

Correlation of Serum Vitamin D and Calcium Levels between Outdoor and Indoor Working Professionals in Dhaka City, Bangladesh

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Abstract

A cross-sectional study was conducted in the Department of Biochemistry, Dhaka Medical College, Dhaka, Bangladesh, from July 2018 to June 2019, to compare serum vitamin D levels and observe its correlation with serum calcium levels among the outdoor and indoor working professionals. A total of 101 indoor and outdoor workers of different sectors living in Dhaka city participated in this study. All study subjects were divided into two groups: outdoor workers were in group A (n=35) and indoor workers were in group B (n=66). Estimation of serum vitamin D₃ was done by chemiluminescent microparticle immunoassay, while serum calcium was measured using Arsenazo-III dye method. The mean age of the study participants was 38.72±6.88 years, ranging between 25 and 52 years. Among the study participants, 64(63.4%) were male and 37(36.6%) were female. Among outdoor workers, most were from Traffic Police Services (83%) and the rest were street hawkers (17%). Indoor workers were recruited from the hospital – nurses (32%), doctors (27%), administrative employees (21%), hospital ward assistants (12%) and medical technicians (8%). Serum vitamin D₃ level was found higher in outdoor workers than that of indoor workers (19.35±5.67 ng/ml vs. 14.17±4.18 ng/ml) and the difference was statistically significant (P<0.001). Serum calcium level was lower in outdoor workers than that of indoor workers (8.82±0.54 mg/dl vs. 9.08±1.08 mg/dl); however, the difference was not statistically significant (P>0.05). Moreover, no significant correlation was observed between serum vitamin D and calcium levels in both outdoor (r= - 0.117; P=0.510) and indoor (r= - 0.163; P=0.196) groups.

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Introduction

Vitamin D is an important nutrient for skeleton growth and bone health. It is obtained from the diet or supplements and also produced in the skin upon sun exposure. However, both children and adults are vulnerable to vitamin D deficiency.¹ Vitamin D plays a key role in maintaining calcium and phosphate homeostasis in the body. It helps to maintain serum calcium levels within its normal limits by acting on various target organs like intestine, kidneys, bones and parathyroids.^{1,2} It increases serum calcium levels by several mechanisms: Vitamin D increases intestinal

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calcium absorption by both transcellular and paracellular pathways. This is accomplished by inducing the formation of various transport proteins involved in intestinal calcium absorption by the genomic actions of vitamin D.^{1,3} The active vitamin D metabolite, 1,25-dihydroxy cholecalciferol binds to its specific receptors in the intestinal cell and stimulates the active calcium transport from the intestine to the circulation.^{1,4}

However, vitamin D is not needed for the paracellular transport of calcium, which depends on the calcium gradient.^{1,2,4} Active calcium absorption decreases when the serum 25-hydroxycholecalciferol (vitamin D₃) concentration is <20 nmol/L.⁴ Hence, this level is also marked as vitamin D deficiency, as per Endocrine Society's Clinical Guidelines.⁵ The serum level of 25-hydroxy cholecalciferol or vitamin D₃ is generally considered as the best marker of a subject's vitamin D status, due to its ease of measurement, long half-life and correlation with known vitamin D effects.^{5,6}

Ethnic variations were observed for serum vitamin D₃ and calcium levels, which may be attributable to food habits and lifestyle differences, skin pigmentation and skin exposure to UVB-containing sunshine.⁷⁻¹¹ Evidence showed that indoor workers are less exposed to sunlight than outdoor workers; therefore, indoor workers might have a significantly negative effect on the biological ability to synthesize vitamin D,¹¹ which is expected to be reflected in their serum vitamin D₃ levels. However, there is a scarcity of such research in our Bangladeshi population. Hence, the present study was designed to compare serum vitamin D levels among the outdoor and indoor working professionals and observe its correlation with serum calcium levels.

Methods

This cross-sectional study was conducted in the Department of Biochemistry, Dhaka Medical College, Dhaka, Bangladesh, from July 2018 to June 2019. A total of 101 indoor and outdoor workers of different sectors living in Dhaka city participated in this study. Apparently healthy individual, aged between 18 and 60 years (of either gender), not having any systemic disease (e.g., diabetes mellitus, chronic kidney or liver disease, endocrine disorder, bone disease, etc.) or pregnancy was selected for the study through a primary scrutiny. They were divided into two groups: outdoor workers were in group A (n=35) and indoor workers were in group B (n=66). Blood samples were collected from all the study participants. Estimation of serum vitamin D₃ was done by chemiluminescent microparticle immunoassay (Access 2 Immunoassay System of Beckman Coulter Inc., USA), while serum calcium was measured using Arsenazo-III dye method (AU680 Clinical Chemistry Analyzer of Beckman Coulter Inc., USA).

Estimation of vitamin D: It was a two-step competitive binding immunoenzymatic assay. In the initial incubation, sample was added to a reaction vessel with a DBP releasing agent and paramagnetic particles coated with a sheep monoclonal anti-25-hydroxy vitamin D antibody. 25-hydroxy vitamin D was released from DBP and bonded to immobilized monoclonal anti-25(OH) vitamin D on the solid phase. Subsequently, a 25-hydroxy vitamin D analogue-alkaline phosphatase conjugate was added which competes for binding to the immobilized monoclonal anti-25hydroxy vitamin D. After a second incubation, materials bound to the solid phase were held in a magnetic field while unbound materials were washed away. Then, the

chemiluminescent substrate Lumi-Phos*530 was added to the vessel and light generated by the reaction was measured with a luminometer. The light production was inversely proportional to the concentration of 25-hydroxy vitamin D in the sample. The amount of analyte in the sample was determined from a stored, multi-point calibration curve. The standard calibration (6 point) procedure was run using test standard calibrators. A calibration curve was prepared taking concentration of 25-hydroxy vitamin D as abscissa and relative luminescence unit (RLU) as ordinate. Calibration ranged between 0.0 and 167.0 ng/ml.

Estimation of serum calcium: It was done using a colorimetric approach after releasing protein-bound calcium by acidification, followed by complexing calcium with a chromogen. Arsenazo-III dye reacted with calcium in an acid solution to form blue-purple complex. The colour developed was measured at 660 nm and was proportional to the calcium concentration in the sample. The standard calibration (3 point) procedure was run using test standard calibrators. A calibration curve was prepared taking concentration of calcium as abscissa and absorbance as ordinate. Calibration ranged between 0.0 and 26.1 mg/dl.

After multiple checking, data were recorded in a predesigned data collection sheet. Continuous variables were expressed as mean \pm SD and compared between groups by unpaired student's t-test. Categorical variables were compared using Chi-square test. Correlation was done by Pearson's correlation coefficient test. Level of significance was defined as P value <0.05 at 95% confidence interval. Statistical analysis was done using Statistical Package for the Social Sciences (SPSS) version 24.0 for windows. The study was approved by the Ethical Review Committee of Dhaka Medical College, Dhaka, Bangladesh.

Results

The mean age of the study participants was 38.72 \pm 6.88 years, ranging between 25 and 52 years. Among the study participants, 64(63.4%) were male and 37(36.6%) were female. Among outdoor workers, most were from Traffic Police Services (83%) and the rest were street hawkers (17%). Indoor workers were recruited from the hospital – nurses (32%), doctors (27%), administrative employees (21%), hospital ward assistants (12%) and medical technicians (8%) (Table-I). Serum vitamin D₃ level was found higher in outdoor workers than that of indoor workers (19.35 \pm 5.67 ng/ml vs. 14.17 \pm 4.18 ng/ml) and the difference was statistically significant (P<0.001). Serum calcium level was lower in outdoor workers than that of indoor workers (8.82 \pm 0.54 mg/dl vs. 9.08 \pm 1.08 mg/dl); however, the difference was not statistically significant (P>0.05) (Table-II). No significant correlation was observed between serum vitamin D and calcium levels in both outdoor (r= - 0.117; P=0.510) and indoor (r= - 0.163; P=0.196) groups (Fig. 1).

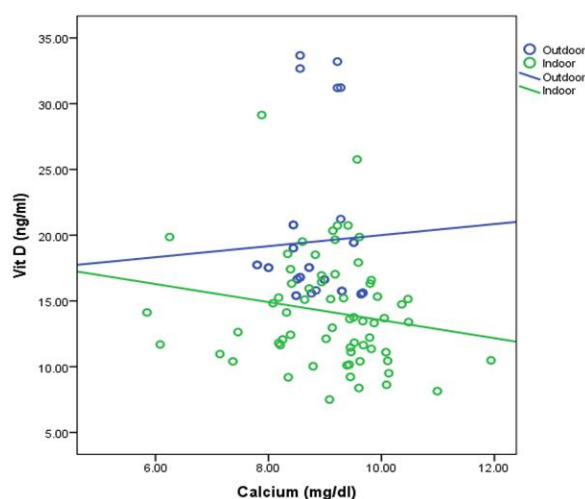
Table-I: Demographic characteristics of the study participants

Variables	Group A (n=35)	Group B (n=66)
Age group (in years)		
21-30	11 (31.4%)	9 (13.6%)
31-40	11 (31.4%)	28 (42.4%)
41-50	12 (34.3%)	27 (40.9%)
51-59	1 (2.9%)	2 (3.0%)
Mean age	38.72 \pm 6.88 years	
Gender		
Male	27 (77%)	37 (56%)
Female	8 (23%)	29 (44%)
Occupation		
Traffic Police	29 (83%)	-
Street Hawker	6 (17%)	-
Nurse	-	21 (32%)
Doctor	-	18 (27%)
Administrative Employee	-	14 (21%)
Ward Assistant	-	8 (12%)
Medical Technician	-	5 (8%)

Table-II: Serum D₃ and calcium levels of outdoor and indoor study participants

Variables	Total (n=101) Mean±SD	Group A (n=35) Mean± SD	Group B (n=66) Mean± SD	P value
Serum D ₃ (ng/ml)	15.96±5.33 (7.50 – 33.67)	19.35±5.67 (13.94 – 33.67)	14.17±4.18 (7.50 – 29.14)	<0.001 ^S
Serum Calcium (mg/dl)	8.99±0.94 (5.85 – 11.94)	8.82±0.54 (7.80 – 9.67)	9.08±1.08 (5.85 – 11.94)	>0.05 ^{NS}

Figures in the parentheses indicate range. P value reached from unpaired Student's t-test; S=significant, NS=not significant.

**Fig. 1.** Correlation between serum vitamin D and calcium levels in both outdoor and indoor study participants

Discussion

As we know that vitamin D synthesis is highly dependent on sunlight, factors and conditions causing lower time spent in outdoor activities, consumption of an imbalanced diet and low-quality (nutrient-poor) food, more covering clothing style, skin exposure to sunlight and widespread use of sun block can be expected to adversely impact vitamin D status in humans.¹⁰⁻¹² Furthermore, in many big cities, air pollution and blockage of sunlight by high-rise buildings also contributes to vitamin D deficiency;¹³ our study place, Dhaka city, is not an exception to this.

Workers with vitamin D deficiency often present with common symptoms such as non-specific weakening of the muscles and myalgia, and these symptoms may be confused for fibromyalgia or chronic fatigue syndrome. In many cases, the musculoskeletal diseases of workers are attributed to the intensity of the work they perform or to their posture at work, whereas, unfortunately, vitamin D deficiency is seldom considered the potential cause of the symptoms.¹⁴ Individuals who work indoors at day may be insufficiently exposed to sunlight irradiation, thereby developing vitamin D deficiency. Our study revealed that difference in vitamin D status between outdoor and indoor working professionals. Similar observations were reported by Sowah *et al.*¹⁰, Nair-Shalliker *et al.*¹⁵, Maruyama-Nagao *et al.*¹⁶, Selvarajan *et al.*¹⁷, Dharmshaktu *et al.*¹⁸, Kawashima *et al.*¹⁹, and Weaver *et al.*²⁰

Low-vitamin diet is independently affected by socioeconomic factors for instance low vitamin D levels have been noted among low-income groups, who are surprisingly more exposed to sunlight than high income groups.⁸ This finding should be taken into consideration, too. Research in the field also reported on vitamin D in relation to lifestyle and menopausal status.¹⁶ Vitamin D metabolism is closely related to calcium and phosphate.^{4,14,18} However, in our study, no correlation was observed between vitamin D and calcium levels.

Although optimal cautions were involved in every step of this study, limitations still exist. We had a relatively small sample size and that was collected from only Dhaka city; the outdoor study subjects were selected only from two professions. Hence, overall, the study findings might not represent the picture of the whole of the country.

Conclusion

Serum vitamin D level was found significantly higher in outdoor workers than that of indoor workers. However, no significant correlation was observed between serum vitamin D and calcium levels in both outdoor and indoor groups. Further studies are recommended involving multiple centres, larger samples and longer durations in different parts of the country.

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