

Vitamin D levels in seven non-identical occupational groups entail redefining of existing vitamin D deficiency diagnostic cut off level for native Bangladeshi population

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Abstract

Background and objectives: Recent publications have reported alarming prevalence of hypovitaminosis D in South Asian countries including Bangladesh. But, data on vitamin D levels in different occupational groups are lacking. This study addressed the prevalence of hypovitaminosis D in different occupational groups of Bangladesh. Additionally, the study estimated parathyroid hormone, phosphate, calcium and metabolic syndrome in these groups to see the effect of hypovitaminosis D on these parameters.

Materials and method: Seven diverse occupational groups (agrarian workers, rickshaw-pullers, young cricketers and footballers, fishermen, dry fish industry workers, garment-workers and medical students) of Bangladesh were selected based on grade of physical activity and level of sun exposure. Blood was collected for the estimation of 25(OH) vitamin D, fasting glucose, lipid profiles, calcium, phosphate, magnesium and intact parathyroid (iPTH) hormone. Multiple comparisons of these variables among the 7 groups were estimated by ANOVA.

Results: A total of 785 (m / f = 359 / 426) participants volunteered. Of them, 54.2% had vitamin D deficiency. Metabolic syndrome was 5% and showed no significant association with hypovitaminosis D ($\chi^2 = 0.9$, $p=0.43$). For biophysical characteristics, the mean (\pm SD) values of age, body mass index, waist to hip ratio and waist to height ratio were 33.8 ± 16.3 y, 22.3 ± 4.1 kg/m², 0.87 ± 0.06 and 0.39 ± 0.16 , respectively. The values for vitamin D (ng/ml), calcium (mg/dl), iPTH (pgm/ml) and phosphate (mg/dl) were 20.25 ± 13.1 , 9.57 ± 1.85 , 38.22 ± 24.54 and 4.18 ± 0.81 , respectively. The comparisons of vitamin D and other related variables among the groups (ANOVA) showed vitamin D level in the garments worker was significantly ($p<0.01$) higher from other 6 groups. Likewise, compared with other six, rickshaw-pullers had significantly higher calcium level. Calcium, phosphate and parathyroid hormone did not show any change with decreasing vitamin D level (high to low quartile: Q4 \rightarrow Q1), though parathyroid hormone increased significantly at the lowest vitamin D level (Q1: <11.8 ng/ml: $p=0.002$).

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Conclusion: The prevalence of hypovitaminosis D was high irrespective of occupations, site (rural/urban), social class and sun-exposure. Overall, vitamin D level was low though varied among the groups. Despite minimum and maximum sun-exposure, the garments workers had the highest and the fishermen had the lowest vitamin D levels, respectively. Calcium level was normal in all groups. Calcium, phosphate and parathyroid hormone did not show any changes with decreasing vitamin D, though parathyroid hormone increased significantly when vitamin D decreased to the lowest quartile. The findings indicate that the specific cut off value for vitamin D deficiency needs to be determined for population of a given geographic area.

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Introduction

Vitamin D deficiency (hypovitaminosis D) has become a pandemic with the concerned implications in both skeletal and extra-skeletal health. It is common in South Asian countries and Bangladesh ranks second in the prevalence of vitamin D deficiency. Around 67% of Bangladeshi adults were reported vitamin D deficient [1,2]. In Bangladesh, vitamin D deficiency is common in all age-groups and higher in females [1]. A recent study reported vitamin D deficiency was 71% among adequately sunlight exposed coastal fisherman of Bangladesh [3]. Despite abundant sunshine, the high prevalence of vitamin D deficiency is a mystery in a subtropical country like Bangladesh. Genetic factors affecting dermal synthesis and sun avoiding behavior may be an explanation [4]. Also, all these reported level of vitamin D at which deficiency has been defined is according to the cut off values recommended by the different international bodies.

Synthesis of vitamin D is affected by many factors including geographical location, season, environmental pollution, sunlight exposure time, exposed body surface and skin color [5]. Several factors including lack of knowledge, inadequate sunlight exposure and low intake of vitamin D rich food and disease conditions were identified as risk factors of vitamin D deficiency in Bangladeshi people [1]. Apart from these, different socio-demographic factors such as old age, female sex, low socio-economic status, urban residence and indoor occupation may be responsible for the low vitamin D level in our population [1,6,7].

The level of vitamin D at which deficiency has been defined is still a debatable issue. The optimal cut-off of vitamin D was determined by several factors

including suppression of parathyroid hormone, calcium absorption, markers of bone formation and resorption, bone mineral density, osteomalacia and rickets. The Institute of Medicine (IOM) proposed 20 ng/ml is an optimal cut off level for vitamin D deficiency [8]. Evaluation of bone markers also showed similar cut-off [9]. However, several societies suggest ≤ 30 ng/ml as an optimal level of risk for vitamin D deficiency [10,11]. The plateau of parathyroid hormone is reached at various Vit D levels. Some studies failed to find a relation between parathyroid hormone and vitamin D [9]. Similarly, two studies conducted among Bangladeshi adults found 15.2 ng/ml in female garment workers and 30.1 ng/ml in apparently healthy population, as the minimum Vitamin D level required for suppressing parathyroid hormone [12,13].

There are several studies on vitamin D with conflicting results, especially regarding the optimum level of vitamin D that is required to maintain bone health. Limited data are available regarding vitamin D status with its association with calcium and parathyroid hormone in different occupational groups of Bangladesh. Therefore, this study was undertaken to measure vitamin D, parathyroid hormone and calcium levels in different occupational groups of Bangladesh and to see the relation between them.

Materials and methods

The study was approved by the Institutional Ethical Review Board and conducted from May 2018 to July 2021.

Study design: Seven occupational groups / workers were selected. The selection was based on grading

of (a) physical activities ranging from sedentary to strenuous, and (b) exposure to sun from none to heavy. Thus, seven occupations considered were: *agrarian workers* and *rickshaw-pullers* (moderate sun-exposure with moderate to heavy physical activities, one was rural and the other was urban), *garment-workers*, and *medical students* (both sedentary and least sun-exposure and urban), *young cricketers* and *footballers* (YCF) from a training institute for athletics and sports (moderate to heavy sun-exposure and physical activities), *fishermen* and *dry fish industry workers (DFIW)* both groups had sun-exposure of 4 to 8 hours everyday with moderate to heavy physical activities.

For the *agrarian workers*, five villages were purposively selected in Nandail sub-district of Mymensingh district about 100 Km north-east of Dhaka city. *Garment-workers* and *rickshaw-pullers* were selected from Dhaka City. The *medical students* of Ibrahim medical college (IMC) in Dhaka City actively participated when they were briefed about the objectives of the protocol. The *young cricketers and footballers* (YCF) from Bangladesh Krira Shikkha Protistan (BKSP), an athlete and sports training institute in Dhaka volunteered. Likewise, the *fishermen* and *dry fish industry workers* agreed to volunteer when discussed with the fishermen's (*motsojibi*) union of the area.

The investigations included - a) socio-economic history, b) clinical history, c) anthropometry (height, weight) d) and estimation of 25-hydroxyvitamin D [25(OH)D] and other biochemical tests namely fasting blood glucose (FBG), lipid profiles, intact parathyroid hormone (iPTH), calcium, phosphate, magnesium, alkaline phosphatase as mentioned in the Figure-1. Algorithm of the study protocol is shown in Figure-1.

For each occupational group the willing participants were enlisted on the day before investigation and were informed about the objectives and procedural details of the study. They were advised to attend an investigation site at 8 AM in the next morning with an overnight fast.

On the investigation day, each participant was interviewed on socio-economic and clinical history. Height, weight and blood pressure were measured by standard procedures. Body mass index was calculated with the formula ($BMI = \text{weight in kg} \div \text{height in meter}^2$). About 5 ml of blood was collected aseptically from each participant. Collected blood samples were centrifuged and sera were separated in different aliquots, which were frozen locally and transported in coldbox to biochemistry laboratory for analysis.

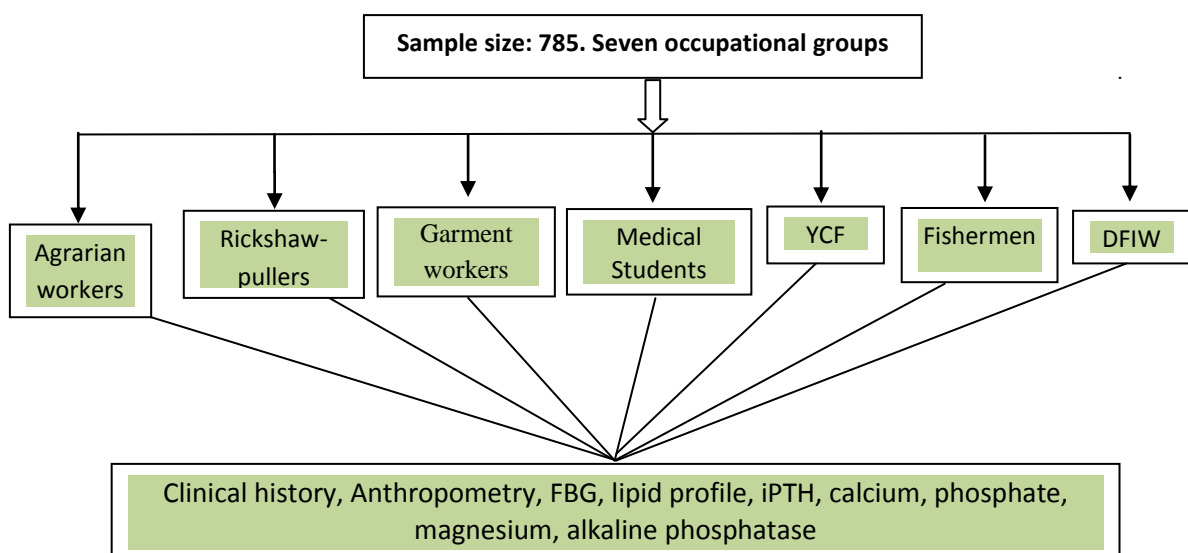


Figure-1: Algorithm of the study protocol. FBG: fasting blood glucose, iPTH: intact parathyroid hormone

The measurements of plasma glucose were done by glucose oxidase- peroxidase method using Technicon M-II auto analyzer. Lipids namely triglyceride, (TG), cholesterol (Chol), high density lipid (HDL) and low density lipid (LDL) were estimated by Hitachi-704 auto-analyzer using enzymatic method. LDL-cholesterol was measured using formula: LDL-C = 0.9 TC- (0.9 TG/5)-28 [14]. Serum 25-hydroxyvitamin D [25(OH)D] was measured by enzyme linked immunosorbent assay (ELISA). Serum iPTH, calcium, albumin, phosphate and magnesium were measured by chemiluminescent enzyme-labeled immunometric assay with Immulite 2000 systems Siemens, USA analyzer. Corrected calcium was calculated from fasting calcium and albumin by using correction formula {corrected calcium (mg/dl) = measured calcium (mg/dl) + 0.8 × (4 –measured albumin in gm/ dl)}.

Diagnostic criteria: Diagnostic cut-off for hypovitaminosis D (or vitamin D deficiency) was <20 ng/ml [8,9] and metabolic syndrome was a constellation of BMI >22.3, SBP >114mmHg, FBG >5.5 mmol/l and TG >165 mg/dl [15].

Statistical analysis: The biophysical characteristics of the seven occupational groups were depicted in mean with standard deviation (SD) and 95% confidence interval (CI). The prevalence rates of vitamin D deficiency of the seven study groups by sex were given in percentages. The characteristics of participants were compared between with and without vitamin D deficiency (vitamin D<20 vs. ≥20 ng/ml) and were estimated by unpaired t-test. Multiple comparisons of variables among different groups were estimated by ANOVA with Scheffe’s Post hoc test.

Results

A total of 785 (m / f = 359 / 426) individuals were enrolled in the study (Table-1a). Of the total 785 participants, 424 (54%) had vitamin D deficiency. Compared to males, females had significantly higher prevalence of hypovitaminosis D (m / f = 43.7% / 62.8%, $\chi^2 = 28.1, p<0.001$). The overall prevalence of metabolic syndrome (MetS) was 5% and not related to hypovitaminosis D ($\chi^2 = 0.9, p=0.43$ NS; Table 1b). Prevalence of metabolic syndrome was highest in fishermen (12.2%) and

lowest in DFI workers [0%; (Table-1c)]. Of them, 45% were non-affluent and 40% were illiterate (data not shown). Regular sun-exposure was found in 41.5%. Agrarian workers, fishermen and young cricketers/footballers had the highest rates of frequent and regular sun exposure.

Table-1a: Prevalence of hypovitaminosis D (Vitamin D <20ng/ml) by gender of the participants (N=785)

Gender	Number	Level	
		<20 ng/ml N (%)	≥20 ng/ml N (%)
Male	359	157 (43.7)	202 (56.3)
Female	426	267 (62.7)	159 (37.3)
Total	785	424 (54)	361 (46)

Table-1b: Prevalence of metabolic syndrome among the study population having normal (≥20 ng/ml) and deficient (<20ng/ml) vitamin D levels

Vitamin D status	Number	Metabolic syndrome present	
		Yes N (%)	No N (%)
Hypovitaminosis D (<20 ng/ml)	424	24 (5.7)	400 (94.3)
Normovitaminosis D (≥20 ng/ml)	361	15 (4.2)	346 (95.8)
Total	785	39 (5.0)	746 (95.0)

Note: Chi-sq=0.9, p =0.43.

Table-1c: Prevalence of metabolic syndrome by occupations (N=785)

Study groups	Number	Metabolic syndrome present	
		Yes N (%)	No N (%)
Agrarian workers	152	12 (7.9)	140 (92.1)
Garment workers	161	8 (5.0)	153 (95.0)
Rickshaw-pullers	80	5 (6.3)	75 (93.8)
Medical students	96	3 (3.1)	93 (96.9)
(YCF)	133	2 (1.5)	131 (98.5)
Fishermen	74	9 (12.2)	65 (87.8)
DFIW	89	0 (0.0)	89 (100.0)
Total	785	39 (5.0)	746 (95.0)

Note: YCF: Young cricketers / footballers; DFIW: Dry-fish industry workers

The characteristics of the study participants are shown in Table 2a, 2b, 2c and 2d. The mean values (\pm SD) and 95% CI of age, BMI, waist to hip ratio (WHR), waist to height ratio (WHtR) are shown in Table-2a; systolic and diastolic blood pressure (SBP, DBP) and FBG in Table-2b, lipids in Table-2c and vitamin D, iPTH, calcium, ALP, Mg in Table-2d. The mean (\pm SD) of age, BMI, WHR, SBP, FBG, Chol and

HDL were 33.8 (\pm 16.3)y, 22.3 (\pm 4.1) kg/m², 0.87 (\pm 0.06), 113.6 (\pm 18.2) mmHg, 5.5 (\pm 1.7) mmol/L, 158 (\pm 43.8) mg/dl and 49.1(\pm 8.5) mg/dl, respectively. The mean (\pm SD) of vitamin D was 20.25 (\pm 13.1) ng/ml and iPTH was 38.22 (\pm 24.5) pg/ml), calcium 9.57 (\pm 1.85) mg/dl), phosphate 4.18 (\pm 0.81) mg/dl and magnesium 1.82 (\pm 0.88) mg/dl.

Table-2a: Mean (\pm SD and 95% CI) values of biophysical characteristics (age, BMI, WHR and WHtR) of the seven occupational groups.

Variables	Occupational groups	N	Mean	SD	95% CI
Age (y)	Agrarian workers	152	47.3	15.7	45.0 – 49.7
	Garments workers	161	27.9	9.2	26.5 – 29.4
	Rickshaw-puller	80	35.8	13.6	32.8 – 38.8
	Medical students	96	24.1	5.9	22.9 – 25.2
	Young cricketers/ footballers	133	15.9	2.5	15.5 – 16.4
	Fishermen	74	46.2	14.9	43.4 – 49.0
	Dry fish industry workers (DIW)	89	41.8	13.5	39.0 – 44.6
	Total		785	33.8	16.3
BMI (kg/m ²)	Agrarian workers	152	20.9	3.9	20.3 – 21.5
	Garments workers	161	23.5	3.6	23.0 – 24.1
	Rickshaw-puller	80	19.0	3.3	18.2 – 19.7
	Medical students	96	25.5	4.5	24.6 – 26.4
	Young cricketers/ footballers	133	21.0	2.3	20.6 – 21.4
	Fishermen	74	24.0	4.7	22.9 – 25.1
	Dry fish industry workers (DIW)	89	22.4	3.6	21.7 – 23.2
	Total		785	22.3	4.1
WHR	Agrarian workers	152	0.88	0.06	0.87 – 0.89
	Garments workers	161	0.88	0.06	0.87 – 0.89
	Rickshaw-puller	80	0.86	0.06	0.85 – 0.87
	Medical students	96	0.85	0.06	0.84 – 0.86
	Young cricketers/ footballers	133	0.88	0.04	0.87 – 0.88
	Fishermen	74	0.87	0.05	0.86 – 0.89
	Dry fish industry workers (DIW)	89	0.88	0.05	0.87 – 0.89
	Total		785	0.87	0.06
WHtR	Agrarian workers	152	0.50	0.06	0.49 – 0.51
	Garments workers	161	0.52	0.06	0.51 – 0.53
	Rickshaw-puller	80	0.46	0.06	0.45 – 0.48
	Medical students	96	0.53	0.06	0.51 – 0.54
	Young cricketers/ footballers	133	0.19	0.01	0.18 – 0.19
	Fishermen	74	0.21	0.03	0.20 – 0.22
	Dry fish industry workers (DIW)	89	0.20	0.03	0.20 – 0.21
	Total		785	0.39	0.16

Note: BMI – body mass index (weight in kg / height in msq), WHR: waist to hip ratio, WHtR: waist to height ratio, SD – standard deviation

Table-2b: Mean (\pm SD and 95% CI) values of biophysical and biochemical characteristics (SBP, DBP and FBG) of the seven occupational groups.

Variables	Occupational groups	N	Mean	SD	95% CI
SBP (mmHg)	Agrarian workers	152	117.3	19.4	114.3 – 120.3
	Garments workers	161	107.1	18.5	104.3 – 109.9
	Rickshaw-puller	80	115.1	15.2	111.8 – 118.5
	Medical students	96	114.0	11.4	111.6 – 116.3
	Young cricketers/ footballers	133	105.5	11.2	103.6 – 107.4
	Fishermen	74	123.0	21.5	119.0 – 127.0
	Dry fish industry workers (DIW)	89	117.6	18.7	113.8 – 121.4
	Total		785	113.6	18.2
DBP (mmHg)	Agrarian workers	152	69.7	12.1	67.9 – 71.6
	Garments workers	161	70.7	11.6	68.9 – 72.5
	Rickshaw-puller	80	69.0	8.5	67.1 – 70.9
	Medical students	96	74.0	8.8	72.2 – 75.8
	Young cricketers/ footballers	133	66.4	9.6	64.8 – 68.1
	Fishermen	74	81.9	12.1	79.6 – 84.2
	Dry fish industry workers (DIW)	89	77.3	12.3	74.8 – 79.8
	Total		785	72.2	12.0
FBG (mmol/L)	Agrarian workers	152	5.4	2.7	4.9 – 5.8
	Garments workers	161	5.9	1.1	5.7 – 6.1
	Rickshaw-puller	80	5.6	1.8	5.2 – 6.0
	Medical students	96	4.9	0.7	4.7 – 5.0
	Young cricketers/ footballers	133	5.5	0.4	5.4 – 5.5
	Fishermen	74	6.0	1.3	5.7 – 6.3
	Dry fish industry workers (DIW)	89	6.1	3.0	4.5 – 7.7
	Total		785	5.5	1.7

Note: SBP / DBP – systolic / diastolic blood pressure, FBG – fasting blood glucose.

Table-2c: Mean (\pm SD and 95% CI) values of biochemical characteristics (chol, HDL, LDL and TG) of the seven occupational groups.

Variables	Occupational groups	N	Mean	SD	95% CI
CHOL (mg/dl)	Agrarian workers	152	173.1	47.7	165.8 – 180.3
	Garments workers	161	156.0	36.4	150.5 – 161.6
	Rickshaw-puller	80	124.5	37.1	116.3 – 132.7
	Medical students	96	144.2	29.7	138.3 – 150.1
	Young cricketers/ footballers	133	152.2	34.2	146.7 – 157.7
	Fishermen	74	188.1	54.1	178.0 – 198.3
	Dry fish industry workers (DIW)	89	156.9	36.2	149.5 – 164.3
	Total		785	158.6	43.8
HDL (mg/dl)	Agrarian workers	152	49.6	8.8	48.3 – 50.9
	Garments workers	161	49.6	3.1	49.1 – 50.1
	Rickshaw-puller	80	41.8	14.2	38.7 – 45.0
	Medical students	96	49.7	5.1	48.6 – 50.7
	Young cricketers/ footballers	133	51.2	7.1	50.1 – 52.4
	Fishermen	74	52.6	8.4	51.0 – 54.1
	Dry fish industry workers (DIW)	89	45.2	8.6	43.5 – 47.0
	Total		785	49.1	8.5
LDL (mg/dl)	Agrarian workers	152	87.8	42.8	81.3 – 94.3
	Garments workers	161	78.5	30.2	73.9 – 83.1
	Rickshaw-puller	80	46.0	30.1	39.4 – 52.6
	Medical students	96	61.0	26.1	55.8 – 66.2
	Young cricketers/ footballers	133	74.7	30.7	69.7 – 79.7
	Fishermen	74	92.4	54.1	82.2 – 102.5
	Dry fish industry workers (DIW)	89	77.8	37.7	70.1 – 85.5
	Total		785	76.3	39.3
TG (mg/dl)	Agrarian workers	152	172.3	50.0	164.8 – 179.9
	Garments workers	161	138.1	32.1	133.2 – 143.0
	Rickshaw-puller	80	177.4	89.9	157.6 – 197.2
	Medical students	96	164.6	75.8	149.5 – 179.6
	Young cricketers/ footballers	133	134.8	29.9	129.9 – 139.6
	Fishermen	74	224.2	83.3	208.6 – 239.8
	Dry fish industry workers (DIW)	89	167.5	49.7	157.3 – 177.7
	Total		785	165.1	64.4

Note: Chol – total cholesterol, HDL / LDL – high / low density lipoproteins, TG – triglycerides.

Table-2d: Mean (\pm SD and 95% CI) values of Vitamin D, serum calcium, iPTH, phosphate, ALP and magnesium level of the seven occupational groups.

Variables	Occupational groups	N	Mean	SD	95% CI
Vitamin D (ng/ml)	Agrarian workers	152	22.83	14.3	20.70 – 25.05
	Garments workers	161	28.47	15.4	26.12 – 30.81
	Rickshaw-puller	80	20.06	5.6	18.81 – 21.31
	Medical students	96	18.90	17.1	15.50 – 22.30
	Young cricketers/ footballers	133	17.21	6.3	16.18 – 18.24
	Fishermen	74	13.69	7.8	12.21 – 15.17
	Dry fish industry workers (DIW)	89	14.88	9.8	12.85 – 16.90
	Total	785	20.25	13.1	19.38 – 21.11
S. calcium (mg/dl)	Agrarian workers	152	8.90	1.20	8.71 – 9.09
	Garments workers	161	9.49	1.52	9.25 – 9.72
	Rickshaw-puller	80	11.41	1.89	11.00 – 11.83
	Medical students	96	8.87	0.77	8.72 – 9.03
	Young cricketers/ footballers	133	9.73	1.54	9.48 – 9.98
	Fishermen	74	9.80	2.58	9.32 – 10.29
	Dry fish industry workers (DIW)	89	9.39	2.30	8.92 – 9.86
	Total	785	9.57	1.85	9.44 – 9.69
iPTH (picogram/ml)	Agrarian workers	24	33.85	11.39	29.04 – 38.66
	¥ Garments workers	0	.	.	.
	Rickshaw-puller	80	30.78	28.40	24.46 – 37.10
	Medical students	96	45.23	21.49	40.97 – 49.50
	¥ Young cricketers/ footballers	0	.	.	.
	¥ Fishermen	0	.	.	.
	¥ Dry fish industry workers (DIW)	0	.	.	.
	Total	200	38.22	24.54	34.84 – 41.61
Phosphate (mg/dl)	¥ Agrarian workers	0	.	.	.
	¥ Garments workers	0	.	.	.
	¥ Rickshaw-puller	0	.	.	.
	¥ Medical students	0	.	.	.
	Young cricketers/ footballers	133	4.11	0.83	3.97 – 4.24
	Fishermen	74	4.17	0.80	4.02 – 4.33
	Dry fish industry workers (DIW)	89	4.28	0.76	4.12 – 4.44
	Total	296	4.18	0.81	4.09 – 4.26
Alk_phos (IU/L)	Agrarian workers	152	146.0	39.8	139.8 – 152.3
	Garments workers	161	142.0	76.1	130.1 – 153.8
	Rickshaw-puller	80	147.4	97.3	126.0 – 168.8
	Medical students	96	203.6	56.1	192.5 – 214.8
	¥ Young cricketers/ footballers	0	.	.	.
	¥ Fishermen	0	.	.	.
	¥ Dry fish industry workers (DIW)	0	.	.	.
	Total	589	156.51	71.2	150.2 – 162.7
Magnesium (mg/dl)	¥ Agrarian workers	0	.	.	.
	¥ Garments workers	0	.	.	.
	¥ Rickshaw-puller	0	.	.	.
	¥ Medical students	0	.	.	.
	Young cricketsr/ footballers	133	1.71	1.07	1.54 – 1.88
	Fishermen	74	2.26	0.66	2.13 – 2.38
	Dry fish industry workers (DIW)	89	1.47	0.52	1.36 – 1.57
	Total	296	1.82	0.88	1.72 – 1.91

Note: ¥ - not done.

The prevalence of hypovitaminosis D (<20ng/ml) according to occupational groups is shown in Table-3a. Regarding occupation, highest prevalence of hypovitaminosis D was found in DFIW (77%) followed by medical students (72.9%), fishermen (71.6%), YCF (69.9%), rickshaw-puller (42.5%) and lowest in garment workers (23.0%).

Table-3a: Prevalence of hypovitaminosis D (vitamin D <20ng/ml) according to occupational groups

Study groups	Vitamin D level		Total
	<20 ng/ml N (%)	≥20 ng/ml N(%)	
Agrarian workers	70 (46.1)	82 (53.9)	152
Garments workers	37 (23.0)	124 (77.0)	161
Rickshaw-puller	34 (42.5)	46 (57.5)	80
Medical students	70 (72.9)	26 (27.1)	96
Young cricketers/ footballers	93 (69.9)	40 (30.1)	133
Fishermen	53 (71.6)	21 (28.4)	74
Dry fish industry workers (DFIW)	67 (75.3)	22 (24.7)	89
Total	424 (54.2)	361 (45.8)	785

Note: Highest to lowest prevalence of hypovitaminosis D (Vitamin D<20ng/ml) were DFIW (77%)> medical students (72.9%)> Fishermen (71.6%) > YCF (69.9%)>rickshaw-puller (42.5%)>garments workers (23.0%)

Table-3b: The prevalence of vitamin D deficiency (<20ng/dl) according to gender among different occupational groups

Occupations	Vitamin D Level Hypo vs normal <20 vs. ≥20 ng/ml	Sex		Total
		Male	Female	
		N (%)	N (%)	
Agrarians workers	<20 ng/ml	18 (25.70)	52 (74.3)	70
	≥20 ng/ml	37 (45.1)	45 (54.9)	82
	Total	55 (36.2)	97 (63.8)	152
Garments workers	<20 ng/ml	6 (16.2)	31 (83.8)	37
	≥20 ng/ml	51 (41.1)	73 (58.9)	124
	Total	57 (35.4)	104 (64.6)	161
Rickshaw-puller¥	<20 ng/ml	34 (42.5)	-	34
	≥20 ng/ml	46 (57.5)	-	46
	Total	80 (100.0)	-	80
Medical students	<20 ng/ml	31 (44.3)	39 (55.7)	70
	≥20 ng/ml	7 (26.9)	19 (73.1)	26
	Total	38 (39.6)	58 (60.4)	96
Young cricketers/ footballers	<20 ng/ml	27 (29.0)	66 (71.0)	93
	≥20 ng/ml	27 (67.5)	13 (32.5)	40
	Total	54 (40.6)	79 (59.4)	133
Fishermen	<20ng/ml	20 (37.7)	33 (62.3)	53
	≥20 ng/ml	19 (90.5)	2 (9.5)	21
	Total	39 (52.7)	35 (47.3)	74
Dry fish industry workers (DIW)	<20 ng/ml	21 (31.3)	46 (68.7)	67
	≥20 ng/ml	14 (63.6)	8 (36.4)	22
	Total	35 (40.2)	52 (59.8)	89
Total	<20 ng/ml	157 (37.0)	267 (63.0)	424
	≥20 ng/ml	202 (56.0)	159 (44.0)	359
	Total	359 (45.7)	426 (54.3)	785

Note: ¥ - no female participants; Bold figures indicate deficiency and parenthesis indicate prevalence in percentages.

Table-3b depicts prevalence of hypovitaminosis D (<20ng/ml) among the male and female of different occupational groups. Prevalence of hypovitaminosis D was significantly ($p < 0.05$) high among the females compared to males of all occupational groups except medical students (male vs. female: 44.3% vs. 55.7%).

Table-4a: Multiple comparisons of means of vitamin D levels among the seven occupational groups using One-way ANOVA: Post hoc Scheffe tests. The occupational group [‘I’] is compared with the others [‘J’]

Dependent Variable –Vit D			
[I] source	[J] source	Mean Difference (I-J)	Sig.
Agrarian workers	Garments	-6.14*	.002
	Rickshaw-puller	2.42	.905
	Medical students	5.11	.100
	Young cricketers/ footballers (YCF)	5.69*	.014
	Fishermen	7.92*	.002
	Dry fish industry workers (DFIW)	7.50*	.002
Garments workers	Agrarian workers	6.14*	.002
	Rickshaw-puller	8.57*	.000
	Medical students	11.25*	.000
	Young cricketers/ footballers	11.84*	.000
	Fishermen	14.07*	.000
	Dry fish industry workers (DFIW)	13.65*	.000
Rickshaw-pullers	Agrarian workers	-2.42	.905
	Garments workers	-8.57*	.000
	Medical students	2.68	.902
	Young cricketers/ footballers	3.26	.716
	Fishermen	5.49	.235
	Dry fish industry workers (DFIW)	5.07	.282
Medical students	Agrarian workers	-5.11	.100
	Garments workers	-11.25*	.000
	Rickshaw-puller	-2.68	.902
	Young crickets/ footballers	0.58	1.000
	Fishermen	2.81	.890
	Dry fish industry workers (DFIW)	2.39	.936
Young cricketers/footballers	Agrarian workers	-5.69*	.014
	Garments workers	-11.84*	.000
	Medical students	-3.26	.716
	Medical students	-.58	1.000
	Fishermen	2.22	.950
	Dry fish industry workers (DFIW)	1.80	.977
Fishermen	Agrarian workers	-7.92*	.002
	Garments workers	-14.07*	.000
	Rickshaw-puller	-5.49	.235
	Medical students	-2.81	.890
	Young cricketers/footballers	-2.22	.950
	Dry fish industry workers (DFIW)	-0.42	1.000

Note: * -indicates significant differences between occupational groups between source (I) and source (J). The sign (-) indicates I source lower than J source. For example, the agrarians workers (I) had significantly lower Vit-D level than Garment workers (J), ($p = 0.002$); whereas significantly higher than YCF ($p = 0.014$), fishermen and DFIW (both $p = 0.002$)

Multiple comparisons of mean (\pm SD) of vitamin D and calcium levels among the seven occupational groups were estimated by One-Way ANOVA and Post-Hoc tests (Table-4a and 4b). The observed estimated figures are self-explanatory. The source ‘I’ denotes an occupation to which other six occupations ‘J’ are

Table-4b: Multiple Comparisons of vitamin D and serum calcium levels among the seven occupational groups using One-way ANOVA: Post hoc Scheffe tests. The occupational group [‘I’] is compared with the others [‘J’]

Dependent Variable - calcium			
[I]source	[J] source	Mean Difference (I-J)	Sig.
Agrarian workers	Garments workers	-0.64	.086
	Rickshaw-puller	-2.57*	.000
	Medical students	-0.02	1.000
	Young cricketers/ footballers	-0.88*	.003
	Fishermen	-0.22	.988
	Fish drying industrial labor	-0.66	.191
Garments workers	Agrarian workers	0.64	.086
	Rickshaw-puller	-1.93*	.000
	Medical students	0.61	.224
	Young cricketers/ footballers	-0.24	.952
	Fishermen	0.41	.792
	Fish drying industrial labor	-0.02	1.000
Rickshaw-puller	Agrarian workers	2.57*	.000
	Garments workers	1.93*	.000
	Medical students	2.54*	.000
	Young cricketers/ footballers	1.68*	.000
	Fishermen	2.34*	.000
	Fish drying industrial labor	1.90*	.000
Medical students	Agrarian workers	0.02	1.000
	Garments workers	-0.61	.224
	Rickshaw-puller	-2.54*	.000
	Young cricketers/ footballers	-0.86*	.019
	Fishermen	-0.20	.996
	Fish drying industrial labor	-0.63	.339
Young cricketers/ footballers	Agrarian workers	0.88*	.003
	Garments workers	0.24	.952
	Rickshaw-puller	-1.68*	.000
	Medical students	0.86*	.019
	Fishermen	0.65	.269
	Fish drying industrial labor	0.22	.987
Fishermen	Agrarian workers	0.22	.988
	Garments workers	-0.41	.792
	Rickshaw-puller	-2.34*	.000
	Medical students	0.20	.996
	Young cricketers/ footballers	-0.65	.269
	Fish drying industrial labor	-0.43	.834
Fish drying industrial labor	Agrarian workers	0.66	.191
	Garments workers	0.02	1.000
	Rickshaw-puller	-1.90*	.000
	Medical students	0.63	.339
	Young cricketers/ footballers	-0.22	.987
	Fishermen	0.43	.834

* - indicates significance of the differences between occupational groups between source (I) and source (J). The sign (-) indicates I source lower than J source. The mean difference is significant at the 0.05 level.

compared. As shown in Table 4a, the agrarian workers (I) had significantly lower vitamin D level than garment workers (J), ($p = 0.002$); whereas significantly higher than YCF ($p=0.014$), fishermen and DFIW (both $p = 0.002$).

Figure-2 shows the comparisons of vitamin D related variables (calcium and iPTH) among the three occupational groups. Vitamin D and iPTH varied strikingly but calcium did not, rather maintained a consistent level.

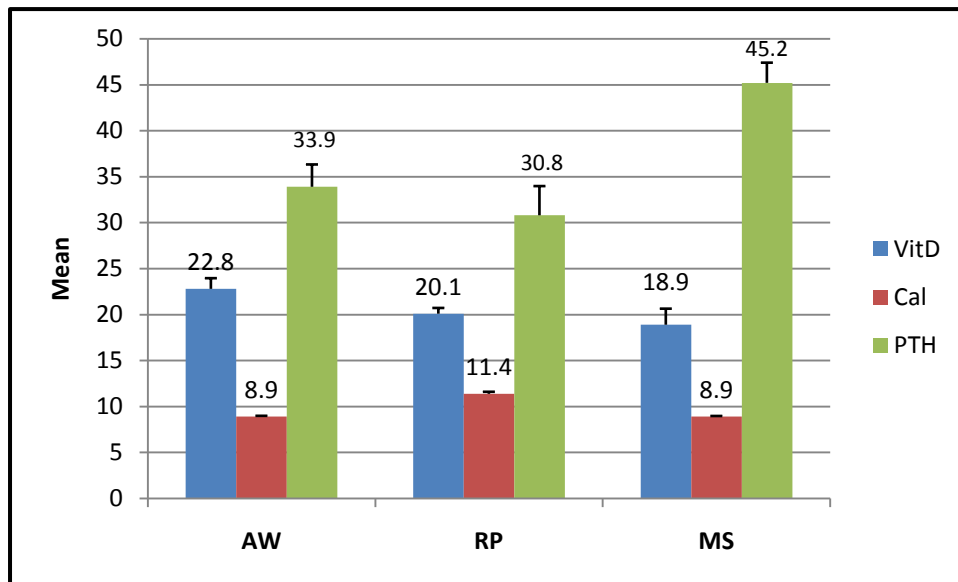


Figure-2: Comparative mean (\pm SE) values of vitamin D (Vit D), calcium (Cal) and parathyroid hormone (iPTH) of agrarian workers (AW), rickshaw-pullers (RP) and medical students (MS).

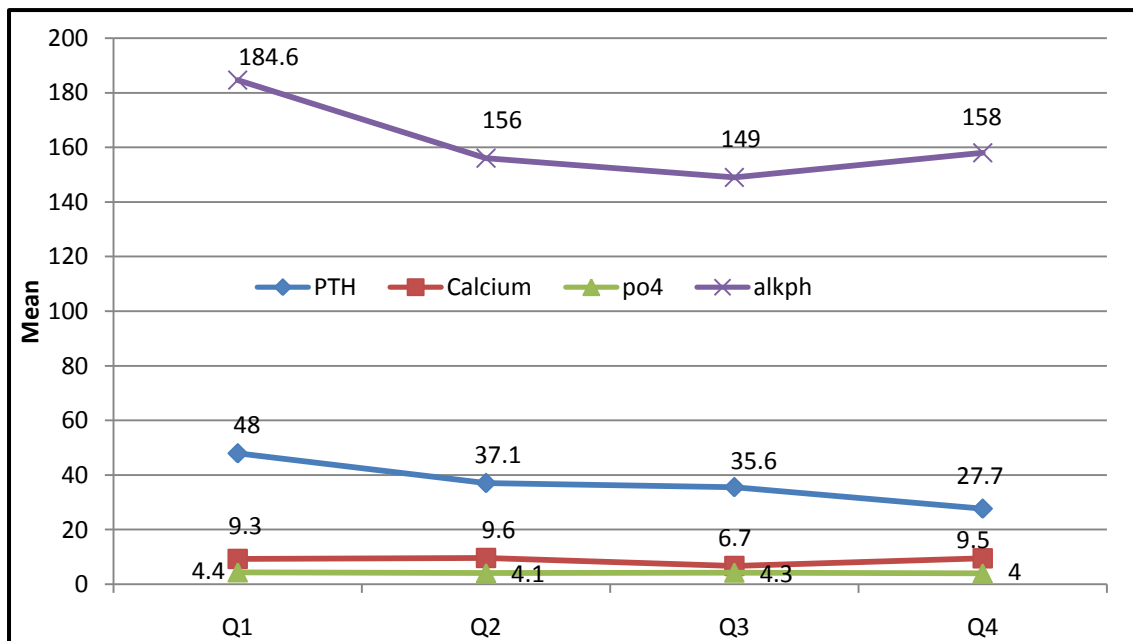


Figure-3: The mean values of iPTH, calcium, phosphate and alkaline phosphatase are shown according to quartiles of vitamin D (Q1<11.8, Q2 11.8 – 17.9, Q3 18 – 24.9 and Q4>25.0 ng/ml), estimated by ANOVA.

A line graph (Figure-3) was constructed according to quartiles of vitamin D (Q1 - 4) to determine whether the mean values of iPTH, calcium, phosphate and alkaline phosphatase show any variation with increasing quartile of vitamin D levels and estimated by ANOVA. Serum calcium and phosphate showed no change with the changed vitamin D levels. Only iPTH showed significant difference between Q1 and Q4 of Vitamin D (48.0 vs. 27.7 ng/ml, $p=0.002$), higher being in the lowest than in the highest quartile. PTH showed significant increase when vitamin D decreased extremely (<11.8 ng/ml: $p=0.002$). There was a significant weak negative correlation between vitamin D and iPTH. Simple linear regression with iPTH as dependent variable showed a significant association with vitamin D ($\beta=-0.608$, $p<0.001$, 95% CI -0.902 to -0.295, $R^2=7.1$). When vitamin D decreased by 1 ng/ml, iPTH increased by 0.608 pg/ml.

Discussion

This study was unique considering the inclusions of several occupations that are distinctively different from one another, each with their own entity and characteristics. For example, at one hand there was the non-affluent rickshaw-pullers who were urban dwellers and heavily exposed to the sun doing strenuous physical activities (BMI=19.0); and on the other hand there was the affluent medical students who were also urban, but rarely exposed to the sun and doing minimum physical activities (BMI=25.5). Thus, each group differed from the other with respect to site (urban/ rural), social class (affluent/non-affluent), grading of sun-exposure (maximum/moderate/minimum) and physical activity (strenuous/moderate/sedentary). The observed biophysical characteristics of different groups (Table 2a-2d) also proved such differences.

There was a high rate of vitamin D deficiency in the study population, with no association with metabolic syndrome. Surprisingly, the highest rate of vitamin D deficiency was seen in occupations with maximum sun exposure. Despite the low levels of vitamin D, iPTH, calcium and phosphate were in the normal range. There was a weak inverse relation between vitamin D and iPTH, which became more apparent below a vitamin D level of

11 ng/ml. Several studies opined in favor of "association between hypovitaminosis D and the metabolic syndrome, its component factors, cardiovascular disease (CVD) and mortality" [16,17]. However, we found no association between vitamin D deficiency and metabolic syndrome (Table-1b), nor was there any correlation with component factors (correlation matrix not shown).

Overall, more than half of the participants had hypovitaminosis D, which is consistent with other South Asian studies [1,-3,7,12,18]. Some unexpected findings were encountered. It is expected that individuals with maximum sun-exposed occupations should have the lowest prevalence of vitamin D deficiency. On the contrary, the garment workers who had minimum sun-exposure had the lowest prevalence (23%) of vitamin D deficiency compared to maximum sun exposed (≥ 8 h/d) occupations – DFIW (77%) and fishermen (71%) (Table3a). Furthermore, the least sun-exposed (garments workers) had the highest vitamin D level, which differed significantly from other groups (Table -4a). Vitamin D level depends on genetic, epigenetic and environmental factors [4]. As the population belonged to low socioeconomic class, poor nutrition may have contributed to the low levels. Bangladesh is a tropical country with abundant sunlight (23.6850° N, 90.3563° E). Lowest level of vitamin D (13.7 ± 7.8 ng/ml) was observed in fishermen despite abundant sun exposure. A study in Hawaii also showed that 51% of the population with mean sun exposure of 28.9 hours/week had vitamin D deficiency [18]. Sun exposure does not increase vitamin D above 60 ng/ml. Authors believe that the skin may restrict production of vitamin D in response to excess sun exposure [19]. Possible mechanisms include decreased production, enhanced breakdown, decreased transport of vitamin D in the skin and increased melanin production [18]. In addition to environmental factors, there is an influence of genetic polymorphism on serum 25(OH)D and 1,25(OH) vitamin D levels. Several steps of vitamin D metabolism are under genetic control [4]. Although the genetic influence of vitamin D is still poorly understood, family studies in different populations have found that genetic factors contribute to 70%

of the variation in serum vitamin D level [20]. Genetic polymorphisms arising from evolutionary responses to the environment may explain different levels of vitamin D in different populations.

The other uncommon finding – despite low vitamin D level, serum iPTH, calcium and phosphate levels were in the normal range. Usually, iPTH maintains inverse association with vitamin D. In this study, inverse relation was found only at extremely low vitamin D level (<11.8ng/ml), when iPTH increased significantly [Figure-3]. We found iPTH did not increase with decreasing vitamin D till it reached <11.8ng/ml, after which iPTH increased significantly. Possibly, this rise was inevitable to maintain dynamic calcium-phosphate homeostasis. There are many studies looking at the relationship between vitamin D and parathyroid hormone. However, they had controversial results. Not all studies found a definite level of vitamin D at which iPTH level increased [9]. However, some of them demonstrated that iPTH reached a plateau below a vitamin D of 30 ng/ml [5,21]. The threshold of vitamin D below which markers of bone resorption and formation start to increase was only 18 ng/dl [9]. A study on 200 young Bangladeshi female garments workers found that iPTH level increased below a vitamin D cut off of 15.22 ng/ml [12]. Another similar study in 130 healthy Bangladeshi adults with a mean age of 37 years showed a cut off of 27.55 ng/ml [13]. Reciprocal association between vitamin D and iPTH may not be simple. Some unexplored determinants might influence calcium-phosphate-magnesium homeostasis. Phosphate homeostasis is under direct influence of calcitriol, iPTH, and phosphatonins, including fibroblast growth factor 23 (FGF-23). Receptors of vitamin D, FGF-23, iPTH, and calcium-sensing receptor (CaSR) also play an important role in phosphate homeostasis [17,22]. It is now clear that there is interplay of FGF-23, Klotho and parathyroid hormone on the calcium and phosphate homeostasis [23,24].

Regarding limitations of the study, we could not ascertain drug history (anyone taking vitamin D or other micronutrients), dietary habits (fat-deficient), steatorrhea and presence of inflammatory bowel syndrome (IBS). Had there been dual energy X-ray

absorptiometry (DEXA scan) we could have shown association between vitamin D deficiency and osteopenia. We could not also investigate iPTH, phosphate, and magnesium for all participants.

Conclusions

We conclude that the prevalence of hypovitaminosis D in the study population was high and was not related to metabolic syndrome (obesity, hyperglycemia, hypertension, dyslipidemia). It was also revealed that sun-exposure had insignificant effect on vitamin D level. Calcium and phosphate showed no association with vitamin D. Also, parathyroid hormone and vitamin D levels showed no significant association except at the lowest quartile of the vitamin D level. Despite very low vitamin D level, the participants were found physically active and mentally healthy with respect to their occupations. We may assume 'hypovitaminosis D' is not the only player in maintaining electrolytes and health. So, our findings reasonably demand a careful evaluation of the existing cut-offs values for hypovitaminosis D based on and including other regulatory substances or secretions namely FGF-23, Klotho, osteocalcin and phosphatonins.

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Author's contributions

TH: designing of the study, wrote the manuscript and analyzed the data; MSM: wrote the introduction of the manuscript; NT, MM, HM, AB, MMHC, KNH, MMT: involved in designing of the study, data collection, organization, computing, editing, and assisting reviewing literatures and laboratory assay; MAS: involved in designing of the study, data collection, data analysis and editing manuscript,

Competing interest

The authors declare no conflict of interest.

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