



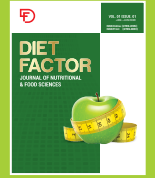
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Original Article



Nutritional and Vitamin D Status of University Students in Khyber Pakhtunkhwa, Pakistan: A Cross-Sectional Study

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ABSTRACT

Data on Vitamin D level and nutritional status of Pakistani university students are limited. **Objective:** To find dietary habits of the university students with special focus on vitamin D levels. **Methods:** This descriptive cross-sectional study included a total of 400 students (272/128 male/female) 18-26 years old students from Bacha Khan University Charsadda were included in this descriptive cross-sectional study. Socio-demographic, selected anthropometrics, dietary food intake, blood chemistry (lipid profile and CRP), and vitamin D status were measured. **Results:** Moderate energy deficiencies, particularly males (73% of RDA), and protein was meeting requirements in females but marginally short in males. Calcium (65% RDA), vitamin A (41% female, 30% male), and vitamin D (20% RDA) intake were significantly low. Iron intake was marginal in females (60%) but over met in males (135%). Low milk/dairy, egg, fish, and fruit/vegetable consumption reflected low micronutrient variety. Regular consumption of fast foods (3.4 times/week) and limited consumption of fish (0.5 servings/week) also indicated a shift toward nutrient-scarce dietary habits. Mean serum 25(OH)D level of 26.1±12.6 ng/mL with a high number (32% ;95% CI: 13.5-27.3) of respondents were vitamin D deficient; 42% (95% CI: 35.3-52.8) were vitamin D insufficient; and only 26% (95% CI: 20.1-32.2) of the participants had normal vitamin D. In addition, high levels of inflammatory and lipid markers were observed. **Conclusion:** In summary, university students exhibited poor vitamin D status with high levels of CRP and blood lipid profile such that interventions are warranted.

INTRODUCTION

University students are a unique and pivotal population, experiencing a life phase of increasing autonomy, academic pressure, and formation of lifelong behaviors [1-3]. This phase, typically synonymous with late adolescence and young adulthood, is marked by dramatic changes in habits of daily life, such as changes in food habits, sleep habits, exercise habits, and psychosocial activities. These changes in lifestyle have the ability to impact highly the level of nutrition among students and expose them to numerous short- and long-term health issues [2-5]. The campus life tends to encourage eating habits of poor quality, greater dependency on fast or convenience foods, and lower intake of highly nutritious meals with good balance [3]. At the same time, sleep deprivation, school

stress of high intensity, and physical inactivity usually enabled by prolonged screen time and absence of organized physical activity contribute to nutritional imbalance and negative health consequences [4-9]. A high body of evidence shows that these elements of lifestyle are associated with higher prevalence of micronutrient inadequacies, overweight, and obesity, dyslipidemia, and inflammation among university students, thus at higher risk of having Non-Communicable Diseases (NCDs) in the long run [1, 9]. On this basis, it is crucial to determine the nutritional and biochemical status among university students. These results not only inform on the current health status of this group, but also inform the development of timely interventions for promoting



healthier behaviors. The main objective of the present study was to evaluate vitamin D status of the university students in Khyber Pakhtunkhwa, Pakistan. Particular emphasis is placed on gender-specific variation in body weight, body mass index (BMI), and significant biochemical parameters such as serum 25-hydroxyvitamin D [25(OH)D], lipid profiles total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglycerides and high-sensitivity C-reactive protein (hs-CRP) as a marker of inflammation. The findings of this research are anticipated to identify common nutritional deficits and biochemical disturbances among university students, putting into perspective early markers of cardiometabolic risk.

Such information may be used as a platform for evidence-based health promotion strategies and campus wellness programs to optimize the overall health and academic achievement of students.

METHODS

A descriptive cross-sectional study was conducted in Bacha Khan University Charsadda in the year 2021. Population was the students of Bacha Khan University. Cross-sectional design was employed since it provides data collection and analysis at a single time-point in time and hence suits to find out prevalence, pattern, or association within a defined population [10]. Students of university were included at 18-26 years age, both male and female and without chronic illnesses. Sample Size was calculated by Cochran's formula as discussed in the following: $n = N / (1 + Ne^2)$ Where, n = Number of sample; N = Whole population and e = Error tolerance (level). Simple random sampling process was utilized. Complete list of enrolled students was obtained from director admission office. The sample as defined was selected at random from the list by using a random number generator. For anthropometrics, height and weight of the participants were recorded [11, 12]. Weight was measured on a calibrated balance with minimal clothing on (Model: 708; Seca, Hamburg, DE). Height was measured with a calibrated stadiometer (Model: 206; Seca, Hamburg, DE). Measurements and testing were carried out by highly trained dietitians at University clinics. BMI was calculated from the formula: $\text{weight (Kg)} / \text{height (m}^2\text{)}$. To analyze the body composition, the participants were requested to go to HND lab at BKUC. Body composition was examined on Bioelectric Impedance Analysis (BIA) following standard operating procedures designed by body composition core-lab. A 10 ml blood sample was obtained and analyzed for the following biochemical parameters: 25-OH vitamin D, C-reactive protein (CRP), blood lipid profile etc. As per standard protocol as supplied by the manufacturer. In short, CRP was measured on immunoturbidimetric (Tina-

quant CRP detection method; Roche Diagnostics) on a Hitachi 717 analyzer with between-assay CV was 2.6% at 4.65 mg/L CRP. Serum 25-OH vitamin D was estimated by ELISA kits (MY Bio-Source, CATLOG # MBS2601819). 25-OH Vitamin D in all the samples was read at the same time with standards and the concentrations were calculated from external standards. Serum Triglycerides (TG), Total Cholesterol (TC), HDL, LDL was determined by automated chemistry analyzer (HumaStar 600- Germany). Cholesterol/HDL and LDL/HDL ratios were calculated by dividing total cholesterol and LDL respectively by HDL. Serum concentration of 25-OH D was measured by taking blood samples. A single blood sample from each subject was taken and examined in Thumbay hospital, Ajman-UAE laboratories. Outcomes were classified into four categories according to vitamin D concentration of 25-hydroxyvitamin: deficiency (<10 ng/dl), insufficiency (10-29 ng/dl), sufficiency (30-100 ng/dl), and likely intoxication (>150 ng/dl). The dietary recall data was analyzed by utilizing valid food composition software. Descriptive statistics were calculated for all variables: Means \pm Standard Deviation (SD) for quantitative variables and frequencies and percentages for categorical variables. Normality was tested with the Shapiro-Wilk test and visual means (histograms, Q-Q plots). Variables satisfied parametric assumptions were tested with parametric tests; otherwise, their non-parametric equivalents were used. Two means comparisons (e.g. male vs female) were performed by student-t-test and a single mean comparison (e.g. mean nutrient intake vs RDA of that nutrient) was performed using one-sample t-test. The participants were grouped according to their hs-CRP, TC, LDL-C and HDL-C levels. Taking into account the hs-CRP (mg/L) levels, the participants were grouped in any of the three groups as follow: Low Risk (< 1.0); Moderate Risk (1.0 - 3.0); High Risk (>3.0); Based on their TC (mg/dL) levels, the participants were grouped in any of these groups; Optimal (<200); Border line high (200 - 239); High (\geq 240). Based on their HDL-C (mg/dL) data, the participants were categorized as following: No Risk (\geq 40 mg/dL); Moderate Risk (35-39 mg/dL); High Risk (<35 mg/dL). Similarly, based on their LDL-C (mg/dL) level, these cutoffs were considered for grouping the participants accordingly into any of the five groups: Optimal: <100; Near Optimum: 100-129; Borderline High: 130-159; High: 160-189; Very High: \geq 190. These cutoffs were taken from well-established clinical guidelines, such as those from the National Cholesterol Education Program (NCEP ATP III) and American Heart Association (AHA) as reported elsewhere [13-17]. They were used to categorize individuals' cardiovascular (TC, LDL-C; HDL-C) and inflammation (hs-CRP) risks. These categories based on the LDL-C levels represent increasing risk for cardiovascular disease, especially when combined

with high hs-CRP, low HDL-C, or elevated TC. Chi-Square Goodness-of-Fit Test was used to test whether the observed frequency distribution for a single categorical variable (e.g., Vitamin D levels or LDL-C categories) significantly differs from an expected distribution (such as a normal or theoretical distribution). Statistical significance was presumed for $P < 0.05$. All the analyses were performed on SPSS Version 20.0.

RESULTS

In the present study, most of the participating students were male (68%, $N = 272$). More than half of the students come from rural areas (64%, $N = 256$). The mean age of the students participating in the study was $M = 21.21 \pm 4.56$. Detailed data are presented in table 1.

Table 1: Mean Anthropometrics and Nutritional Status by Gender

Variables	Male Mean \pm SD	Female Mean \pm SD	Overall Mean \pm SD
Weight (kg)	$M = 75.63 \pm 13.64$	$M = 56.55 \pm 7.66$	$M = 66.2 \pm 7.31$
BMI *	$M = 24.43 \pm 3.44$	$M = 20.11 \pm 3.33$	$M = 22.24 \pm 2.4$
Underweight (n = 40)	10% (N = 27)	10% (N = 13)	10% (N=40)
Normal weight (n = 244)	60% (N = 163)	62% (N = 79)	61% (N=244)
Overweight (n=116)	30% (N = 82)	28% (N = 36)	29% (N=116)

Data were presented as mean \pm SD or n (%). * According to Diagnostic and Statistical Manual of Mental Disorders (DSM)-V, a body mass index less than or equal to 17.5 kg/m² is considered as a diagnostic criterion for anorexia nervosa.

Dietary and Nutrients Intake

Table 2 showed the dietary intake of the students. The daily energy intake was 1834 ± 340 kcal, covering about 81% of the RDA for female and 73% of that for male University students, indicating a general state of moderate caloric deficit, particularly in male. Protein consumption was 52 ± 11 grams/day, covering 103% of the RDA for female and 83% for male students, indicating that protein requirements were sufficiently met in female students but that men might be at marginal risk of insufficient supply. Calcium consumption was significantly lower than recommended, with a mean consumption of 650 ± 180 mg/day, amounting to only 65% of the RDA, creating potential concerns about bone status and vitamin D metabolism. Iron consumption averaged 10.8 ± 3.2 mg/day, being equivalent to 60% of the female requirement but more than 135% in men, indicating gender differences in iron needs and potential dietary choices. The intake of Vitamin A was somewhat low (525 ± 110 μ g RAE/day), covering only 41% of the RDA for female students and 30% for male. The intake of Vitamin D was particularly low, averaging 122 ± 60 IU/day, representing merely 20% of the suggested 600 IU/day, reflecting a very important deficiency matching general national concerns about hypovitaminosis D. In terms of food group intakes, milk and dairy intakes were 0.8 ± 0.5 servings/day, reaching

only 41% of the minimum required intake, and egg consumption was 2.4 ± 1.7 per week, or 49% of the recommended intake. Fish intakes were very low (0.5 ± 0.8 servings/week), reaching only 21% of the recommendation, which could contribute to both low vitamin D and omega-3 fatty acid intakes. Individuals ate 2.3 ± 1.3 servings daily of fruits and vegetables, just 40% of the five recommended servings, reflecting inadequate micronutrient and fiber intake. Fast food, on the other hand, was eaten on a regular basis, with a mean of 3.4 ± 1.0 times weekly, reflecting a predisposition to convenience-dominant, nutrient-depleted diets. Sugar-sweetened beverage intake was 178 ± 78 ml/day, which fell within recommended parameters for the majority of the participants, although the cumulative effect on nutrient displacement deserves attention.

Table 2: Dietary and Nutrients Intake

Food/Nutrient	Mean \pm SD	Percentage of RDA Met*	p-Value
Energy (kcal)	1834 ± 340	~81% (F), ~73% (M)	0.358
Protein (g)	52 ± 11	~103% (F), ~83% (M)	0.344
Calcium (mg)	650 ± 180	65%	0.03
Iron (mg)	10.8 ± 3.2	60% (F), 65% (M)	0.02; 0.02
Vitamin A (μ g RAE)	525 ± 110	41% (F), 30% (M)	0.003; 0.003
Vitamin D (IU)	122 ± 60	20%	0.002
Milk and Dairy (servings/day)	0.8 ± 0.5	41%	0.003
Eggs (number/week)	2.4 ± 1.7	49%	0.004
Fish (servings/week)	0.5 ± 0.8	21%	0.0003
Fruits and Vegetables (servings/day)	2.3 ± 1.3	40%	0.003
Fast Food (times/week)	3.4 ± 1.0	—	—*
Sugar-Sweetened Beverages (ml/day)	178 ± 78	Within limit	—*

*there is no data on RDA available; $P < 0.05$; All analysis were done using one-sample-t-test by comparing the mean of nutrient with its RDA; RDA values are based on WHO/FAO and NIH dietary guidelines for 18-25-year-olds: F=Female Students; M=Male Students

Biochemical Status

The biochemical status of the students is shown in Table 3. Male and female students had the same vitamin D level ($p > 0.05$). However, significant differences were observed in the concentration of other biochemicals between male and female students. The mean serum 25(OH)D level of the participants was 26.1 ± 12.6 ng/mL showing an overall insufficiency of vitamin D nutrition.

Table 3: Biochemical Status (n=50)

Variables	Male Mean \pm SD	Female Mean \pm SD	Total Mean \pm SD	p-Value
Serum 25(OH)D ng/mL	26.5 ± 12.3	25.7 ± 13.1	27.1 ± 12.6	0.156
CRP (mg/dL)	7.4 ± 1.55	6.51 ± 2.11	6.62 ± 2.66	0.001
TC (mL / dL) M \pm DE	178.8 ± 54.3	197.7 ± 46.3	191.3 ± 50.3	0.002
HDL-C (mg / dL) M \pm DE	47.6 ± 21.7	47.2 ± 22.31	47.4 ± 21.7	0.004
LDL-C (mg / dL) M \pm DE	141.4 ± 13.1	141.3 ± 13.4	141.4 ± 13.3	0.034
Triglycerides (mg / dL) M \pm DE	132.7 ± 69.8	149.4 ± 61.8	140.1 ± 66.1	0.000

Distribution of Serum 25(OH) D Levels among Participants Students

The distribution of different serum 25(OH) D levels among the participants is presented in Table 4. A high number (32.%; 95% CI: 13.5–27.3) of respondents were vitamin D deficient; 42% (95% CI: 35.3–52.8) were vitamin D insufficient; and only 26% (95% CI: 20.1 – 32.2) of the participant had normal vitamin D. The χ^2 (Chi-square) tests results show Significant results ($p < 0.05$) for HDL-C and LDL-C only, indicating that the observed frequencies in these categories deviate significantly from what would be expected by chance, reflecting a true underlying pattern (e.g., higher frequency in "high risk" LDL-C).

Table 4: Biomarkers Frequency (n=50)

Variables	Frequency (%)	95% CI*	χ^2 (Chi-square)	p-Value
Vitamin D deficiency (<10 ng/dl**)	16 (32)	13.5-27.3	1.96	0.375
Vitamin D insufficiency (10-29 ng/dl**)	21 (42)	35.3-52.8		
Normal (30-100 ng/dl**)	13 (26)	20.1-32.2		
hs-CRP (mg/L)				
Low Risk (<1.0)	12 (24)	19.2-28.9	3.04	0.219
Moderate Risk (1.0 – 3.0)	22 (44)	32.4 –52.3		
High Risk (>3.0)	16 (32)	28.4 –37.2		
TC (mg/dL)				
Optimal (<200)	13 (26)	20.–37.1	1.96	0.375
Border line high (200 – 239)	16 (32)	28.7-38.2		
High (≥ 240)	21 (42)	32.9-49.2		
HDL-C (mg/dL)				
No Risk (≥ 40)	10 (20)	15.9-27.3	7.0	0.03
Moderate Risk (35 – 39)	15 (30)	26.2-38.2		
High Risk (< 35)	25 (50)	41.1-56.3		
LDL-C (mg/dL)				
Optimal (< 100)	10 (20)	16.3-28.4	25.8	0.0003
Near Optimum (100 – 129)	2 (4)	2.1-7.5		
Border Line High (130 – 159)	10 (20)	15.4- 26.3		
High (160 – 189)	5 (10)	6.5-18.7		
Very high (≥ 190)	23 (46)	35.9- 50.1		

Ng/DL= Nanograms per deciliter; CI, confidence interval.

DISCUSSION

The findings reveal 32% of students were Vitamin D deficient, a health condition also evidenced by other UAE and Iranian research [13, 14]. Confidence interval (CI: 13.5–27.3%) indicates a substantial number of this group might experience severe vitamin D deficiency. Moreover, 42% had low levels, indicating extensive deficiency in vitamin D but not being deficient. This continues to represent health risk, especially to immune and bone health [15, 16]. Vitamin D status in the current study was in the normal category in only 26% of the students. This indicates the prevailing trend of insufficiency in the population under study. These data indicate that most

(74%) of the students had inadequate or deficient status of Vitamin D, indicating possible lack of sufficient exposure to sunlight, inappropriate dietary intake or both [16–18]. Elevated levels of hs-CRP, LDL and TC and decreased level of HDL are risk markers for several diseases [19–22]. Several regional studies have highlighted significant gaps in the nutritional knowledge and behaviors of university students in Khyber Pakhtunkhwa, Pakistan. Khan et al., reported that a large proportion of students in Peshawar had limited awareness regarding nutritional supplements, reflecting a broader deficiency in basic nutrition literacy among university populations [19]. Similarly, Haq et al., assessed knowledge, attitudes, and practices about vitamin D and found that many medical students in Swat lacked adequate understanding of its importance, sources, and deficiency consequences [20]. Zeb's study further explored eating attitudes and found that disordered eating behaviors were prevalent among university girls in Peshawar, closely linked to their nutritional status [21]. Moreover, Khan et al., evaluated the diet and physical activity habits of medical students and identified concerning patterns such as irregular meal timings, inadequate intake of nutrient-dense foods, and sedentary lifestyles, all of which contribute to poor nutritional status [22]. Lastly, Hussain et al., conducted a population-based preprint study and proposed region-specific reference ranges for vitamin D levels in Peshawar, stressing the need for localized standards to better interpret deficiency risks in this demographic [23]. The findings of the current study on hs-CRP are that 24% were with low risk, i.e., comparatively low systemic inflammation; 44% were with moderate risk and 32% with high risk, indicating the existence of underlying inflammation or chronic health stressors. More than half of the children (76%) had moderate to severe inflammation, which may have been triggered by infection, dietary imbalance, or psychosocial stress. Results for Total Cholesterol (TC) indicate that 26% were normal (<200 mg/dL); 32% were mildly elevated cholesterol (200–239 mg/dL) and 42% in high cholesterol levels (≥ 240 mg/dL). A staggering 74% of the children were borderline or high in total cholesterol, indicating nutrition imbalance, presumably high in saturated fats or processed foods. HDL-C 20% of the children had protective HDL levels (≥ 40 mg/dL), 30% were moderately at risk (35–39 mg/dL), and 50% were highly at cardiovascular risk with low HDL (less than 35 mg/dL). Poor HDL-C status was found in half of the children and is most likely a marker of increased cardiovascular risk as well as unhealthy lipid profiles due to inappropriate dietary intake and an inactive lifestyle. LDL-C 20% had normal (<100 mg/dL) LDL levels; 4% were in this category (100–129 mg/dL) and 20% were slightly high

(130–159 mg/dL) and 10% were high (160–189 mg/dL) and 46% had very high (>190 mg/dL) LDL levels. Alarmingly, almost half (46%) of them had very high LDL-C, indicating an excess risk for future cardiovascular complications. Along with other cholesterol derangement, this is a picture of dismal dyslipidemia [22, 23]. Findings of the present study indicate very high malnutrition and cardiometabolic status of Pakistani university students. Large concerns are 1) very high prevalence of deficiency/insufficiency of vitamin D; 2) elevated prevalence of inflammatory markers (hs-CRP); 3) elevated prevalence of dyslipidemia (low HDL and high LDL). The data underscore the necessity of introducing nutritional intervention, better meal planning, physical exercise, and potentially supplementation to correct such deficiency states and avoid long-term health effects [16].

CONCLUSIONS

The current research reports considerable nutritional and biochemical imbalances among Pakistani university students, and found that a high number were vitamin D deficient and/or had inadequate levels, suggesting that almost three-quarters (74%) of the participants were at risk based on less-than-ideal vitamin D status. This lack is of great concern to immune and bone health, and could be due to a lack of sunlight exposure, poor dietary consumption, or a combination of the two.

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Authors Contribution

Conceptualization: IA

Methodology: HUR, IA

Formal analysis: HUR, IA

Writing, review and editing: HUR, IA

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

All the authors declare no conflict of interest.

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