

Burden of Vitamin D and Vitamin B₁₂ Deficiency among Older Patients in North India: a Single-Centre Study

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ABSTRACT

Objectives: Vitamin D and B₁₂ deficiency in older adults is associated with osteoporosis and dementia, respectively. The objective of this study is to estimate the prevalence of vitamin D and B₁₂ deficiency among older patients, assess gender-specific differences and analyse seasonal variations in serum levels.

Materials and methods: This is a retrospective hospital record-based study. All patients undergoing vitamin D and/or B₁₂ estimation from October 2022 to September 2023 in the Biochemistry laboratory of AIIMS Raebareli, India, were included. Serum vitamin D and B₁₂ assays were performed using chemiluminescence immunoassays on VITROS ECIQ Immunodiagnostic Systems. For vitamin D, ≥30 ng/mL was considered normal, 20-30 ng/mL as insufficient and <20 ng/mL as deficiency. For vitamin B₁₂, ≥300 pg/mL was considered normal, 200-300 pg/mL as borderline and <200 pg/mL as deficiency. Statistical analysis was performed in IBM SPSSv26.

Results: We estimated the levels of vitamin D in 1 647 older patients and those of vitamin and B₁₂ in 2 298 older patients. The prevalence of vitamin D insufficiency (20-30 ng/mL), vitamin D deficiency (<20 ng/mL), borderline B₁₂ (200-300 pg/mL) and B₁₂ deficiency (<200 pg/mL) was seen in 31.9%, 28.9%, 17.1% and 17.1% of subjects, respectively. Female participants had significantly lower vitamin D and higher vitamin B₁₂ levels than male subjects. Significant seasonal variations in levels of vitamins D and B₁₂ were observed, with the lowest levels being detected in March (F=4.98, p=0.00) and August (F=2.51, p=0.04), respectively.

Conclusion: Older patients in North India suffer from a high burden of vitamin D and B₁₂ deficiency. Introducing a nutritional supplementation programme for older persons and liberalisation of non-vegetarian food sales may be helpful.

Keywords: vitamin D, vitamin B₁₂, aged, deficiency, micronutrients, India.

INTRODUCTION

Vitamin D is a fat-soluble vitamin that plays a major role in maintaining health (1). The human skin when exposed to solar ultraviolet B radiation (290-320 nm range) converts 7-dehy-

drocholesterol to vitamin D₃. Thereafter, it is sequentially metabolised in the liver and kidney to form 1,25-dihydroxyvitamin D (2). Vitamin D can also be obtained from dietary sources. About 90% of it is present in the circulation and is derived from endogenous synthesis in the skin (3). Atrophic skin changes, which are common in the

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older population, interfere with the dermal production of vitamin D (4). A low dietary vitamin D, impaired absorption from the gastrointestinal tract and reduction in renal synthesis of 1,25-dihydroxyvitamin D increases the risk of vitamin D deficiency in older adults (3).

Vitamin D maintains calcium and phosphorus homeostasis in our body (2). It is utilised by non-skeletal tissues via the various vitamin D receptors in the body (5). It is significant for bone metabolism. Its deficiency causes rickets in children and osteoporosis in adults (2). Vitamin D insufficiency is reported to be associated with numerous disease conditions such as hypertension (3), dyslipidaemia (6), cardiovascular diseases (7), insulin resistance (8), metabolic syndrome (9), obesity (10), diabetes mellitus (11), peripheral vascular disease (12) and Alzheimer disease (13). A recent report highlights that vitamin D deficiency is associated with an increased risk of breast, colorectal and prostate cancers (14).

Vitamin D deficiency in the older population is reported to be associated with muscle weakness, sarcopenia and limitation in the capacity to perform daily activities. All these result in increased body sway, risk of falls and occurrence of fractures (3, 15, 16, 17). The vitamin D status of an individual is usually evaluated by estimating the serum 25-hydroxyvitamin D level, as it is the major form of this vitamin in the circulation (18). Different studies conducted across India in various populations report a prevalence of vitamin D deficiency from 40% to 99% (19). Till date, only two studies assessed the burden of vitamin D among older persons in India (20, 21), but both of them were conducted on a South Indian population. Therefore, there is a paucity of data on the prevalence of vitamin D deficiency among older persons living in Northern India.

Vitamin B₁₂ is a water-soluble vitamin that is present exclusively in animal-origin food such as eggs, meat, seafood and dairy products (22). During the process of digestion in the stomach, hydrochloric acid and pepsin release vitamin B₁₂ available in the food. The free vitamin B₁₂ then binds with intrinsic factor and is absorbed when it reaches the distal part of the ileum. Vitamin B₁₂ deficiency in the older population may be due to either an inadequate consumption of B₁₂ rich food or an impaired absorption of this vitamin (23). Atrophic gastritis is a prime cause of B₁₂ malabsorption among older people; it leads to

hypochlorhydria, which in turn interferes with the release of vitamin B₁₂ from the food. This further results in intestinal bacterial overgrowth, which hampers vitamin B₁₂ uptake. The prevalence of atrophic gastritis increases with age and is reported to be present in 20-50% of older population (24, 25).

Restricted capacity to make food choices due to financial restrictions and practices of adhering to a strict vegetarian diet due to socio-cultural and religious issues also puts an older person at risk of vitamin B₁₂ deficiency (26, 27). In India, a large proportion of the population consumes a lacto-vegetarian diet, in which milk and milk products provide the vitamin B₁₂ (27). However, the practice of boiling milk before consumption decreases its vitamin B₁₂ content (28). As no "gold standard" test exists to detect B₁₂ deficiency, diagnosis is based on low levels of vitamin B₁₂ in the serum together with clinical symptoms, which responds to treatment (23). Some studies have suggested that serum homocysteine and methylmalonic acid can be useful indicators in those with marginal levels of serum vitamin B₁₂ (29, 30).

The clinical symptoms of B₁₂ insufficiency may be non-specific in the early stages. It frequently causes macrocytosis and on a few occasions, is reported to cause isolated thrombocytopenia and neutropenia (23). An increase in serum homocysteine due to vitamin B₁₂ deficiency increases the risk of ischaemic heart disease and stroke (31). Neuropsychiatric manifestations like weakness, paraesthesia, altered gait, cognitive impairment, depression and Alzheimer disease are also reported in older person with vitamin B₁₂ deficiency (32, 33). In two different studies conducted on South and West Indian older people, the reported prevalence of vitamin B₁₂ deficiency was 42.3% and 63.3%, respectively (21, 34). There is a paucity of data on the prevalence of vitamin B₁₂ deficiency in the North Indian older population.

As Northern India is located in higher latitudes compared to South India, it receives relatively fewer sunshine hours during the winter months. Further, unlike South India, the Gangetic plains of North India are covered with dense fog during the winter months, which restricts sunlight exposure (35). Uttar Pradesh (in North India) has a lower per capita income, lower female literacy rate, higher birth rate and higher infant

mortality as compared to the South Indian states (36, 37). Due to sociocultural issues, a large proportion of the population living there consume a lacto-vegetarian diet (38). Unlike the South Indian states, there is a lack of industrialisation in Uttar Pradesh. Therefore, most people lead a traditional lifestyle, where agriculture is the primary occupation and livestock rearing is an additional income source (39).

The present study aims to estimate the prevalence of vitamin D and B₁₂ deficiency among older patients, identify gender-specific differences and assess seasonal variations. □

METHODS

Our study was conducted in the biochemistry laboratory of All India Institute of Medical Sciences (AIIMS), Raebareli, India. The institute receives around 2 000 patients in the outpatient department daily, mainly from Raebareli and adjoining districts of Uttar Pradesh, India. The biochemistry laboratory is fully automated and provides round-the-clock services to patients.

This is a retrospective hospital record-based cross-sectional study. All older (≥ 60 years of age) patients who had attended the institute for treatment and had undergone vitamin D and/or B₁₂ estimation as a part of their treatment process were included in the present study. There were no exclusion criteria. The study included vitamin D and B₁₂ investigation reports of all older patients from October 2022 to September 2023.

The sample size was calculated based on the formula, $n = z^2 p(1-p)/d^2$, with n representing the required sample size, z the level of confidence at 95% (1.96 standard value), p the prevalence, d the error margin at 5% (0.05 standard value). Based on a previous study (21), we considered a prevalence of vitamin D and B₁₂ deficiency of 39.1% and 42.3%, respectively in older people. To estimate the prevalence of vitamin D and vitamin B₁₂ deficiency in the older population, we calculated a sample size of 366 and 374, respectively.

About 5 mL of venous blood was collected in a gold-top serum separator tube. The separated serum was utilised for analysing vitamin D and B₁₂ levels. The estimation of the two vitamins was based on the competitive immunoassay principle and carried out using the Vitros Eciq immunodiagnostic system. The reagent packs,

calibrators and quality control materials were obtained from Vitros Immunodiagnostics. Internal quality control was run daily before running patient samples. The assays were performed as per the instructions provided in the kit literature. The reportable range was 8 to 150 ng/mL for vitamin D assay and 159 to 1 000 pg/mL for vitamin B₁₂ assay.

Vitamin D levels ≥ 30 ng/mL were considered normal, 20-30 ng/mL insufficient and < 20 ng/mL deficient (19). Vitamin B₁₂ levels ≥ 300 pg/mL were considered normal, 200-300 pg/mL borderline and < 200 pg/mL deficient (40).

Data of all older patients undergoing the vitamin D and/or B₁₂ estimation, including their age, sex, hospital registration number, date of sample collection and the test value, were extracted from the hospital management information system. Two separate Excel spreadsheets were prepared, one for vitamin D and another one for vitamin B₁₂. In patients who had undertaken a test more than once during the study period, the result of the first test was considered for further analysis.

Ethical clearance was taken from the Institutional Ethics Committee prior to starting the study (Ethical approval number: 2024-2-OTH-EXP-7 dated 8 June 2024). Owing to the retrospective nature of our study, the requirement of informed consent was waived by the Institutional Ethics Committee. Confidentiality of participant data was maintained at all levels. The study was conducted according to the guidelines of the Declaration of Helsinki. Strict confidentiality was maintained throughout the study regarding the patient data utilised for the current study.

All categorical data were presented as absolute frequency and percentage (relative frequency). Continuous data were checked for normality using the Kolmogorov-Smirnov test. All parametric data were presented as mean $\pm$ standard deviation (SD) and non-parametric data as median with interquartile range. An independent sample t-test was used to analyse the significance of study parameters between two groups for parametric data and a one-way ANOVA test was utilized to analyse the significance of study parameters between three or more groups. Patients were divided into three categories based on their age (60-69 years, 70-79 years, and 80 years and above) for further analysis. A p-value < 0.05

was considered as a level of significance. Data was analysed using IBM SPSS version 26. $\square$

RESULTS

During the study period of one year, 1 647 older patients had undergone vitamin D estimation. Participants had an age range of 60-95 years and a median age of 65 years (inter-quartile range 62-70 years). Of the 1 647 subjects, 854 (51.9%) were men and 793 (48.1%) women. On analysing the serum vitamin D levels of the 1 647 older patients, we observed that they were deficient in 476 (28.9%) of subjects, insufficient in 525 (31.9%) and adequate in 646 (39.2%) of participants (Table 1).

On analysing vitamin D levels gender-wise, we observed a significantly lower level in female participants compared to male ones (27.64 ± 17.17 vs 32.38 ± 16.24 ng/mL, $t = -5.64$,

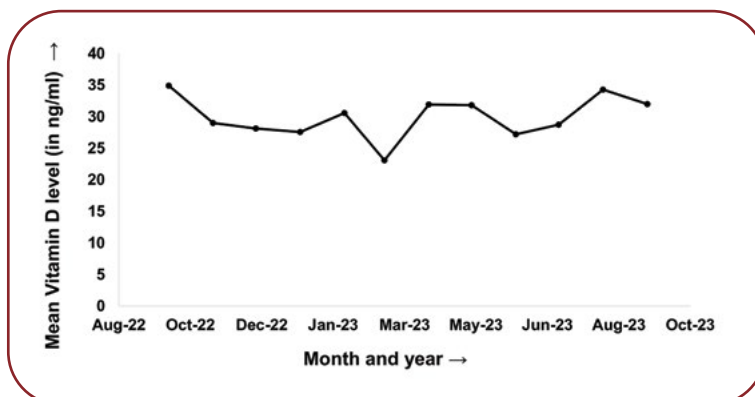


FIGURE 1. Seasonal variation of vitamin D level in older patients

$p = 0.00$). This was evident in older female patients of all age groups (Table 2).

The seasonal trend highlighted that the serum vitamin D level was the highest in October and the lowest in March (Figure 1). This seasonal difference was found to be statistically significant ($F = 4.98$, $p = 0.00$).

TABLE 1. Age-wise prevalence of vitamin D deficiency in the older patients

	Serum vitamin D level (in ng/mL)			Total
	0-19.99	20-29.99	≥ 30	
Age group (years)				
60 – 69	321 (28.3%)	388 (34.27%)	423 (37.36%)	1 132 (100%)
70 – 79	123 (30.0%)	110 (26.83%)	177 (43.17%)	410 (100%)
≥ 80	32 (30.47%)	27 (25.71%)	46 (43.81%)	105 (100%)
Total	476 (28.9%)	525 (31.9%)	646 (39.2%)	1 647 (100%)

TABLE 2. Serum vitamin D and vitamin B₁₂ levels in male and female patients

Parameter	Age group (years)	Gender		Female vs male patients (t-test)	
		Female	Male	t	p
Serum vitamin D (ng/mL)	60 – 69	27.57 ± 16.35	31.37 ± 15.18	- 3.94	0.00
	70 – 79	28.39 ± 19.61	34.32 ± 18.62	- 3.09	0.00
	≥ 80	25.94 ± 18.19	33.33 ± 14.49	- 2.32	0.00
	Overall	27.64 ± 17.17	32.38 ± 16.24	- 5.64	0.00
Serum vitamin B ₁₂ (pg/mL)	60 – 69	603.96 ± 353.02	534.16 ± 356.67	3.89	0.000
	70 – 79	660.19 ± 372.56	573.21 ± 359.33	2.84	0.005
	≥ 80	691.90 ± 378.82	730.93 ± 356.24	-0.652	0.515
	Overall	621.30 ± 359.90	559.43 ± 360.72	4.11	0.000

TABLE 3. Age-wise prevalence of vitamin B₁₂ deficiency in older patients)

	Serum vitamin B ₁₂ level (pg/mL)			Total
	< 200	200-299.9	≥ 300	
Age group (years)				
60 – 69	284 (15.78 %)	295 (18.77 %)	992 (63.14 %)	1571 (100 %)
70 – 79	94 (16.23 %)	82 (14.16 %)	403 (69.60 %)	579 (100 %)
≥ 80	16 (10.81 %)	16 (10.81 %)	116 (78.38 %)	148 (100 %)
Total	394 (17.1%)	393 (17.1%)	1511 (65.8%)	2298 (100%)

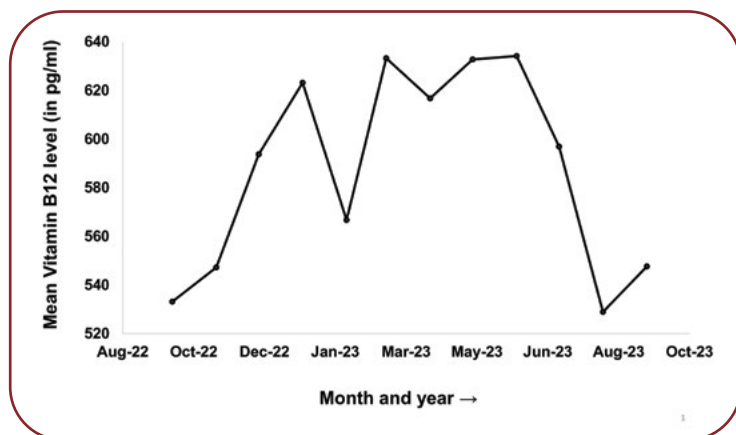


FIGURE 2. Seasonal variation of vitamin B₁₂ in older patients

During our study period, 2 298 older patients had undergone vitamin B₁₂ estimation. Participants' age range was 60–95 years and their median age 65 years (interquartile range 62–70 years). Of the 2 298 subjects, 854 (51.9%) were men and 793 (48.1%) women. Analysis of serum vitamin B₁₂ showed that its levels were deficient in 394 (17.1%) of subjects, insufficient in 393 (17.1%) of patients and adequate in 1 511 (65.8%) of subjects (Table 3).

On analysing vitamin B₁₂ levels gender-wise, a significantly higher B₁₂ level in females compared to males was observed in the age groups of 60–69 and 70–79 years. However, there was no significant difference in vitamin B₁₂ levels between the two sexes among those aged 80 years and above. Overall, vitamin B₁₂ level was significantly higher in females than males (621.30 ± 359.90 vs 559.43 ± 360.72 , $t = 4.11$, $p = 0.00$) (Table 2).

The seasonal trend highlighted that the serum vitamin B₁₂ level was higher from March to June and the lowest in August (Figure 2). This seasonal difference was statistically significant ($F = 2.507$, $p = 0.04$). □

DISCUSSION

In our study, we identified vitamin D deficiency in 28.9% of participants and insufficiency in 31.9% of subjects (Table 1). Suryanarayana *et al* (20) reported that the prevalence of vitamin D deficiency among the older people in Hyderabad metropolitan area of South India was 56.3%. However, this study had a small sample size ($n=298$). Marwaha *et al* (41) performed a statistical analysis on a subset of their study popula-

tion and reported that the prevalence of vitamin D deficiency among persons aged 65 and above living in Delhi (North India) was 91.2%. A meta-analysis carried out by Meshkin *et al* (42) showed that the global prevalence of vitamin D deficiency in the older population was 59.7%. Most patients who attend our institute live in Raebareli and adjoining districts of Uttar Pradesh in North India. For the majority of people living in these rural areas, agriculture is the primary occupation, with dairy farming being a subsidiary activity which helps to increase the farmers' family income (43). Exposure to sunlight during agricultural activity helps in the endogenous synthesis of vitamin D. As a significant proportion of people living in this region have a lacto-vegetarian diet due to social and religious issues (38), they regularly consume fresh milk from their cattle, which may explain the lower prevalence of vitamin D deficiency in this rural area compared to metropolitan cities like Delhi (in North India) and Hyderabad (in South India).

Older female patients had a significantly lower vitamin D levels than male ones (Table 2). This may be due to the fact that, in rural India, men perform outdoor activities such as agriculture, while women stay indoors performing household work and raising their children and grandchildren (44). Sunlight exposure during outdoor agricultural activities is likely to lead to higher vitamin D levels in men. Our study highlights a significant seasonal trend of vitamin D levels in patients in this region, with the highest levels being observed in August and the lowest in March. This may be due to adequate sunlight exposure during the paddy cultivating season (July–September) and inadequate sunlight exposure due to foggy days in winter (December–January).

In our study, we identified vitamin B₁₂ deficiency in 17.1% and insufficiency in 17.1% of older patients (Table 3). Sundarakumar *et al* (21) reported that the prevalence of vitamin B₁₂ deficiency among older people in rural Karnataka (in South India) was 42.3%. A review highlighted that B₁₂ deficiency was up to 61.7% among the older population in India (45). In the United States and the United Kingdom, around 6% of older people are reported to have B₁₂ deficiency (46). The lower prevalence of B₁₂ deficiency in the older patients in our study compared to other regions in India may be explained by the fact that a significant proportion of the people living

in this region consumed a lacto-vegetarian diet (38), including fresh milk derived from their dairy farm (43). Fresh milk has a higher B₁₂ quality than pasteurised milk (47), which is mostly consumed in urban India. However, the prevalence of B₁₂ deficiency is higher than in developed nations like the United States and the United Kingdom (46), which may be because a proportion of the North Indian population avoids the consumption of eggs, fish and meat.

In our study, we found a higher B₁₂ level in females compared to males (Table 2). Similar findings were reported in previous studies (45, 48). Margalit *et al* (48) showed that this gender-based difference in the B₁₂ level could not be explained by dietary habits or hormonal differences. This is an area of future research. The low level of B₁₂ during July-August corresponds to the month of Shravana (the fifth month of Hindu calendar) (Figure 2). Every year during this month there is a restriction on the sale of non-vegetarian food such as meat in the Uttar Pradesh province of India (49). So, a decreased opportunity to consume non-vegetarian food due to restricted sales may be related to the lower serum B₁₂ level.

The strengths of our study are its large sample size, study participants representative of the rural population, monthly data collection depicting seasonal trends and assays conducted by standardised laboratory methods.

The present study has some limitations too. As it was a hospital-based single-centre study, its findings may not always be generalised to the whole community in Northern India. We did not have the facility to assess serum holo-transcobal-

amin homocysteine or methylmalonic acid levels in subjects with marginal B₁₂ levels. We also did not have the facility to test intrinsic factor antibodies. It is not known if a patient was taking micronutrient supplementation prior to giving the blood sample. As people attending the outpatient department of our institution are provided with offline consultations, our Hospital Information Management System did not have a record of patients' provisional diagnosis. Hence, we cannot relate the vitamin D and B₁₂ levels with the disease pathology in our study. □

CONCLUSION

A significant burden of micronutrient deficiency, such as vitamin D and B₁₂ deficiency, is prevalent among older patients living in rural areas of North India. A government funded nutritional supplementation programme targeting the older population may be helpful. Promoting dairy farming in rural areas and vitamin D fortification of packaged milk in urban areas can help overcome vitamin D deficiency to a large extent. Liberalisation of non-vegetarian food sales during July-August (Shravan) may provide more food choices, thereby improving B₁₂ levels. Food fortification and promoting the cultivation of genetically modified crops, in which inhibitors of trace element absorption (eg, phytate) are substantially low, can help reduce the micronutrient deficiency of the population. □

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