

## Reference interval of Serum 25-Hydroxyvitamin D of adult Bangladeshi population

Wasim Md Mohosin Ul Haque<sup>1\*</sup>, Md Faruque Pathan<sup>2</sup>, M Abu Sayeed<sup>3</sup>

<sup>1</sup>Department of Nephrology, Bangladesh Institute of Research and Rehabilitation in Diabetes, Endocrine and Metabolic Disorders, Dhaka, Bangladesh;

<sup>2</sup>Department of Endocrinology, Bangladesh Institute of Research and Rehabilitation in Diabetes, Endocrine and Metabolic Disorders, Dhaka, Bangladesh;

<sup>3</sup>Department of Community Medicine, Ibrahim Medical College, Dhaka, Bangladesh.

### Abstract

**Background and objectives:** Vitamin D deficiency is a significant public health concern globally including Bangladesh. Currently, Bangladeshi population is being evaluated for vitamin D status using reference interval derived from western studies. Reference interval derived from western populations may not reflect the actual status of vitamin D status of Bangladeshi population due to differences in sun exposure, ethnicity and dietary habits. Therefore, this study aimed to find out the reference intervals and the lower cutoff value of serum 25-hydroxyvitamin D [25(OH)D] deficiency in healthy Bangladeshi adult population.

**Materials and methods:** This cross-sectional study was conducted involving two population groups. Group-1 comprised of adequately sun exposed healthy coastal fishermen and Group-2 included healthy urban dwellers. Group-1 represented an ideal population for assessing the reference serum vitamin D level while Group-2 population was used to detect the cut-off value of vitamin D deficiency level in adult Bangladeshi population. Chemiluminescent microparticle immunoassay (CMIA) was used to estimate serum 25(OH) D. Serum iPTH and other biochemical parameters were analysed by standard methods. The reference interval of vitamin D was determined according to Clinical and Laboratory Standards Institute (CLSI) Guidelines C28-A3. The lower cut-off value of vitamin D deficiency level was determined by detecting deflection point of parathyroid hormone (PTH) level compared to serum 25(OH)D level.

**Results:** Total 125 and 371 individuals were enrolled in Group 1 and Group 2 respectively. The mean age of the Group-1 and 2 study populations were 37.98±11.61 and 44.19 ± 11.48 years respectively. The mean serum vitamin D levels of Group-1 and 2 study population were 27.36±7.27 ng/ml (95% CI: 26.08, 28.65) and 21.53±15.98 ng/ml (95% CI: 19.9, 23.16) respectively. Serum reference interval of vitamin D of healthy adults (Group-1) was found as 15.88 to 45.27 ng/ml. The cut-off value for vitamin D deficiency in Group-2 adult population was 12.16 ng/ml (95% CI: 11.04, 13.28) as depicted by first upward deflection of serum iPTH when serum 25(OH)D level fell below 12.16 ng/ml.

**Conclusion:** The findings suggest that current Western-based vitamin D reference intervals may not be appropriate for the Bangladeshi population. The results of our study would help the clinicians and policymakers in developing strategies to address vitamin D deficiency in Bangladesh.

\*Correspondence: Wasim Md Mohosin Ul Haque, Department of Nephrology, Bangladesh Institute of Research and Rehabilitation in Diabetes, Endocrine and Metabolic Disorders, Dhaka, Bangladesh. E-mail: [wmmhaque@live.com](mailto:wmmhaque@live.com).

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## Introduction

Vitamin D, a crucial fat-soluble vitamin, plays a vital role in various physiological functions, including calcium and phosphorus homeostasis, immune function, and cell growth modulation [1-4]. Consequently, its deficiency can lead to a range of diseases and disabilities [3,5,6]. Despite Bangladesh's favourable geographical location and climatic condition, the prevalence of vitamin D deficiency remains high even among seemingly healthy individuals [7,8].

Current reference intervals for vitamin D are predominantly derived from studies conducted in Western populations and may not accurately reflect the physiological needs or health outcomes of diverse global populations [9,10]. Variations in genetics, skin pigmentation, dietary habits, and sun exposure significantly impact vitamin D levels, rendering universal reference intervals potentially inappropriate for many non-Western populations. Genetic polymorphisms affecting vitamin D metabolism and receptor activity vary among ethnic groups, leading to different optimal levels of serum 25-hydroxyvitamin D required for health [11,12]. Consequently, a one-size-fits-all approach can result in incorrect diagnoses, unnecessary supplementation, potential toxicity, or undertreatment and persistence of deficiency-related health issues. Therefore, establishing a vitamin D reference interval specific to Bangladeshi population is crucial for accurate diagnosis and effective management of vitamin D deficiency. This study aimed to establish the reference interval of serum 25-hydroxyvitamin D levels of the adult population of Bangladesh by examining two distinct groups: adequately sun-exposed healthy coastal fishermen and urban individuals with limited sun exposure. Also, the study determined the lower cut-off value of vitamin D deficiency level in Bangladeshi adults by detecting the deflection point of serum intact parathyroid hormone (iPTH) level compared to serum 25(OH)D level. Vitamin D deficiency physiologically induces increased synthesis/production of parathyroid hormone.

## Materials and methods

The study was conducted from January 1, 2017, to December 31, 2021 on adult population living in

two geographical locations of Bangladesh. Cross-sectional study design was employed to assess the vitamin D levels in study participants.

**Study site and population:** The study was conducted in two geographical locations: Cox's Bazar, a coastal district, about 298 km south of Dhaka city along the coast of Bay of Bengal with abundant sunlight; and capital city Dhaka, characterized by limited sun exposure of its inhabitants due to urbanization. Group-1 consisted of healthy adult coastal fishermen aged 18 years and above from Cox's Bazar, who were regularly and adequately exposed to sunlight and consumed marine fish, a rich source of vitamin D. Adequate sun exposure was defined as exposure to direct sunlight at least for 30 minutes, between 11 am and 2 pm, three times a week [13]. Group-1 individuals (coastal fishermen) were considered as reference population for determining the serum vitamin D reference interval of adult healthy Bangladeshi population. Group-2 comprised of ostensibly healthy urban residents from Dhaka, aged 18 years and above, with limited sun exposure due to lifestyle and environmental factors.

**Exclusion criteria:** Any individual who refused to participate in the study or who were physically and psychologically handicapped, had known acute or chronic illnesses like diabetes mellitus, hypertension, liver disease or gastrointestinal disorders, kidney disease, malignancy, metabolic bone disease, primary hyperparathyroidism or lactose intolerance were excluded. Anyone with relevant biochemical abnormalities and on anti-epileptic drugs was also excluded. For Group-1, any one who was consuming either vitamin D or calcium-containing products or who had ever consumed such drugs in the past was excluded.

**Sampling technique and sample size:** Convenience sampling was used to select participants from both groups. The sample size was determined based on the desired confidence limits and the variability in vitamin D levels observed in preliminary studies.

**Data collection tools and procedures:** Socio-demographic and clinical data were collected using structured questionnaires. Height, weight and blood pressure were measured. Blood samples were collected for estimation of serum 25(OH)D, iPTH levels and relevant biochemical parameters.

**Collection of blood and biochemical investigations:**

About 10 ml of venous blood was collected aseptically from each participant after counselling and thorough clinical evaluation. Serum was separated immediately and was preserved at  $-60^{\circ}\text{C}$  for biochemical analysis. Chemiluminescent micro-particle immunoassay (CMIA) was used to estimate serum 25(OH)D level by Architect analyser [14]. Based on the Endocrine Society guideline [9] (9), vitamin D level was categorised as: adequate/normal range: 75-100 nmol/L (30-40ng/ml), insufficient: 50 – 74.9 nmol/L (20ng/ml-29.99ng/ml) and deficient: <50 nmol/L (<20ng/ml). Samples were tested for random blood glucose (RBG), serum creatinine, calcium, phosphate (PO<sub>4</sub>), albumin, alkaline phosphatase and iPTH by Architect autoanalyser at the biochemistry laboratory of BIRDEM.

**Statistical analyses:** Statistical analyses were conducted using descriptive and inferential methods to summarize the data and assess factors influencing vitamin D levels. Chi-square tests, correlation analyses, and regression models were employed to explore these relationships. MedCalc version 14 statistical software was utilized to calculate reference intervals (95%, double-sided) following parametric, non-parametric, and robust methods as outlined in the CLSI C28-A3 guideline [15]. A 90% confidence interval (CI) was used for these reference intervals, applying the appropriate statistical techniques.

To determine the lower cut-off value of vitamin D deficiency, the deflection point of intact parathyroid hormone (iPTH) levels was identified using a non-linear model. Piecewise linear regression, applied through the Python plugin, was used to fit two separate lines before and after the deflection point (or “break” point), where the relationship between vitamin D and PTH shifts.

The equation used in this case represented as follows:

$$Y = \begin{cases} k_1 \cdot x + y_0 - k_1 \cdot x_0 & \text{for } x < x_0 \\ k_2 \cdot x + y_0 - k_2 \cdot x_0 & \text{for } x \geq x_0 \end{cases}$$

In this model:

- $x$  = Vitamin D level (independent variable).
- $y$  = PTH level (dependent variable).
- $x_0$  = deflection point where the two lines meet.
- $y_0$  = i PTH level at the deflection point.

- $k_1$  = slope of the line before the deflection point (for  $x < x_0$ ).
- $k_2$  = slope of the line after the deflection point (for  $x \geq x_0$ ).

The steps for calculating the deflection point included:

1. Initial estimation: A starting guess for  $x_0$  is made based on visual inspection or prior knowledge.
2. Curve fitting: The SciPy curve fit function optimizes  $x_0$ ,  $y_0$ ,  $k_1$  and  $k_2$  by minimizing the difference between the model and observed data.
3. Confidence intervals: Standard errors are used to calculate 95% confidence intervals for the estimates.

This approach effectively identified the point where the relationship between vitamin D and iPTH levels changes.

**Ethical Consideration:** The protocol was approved by the Ethics Review Committee (ERC) of the Centre for Higher Studies and Research, Bangladesh University of Professionals and Diabetic Association of Bangladesh. Written informed consent was obtained from each participant prior to enrolment in the study and collection of blood sample. Participants had the right to withdraw from the study at any time.

**Result**

Group-1 and Group-2 consisted of 125 coastal fishermen and 371 urban residents respectively. Out of 371 Group 2 participants, 47.2% were male and 52.8% were females. The mean age of Group-1 and 2 study population was  $37.98 \pm 11.61$  and  $44.19 \pm 11.48$  years respectively. Mean BMI of both groups was not significantly ( $p > 0.05$ ) different ( $22.22 \pm 3.15 \text{ kg/m}^2$  and  $26.28 \pm 3.72 \text{ kg/m}^2$ ). Detail socio-demographic and anthropometric characteristics of Group-1 and 2 study populations are shown in Table-1. All the biochemical parameters of Group-1 study populations were within normal ranges (Table-2).

The mean serum vitamin D level of Group-1 study population was  $27.36 \pm 7.27 \text{ ng/ml}$ , (95% CI: 26.08, 28.65). No significant difference ( $F = 0.506$ ,  $p = 0.679$ ) and correlation ( $r = -0.05511$ ,  $p = 0.5416$ , 95% CI: -0.2285, 0.1217) were found in vitamin D

levels amongst different age groups. However, there was a significant difference among BMI groups ( $F = 3.377, p = .037$ ). Post-hoc testing using Bonferroni correction revealed that individuals with the highest BMI (25-29.9 kg/m<sup>2</sup>) had significantly ( $p = 0.037$ ) lower serum 25(OH)D levels compared to those with the lowest BMI (<18.5 kg/m<sup>2</sup>), with a mean difference of -6.82 ng/ml. However, No significant differences of vitamin D levels were observed between other BMI groups (Table-3).

Based on the Endocrine Society cut-off value [9], out of total 125 participants, 39 (31.2%), 63 (50.4%)

and 23 (18.4%) participants had adequate ( $\geq 30$  ng/ml), insufficient (21-29.9 ng/ml) and deficient ( $< 20$  ng/ml) levels of vitamin D respectively (Table-4). There was no significant difference in serum iPTH level and other relevant biochemical parameters amongst these three vitamin D status groups (Table-5). Also, overall no significant correlation was observed between serum 25(OH)D level and serum iPTH or other relevant biochemical parameters of the total Group-1 study population (Table-6).

**Table-1:** Socio-demographic and anthropometric parameters of Group-1 (n=125) and Group-2 (n=371) study population

Parameters	Group 1			Group 2		
	n (%)	Mean $\pm$ SD	95% CI of mean	n (%)	Mean $\pm$ SD	95% CI of mean
<b>Gender</b>						
Male	121	-	-	175 (47.2)	-	-
Female	-	-	-	196 (52.8)	-	-
<b>Age (years)</b>						
18-30	40 (32.0)	25.48 $\pm$ 3.68	24.3, 26.65	173 (46.6)	34.34 $\pm$ 6.68	33.34, 35.34
31-40	40 (32.0)	35.90 $\pm$ 2.97	34.95, 36.85	182 (49.1)	51.65 $\pm$ 4.69	50.97, 52.34
41-50	28 (22.4)	46.68 $\pm$ 3.32	45.39, 47.97	14 (3.8)	68.21 $\pm$ 2.75	66.63, 69.80
>50	17 (13.6)	58.00 $\pm$ 3.35	56.28, 59.72	1 (0.3)	80	-
All	125	37.98 $\pm$ 11.61	35.93, 40.04	371	44.19 $\pm$ 11.48	43.02, 45.36
Height(cm)	125	161.10 $\pm$ 7.57	159.76, 162.44	371(100)	161.65 $\pm$ 7.86	160.85, 162.45
Weight(kg)	125	57.52 $\pm$ 8.37	56.04, 59.00	371(100)	68.61 $\pm$ 10.86	67.51, 69.72
<b>BMI (kg/m<sup>2</sup>)</b>						
<18.5	10 (8)	16.69 $\pm$ 1.69	15.48, 17.90	4(1.1)	17.55 $\pm$ 0.85	16.20, 18.91
18.5-24.9	91 (72.8)	21.57 $\pm$ 1.74	21.21, 21.93	146(39.4)	23.08 $\pm$ 1.45	22.84, 23.32
25-29.9	24 (19.2)	26.97 $\pm$ 1.46	26.35, 27.58	164(44.2)	27.18 $\pm$ 1.36	26.98, 27.39

Note: CI = confidence interval

**Table-2:** Biochemical parameters of Group-1 study population (n=125)

Parameters	Number	Mean $\pm$ SD	95% CI of mean
RBS (mmol/l)		6.01 $\pm$ 0.87	5.85, 6.16
S creatinine (mg/dl)		.8992 $\pm$ 0.11	0.88, 0.92
S albumin (g/L)		45.4 $\pm$ 2.92	44.89, 45.92
S calcium (mg/dl)	125	9.37 $\pm$ 0.44	9.29, 9.45
S PO4 (mg/dl)		3.29 $\pm$ 0.54	3.19, 3.38
S AlkPhos (U/L)		79.86 $\pm$ 16.38	76.96, 82.76
S iPTH (pg/ml)		20.63 $\pm$ 8.85	19.06, 22.19

Note: RBS= random blood sugar, AlkPhos= alkaline phosphatase (Normal:44 to 147 IU/L), iPTH= intact parathyroid hormone. (Normal: 10 - 65 pg/ml); CI = confidence interval

**Table-3:** Age and BMI-specific serum Vitamin D levels of Group-1 study population (n=125)

Parameters	Number	25(OH)D level Mean ± SD (ng/ml)	95% CI	p value by ANOVA
<b>Age (years)</b>				
18-30	40	28.30 ± 7.77	25.82, 30.78	F = 0.506 p = .679
31-40	40	27.21 ± 7.48	24.82, 29.60	
41-50	28	26.10 ± 6.37	23.63, 28.57	
>50	17	27.59 ± 7.30	23.84, 31.34	
<b>BMI (kg/m<sup>2</sup>)</b>				
≤18.5	10	32.76 ± 8.71	26.53, 39.00	F = 3.37 p = .037
18.6 - 24.9	91	27.14 ± 7.14	25.66, 28.63	
25 - 29.9	24	25.95 ± 6.43	23.23, 28.66	
<b>All</b>	125	27.36 ± 7.27	26.08, 28.65	

CI = confidence interval

**Table-4:** Serum Vitamin D status of the Group-1 (coastal fishermen) study population based on the Endocrine Society cut-off value [9] (9)

Vitamin D status [9]	Number (%)	Serum 25(OH)D level Mean ± SD (ng/ml)
Deficient (<20 ng/ml)	23 (18.4)	17.96 ± 1.69
Insufficient (21 ng/ml - 29.9 ng/ml)	63 (50.4)	25.52 ± 2.53
Adequate (≥30 ng/ml)*	39 (31.2)	35.90 ± 5.16
All cases	125	27.36 ± 7.27

Adequate = Normal + above normal

**Table-5:** Levels of serum iPTH and other relevant biochemical markers related to vitamin D status of Groups-1 study population (n=125)

Parameters	Serum 25-hydroxy vitamin D status groups [9]			ANOVA
	Deficient	Insufficient	Adequate	
S. Calcium (mg/dl)	9.42±0.37	9.37±0.42	9.34±0.51	F = .202, p = .818
S. PO4 (mg/dl)	3.25±0.67	3.26±0.52	3.36±0.48	F = .483, p = .618
S. AlkPhos (U/L)	80.61±20.14	80.89±12.17	77.77±19.24	F = .462, p = .631
S. iPTH (pg/ml)	21.63±7.49	20.81±9.46	19.73±8.68	F = .361, p = .698

**Table-6:** Correlation between serum 25(OH)D levels and relevant biochemical parameters of Group-1 study population

Parameters	r	p-value	95% CI for r
S. Calcium (mg/dl)	-.06803	.4510	-0.2408, 0.1089
S. PO4 (mg/dl)	.06004	.5060	-0.1168, 0.2332
S. AlkPhos (U/L)	-.1612	.0725	-0.3275, 0.01484
S. iPTH	-.1635	.0685	-0.3296, 0.01249

CI = confidence interval

The reference interval for serum vitamin D levels of our Group-1 reference population (coastal fishermen) was calculated in accordance with the CLSI Guidelines C28-A3 [15] using a 90% confidence interval (CI). The calculated reference interval of serum vitamin D value by parametric, non-parametric and robust methods were 15.78-44.29, 15.88-45.27 and 15.69-44.68 ng/ml, respectively. Detail is shown in Table-7. Figure-1 displays the

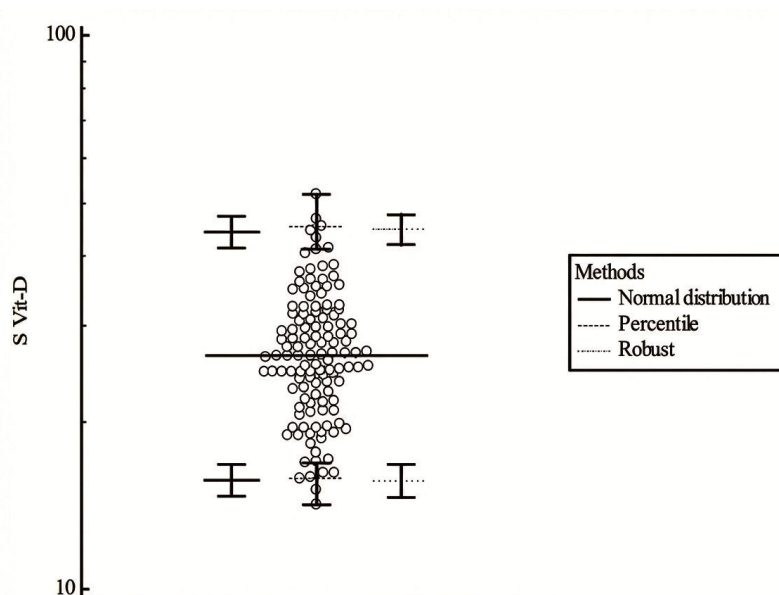
95% reference interval for vitamin D, visually representing the ranges defined by the aforementioned methods, ensuring a comprehensive understanding of the expected vitamin D levels within this population group.

This structured approach enhances the reliability of the reference intervals, providing a solid foundation for further comparative analyses within the study.

**Table-7:** Reference interval of vitamin D levels calculated by different methods using serum 25(OH)D level of Group-1 study population

Methods	Serum 25(OH)D level (ng/ ml)
<b>A. Based on Normal distribution</b>	
Lower limit (90% CI)	15.78 (14.758116.8828)
Upper limit (90% CI)	44.29 (41.4114, 47.3733)
<b>B. Non-parametric percentile method (CLSI C28-A3)</b>	
Lower limit (90% CI)	15.87 (14.2600 to 16.9200)
Upper limit (90% CI)	45.2670 (41.1100, 51.8500)
<b>C. Robust method (CLSI C28-A3)</b>	
Lower limit (90% CI)	15.6855 (14.6794,16.8548)
Upper limit (90% CI)	44.6759 (41.8912, 47.5948)

Note: 90% CI = 90% confidence interval.



**Figure-1:** Reference interval of vitamin D. (95% Reference interval, Double-sided)

In the second part of the study, Group 2 population, consisting of 371 adult urban residents, was used to determine the lower cutoff value for serum 25-hydroxyvitamin D [25(OH)D] deficiency levels in healthy Bangladeshi adult population. Urban residents, exhibited a mean serum vitamin D level of 21.53±15.98 ng/ml (95% CI: 19.90, 23.16), with a median level of 17.18 ng/ml, ranging from 3.48 to 147.32 ng/ml. Parametric tests showed no significant differences in vitamin D levels between males and females (male: 20.16±16.18 ng/ml and females: 22.76±15.74 ng/ml ( $p > .05$ , 95% CI: -5.86,

0.66). However, nonparametric tests indicated significantly higher levels in females ( $p = .008$ ). An analysis of age-related trends revealed that older age groups in the urban population exhibited significantly higher mean serum vitamin D levels, demonstrating a clear positive correlation with age ( $r = .174$ ,  $p = .001$ , 95% CI: 0.073, 0.271). The distribution of vitamin D levels across different age groups and BMI categories is detailed in Table-8. Despite these age-related variations, no significant differences were found in vitamin D levels across different BMI categories ( $F = 0.173$ ,  $p > .05$ ).

**Table-8:** Age and BMI-specific Serum Vitamin D Levels of Group-2 Study Population (n=371)

Variable	n (%)	Mean±SD (ng/ml)	95% CI for Mean	t-test/ANOVA
<b>Gender</b>				
Male	175	20.16±16.18	-5.86, 0.66	$p = .118$
Female	196	22.76±15.74		
<b>Age groups (years)*</b>				
19 to 44	174(46.9)	18.35±15.67	15.99, 20.70	$F=5.990, p=.001$
45 to 64	182(49.1)	23.46±15.32	21.21, 25.70	
65 to 79	14(3.8)	30.15±15.00	21.49, 38.81	
80 years and over	1(.3)	50.49.	-	
<b>BMI (kg/m<sup>2</sup>)</b>				
<18.5	4(1.1)	19.29±10.57	2.47, 36.12	$F = .173, p = .915$
18.5-24.9	146(39.4)	20.88±15.67	18.32, 23.44	
25-29.9	164(44.2)	21.94±17.67	19.21, 24.66	
>30	57(15.4)	22.18±11.60	19.10, 25.26	
Total Gr-2 study population	371(100)	21.53±15.98	19.90, 23.16	

Note: Parametric (independent-samples t-test) and non-parametric (independent-samples Mann-Whitney U test) methods were used as appropriate

According to the Endocrine Society cut-off value [9], 83.6% of the urban population had below-normal (inadequate) vitamin D levels. However, using the fishermen's reference intervals established in the first phase of this study, only 44.2% of the urban population were found to have below-normal vitamin D levels (Table-9). This discrepancy highlights that nearly half of the urban residents categorized as vitamin D deficient by conventional standards might actually fall within an adequate range when using our fishermen's reference intervals. The mean serum vitamin D level was significantly ( $p < .001$ ) lower in the urban group (21.53±15.98 ng/ml) compared to fishermen (27.36±7.27 ng/ml).

The average serum intact parathyroid hormone (iPTH) level of Group-2 urban population was 62.80 ±31.67 pg/ml, ranging widely from 10.20 to 227.54 pg/ml, with a median value of 56.4 pg/ml. A significant negative correlation was observed between serum vitamin D and iPTH levels, evidenced by a correlation coefficient ( $r$ ) of -0.233, indicating that iPTH levels generally rise as vitamin D levels fall. This relationship was statistically significant, with a p-value less than .001 and a 95% confidence interval ranging from -0.3273 to -0.1347. The analysis of iPTH levels across different vitamin D status segments, as defined by the Endocrine Society, is shown in Table-10.

**Table-9:** Vitamin D status of the urban people based on the Endocrine society cut-off value and fishermen's reference interval

Vitamin D status of the Gr-2 people	Below normal n (%)	Normal n (%)	Above normal n (%)
Based on ES cut-off value	310 (83.6) Deficiency 221(59.6) Insufficiency 89 (24)	27 (7.3)	34 (9.2)
Based on FM cut-off value	164(44.2)	184 (49.6)	23 (6.2)

ES = Endocrine society, FM = coastal fishermen (Gr-1)

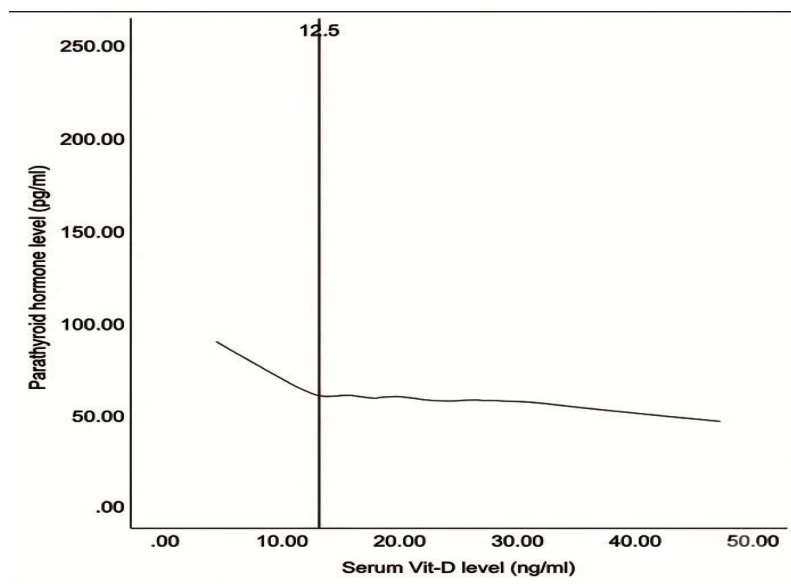
**Table-10:** Mean PTH level in different vitamin D segments based on Endocrine society cut-off value

Vitamin D status	25(OH)D level ng/mL	Number	iPTH (pg/ml) Mean*	95% CI of mean
Vitamin D deficiency	<20	221	66.94	62.68, 71.21
Vitamin D insufficiency	20 - 29.9	89	60.47	53.68, 67.26
Vitamin D normal range	30-40	27	59.89	49.89, 69.90
Vitamin D above normal range	>40	34	44.33	35.90, 52.76

\* Difference of means is significant at <1% level of significance (F=5.55, p=.001);

These findings suggest a nonlinear relationship between vitamin D and iPTH, where iPTH levels decrease as vitamin D increases, with notable differences in mean iPTH levels across vitamin D segments (F=5.55, p=.001;Figure-2). The LOWESS (locally weighted scatter plot smoothing) method was employed to illustrate the relationship between iPTH and 25(OH)D. It depicts the dynamic

relation between iPTH and 25(OH)D levels. The scatterplot highlights a deflection point in the relationship, initially estimated at 12.5 ng/ml, and refined to 12.16 ng/ml (95% CI: 11.04, 13.28) through piecewise linear regression. This deflection point marks a new lower cut-off for optimal serum vitamin D levels based on physiological responses observed in iPTH.



**Figure-2:** The scatterplot with LOESS fitting (50%) illustrates the relationship between vitamin D and PTH, with vitamin D (ng/ml) on the x-axis and serum iPTH (pg/ml) on the y-axis. The vertical line indicates the PTH deflection point using the raw data.

## Discussion

Presently, reference intervals used to assess the vitamin D status of Bangladeshi population, and many other similar non-Western populations, are predominantly derived from studies conducted in Western populations and therefore, may not accurately reflect the physiological needs or health outcomes of these diverse population [9,10]. Variations in genetics, skin colour, dietary habits, and sun exposure significantly impact vitamin D levels, rendering universal reference intervals potentially inappropriate for Bangladeshi as well as many non-Western populations. Therefore, in the present study was conducted to establish a reference interval for serum 25-hydroxyvitamin D of adult Bangladeshi population using a defined reference population groups. The result of this study is expected to prevent over estimation of low vitamin D status among our local population.

In our study, coastal fishermen group constituted the reference population for vitamin D estimation. The coastal environment, with its pollution-free air, excellent sun exposure, and consumption of marine fish, ensured vitamin D adequacy for our reference group. Prior studies, such as the 1958 birth cohort study in Scotland, have also linked coastal climates with higher levels of 25(OH)D due to increased sun exposure [16]. In our study, only male participants were enrolled in Group-1 to ensure adequate sun exposure. Though this might be considered as a limitation, other studies indicate no significant gender-specific differences in vitamin D levels [8,17].

Overweight subjects were excluded from our Group-1, yet consistent with findings of previous studies, a significant negative correlation between vitamin D and BMI was observed [18-21]. Studies consistently show an inverse relationship between BMI and vitamin D levels, highlighting the impact of body weight on vitamin D status [22-24]. The lack of correlation between age and serum vitamin D levels in Group-1 suggests the overall good health of the study population. This might obscure the typical association between lower vitamin D levels and older age, often attributed to reduced outdoor activity and impaired kidney function in older adults [25].

In the present study, health status of the Group-2 participants was not strictly controlled. Only those

with acute illnesses were excluded. This approach aligns with other studies examining the correlation between iPTH and vitamin D, which often include people with a broader range of health statuses [26-32].

In the present study, irrespective of the adequacy of sun exposure, most participants in both the fishermen and urban groups had low vitamin D levels, with means of  $27.36 \pm 7.27$  ng/ml and  $21.53 \pm 15.98$  ng/ml, respectively. This high prevalence of vitamin D inadequacy aligns with recent studies reporting widespread vitamin D insufficiency or deficiency in Bangladesh [8,33-39]. For instance, Mahmood et al. (2017) found that 100% of garment workers and 97% of agricultural and construction workers had low vitamin D levels. Garment workers, in particular, are at high risk due to limited sun exposure and poor nutritional status [40].

Within urban participants, hospital staff had lower vitamin D levels and a higher prevalence of inadequacy compared to healthy volunteers. This finding is supported by multiple studies indicating a high prevalence of vitamin D inadequacy among health personnel [41-46]. The positive correlation between vitamin D and age in the urban population may be explained by higher supplement use among older individuals [43,47,48]. Vitamin D supplementation, as shown in various studies, significantly increases serum 25(OH)D levels [43,49].

Higher vitamin D levels in our reference population (coastal fishermen) compared to urban dwellers underscores the importance of sunlight exposure. Similar studies, such as those by Lee et al. [50] and Haddad and Chyu [51], also reported higher vitamin D levels in individuals with significant sun exposure. Despite this, according to the Endocrine Society's cut-off, around 70% of coastal fishermen in this study had low vitamin D levels, raising questions about the validity of the use of current threshold values. Previous studies in heavily sun-exposed regions in India, report similar findings of low vitamin D levels despite abundant sun exposure [52,53]. Thus, the reference intervals for vitamin D determined in this study using healthy coastal fishermen as reference population represent the optimal levels for the Bangladeshi population. This conclusion is based on the nature of the studied group and the limitations of previous definitions of "optimal" vitamin D status.

In this study, a reference interval of 15.88 ng/mL (90% CI: 14.26–16.92) to 45.27 ng/mL (90% CI: 41.11–51.85) was determined using non-parametric methods aligning with the Clinical and Laboratory Standards Institute (CLSI) Guidelines [54]. The non-parametric approach is appropriate given the asymmetry of the data, providing a robust understanding of the distribution in the fishermen population. The upper limit of 45.27 ng/mL aligns with the commonly accepted upper limit proposed by the Endocrine Society, suggesting the upper threshold of vitamin D status is consistent across various populations.

However, the lower limit of 15.88 ng/mL stands below the traditionally accepted threshold levels of 20-30 ng/mL. Other studies also proposed lower cut-off values for vitamin D sufficiency. Studies from South Korea [50], India [30], China [28], and Greece [29] have consistently shown lower thresholds of 13-20 ng/mL. The range in this study is also closer to those findings, implying that vitamin D requirements are influenced by both environmental and genetic factors, and that a universal cut-off point may not be applicable globally.

Moreover, iPTH dynamics provide additional support for our observed lower thresholds of vitamin D. Vitamin D inadequacy is often marked by increased iPTH levels, which serve as a surrogate marker for deficiency. However, in this study, no significant correlation between vitamin D and iPTH levels was found in the coastal fishermen group, unlike in the urban population, where a deflection point in iPTH occurred at levels far below the Endocrine Society's recommended level of 30 ng/mL. Studies involving other population have noted similar findings: iPTH rises as vitamin D level drops below 8 - 21.1ng/mL [26, 28 -30, 32, 55-58]. Recently, a study involving seven non-identical occupational groups, also reported significant increase in iPTH when vitamin D level dropped below <11.8ng/ml [59]. These studies suggest that a threshold for iPTH deflection, and thus vitamin D sufficiency, may indeed be lower than previously thought. The absence of iPTH deflection in our coastal fishermen is indicative of overall vitamin D adequacy in this population.

Additional support in favour of our low reference interval and lower cut-off values for vitamin D sufficiency comes from recent randomized controlled trials (RCTs) and cohort studies like VITAL and ViDA [60-62]. Post hoc analyses from trials like VITAL and ViDA revealed that vitamin D supplementation benefited participants only when baseline levels were below 12-15 ng/mL [60-62]. Also, Martineau et al. (2017) noted that vitamin D supplementation was most effective in preventing respiratory infections in participants with baseline levels below 10 ng/mL [63]. These findings argue against higher supplementation thresholds and support a more moderate cut-off for deficiency. Furthermore, based on epidemiological evidence, Manson et al. and the Institute of Medicine (IOM), support a deficiency threshold closer to 12.5 ng/mL[62,64] Studies on pregnancy and diabetes prevention also point toward 15 ng/mL as a sufficient level of vitamin D for avoiding adverse outcomes [31,65].

In view of the above, it is concluded that the reference interval identified in the current study (15.88–45.27 ng/mL) truly reflects the optimal range of vitamin D level for the adult Bangladeshi population. The high sun exposure in coastal fishermen makes this population an ideal reference group, better reflecting the biological requirements of individuals in sun-rich environments like Bangladesh. Moreover, the lower limit of this range is consistent with thresholds suggested by contemporary studies that challenge higher vitamin D cut-off points. Thus, considering the iPTH dynamics and recent trial data, the present study provides a more region-specific, evidence-backed approach regarding optimal vitamin D levels.

The strength of our study lies in the fact that the study used a defined population as 'reference population' with supposedly adequate vitamin D levels. Also, reference intervals were determined following the Clinical and Laboratory Standards Institute (CLSI) Guidelines C28-A3 for precise comparison across populations, and the lower cutoff value was identified using the iPTH deflection point. However, the study did not employ strict random sampling and gold-standard LC-MS/MS vitamin D assay.

## Conclusion

This study's findings on healthy coastal fishermen provide critical insights into the optimal serum vitamin D levels for adult Bangladeshi population. Additionally, the study has defined the lower normal limit of vitamin D sufficiency by determining the iPTH deflection point, suggesting a lower optimal range than currently used. Thus, the findings of the present study would help in guiding clinicians and policymakers in developing appropriate treatment and supplementation guidelines for Bangladeshi population.

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## Authors' contributions

WMMUH was the primary researcher, and mainly involved in data collection, analysis, and drafting of the manuscript. MFP and MAS contributed equally to the conception, design, interpretation of the data, and critical revision of the manuscript.

## Conflict of interest

The authors declare that they have no conflict of interest.

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## Data availability statement

The dataset of this study is available in the Zenodo repository, DOI: [10.5281/zenodo.13950605](https://doi.org/10.5281/zenodo.13950605). Additional materials, such as the technical appendix and statistical code, can also be accessed at this DOI.

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