilization and explains why their relationships with vitamin D are measurable in some contexts but not in others. Calcium absorption is almost entirely dependent on active transport mediated by calcitriol, and therefore falls sharply when vitamin D levels are low. By contrast, phosphate absorption remains relatively efficient even without elevated calcitriol, as serum phosphate is primarily regulated by parathyroid hormone and fibroblast growth factor 23 (FGF23) acting at the kidney rather than

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by vitamin D itself. This discrepancy is evident in the literature: Medhi et al.[10] reported a significant association between vitamin D and calcium in osteoporotic adults, whereas Mohammed et al.[11], studying patients with osteomalacia, found no meaningful relationship between vitamin D and the calcium-phosphate product.

Despite extensive data from Pakistan's major cities and provinces, the rural districts of interior Sindh remain underexplored. Khairpur Mir's, like much of the country, exhibits well-documented risk factors, including limited sun exposure among women due to dress conventions, low dairy intake, minimal awareness of vitamin D supplementation, and reduced calcium and phosphate levels in adults. This gap is significant because national and provincial averages can obscure district-level variations, which should inform the prioritization and location of screening and supplementation programs.[6,12]

The purpose of this study was to establish correlation between the Vitamin D level with serum calcium and phosphate level in adults of Khairpur Mir's and to find out the independent factors predicting Vitamin D deficiency in adults of Khairpur Mir's. The descriptive foundation reported for the prevalence and severity of deficiency in all three parameters.

Objectives:

To determine the correlation between vitamin D level and serum calcium and phosphate in the adult population of Khairpur Mir's, and to identify independent predictors of vitamin D deficiency.

Methodology:

For this cross-sectional study, approval was obtained from the ethical review committee of the Department of Biochemistry, Peoples University of Medical and Health Sciences for Women (PUMHS-W), Shaheed Benazirabad (Nawabshah) in collaboration with Khairpur Medical College, Khairpur Mir's, for six months from January 01, 2026 to June30, 2026. A total of 210 participants were recruited from collaborating centers who attended the center and were asymptomatic adult residents of Khairpur Mir's and were recruited consecutively (non-probability sampling method). The size of this sample, when applying Fisher z-transformation for Pearson's correlation coefficient, would have 80% power (α=0.05, two tailed) to detect a Pearson's correlation coefficient of r≈0.19, which was considered adequate for this study's purpose, given the wide range between the 0.125 reported by Çaykara et al.[13] in a large comparator cohort and 0.4403 reported by Medhi et al.[10] in a clinically enriched cross-sectional cohort.

Eligible participants were men and women 20-39 years old who were permanently residing in the district. Individuals outside this age group, or with a known musculoskeletal, skeletal, or periodontal disease were excluded because these could independently affect calcium-phosphate status, as could diabetes, malignancy, tuberculosis, chronic kidney disease, chronic liver disease, and cardiovascular disease. Ethics Review Committee of the institute approved the study before data collection. Written informed consent was gained from all participants in either English or Sindhi as preferred and participant data was kept confidential throughout

All participants were given a structured proforma in English and Sindhi to record their demographic and history data, which were used to determine the possible causes of vitamin D deficiency primarily sun exposure and diet. Venous blood (5 mL) was collected by taking puncture of antecubital vein into plain gel tube, clot it at room temperature for 30 minutes and sent to the laboratory on ice. Serum was centrifuged, (4,000 rpm for 15 minutes) and stored at ≤ −20°C for analysis. For all samples calcium and phosphate were measured using standard colorimetric/photometric techniques and 25-hydroxyvitamin D was measured in all samples. According to Mahar et al.[6] and Aoun et al.[12] vitamin D deficiency was considered to be < 20 ng/mL, insufficiency was 20-29.9 ng/mL, and sufficiency was > 30 ng/mL. Calcium level < 8.5 mg/dL was considered as deficient, and phosphate level < 3.0 mg/dL was considered as deficient, which is compatible with the reference interval used by Çaykara et al.[13]

Data analysis was done using SPSS software version 25. The Shapiro-Wilk test revealed that age, calcium, and phosphate were not normally distributed in the entire sample, but they were approximately normally distributed in the vitamin D deficient and non-deficient subgroups (and, of course, were normally distributed in the non-deficient group), for which the t-test for independent samples was used, and, as a check, Spearman's correlation was used in addition to Pearson's correlation for each of those two comparisons. Continuous variables are summarized as mean ± SD (range), and categorical variables as frequency and percentages. If differences existed between groups in categorical variables, these were tested using chi-square (or Fisher's exact test if necessary), and if differences existed between groups on continuous variables, they were tested using the independent-samples t-test. Pearson's and Spearman's correlation coefficients were used to determine the relationship between vitamin D-calcium and vitamin D-phosphate. Univariate and multivariable binary logistic regression was used to identify independent predictors of vitamin D deficiency; those with a significance of $p<0.25$ in univariate analysis were carried forward into multivariable analysis. The significance level used was $p<0.05$ (two-tailed).

Results:

A total of 220 adults from Khairpur Mir's were recruited, of whom 10 were excluded after being found symptomatic. The final sample comprised 210 asymptomatic adults aged 20–39 years, with a mean age of 30.75 ± 4.67 years; more than half were older than 30 years. The gender distribution was approximately three females for every two males (Table 1). Nearly half of the participants were illiterate, employment was almost evenly distributed, and the majority reported insufficient sunlight exposure and inadequate dietary intake of vitamin D.

Mean serum vitamin D was 19.54 ±8.00 ng/mL. When the vitamin D levels were compared to standard cut-offs, there were 112 participants with hypovitaminosis D (53.3), 77 less than hypovitaminosis D (36.7), and 21 at or above the hypovitaminosis D level (10.0), which means that about nine in ten people were Vitamin D deficient. The severity distribution (severe, moderate, mild, normal) had a general similarity between men and women, and the moderate severity group was the largest among both sexes (Figure 1). The mean level of serum calcium was 8.75 ±0.98 mg/dL and 77 participants (36.7%) were hypocalcemia. Mean serum phosphate was 3.31 ±0.73 mg/dL; 68 (32.4%) were hypophosphatemia (Table 1). There was no significant association of Vitamin D deficiency with sex, education and dietary intake on bivariate analysis (Table 2, all $p>0.1$). It was, however, clearly related to both employment and sun exposure – close to seven in ten employed participants were deficient compared to under four in ten of the unem-

ployed, and about two-thirds of those with inadequate sun exposure were deficient compared to just over a third of those with adequate sun exposure (both p<0.001). Deficiency was also associated with hypocalcemia (p<0.001) and with reduced mean calcium across all groups of deficient (8.39 mg/dL) compared to non-deficiency (9.17 mg/dL) (p<0.001). A smaller, but significant, difference was found between the group means for the phosphate comparison (3.22 vs. 3.42 mg/dL, p=0.047); the categorical comparison for hypophosphatemia itself was not significant (p=0.122). Vitamin D correlated moderately with calcium (r=0.451, p<0.001; Spearman's ρ=0.439, p<0.001) but only weakly with phosphate, and not significantly (r=0.125, p=0.071; ρ=0.117, p=0.090) (Figure 2).

Table No 1. Demographic and biochemical profile of participants (n=210)

Variable	Category	n (%) / Mean ± SD
Age (years)	Mean ± SD (range)	30.75 ±4.67 (20-39)
	20-30 years	97 (46.2%)
	>30 years	113 (53.8%)
Sex	Male	83 (39.5%)
	Female	127 (60.5%)
Educational status	Illiterate	103 (49.0%)
	Literate	107 (51.0%)
Employment status	Unemployed	108 (51.4%)
	Employed	102 (48.6%)
Sun exposure	Adequate	83 (39.5%)
	Inadequate	127 (60.5%)
Dietary vitamin D intake	Adequate	79 (37.6%)
	Inadequate	131 (62.4%)
Serum vitamin D (ng/mL)	Mean ± SD	19.54 ± 8.00
	Deficient (<20)	112 (53.3%)
	Insufficient (20-29.9)	77 (36.7%)
	Sufficient (≥30)	21 (10.0%)
Serum calcium (mg/dL)	Mean ± SD	8.75 ± 0.98
	Deficient (<8.5)	77 (36.7%)
Serum phosphate (mg/dL)	Mean ± SD	3.31 ± 0.73
	Deficient (<3.0)	68 (32.4%)

Figure No1. Severity distribution of vitamin D status by sex (n=210).

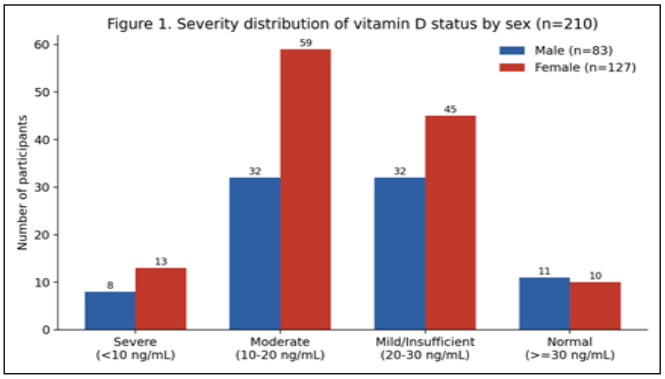
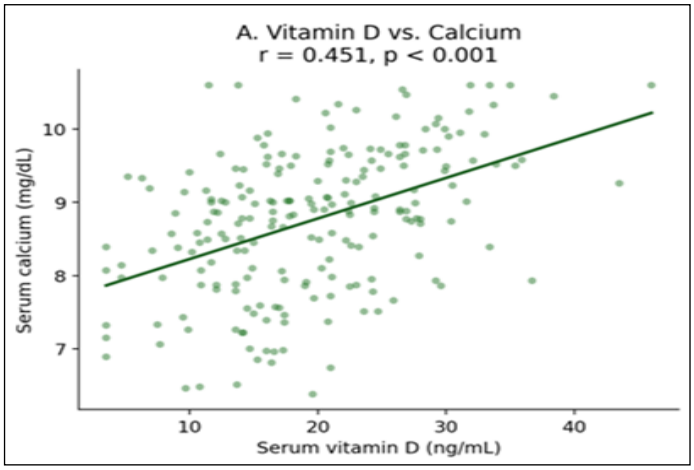


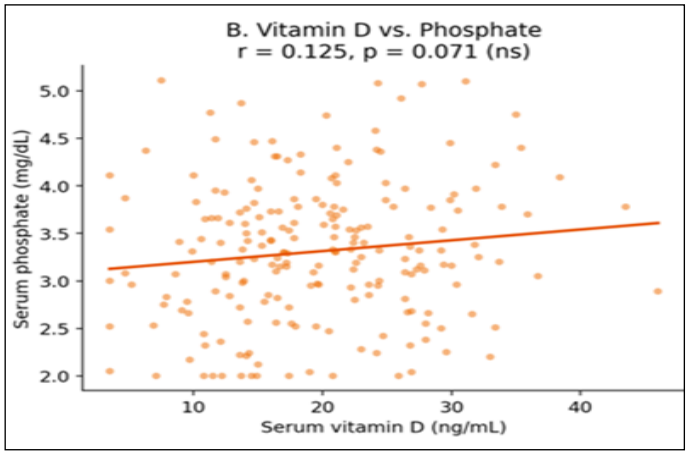
Table No 2. Association of vitamin D deficiency with demographic and biochemical parameters (n=210)

Variable	Vit D deficient (n=112)	Vit D normal (n=98)	p
Sex: Female	72/127 (56.7%)	55/127 (43.3%)	0.287[a]
Education: Illiterate	59/103 (57.3%)	44/103 (42.7%)	0.324[a]
Employment: Employed	71/102 (69.6%)	31/102 (30.4%)	<0.001[a]
Sun exposure: Inadequate	83/127 (65.4%)	44/127 (34.6%)	<0.001[a]
Diet: Inadequate	71/131 (54.2%)	60/131 (45.8%)	0.857[a]
Calcium deficient	55/77 (71.4%)	22/77 (28.6%)	<0.001[a]
Phosphate deficient	42/68 (61.8%)	26/68 (38.2%)	0.122[a]
Mean serum calcium (mg/dL)	8.39 ± 0.95	9.17 ± 0.86	<0.001[b]
Mean serum phosphate (mg/dL)	3.22 ± 0.72	3.42 ± 0.72	0.047[b]

[a]Chi-square test. [b]Independent-samples t-test.

Figure No 2. Correlation of serum vitamin D with serum calcium (A) and serum phosphate (B) (n=210).





Logistic regression analysis identified employment status and inadequate sun exposure as independent risk factors for vitamin D deficiency (Table 3). Individuals who were

unemployed were nearly four times more likely to be deficient (OR = 3.75, 95% CI 2.11-6.62), and a similar association was observed among those reporting insufficient sun exposure (OR = 3.51, 95% CI 1.96-6.29). Age, sex, education, and diet showed no significant unadjusted associations. In the multivariable model (including sex, employment, and inadequate sun exposure, screened at univariate $p < 0.25$), sex was not significant, but both sun exposure and employment remained strongly associated with deficiency. After adjustment, inadequate sun exposure was linked to more than a four-fold increase in risk (OR = 4.41, 95% CI 2.32-8.40, $p < 0.001$), while unemployment was similarly associated (OR = 4.52, 95% CI 2.41-8.48, $p < 0.001$).

Table 3. Univariate and multivariable logistic regression predicting vitamin D deficiency (n=210).

Predictor	Unadjusted OR (95% CI)	p	Adjusted OR (95% CI)[a]	p
Age (years)	0.97 (0.92-1.03)	0.369	–	–
Sex: Female vs. Male	1.41 (0.81-2.45)	0.228	1.55 (0.83 -2.88)	0.167
Education: Illiterate vs. Literate	1.37 (0.79-2.35)	0.261	–	–
Employment: Employed vs. Unemployed	3.75 (2.11-6.62)	<0.001	4.52 (2.41 -8.48)	<0.001
Sun exposure: Inadequate vs. Adequate	3.51 (1.96-6.29)	<0.001	4.41 (2.32 -8.40)	<0.001
Dietary vitamin D: Inadequate vs. Adequate	1.10 (0.63-1.92)	0.746	–	–

[a]Adjusted model retains only predictors with univariate p<0.25 (sex, employment, sun exposure); age, education, and diet were not retained.

Discussion:

The objective of this study was to find out the prevalence of vitamin D deficiency in adults of Khairpur Mir's and its relationship with serum calcium and phosphate levels and predict it. Only two factors (employment and inadequate sun exposure) predicted deficiency independently and deficiency was common, whereas calcium was more closely tracked with vitamin D status, and phosphate was only slightly tracked with vitamin D status. Let's look at each of these in relation to other reports. Although the city of Khairpur Mir is located in the sun belt of Pakistan, just over half of the adults sampled here were vitamin D deficient and additional 36.7% were insufficient, and therefore 189 out of 199 people (or roughly 9 in 10) were either vitamin D deficient or insufficient. That is, if anything, the lowest of any other part of the country. A meta-analysis by Mahar et al. of 37 studies conducted in Pakistan, revealed a national pooled prevalence of 78%, which increased to 80% among Sindh population specifically.[6] In another study, carried out by Sheikh et al., in the year 2025, the prevalence of deficiency among asymptomatic adults of the city of Karachi was found to be 84.3%[14] while another local survey in Peshawar had reported a combined prevalence of around 60%.[15] However, our findings are slightly higher but far less than the 57 % reported in pre- and postmenopausal women of Karachi, Pakistan specifically.[16] This is speculative, as exposure to sunlight was self-reported and not measured, but one possible explanation is that the rural nature of Khairpur Mir provides more incidental exposure to the outdoors, due to agricultural activities, than the largely indoor-oriented work habits of Karachi and Peshawar. The two factors which did independently predict deficiency employment (adjusted OR=4.52) and inadequate sun exposure (adjusted OR=4.41) tell a coherent, albeit somewhat counter-intuitive, story. In many contexts, employment is associated with higher outdoor time and consequently, lower deficiency risk in this context, the opposite was true. As in all studies reported, Sowah et al. in their systematic review reported the same general pattern, indoor occupations having higher deficiency risk than outdoor occupations, irrespective of where the study was conducted[17] and a study in Jamshoro in the same province reported that 62.7% of housewives were in danger of being deficient compared to 16.9% of outdoor workers.[8] Taken together, this indicates that in rural Sindh, employment is more likely to be in the field or courtyard (e.g., outside) than to be in the shop or office (e.g., inside) and, conversely, unemployment is more likely to occur in the field or courtyard (e.g., outside) than to occur in the shop or office (e.g., inside).Both employment and sun exposure are independently associated with the risk of NHANES, indicating that they are not just proxies for the same risk, but each has their own risk. There was no independent effect of age, sex or education, nor diet, which is consistent with the observation of AlQuaiz et al. that demographic factors predicting vitamin D deficiency don't always generalize well.[18]

The correlation of calcium with this study (r=0.451) was close to the value obtained by Medhi et al. in an osteoporotic Assamese population (r=0.4403) [10] but greater than the r=0.125 reported by Çaykara et al. in a large general Turkish population.[13] The most probable cause is the amount of hypocalcaemia in each population: the mean calcium (8.75 mg/dL) was near the low end of the normal range for our participants, while almost all (97.1%) of those of Çaykara et al.,[13] were normocalcaemic. Such a relationship is more easily observed in terms of statistics when a significant portion of a population falls into the deficient range of both measures,[19] as Bikle's account of the role of calcitriol in calcium absorption predicts, and when the vitamin D-calcium connection is most clearly seen at its deficient end, as classical rickets and Osteomalacia do.[20]

Phosphate was a different story: the correlation coefficient was weak and not significant (r=0.125, p=0.071; ρ=0.117, p=0.090), which is similar to almost all of the comparable studies we found: in Osteomalacia patients, Mohammed et al. found no significant link between vitamin D and the calcium-phosphate product despite low mineral values on all counts (r=0.114, p=0.332)[11] and Çaykara et al. found essentially nothing (r=0.016) [13]. This physiology is also why: phosphate is absorbed reasonably efficiently even in the absence of high calcitriol; and parathyroid hormone and FGF23 act primarily on the kidney to regulate blood phosphate levels, with only minor effects on the gut. Despite this, 1/3 of our participants were hypophosphatemia, and this was not vitamin D dependent, but rather was related to renal regulation and/or dietary phosphate intake,

which were more likely to be the factors determining phosphate status in this population.[21,22]

It's important to compare these general population numbers with a comparison for the disease. Here, vitamin D deficiency was the most prevalent abnormality (45%), but calcium and phosphate abnormalities were more prevalent (34% and 78%, respectively) than vitamin D deficiency alone (18%), which is almost opposite to what Jamil et al. found in type-2 diabetic patients, where vitamin D deficiency itself was less prevalent (18%) than calcium and phosphate abnormalities (34% and 78% respectively).[23]This difference may plausibly be attributed to diabetes-specific mechanisms which affect renal phosphate handling, for which the direct effect of insulin do not apply to a general, non-diabetic population.

These findings suggest a targeted approach as opposed to a general approach practically. Nearly 9 out of 10 participants had some vitamin D deficiency; exposure patterns that are modifiable and identifiable (occupational and lifestyle sun avoidance) are responsible for a large proportion of the groups that were vitamin D deficient; vitamin D screening of the general population is unlikely to have more impact than targeted messages to indoor workers, food fortification, or supplementation. This is consistent with the other calcium and vitamin D fracture prevention evidence [24,25] latitude, winter sampling, older age and higher BMI but not every dietary intake predicted hypovitaminosis D in the French VARIETE study[26]and underscores the fact that, in general, vitamin D status varies between populations due to factors of environment and lifestyle, rather than dietary intake. Considering the burden observed in this area, Khairpur Mir and other under studied interior districts of the province deserve to be included in the future provincial/National vitamin D surveillance program.[27]

Several caveats should be noted. First, this was a cross-sectional study and therefore cannot establish causality between employment, sun exposure, and vitamin D deficiency, only associations. Second, sun exposure and dietary intake were assessed using simple self-reported yes/no questions, which may have introduced misclassification, most likely leading to underestimation rather than overestimation of the observed associations. Third, the findings may not be generalizable to older adults, who are more prone to vitamin D deficiency and calcium loss, given the age restriction of the sample. Additionally, important variables such as parathyroid hormone, body mass index, and skin pigmentation were not measured, all of which could influence the relationship studied. Finally, as this was a non-random sample drawn from a single district, caution is warranted in extrapolating the results to other interior districts, including Khairpur Mir's.

Conclusion:

Vitamin D deficiency was highly prevalent among adults in Khairpur Mir's, affecting more than half of the study population. Employment status and inadequate sun exposure independently predicted deficiency, while age, sex, education, and diet showed no significant associations. Serum vitamin D levels correlated significantly with calcium but not with phosphate, consistent with physiological regulation and findings from similar studies in Pakistan and elsewhere. These results suggest that exposure-based screening and supplementation strategies may be more effective than universal approaches in under-studied districts, and that serum calcium serves as a more reliable proxy for vitamin D status than serum phosphate in these populations.

Author's Contributions:

We strongly encourage authors to include author contributions and recommend using CRediT (https://casrai.org/credit/) for standardized contribution descriptions. Please refer to our general author guidelines (Hyperlink)for more information about authorship.

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Authors' Contribution	
Shafique Ahmed Memon	Study design, data collection, results, references
Habibullah Shaikh	Discussion, data analysis, results
Yasmin Shaikh	Introduction, study design, methodology
Shumaila Shaikh	Discussion, results, introduction
Bakhtawar Phulpoto	Introduction, data collection, discussion
Maria Eram Sahito	Results, data analysis, references,