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URBAN-RURAL DISPARITIES IN CALCIUM AND VITAMIN D DEFICIENCY: A POPULATION-BASED STUDY IN FAISALABAD, PAKISTAN

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Abstract
Vitamin D deficiency is a global public-health problem that remains widespread in South Asian countries, including Pakistan, despite abundant sunlight throughout the year. The present cross-sectional study was conducted to assess the prevalence of vitamin D and calcium deficiency and to evaluate complete blood count (CBC) parameters among urban and rural populations of Faisalabad, Pakistan. A total of 68 volunteers (34 urban and 34 rural; 35 males and 33 females; aged 20-50 years) were enrolled. Venous blood samples were collected and analysed for serum calcium, 25-hydroxyvitamin D (by enzyme-linked immunosorbent assay, ELISA) and CBC parameters, including haemoglobin (Hb), total leukocyte count (TLC), haematocrit (HCT), mean corpuscular volume (MCV), red blood cell count (RBC), platelets, mean corpuscular haemoglobin concentration (MCHC) and mean corpuscular haemoglobin (MCH). A structured questionnaire recorded dietary intake, sun exposure, physical activity and body mass index. Mean serum calcium was 8.59 ± 1.58 mg/dL in the 20-34-year group and 8.54 ± 1.77 mg/dL in the 35-50-year group, while mean vitamin D was 8.85 ± 2.96 ng/mL and 9.44 ± 4.67 ng/mL, respectively, indicating widespread deficiency (reference range 20-30 ng/mL). Males had significantly higher vitamin D than females (10.03 ± 4.69 vs. 8.17 ± 2.39 ng/mL; P = 0.0451). Most CBC parameters did not differ significantly between age groups, although MCH was significantly higher in the older group (P = 0.0426). Correlation analysis showed a significant positive association between calcium and vitamin D (P < 0.01) and between HCT and MCV (P < 0.01). Vitamin D and calcium deficiencies were highly prevalent in both urban and rural areas of Faisalabad, with females at greater risk. Despite ample sunshine, cultural practices, limited effective sun exposure and dietary factors appear to drive this deficiency, underscoring the need for routine screening and targeted public-health interventions.

INTRODUCTION

Vitamin D is a fat-soluble secosteroid that functions principally as a hormone and is central to the maintenance of calcium homeostasis and skeletal integrity in the human body. Its deficiency impairs bone mineralisation and predisposes to skeletal deformities; in children it causes rickets,

characterised by defective ossification at the growth plates and bowing of the long bones, whereas in adults it manifests as osteomalacia with diffuse bone pain and muscle weakness (Pettifor, 2003; Holick, 2007). Beyond the skeleton, low vitamin D status has been linked to an increased risk of osteoporosis, diabetes mellitus, cardiovascular disease, tuberculosis and certain malignancies (Liu et al., 2006; Garland, 2009).Two principal forms of the vitamin are recognised: vitamin D2 (ergocalciferol), obtained largely from plant and fungal sources, and vitamin D3 (cholecalciferol), synthesised in the skin upon exposure to ultraviolet-B radiation and also available from animal foods (Holick, 2004; Armas, 2004). Cutaneous synthesis is generally the dominant source, with dietary intake from oily fish, egg yolk, fortified dairy products and supplements contributing the remainder (Chen et al., 1993). Once formed, vitamin D undergoes hepatic hydroxylation to 25-hydroxyvitamin D [25(OH)D], the major circulating metabolite and the most reliable biomarker of vitamin D status (Lips, 2006; Heijboer, 2013).

In the absence of adequate vitamin D, intestinal calcium absorption falls from 50-60% to only 9-15%, triggering a compensatory rise in parathyroid hormone that mobilises skeletal calcium and accelerates bone loss, eventually leading to osteopenia, osteoporosis and an elevated fracture risk (Meunier and Vieth, 2005; Grant, 2006). Adequate vitamin D intake, together with sensible sun exposure and supplementation of 700-800 IU per day, can reduce the incidence of falls and fractures in adults (Broe et al., 2009).

Globally, vitamin D deficiency affects an estimated one billion people, and the burden is especially heavy across South Asia, where 65-70% of populations are reported to be deficient (Akhtar et al., 2016; Nimitphong and Holick, 2013). Pakistan exhibits some of the highest prevalence figures, with deficiency reported in up to 85% of certain groups despite year-round sunshine (Akhtar, 2016). Several factors converge to produce this paradox, including limited effective sun exposure, concealing dress and veiling practices, low intake of vitamin D-rich or fortified foods, air pollution, obesity and broader socio-economic constraints (Mishal, 2001; Babu and Calvo, 2010; Al-Harib and Singh, 2013). Even pregnant and lactating women who consume multivitamins frequently remain deficient (Alsuwaida et al., 2013).Serum 25(OH)D concentrations below 20 ng/mL are widely accepted as indicating deficiency, whereas optimal health is generally associated with levels above 30 ng/mL (Holick, 2007; Rosen, 2012). Obesity further lowers measured serum concentrations because the vitamin is sequestered in adipose tissue, and assay variability related to vitamin D-binding protein can complicate interpretation (Atabek et al., 2006; Heijboer, 2013). Despite the recognised public-health significance of deficiency, large-scale comparative data contrasting urban and rural populations within Pakistan remain limited (Adams and Hewison, 2010; Absoud et al., 2011).

Faisalabad, the third-largest city of Pakistan, comprises both densely populated urban districts and surrounding agrarian rural communities whose lifestyles, diets and patterns of sun exposure differ markedly. The present study was therefore designed to determine the prevalence of vitamin D and calcium deficiency, to examine their relationship with dietary intake, body mass index, sun exposure, skin pigmentation and physical activity, and to evaluate associated complete blood count parameters among urban and rural residents of Faisalabad.

2. Materials and Methods

2.1 Study design and population

This survey-based, cross-sectional study investigated urban-rural disparities in calcium and vitamin D deficiency among the population of Faisalabad, Pakistan. Two groups of volunteers were recruited from urban and rural areas of the district. A total of 68 participants (34 urban and 34 rural; 35 males and 33 females) aged between 20 and 50 years were enrolled. Participants with no documented history of vitamin D supplementation were preferred, and a structured questionnaire was administered to each volunteer.

2.2 Questionnaire and anthropometry

A structured questionnaire was designed in accordance with the daily routine of the volunteers. It captured dietary intake of vitamin D-rich foods (fish, milk, egg yolk and supplements), body mass index, overall diet, skin pigmentation, physical activity and the extent of daily sun exposure. Additional items

recorded any previous intake of calcium or vitamin D supplementation. Body weight and height were measured with a calibrated weighing scale and measuring tape, respectively, and body mass index (BMI) was calculated as weight in kilograms divided by the square of height in metres.

2.3 Blood sampling and processing

Blood samples were collected from the antecubital vein using sterilised disposable syringes following standard venipuncture technique. Approximately 3 mL of blood was dispensed into each of two labelled tubes, one containing the anticoagulant ethylenediaminetetraacetic acid (EDTA) for haematological analysis and one without anticoagulant for serum separation. EDTA tubes were mixed gently by inversion. For serum, samples were allowed to clot and then centrifuged at 5000 rpm for 5-10 minutes to separate serum from the cellular fraction. Serum was transferred into sterilised Eppendorf tubes, labelled by participant, and stored at approximately -10 to -15 degrees Celsius until analysis (Brock et al., 2010). Samples were analysed within 24 hours of collection wherever possible (Visser et al., 2006).

2.4 Estimation of serum vitamin D by ELISA

Serum 25-hydroxyvitamin D was estimated using a solid-phase competitive enzyme-linked immunosorbent assay (ELISA). Microwell plates pre-coated with anti-vitamin D antibody were used. Briefly, 10 microlitres of serum, standards and controls were dispensed into the wells, followed by the biotinylated 25(OH)D reagent; the mixture was incubated at room temperature on a plate shaker. Endogenous vitamin D competed with the biotin-labelled vitamin D for a fixed number of antibody-binding sites. After incubation, wells were washed with 1X wash buffer to remove unbound material, streptavidin-horseradish peroxidase (SA-HRP) conjugate was added, and tetramethylbenzidine (TMB) substrate was introduced and incubated in the dark. The enzymatic reaction was terminated with stop solution, and absorbance was measured spectrophotometrically. The intensity of the developed colour was inversely proportional to the concentration of 25(OH)D in the sample, and concentrations were read from a standard curve (Looker et al., 2008; Holick et al., 2018).

2.5 Serum calcium and complete blood count

Complete blood count parameters, including haemoglobin (Hb), total leukocyte count (TLC), haematocrit (HCT), mean corpuscular volume (MCV), red blood cell count (RBC), platelet count, mean corpuscular haemoglobin concentration (MCHC) and mean corpuscular haemoglobin (MCH), were determined from EDTA-anticoagulated samples using an automated haematology analyser based on the electrical impedance principle (Mehta, 2018). Serum calcium was measured by standard colorimetric biochemical assay.

2.6 Statistical analysis

Data were analysed using Student's t-test and analysis of variance (ANOVA), and relationships between variables were examined using Pearson's correlation coefficient (Montgomery, 2013). A probability value of $P < 0.05$ was considered statistically significant and $P < 0.01$ highly significant; values of $P > 0.05$ were regarded as non-significant. Results are expressed as mean ± standard deviation (SD), with standard error (SE) reported where appropriate.

3. Results

3.1 Characteristics of respondents

A total of 68 volunteers participated in the study, equally divided between urban (n = 34, 50%) and rural (n = 34, 50%) areas (Table 1). The cohort comprised 35 males and 33 females distributed across two age strata, 20-34 years (n = 36) and 35-50 years (n = 32). The age- and gender-wise distribution of respondents is shown in Figure 1. Anthropometric assessment indicated that the majority of participants fell within the normal BMI range, with smaller proportions classified as overweight or underweight; consistent with previous reports, obesity was associated with a tendency toward lower vitamin D status (Brock et al., 2010).

Table 1. *Distribution of respondents according to area of residence.*

Area	Frequency	Percent	Valid Percent	Cumulative Percent
Rural	34	50.0	50.0	50.0
Urban	34	50.0	50.0	100.0
Total	**68**	**100.0**	**100.0**	

Figure 1. *Distribution of respondents by age group (20-34 and 35-50 years) and gender.*

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3.2 Serum calcium status

Mean serum calcium concentrations were comparable between the two age groups, at 8.59 ± 1.58 mg/dL in respondents aged 20-34 years and 8.54 ± 1.77 mg/dL in those aged 35-50 years, a difference that was not statistically significant (P = 0.9018; Table 5). When stratified by gender, males showed slightly higher mean calcium (8.75 ± 1.52 mg/dL) than females (8.36 ± 1.79 mg/dL), although the difference did not reach significance (P = 0.3413; Table 4). The distribution of mean calcium across age and gender groups is presented in Figure 2. Overall, calcium values clustered toward the lower end of the reference interval, consistent with the widespread vitamin D deficiency observed in this population (Grant, 2006).

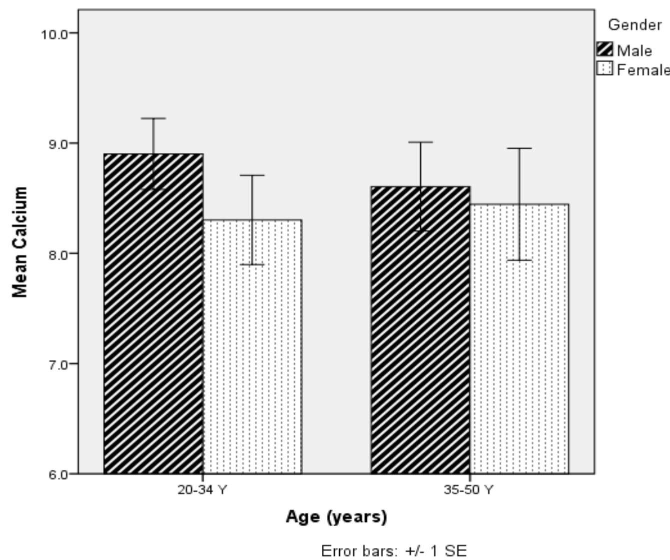

Figure 2. *Mean serum calcium (mg/dL) for males and females within the 20-34 and 35-50-year age groups.*

3.3 Serum vitamin D status

Serum 25-hydroxyvitamin D concentrations were markedly low across the entire cohort. Mean vitamin D was 8.85 ± 2.96 ng/mL in the 20-34-year group and 9.44 ± 4.67 ng/mL in the 35-50-year group, with no significant difference between the age strata (P = 0.5275; Table 5). Because the accepted threshold for deficiency is below 20 ng/mL, essentially all participants were vitamin D deficient, mirroring the 80-90% deficiency rates previously reported for the region (Akhtar, 2016; Nimitphong and Holick, 2013).

A clear gender difference emerged: males had significantly higher mean vitamin D (10.03 ± 4.69 ng/mL) than females (8.17 ± 2.39 ng/mL; P = 0.0451; Table 4 and Figure 3). This finding is consistent with reports that concealing dress and reduced effective sun exposure place women at heightened risk of deficiency (Mishal, 2001; Al-Harib and Singh, 2013). Within the male subgroup, vitamin D tended to be higher in the older age band (10.78 ± 5.70 ng/mL) than the younger band (9.23 ± 3.31 ng/mL), whereas the reverse was seen in females, although neither age-related difference was statistically significant (Tables 2 and 3).

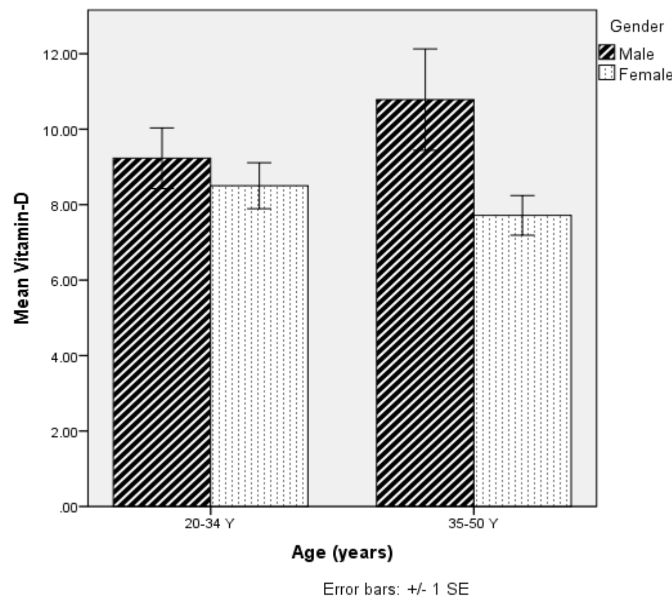

Figure 3. *Mean serum vitamin D (ng/mL) for males and females within the 20-34 and 35-50-year age groups.*

3.4 Complete blood count parameters

Mean haemoglobin was 11.92 ± 2.41 g/dL in the younger group and 13.01 ± 2.18 g/dL in the older group; the difference approached but did not reach significance (P = 0.0565; Table 5, Figure 4). Total leukocyte count was essentially unchanged between age groups (7613.58 ± 1694.71 vs. 7624.06 ± 1935.83 per microlitre; P = 0.9811; Figure 5), a finding in keeping with reduced leukocyte counts reported in some calcium- and vitamin D-deficient patients (Streiff et al., 2002).

Haematocrit (40.36 ± 7.04% vs. 41.10 ± 5.77%; P = 0.6377; Figure 6), mean corpuscular volume (87.68 ± 9.49 vs. 85.42 ± 9.62 fL; P = 0.3350; Figure 7), red blood cell count (4.43 ± 0.55 vs. 4.40 ± 0.61 million per microlitre; P = 0.8054; Figure 8), platelet count (286.19 ± 68.66 vs. 286.06 ± 81.31 thousand per microlitre; P = 0.9942; Figure 9) and MCHC (34.53 ± 1.69 vs. 33.95 ± 1.74 g/dL; P = 0.1727; Figure 10) all showed non-significant differences between the two age groups. Of the haematological indices, only mean corpuscular haemoglobin differed significantly, being higher in the older group (31.67 ± 3.96 pg) than the younger group (30.01 ± 2.57 pg; P = 0.0426; Figure 11). On gender-wise comparison of the overall data, haematocrit was significantly higher in males than females (42.28 ± 6.37% vs. 39.03 ± 6.17%; P = 0.0365; Table 4), consistent with established physiological differences (Laura et al., 2016).

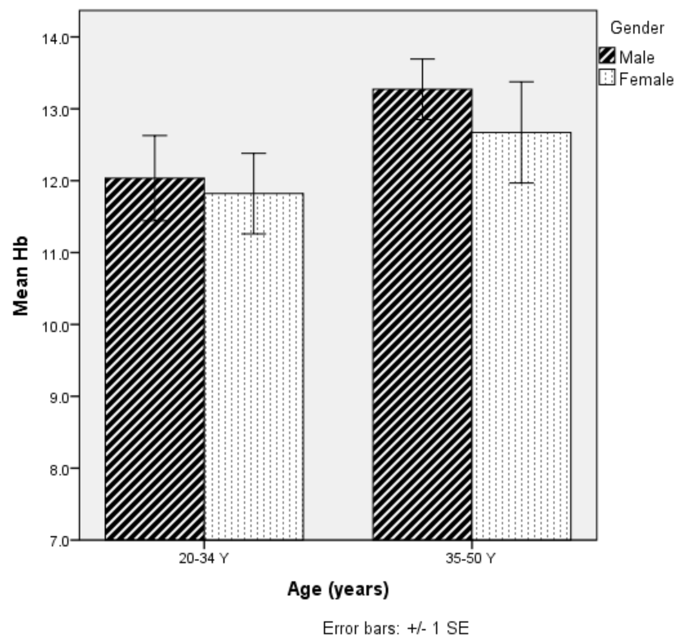

Figure 4. *Mean haemoglobin (Hb) for males and females within the 20-34 and 35-50-year age groups.*

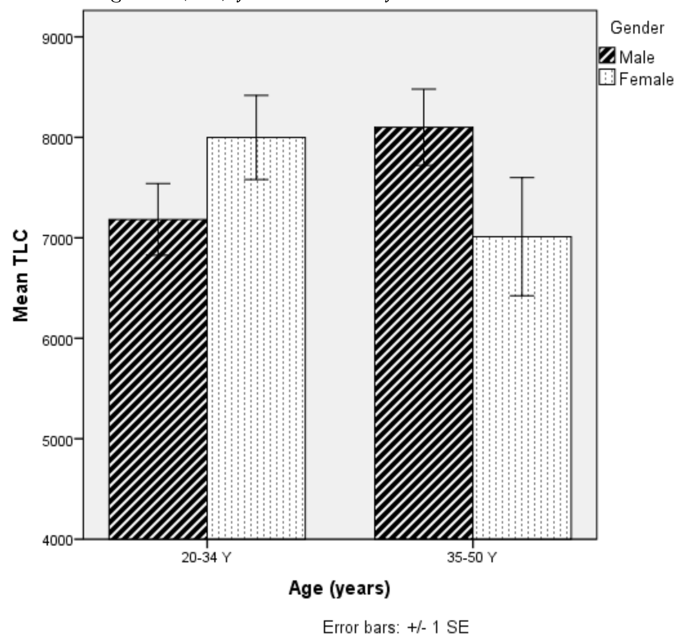

Figure 5. *Mean total leukocyte count (TLC) for males and females within the 20-34 and 35-50-year age groups.*

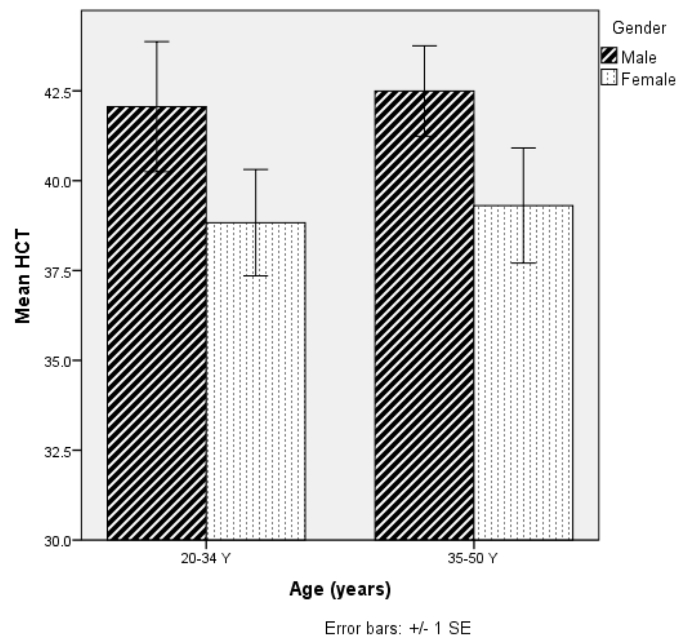

Figure 6. *Mean haematocrit (HCT) for males and females within the 20-34 and 35-50-year age groups.*

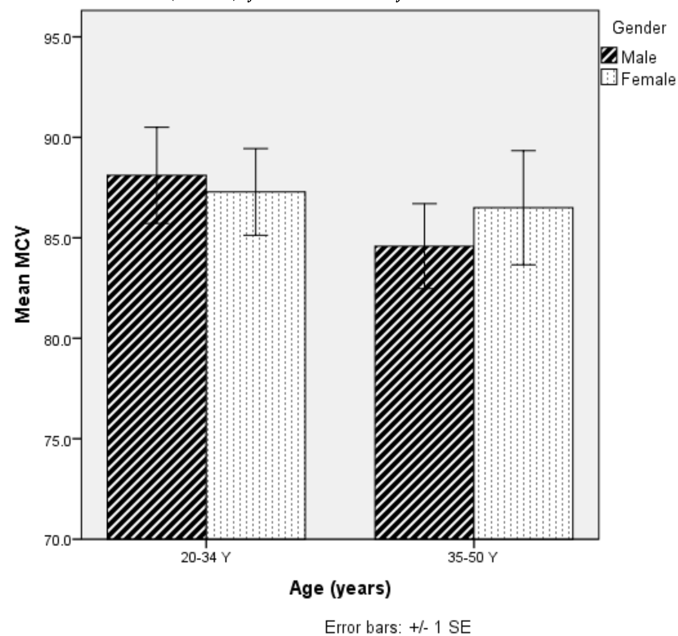

Figure 7. *Mean corpuscular volume (MCV) for males and females within the 20-34 and 35-50-year age groups.*

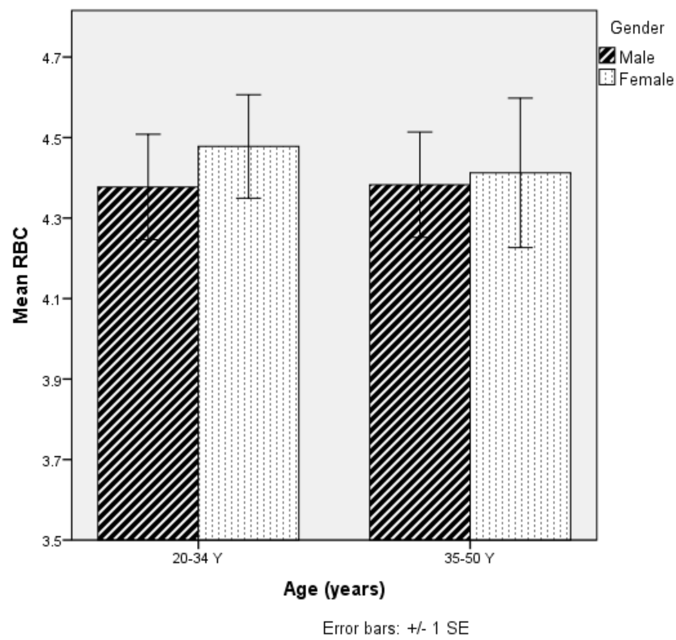

Figure 8. *Mean red blood cell count (RBC) for males and females within the 20-34 and 35-50-year age groups.*

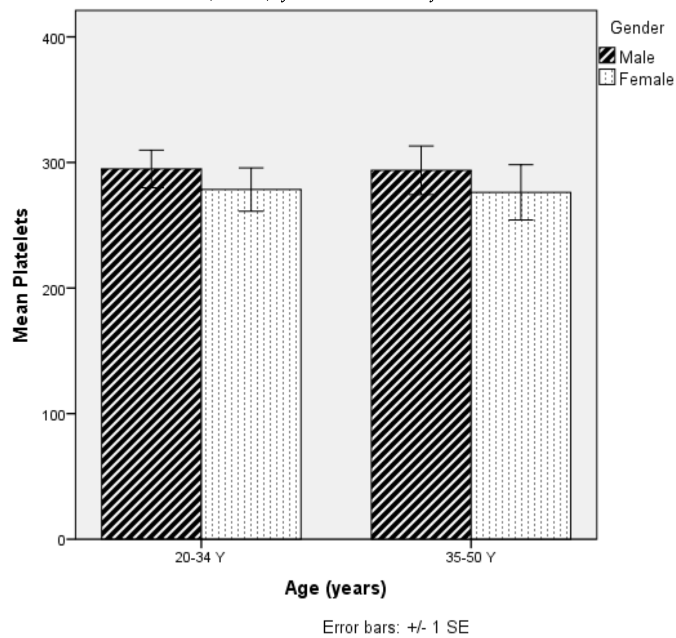

Figure 9. *Mean platelet count for males 20-34 and 35-50-year age groups. and females within the*

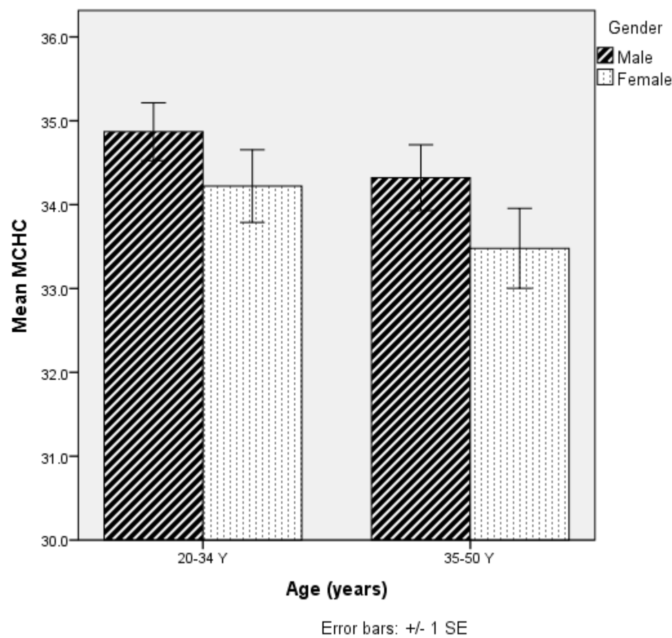

Figure 10. *Mean corpuscular haemoglobin concentration (MCHC) for males and females within the 20-34 and 35-50-year age groups.*

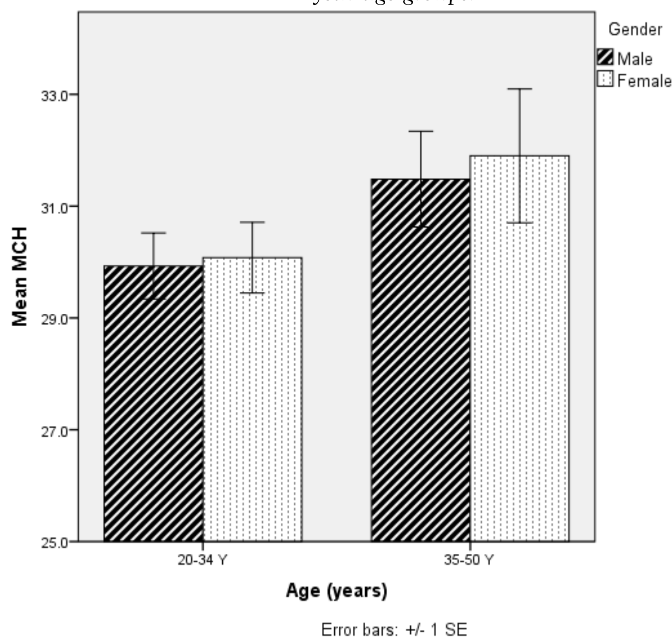

Figure 11. *Mean corpuscular haemoglobin (MCH) for males and females within the 20-34 and 35-50-year age groups.*

Parameter	Age	N	Mean	SD	SE	t-value	P-value
alcium	20-34 Y	17	8.9	1.33	0.32	0.57	0.5724
	35-50 Y	18	8.61	1.71	0.4		
itamin D	20-34 Y	17	9.23	3.31	0.8	-0.98	0.3352
	35-50 Y	18	10.78	5.7	1.34		

Parameter	Age	N	Mean	SD	SE	t-value	P-value
b	20-34 Y	17	12.04	2.44	0.59	-1.72	0.0954
	35-50 Y	18	13.27	1.79	0.42		
LC	20-34 Y	17	7182.94	1471.91	356.99	-1.76	0.0881
	35-50 Y	18	8100.56	1608.6	379.15		
CT	20-34 Y	17	42.06	7.47	1.81	-0.2	0.8432
	35-50 Y	18	42.49	5.34	1.26		
CV	20-34 Y	17	88.11	9.85	2.39	1.11	0.2756
	35-50 Y	18	84.58	8.98	2.12		
BC	20-34 Y	17	4.38	0.54	0.13	-0.03	0.9756
	35-50 Y	18	4.38	0.56	0.13		
atelets	20-34 Y	17	294.88	61.62	14.95	0.05	0.9628
	35-50 Y	18	293.72	82.15	19.36		
CHC	20-34 Y	17	34.87	1.42	0.34	1.05	0.3036
	35-50 Y	18	34.32	1.67	0.39		
CH	20-34 Y	17	29.93	2.43	0.59	-1.48	0.1492
	35-50 Y	18	31.48	3.63	0.86		

Table 2. *Comparison of blood characteristics between the two age groups (20-34 and 35-50 years) among male respondents.*

*NS = Non-significant (P>0.05); * = Significant (P<0.05); ** = Highly significant (P<0.01). SD = standard deviation; SE = standard error*

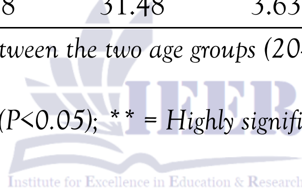

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Table 3. *Comparison of blood characteristics between the two age groups (20-34 and 35-50 years) among female respondents.*

Parameter	Age	N	Mean	SD	SE	t-value	P-value
alcium	20-34 Y	19	8.3	1.76	0.4	-0.22	0.8259
	35-50 Y	14	8.45	1.9	0.51		
itamin D	20-34 Y	19	8.5	2.66	0.61	0.93	0.3577
	35-50 Y	14	7.72	1.96	0.52		
b	20-34 Y	19	11.82	2.44	0.56	-0.96	0.3462
	35-50 Y	14	12.67	2.64	0.7		
LC	20-34 Y	19	7998.89	1823.97	418.45	1.41	0.1688
	35-50 Y	14	7011.43	2198.61	587.6		
CT	20-34 Y	19	38.83	6.46	1.48	-0.22	0.8308
	35-50 Y	14	39.31	5.99	1.6		
CV	20-34 Y	19	87.28	9.41	2.16	0.22	0.8242
	35-50 Y	14	86.5	10.63	2.84		

Parameter	Age	N	Mean	SD	SE	t-value	P-value
BC	20-34 Y	19	4.48	0.56	0.13	0.3	0.7655
	35-50 Y	14	4.41	0.69	0.19		
atelets	20-34 Y	19	278.42	75.2	17.25	0.08	0.9367
	35-50 Y	14	276.21	82.19	21.97		
CHC	20-34 Y	19	34.22	1.89	0.43	1.14	0.2619
	35-50 Y	14	33.48	1.78	0.48		
CH	20-34 Y	19	30.08	2.75	0.63	-1.44	0.1587
	35-50 Y	14	31.9	4.48	1.2		

*NS = Non-significant (P>0.05); * = Significant (P<0.05); ** = Highly significant (P<0.01). SD = standard deviation; SE = standard error.*

Table 4. *Gender-wise comparison (male vs. female) of blood characteristics for the overall data.*

Parameter	Gender	N	Mean	SD	SE	t-value	P-value
alcium	Male	35	8.75	1.52	0.26	0.96	0.3413
	Female	33	8.36	1.79	0.31		
tamin D	Male	35	10.03	4.69	0.79	2.04	**0.0451***
	Female	33	8.17	2.39	0.42		
b	Male	35	12.67	2.19	0.37	0.86	0.3948
	Female	33	12.18	2.52	0.44		
LC	Male	35	7654.86	1590.55	268.85	0.17	0.8652
	Female	33	7579.97	2020.08	351.65		
CT	Male	35	42.28	6.37	1.08	2.13	**0.0365***
	Female	33	39.03	6.17	1.07		
CV	Male	35	86.3	9.44	1.6	-0.28	0.7799
	Female	33	86.95	9.79	1.7		
BC	Male	35	4.38	0.54	0.09	-0.5	0.6180
	Female	33	4.45	0.61	0.11		
atelets	Male	35	294.29	71.84	12.14	0.93	0.3553
	Female	33	277.48	76.99	13.4		
CHC	Male	35	34.59	1.55	0.26	1.65	0.1039
	Female	33	33.91	1.85	0.32		
CH	Male	35	30.73	3.16	0.53	-0.15	0.8821
	Female	33	30.85	3.64	0.63		

*NS = Non-significant (P>0.05); * = Significant (P<0.05); ** = Highly significant (P<0.01). SD = standard deviation; SE = standard error.*

Table 5. *Comparison of blood characteristics between the two age groups (20-34 and 35-50 years) for the overall data.*

Parameter	Age	N	Mean	SD	SE	t-value	P-value
ılcium	20-34 Y	36	8.59	1.58	0.26	0.12	0.9018
	35-50 Y	32	8.54	1.77	0.31		
ıtamin D	20-34 Y	36	8.85	2.96	0.49	-0.64	0.5275
	35-50 Y	32	9.44	4.67	0.83		
b	20-34 Y	36	11.92	2.41	0.4	-1.94	0.0565
	35-50 Y	32	13.01	2.18	0.39		
LC	20-34 Y	36	7613.58	1694.71	282.45	-0.02	0.9811
	35-50 Y	32	7624.06	1935.83	342.21		
CT	20-34 Y	36	40.36	7.04	1.17	-0.47	0.6377
	35-50 Y	32	41.1	5.77	1.02		
CV	20-34 Y	36	87.68	9.49	1.58	0.97	0.3350
	35-50 Y	32	85.42	9.62	1.7		
BC	20-34 Y	36	4.43	0.55	0.09	0.25	0.8054
	35-50 Y	32	4.4	0.61	0.11		
atelets	20-34 Y	36	286.19	68.66	11.44	0.01	0.9942
	35-50 Y	32	286.06	81.31	14.37		
CHC	20-34 Y	36	34.53	1.69	0.28	1.38	0.1727
	35-50 Y	32	33.95	1.74	0.31		
CH	20-34 Y	36	30.01	2.57	0.43	-2.07	**0.0426***
	35-50 Y	32	31.67	3.96	0.7		

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*NS = Non-significant (P>0.05); * = Significant (P<0.05); ** = Highly significant (P<0.01). SD = standard deviation; SE = standard error*

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3.5 Correlation analysis

Pearson correlation analysis of the overall data revealed a highly significant positive correlation between serum calcium and vitamin D (r = 0.442; P < 0.01; Table 6), reinforcing the physiological interdependence of these two parameters (Meunier and Vieth, 2005). Haematocrit

	Age	Ca	VitD	Hb	TLC	HCT	MCV	RBC	Plt	MCHC	MCH
Age	1.000										
Ca	0.100	1.000									
VitD	0.163	**0.442****	1.000								
Hb	0.156	0.030	0.192	1.000							
TLC	-0.038	-0.192	-0.100	0.179	1.000						
HCT	0.044	-0.097	-0.008	0.112	-0.040	1.000					
MCV	-0.156	-0.096	-0.112	-0.072	-0.055	**0.343****	1.000				
RBC	-0.045	-0.024	0.195	0.155	0.103	0.016	**0.286***	1.000			
Plt	-0.127	-0.028	-0.153	0.143	0.148	**0.244***	0.157	-0.033	1.000		
MCHC	-0.036	0.071	0.102	-0.046	0.151	0.007	0.038	-0.044	0.190	1.000	
MCH	0.223	0.031	-0.015	**0.275***	-0.200	**0.302***	0.191	0.055	-0.113	-0.177	1.000

was positively correlated with mean corpuscular volume (r = 0.343; P < 0.01) and with platelet count (r = 0.244; P < 0.05), while MCV showed a significant negative correlation with RBC count (r = -0.286; P < 0.05). MCH correlated positively with haemoglobin (r = 0.275; P < 0.05) and haematocrit (r = 0.302; P < 0.05).

In the female subgroup, the calcium-vitamin D correlation was particularly strong (r = 0.725; P < 0.01) and HCT correlated significantly with MCV (r = 0.505; P < 0.01; Table 6 footnote applies). Among younger respondents (20-34 years), vitamin D was significantly associated with both calcium (r = 0.480; P < 0.01) and RBC count (r = 0.376; P < 0.05), suggesting that vitamin D status may influence erythropoietic indices in this population (Raje, 2013).

Table 6. *Pearson correlation matrix for the overall data (Pearson's r; significance markers shown).*
*Values are Pearson's correlation coefficients (r). * = significant (P<0.05); ** = highly significant (P<0.01). Ca = calcium; VitD = vitamin D; Plt = platelets.*

4. Discussion

The present study documents a strikingly high prevalence of vitamin D deficiency among both urban and rural residents of Faisalabad, with mean serum 25(OH)D concentrations of roughly 9 ng/mL, far below the 20 ng/mL threshold that defines deficiency (Holick, 2007; Rosen, 2012). This is consistent with the broader South Asian picture, where 65-70% of the population is reported to be deficient, and with Pakistan-specific data indicating deficiency in as many as 85% of certain groups (Akhtar et al., 2016; Akhtar, 2016). The persistence of such widespread deficiency despite Faisalabad's latitude and abundant sunshine underlines that ultraviolet availability alone does not guarantee adequate cutaneous synthesis.

The most consistent demographic signal in our data was the significantly lower vitamin D status of women compared with men (8.17 vs. 10.03 ng/mL; P = 0.0451). This gender gap is well described in Muslim-majority and South Asian settings and is generally attributed to concealing dress, predominantly indoor activity and limited skin exposure to sunlight (Mishal, 2001; Al-Harib and Singh, 2013; Alsuwaida et al., 2013). The exceptionally strong calcium-vitamin D correlation observed in the female subgroup (r = 0.725) suggests that women in this population may be particularly vulnerable to the coupled deficiency of both micronutrients, a combination that heightens the risk of osteomalacia and osteoporosis (Lamont and Jorgensen, 2012).

Calcium concentrations clustered at the lower end of the reference range across all groups, in keeping with the dependence of intestinal calcium absorption on adequate vitamin D (Meunier and Vieth, 2005; Grant, 2006). The highly significant positive correlation between calcium and vitamin D in the overall data ($P < 0.01$) provides direct biochemical confirmation of this interdependence in the studied cohort. Dietary factors typical of plant-based South Asian diets, including high phytate and fibre intake, can further impair calcium bioavailability and deplete vitamin D stores (Babu and Calvo, 2010).

The complete blood count indices were largely within physiological limits and showed few significant differences between age groups. The borderline elevation of haemoglobin and the significant increase in mean corpuscular haemoglobin with age, together with the vitamin D-RBC association seen in younger participants, are consistent with emerging evidence that vitamin D influences erythropoiesis and bone-marrow function (Raje, 2013). The significantly higher haematocrit in males reflects expected sex-related haematological variation rather than a disease effect (Laura et al., 2016). The non-significant reduction in total leukocyte count among deficient individuals aligns with reports linking low vitamin D to altered immune-cell profiles (Streiff et al., 2002).

Several determinants likely converge to produce the observed deficiency. Cutaneous synthesis is constrained by cultural dress, indoor lifestyles, skin pigmentation and, in urban areas, air pollution that attenuates ultraviolet-B penetration (Holick, 2016; Babu and Calvo, 2010). Dietary intake of vitamin D-rich or fortified foods remains low, and obesity, where present, sequesters the vitamin in adipose tissue and lowers circulating concentrations (Brock et al., 2010; Atabek et al., 2006). The comparable deficiency observed in both urban and rural groups suggests that these influences operate across the rural-urban divide, albeit through partly different pathways, dietary inadequacy and indoor occupation in rural settings versus pollution and concealment in urban ones (Adams and Hewison, 2010; Absoud et al., 2011).

The principal limitations of this study are its modest sample size and cross-sectional design, which preclude causal inference, and the absence of direct measurement of dietary calcium intake and seasonal variation in sun exposure. Assay-related variability in 25(OH)D measurement and the influence of vitamin D-binding protein should also be acknowledged (Heijboer, 2013). Nonetheless, the consistency of the findings with regional literature, and the clear biochemical correlations recovered, support their validity. Larger longitudinal studies incorporating parathyroid hormone, dietary recall and seasonal sampling are warranted to define the determinants of deficiency more precisely and to guide intervention (Ginde et al., 2009; Visser et al., 2006).

5. Conclusion

Vitamin D and calcium deficiencies are highly prevalent among both urban and rural residents of Faisalabad, Pakistan, with women bearing a disproportionate burden. The strong positive correlation between serum calcium and vitamin D confirms their physiological coupling, while the largely preserved complete blood count indices indicate that the deficiency had not yet produced overt haematological derangement in this cohort. Despite year-round sunshine, cultural practices, limited effective sun exposure, dietary inadequacy and obesity appear to sustain this deficiency. Routine screening of at-risk groups, food fortification, sensible sun-exposure guidance and targeted supplementation are urgently needed to address what is clearly a major, and largely preventable, public-health problem in this region.

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