



Vitamin D status among Kashmiri tribal population: A cross-sectional community-based study

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Background & objectives: Vitamin D deficiency (VDD) is prevalent across all age groups in general population of India but studies among tribal populations are scanty. This study aimed to evaluate the prevalence of VDD in the indigenous tribal population of the Kashmir valley and examine associated risk factors.

Methods: In this cross-sectional investigation, a total of 1732 apparently healthy tribal participants (n=786 males and n=946 females) were sampled from five districts of Kashmir valley by using probability proportional to size method. Serum 25-hydroxy vitamin D (25(OH)D) levels were classified as per the Endocrine Society (ES) recommendations: deficiency (<20 ng/ml), insufficiency (20-30 ng/ml) and sufficiency (>30 ng/ml). The serum 25(OH)D levels were assessed in relation to various demographic characteristics such as age, sex, education, smoking, sun exposure, body mass index and physical activity.

Results: The mean age of the male participants was 43.79±18.47 yr with a mean body mass index (BMI) of 20.50±7.53 kg/m², while the mean age of female participants was 35.47±14.92 yr with mean BMI of 22.24±4.73 kg/m². As per the ES guidelines 1143 of 1732 (66%) subjects had VDD, 254 (14.71%) had insufficient and 334 (19.3%) had sufficient serum 25(OH)D levels. VDD was equally prevalent in male and female participants. Serum 25(OH)D levels correlated positively with serum calcium, phosphorous and negatively with serum alkaline phosphatase. Gender, sun exposure, altitude, physical activity and BMI did not seem to contribute significantly to VDD risk.

Interpretation & conclusions: VD deficiency is highly prevalent among Kashmiri tribals, although the magnitude seems to be lower as compared to the general population. These preliminary data are likely to pave way for further studies analyzing the impact of vitamin D supplementation with analysis of functional outcomes.

Key words 25 OHD - 25(OH)D – Bakarwal - Gujjar - Kashmiri - tribals - vitamin D deficiency

Vitamin D deficiency (VDD) is a common epidemic affecting around one billion people globally¹.

Despite being a tropical country with adequate day light and sunshine, 70-100 per cent of both rural and

urban Indian population is reported to have VDD². In India, VDD has been reported among school children, adults, young girls as well as pregnant women^{2,3}. Besides bone mineral homeostasis, VDD has been associated with a wide range of non-skeletal effects including predisposition to different morbidities⁴ such as hypertension, obesity, hypertriglyceridemia, auto-immune disorders like rheumatoid arthritis⁵, multiple sclerosis⁶, ovarian, breast, and colorectal cancer⁷ and infections like tuberculosis⁸.

Kashmir valley located 1574-5425 feet above sea level (latitude: 32°44'N, longitude: 74°54'E) with an average ultraviolet (UV) index of seven, requiring 15-20 min of sunlight exposure for adequate vitamin D synthesis. Despite this, VDD is highly prevalent in Kashmir, with reported prevalence of 83 per cent in healthy adults⁹, 88.6 per cent in adolescent girls¹⁰ and 74 per cent in pregnant women¹¹. Tribals constitute around 11.9 per cent of the total population of Jammu and Kashmir (J&K) and 1.43 per cent of the total tribal population of the country¹², and experience considerable disparity compared to general population in terms of health facilities, education and economic pursuits. Cultural and social taboos dictate their lifestyle patterns such as clothing, nutrition and physical activity that may limit sun exposure. The lack of any fortification of staple foods, awareness about importance of sunshine and increasing cost of supplements *etc.* further increases their risk of VDD among these tribals.

While there is abundant data from studies assessing vitamin D status across various parts of India², there is no comprehensive study to assess vitamin D among the tribals in general and those of J&K in particular. Therefore, the present study was undertaken to estimate vitamin D levels among tribal population of Kashmir valley and identify risk factors associated with it.

Material & Methods

This was a cross-sectional study conducted on the tribal population of Kashmir valley during August 2015 to June 2018, assessing the status of non-communicable diseases (NCDs). The study was carried out at Sher-I-Kashmir Institute of Medical Sciences, Kashmir, in collaboration with the All India Institute of Medical Sciences, New Delhi, India. The study protocol was approved by the respective Institutional Ethics Committees. The study was conducted as per Helsinki declaration 1975 and an informed consent was obtained from all the study

participants prior to enrollment. For children below 18 yr, the consent was taken and questionnaire was completed with their parents' assistance. Individuals who were mentally or physically challenged, pregnant women and those who refused to provide consent were excluded from the study.

Study design and participant selection: Of the 12 districts housing tribals (Gujjar and Bakarwal) in the valley of Kashmir, five (Anantnag, Pulwama, Ganderbal, Kupwara and Srinagar) were selected based on multi-stage cluster sampling with probability proportional to size by using random number table. From each district one tehsil, from each tehsil one block and from each block two villages with a cap of 1000 subjects per village were selected. The present study was a sub-study of a larger health survey of NCDs among the tribal population of Kashmir valley¹³. The villages were approached through local health services and village heads. Study protocol, information brochures, and pamphlets were translated in different languages (in English, Pahadi, Gojri and Urdu) for circulation in selected villages, in addition to verbal instructions delivered through village or religious heads or health workers.

Data collection and administration of study questionnaire: On the day of commencement of the study, the research team visited the selected villages along with the clinicians and participants were recruited through house-to-house survey after giving a talk about general health awareness and the purpose of the study. The modified WHO-STEPs questionnaire¹⁴ was used as a STEP-wise approach to collect data and measure NCD risk factors. Information were obtained on demographic profile socioeconomic condition, lifestyle issues, nutritional as well as health status of the population. Validation of the questionnaire was carried out by using a pretest in 100 subjects to assess its suitability and reproducibility. The questionnaire was administered through, face-to-face interview in local vernacular language by a limited number of trained researchers. Wherever applicable, pictures/show cards were used to enhance the comprehension of the questionnaire.

The anthropometric measurements (weight, body mass index (BMI), height, waist circumference) of the study participants were recorded. Standardized digital instruments used for the study with respective specifications were: digital body weight scale (range 0.1-200 kg), stadiometer and measuring tape

(Seca-213, Hamburg Germany). Measurement was recorded in triplicate and the final values were calculated as the average of the three readings. Similarly, blood pressure (BP) of the study participants was measured by a trained physician on the left arm and the average of three readings of systolic BP and diastolic BP was taken. The BP apparatus used for measuring the BP was calibrated according to the manufacturer's instructions and Cronbach's alpha of more than 0.8 was considered as acceptable inter- and intra-observer variations. In addition to brief general physical examination, features of rickets such as bowed legs, widening of wrists and large forehead were recorded.

Laboratory evaluation: After overnight fast, 10 ml venous blood sample was withdrawn from each participant for measurement of hemogram, calcium, phosphorus, glucose, lipids, liver and kidney function in addition to serum 25(OH)D. Blood samples were collected in a plain, ethylenediaminetetraacetic acid or fluoride containing vacutainer depending on the assay. Blood samples were centrifuged at the study site and aliquots were transported within 2 h in cold boxes to the storage sites. The samples for biochemical estimations were separated immediately for same day assays while those for hormonal and inflammatory marker estimations were stored at -80°C until the assay. Biochemical assays were conducted on autoanalyzer (Response 920, DIASYS Germany). Serum 25(OH)D levels were measured using fully automated high-throughput immunoassay system (ADVIA Centaur, Seimens Healthcare Diagnostics, Erlangen, Germany). The inter and intra-assay coefficients of variation were within the prescribed limits as per the manufacturer's protocol.

Sample size calculation: With reference from a previous study⁹ and taking an estimated VDD prevalence of 60, 70, 80 and 90 per cent in age groups 0-20, 21-40, 41-60 and >60 yr respectively and a design effect of 1.5, we arrived at a sample size of 1608 participants. Taking into account a non-response rate of five per cent, a total of 1732 participants were enrolled. In order to reach this sample size, every fifth individual of the total population was invited for vitamin D estimation. Participants were recruited in equal proportion among age groups 21-40, 41-60 and >60 yr. There was however a low response rate (74%) in the 0-20 yr age group.

Statistical analyses: Data were analyzed using SPSS software, version 22 (SPSS Inc., Chicago, IL, USA)

and are presented as percentage for nominal variables or mean \pm standard deviation (SD) for continuous variables. Normality assessment was examined using Shapiro-Wilk test and data were transformed wherever necessary. Serum 25(OH)D levels were classified as: deficiency (<20 ng/ml), insufficiency (20-30 ng/ml) and sufficiency (>30 ng/ml) as per Endocrine Society (ES) recommendations¹⁵. Baseline mean vitamin D levels were compared using one-way ANOVA. Pearson correlation was used to assess the relation between various demographic and environmental factors. Two-sided $P < 0.05$ was considered significant.

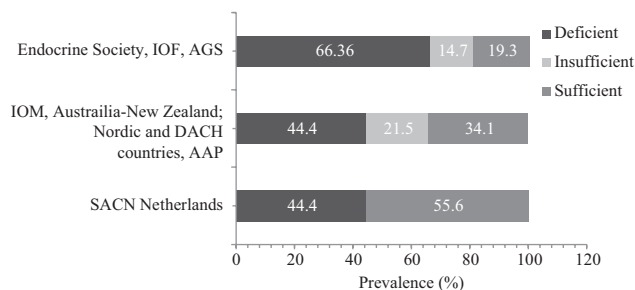
Results

The mean age of male participants was 43.79 ± 18.47 yr (minimum 10 - maximum 80 yr) with a mean BMI of 20.50 ± 7.53 kg/m² while the mean age of female participants was 35.47 ± 14.92 yr (minimum 10 - maximum 80 yr) with mean BMI of 22.24 ± 4.73 kg/m². Table I shows age-wise baseline clinical and biochemical parameters of the tribal study participants. The mean BMI as well as mean BP were significantly high in the higher age group among both genders. Around 12.42, 15.33, 31.25, 35.54, 46.22, 58.03 per cent subjects among age groups 0-20, 21-30, 31-40, 41-50, 51-60 and >60 yr respectively were having BP above normal. Baseline mean serum phosphorus, calcium and alkaline phosphatase levels were comparable among males and females. The mean blood glucose showed an increasing trend with age in both males and females.

As per ES cut-offs, 66 per cent of the entire study population had VDD, 14.7 per cent had insufficient and 19.3 per cent had sufficient serum 25(OH)D levels (Fig. 1). As per the Scientific Advisory Committee for Nutrition and Institute of Medicine (IOM) guidelines¹⁶, the percentage prevalence of VDD was 44.4 per cent. The IOM guidelines define VDD as serum 25(OH)D levels < 20 ng/ml. The mean (\pm SD) serum 25(OH)D status of the cohort was 24.92 ± 22.97 ng/ml and the values improved significantly with increasing age ($P \leq 0.01$) thereby decreasing the percentage prevalence of VDD (Fig. 2). Serum 25(OH)D levels did not differ significantly between two genders ($P = 0.972$), but was lower in those with a higher level of education ($P = 0.03$). There were no marked differences in serum 25(OH)D levels in relation to altitude, food habits, physical activity, sun exposure, smoking practice and BMI (Fig. 3). Serum 25(OH)D levels correlated positively

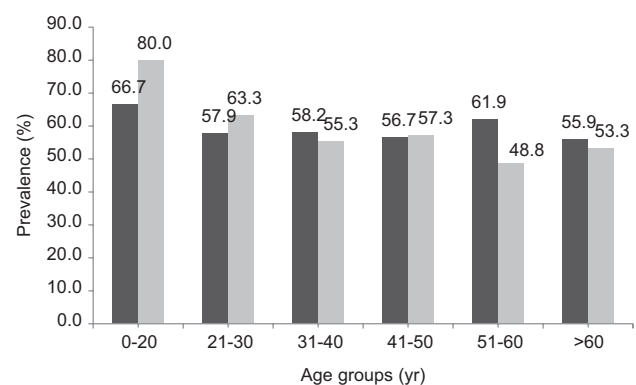
Table I. Age-wise mean clinical parameters of the study population

	Age groups in years					
	0-20	21-30	31-40	41-50	51-60	>60
Total, n (%)	272 (15.7)	330 (19.1)	400 (23.1)	296 (17.1)	234 (13.5)	200 (11.5)
Weight (kg)						
Male	48.18±11.58	58.28±7.84	59.37±9.86	61.89±9.59	60.29±10.87	60.23±10.80
Female	47.48±10.12	54.27±9.75	56.08±11.39	55.66±12.04	56.47±12.78	53.09±11.85
Height (cm)						
Male	165.53±13.98	171.43±11.81	170.09±11.53	170.38±10.14	168.62±11.30	168.21±9.61
Female	155.76±9.41	156.76±8.14	156.85±7.53	154.26±8.01	154.11±8.60	151.32±7.58
BMI (kg/m ²)						
Male	17.49±3.13	20.08±4.46	20.63±3.65	21.44±3.62	21.19±3.84	21.38±3.92
Female	19.62±3.75	22.13±4.11	22.82±4.56	23.47±5.21	23.70±5.32	23.31±5.60
Waist (cm)						
Male	73.21±13.34	74.47±12.23	75.85±12.31	78.59±15.07	76.80±14.43	80.31±16.67
Female	73.79±11.08	80.26±11.82	81.88±12.81	85.29±14.53	82.83±13.89	80.21±14.34
SBP (mmHg)						
Male	117.66±11.90	120.80±12.97	123.05±17.86	128.41±18.09	133.02±21.93	137.38±22.41
Female	116.47±14.81	117.50±14.76	122.75±16.97	125.23±19.92	133.53±20.68	140.00±20.88
DBP (mmHg)						
Male	75.80±9.74	76.89±10.23	80.91±11.35	83.95±13.01	85.70±13.84	87.99±14.50
Female	76.15±10.50	78.08±10.92	79.17±11.77	81.20±12.44	87.75±12.51	89.52±14.82
Data presented as mean±SD. SD, standard deviation; BMI, body mass index; DBP, diastolic blood pressure; SBP, systolic blood pressure						

**Fig. 1.** Vitamin D status in the tribal population according to various guidelines. SACN, Scientific Advisory Committee for Nutrition; IOM, Institute of Medicine; AAP, American Academy of Pediatrics.

with serum calcium ($r=0.065$, $P=0.056$), and serum phosphorus levels ($r=0.07$, $P=0.02$) but correlated negatively with serum alkaline phosphatase ($r=-0.063$, $P=0.04$), serum hs-CRP ($r=-0.176$, $P=0.01$) and serum insulin levels ($r=-0.079$, $P=0.01$).

VDD was prevalent across all age groups with highest prevalence in younger subjects (<20 yr). Thereafter, the prevalence of VDD did not vary

**Fig. 2.** Prevalence of VDD across age-groups.

significantly as age increased. Further, the risk of VDD also did not vary significantly with increasing age (Table II). Around 75.4 per cent males and 68.4 per cent females with BMI of <18.5 kg/m² had serum 25(OH)D concentration <20 ng/ml. Graduates were found at higher risk of VDD as compared to illiterates ($P<0.05$). Smoking increased the risk, while physical activity had no effect on VDD. Sun exposure (<2 h/day)

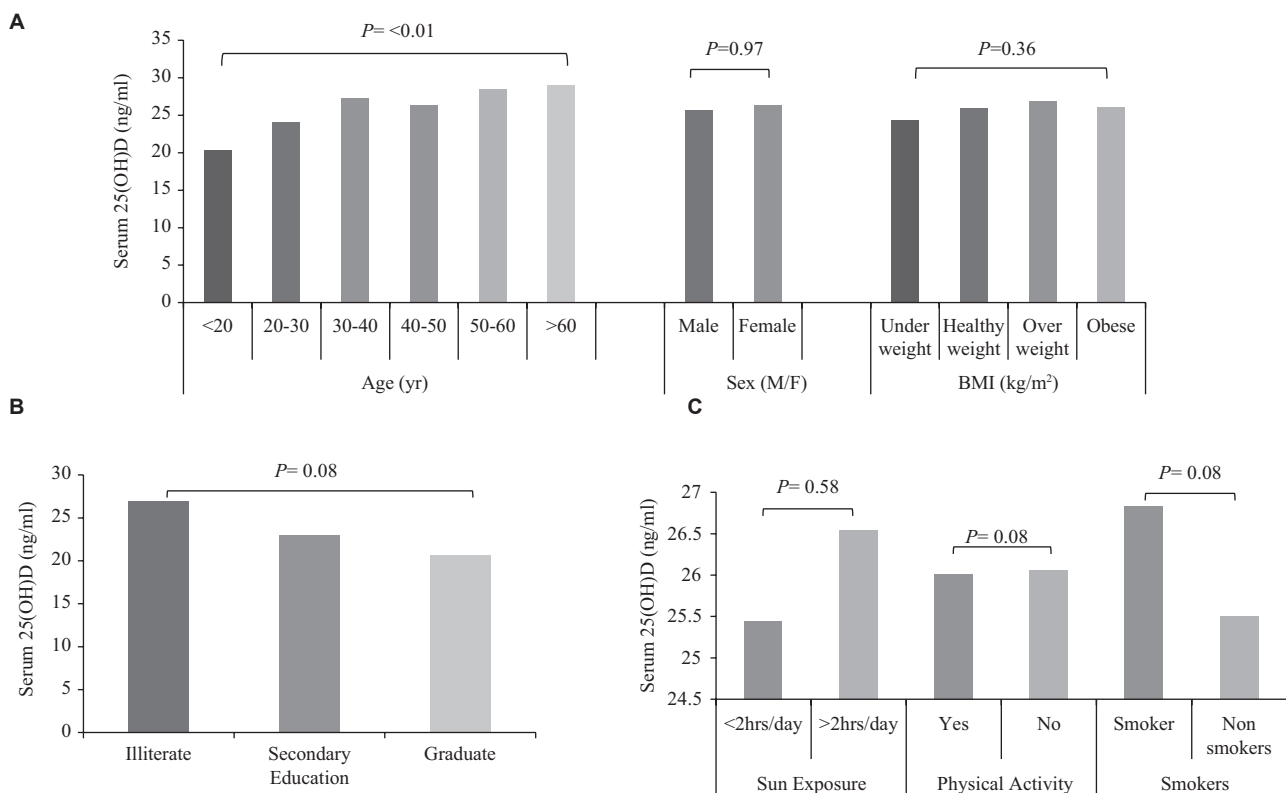


Fig. 3. Relation of various factors to vitamin D status in tribal population, (A) anthropometric parameters; (B) educational status; (C) lifestyle factors.

marginally decreased the risk of VDD among both males and females (Table III).

Discussion

VDD has been reported in considerable proportions in Indian sub-continent by many researchers². These studies attribute such findings to various ethnic and lifestyle factors such as increasingly indoor stay, socio-cultural and nutritional practices, and skin pigmentation. However, sparse information is available on the prevalence of VDD in tribal communities. The present study estimated the prevalence of VDD among apparently healthy tribal subjects (Gujjar and Bakarwal) of Kashmir valley and found that 66 per cent are deficient using ES criteria, which is better than that reported from general population⁹. Till date, only a few studies have been conducted to assess the prevalence of VDD and associated risk factors among tribal population in India. Noticeably studies conducted on rural population across India, reported a VDD prevalence of 73.91 per cent in healthy adults of Potheri Village of Kancheepuram district, Tamil Nadu¹⁷, 44 and 70 per cent in rural adult males and females of Tirupati, Andhra Pradesh¹⁸ and

70 per cent in rural population of Agota Village, Uttar Pradesh¹⁹. The prevalence of VDD was almost same as reported from the urban areas, with 62 per cent from Uttar Pradesh¹⁹ and 87 per cent from New Delhi²⁰ being vitamin D deficient. Despite the study participants belonging to low socio-economic indices, being undernourished, living in poorly ventilated houses with little or no access to pure drinking water *etc.*, their VD status was relatively better compared to non-tribal Kashmiri population⁹. Although we have not quantitated sun exposure, such differences could be attributed to the differential habit of engaging in outdoor physical activities such as farming, wood cutting and herding animals and could also be linked with higher consumption of dairy products.

Among the risk factors of VDD, obesity was unlikely a contributor as most of our study participants were lean. However, BMI is not the only predictor of adiposity and the association between adiposity and serum 25(OH)D levels has been found to be weaker if adiposity is defined by anthropometric measures only, rather than precisely measuring total body fat percentage²¹⁻²³. Further, other factors such as diet,

Table II. Age-wise mean biochemical parameters of the study population

	Age groups in years					
	0-20	21-30	31-40	41-50	51-60	>60
Total, n (%)	272 (15.7)	330 (19.1)	400 (23.1)	296 (17.1)	234 (13.5)	200 (11.5)
Phosphorus (mg/dl)						
Male	3.47±0.54	3.38±0.48	3.43±0.45	3.47±0.42	3.33±0.50	3.38±0.46
Female	3.59±0.45	3.38±0.51	3.43±0.44	3.75±0.41	3.44±0.49	3.25±0.46
ALP (IU/L)						
Male	136.44±32.38	129.72±34.39	125.83±33.58	122.62±31.62	128.17±33.13	124.53±31.00
Female	134.42±33.69	131.46±33.42	128.92±33.80	130.89±34.38	131.79±35.05	130.95±33.89
Serum 25(OH)D (ng/ml)						
Male	24.16±22.63	29.38±30.34	25.68±23.60	24.24±20.76	27.63±28.11	26.45±24.21
Female	17.81±14.47	21.30±16.71	25.64±22.45	24.74±20.54	25.01±22.12	28.77±25.18
Blood sugar random (mg/dl)						
Male	99.71±18.53	103.80±16.83	107.53±38.90	108.49±22.32	111.63±26.31	114.32±37.79
Female	106.45±22.38	102.22±18.24	109.73±21.62	117.12±50.07	112.81±25.41	113.50±25.52
Insulin (μIU/ml)						
Male	12.36±10.24	12.51±11.91	12.64±13.16	12.95±14.78	13.25±15.58	11.32±10.14
Female	14.51±11.21	15.84±14.96	14.63±12.79	17.26±15.37	15.76±14.44	14.23±13.12
hs-CRP (mg/L)						
Male	3.64±3.75	2.85±3.04	3.43±3.52	3.38±3.72	3.81±1.04	3.48±2.38
Female	4.16±3.88	3.21±3.28	3.64±3.76	3.42±3.54	4.33±3.49	4.28±3.86

Data presented as mean±SD. ALP, alkaline phosphatase; hs-CRP, high-sensitivity C-reactive protein

ethnicity and lifestyle could be potential contributors of VDD in our study population. The level of education had an inverse relation with serum 25(OH)D levels. Similar observations were made by an earlier study on Tunisian women demonstrating that higher education was associated with more VDD²⁴. A plausible explanation for this observation could be that educated individuals are more likely to take up indoor jobs that decreases their exposure to sun and may also engage in some sun protection behaviour. Even though educated people are believed to be more health oriented, a study from India revealed lack of awareness in them regarding VDD²⁵. Categorization of exposure to sun to < 2 or > 2 h did not show any significant difference in serum 25(OH)D levels in the present investigation.

Studies have confirmed smoking as an independent lifestyle factor which is associated with low 25(OH)D levels both in men and women. As per published reports it is suggested that chemicals in tobacco smoke may in fact have a direct effect on vitamin D metabolism and function²⁴. The depression of the (vitamin D-PTH) axis is reportedly seen among chronic smokers and eventually affects bone metabolism and contributes

to increased risk of fractures and osteoporosis. Smoking has also been shown to alter expression of several genes, among which are some genes involved in the metabolic pathway of vitamin D²⁴. We found lower vitamin D levels in smokers as compared to non-smokers (although not significant) which could be attributed to the low response rate for some of the questions pertaining to smoking. This could also be attributed to other confounding factors such as specific medications and dosage of supplements used, which, however, were not captured in our investigation.

Dietary factors such as milk, fish or non-vegetarian food consumption did not have significant association with VD status. Although detailed calculation of macronutrient and micronutrient intake including calcium intake could have provided better insights, the practice of consuming a significant amount of dairy products did not seem to have much relation with vitamin D status. This may be explained by the fact that most of the dietary sources are very low in vitamin D content, for example, unfortified milk, which remains the main source of VD among vegetarians, contains only 2 IU of VD/100 ml, which is further reduced

Table III. Prevalence of vitamin D deficiency with respect to risk factors

Study Parameters	Males		Females	
	OR (95% CI)	P	OR (95% CI)	P
Age group (yr)				
0-20	Reference		Reference	
21-30	0.543 (0.206-1.433)	0.068	0.238 (0.103-0.551)	0.075
31-40	0.604 (0.242-1.508)	0.613	0.131 (0.053-0.325)	0.572
41-50	0.370 (0.144-0.949)	0.794	0.215 (0.081-0.573)	0.649
51-60	0.648 (0.246-1.701)	0.119	0.147 (0.051-0.426)	0.175
61+	0.405 (0.157-1.046)	0.348	0.116 (0.034-0.393)	0.060
BMI				
Underweight	Reference		Reference	
Healthy	0.514 (0.305-0.866)	0.034	1.273 (0.709-2.287)	0.170
Overweight	0.572 (0.294-1.114)	0.032	1.584 (0.807-3.107)	0.918
Education level				
Illiterate	Reference		Reference	
Secondary	1.068 (0.495-2.306)	0.085	1.104 (0.404-3.019)	0.061
Graduate	1.406 (0.340-5.819)	0.110	5.342 (0.548-12.045)	0.695
Smoking				
Non-smoker	Reference		Reference	
Smoker	1.272 (0.616-2.630)	0.384	1.512 (0.532-4.298)	0.631
Physical activity				
Yes	Reference		Reference	
No	1.032 (0.610-1.744)	0.384	1.496 (0.875-2.558)	0.198
Dairy products				
Never	Reference		Reference	
One times a month	0.381 (0.089-1.627)	0.075	1.616 (0.456-5.724)	0.004
Two-three times a month	0.353 (0.087-1.432)	0.572	1.884 (0.593-5.990)	0.018
One-two times a week	0.667 (0.162-2.740)	0.649	1.455 (0.452-4.689)	0.021
Three-four times a week	0.357 (0.093-1.372)	0.175	0.977 (0.320-2.977)	0.027
Five times a week	0.387 (0.100-1.508)	0.060	1.814 (0.598-5.507)	0.070
Sun exposure (hour/day)				
<2	Reference		Reference	
>2	0.936 (0.651-1.485)	0.233	0.776 (0.512-1.175)	0.623

OR, odds ratio; CI, confidence interval

by dilution and adulteration of milk. Besides, high phytate content in Indian diet leads to poor absorption of dietary calcium¹⁸. In addition, deep frying of foods, which is very popular in India, depletes vitamin D by thermal degradation².

Although sun exposure is the primary determinant of vitamin D status in humans, we did not find an association between duration of sunlight exposure (<2 h) and serum 25(OH)D levels, which could be

partly attributed to cultural practices of heavy clothing in these indigenous groups. Moreover, sun exposure was not recorded in detail as a continuous variable in this study. Kashmir valley is located in the high latitude Himalayan belt of northern India. The low solar zenith angle, during prolonged winters affects the amount of UV-B radiation that becomes available for cutaneous vitamin D synthesis in the body^{25,26}. This could further increase the risk of VDD in our study population. Higher amount of physical activity was associated

with higher 25(OH)D levels (Table III). While it is considered as a surrogate for sun exposure, physical activity may affect vitamin D concentrations through other mechanisms²⁷.

In the present study, serum 25(OH)D positively correlated with serum calcium level and negatively with serum alkaline phosphatase. These findings are consistent with the fact that vitamin D facilitates absorption of calcium across the intestinal mucosa by both genomic and non-genomic mechanisms²⁸. VDD state leads to secondary hyperparathyroidism resulting in defective bone mineralization while raised alkaline phosphatase levels cause rickets in children and osteomalacia in adults²⁹.

Despite the large sample size and assessment of a considerable number of risk factors including anthropometric measurements (age, gender, BMI), lifestyle issues (education level, smoking status, income) as well as environmental factors such as exposure to sunlight in this study, there were a few limitations. These included lack of data on serum PTH and diet. Despite this limitation, our study provides important preliminary evidence on a socio-economically deprived population group. Furthermore, our results could be generalized for adult age groups except for younger children and adolescents where the response rate was low owing to a higher rate of refusals for sampling.

In conclusion, this is the first study to assess the vitamin D status of tribal population of Kashmir valley. As compared to the general population, a lower prevalence of VDD was observed across all age groups irrespective of gender and BMI. Given the deleterious consequences of VDD on both skeletal and extra-skeletal health, there is an urgent need for planning well-controlled interventional studies to pave the path for relevant prevention or supplementation programmes.

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Conflicts of Interest: None.

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