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Profile of Serum Vitamin D Levels Among Individuals in Mizoram: A Retrospective Study**Hmingthanzuali Ralte¹, John Zohmingthanga², Chawngthu Vanlalhlua^{3*}, Zothansangi⁴ and Babie Zirnunsangi⁵**¹Assistant Professor, Department of Pathology, Zoram Medical College, India² Medical Superintendent, State Referral Hospital, Fakawn, India³Associate Professor, Department of Surgery, Zoram Medical College, India⁴Assistant Professor, Department of Pathology, Zoram Medical College, India⁵Demonstrator, Department of Pathology, Zoram Medical College, India**ABSTRACT**

Although there are innumerable studies on vitamin D deficiency in India, there is limited data in Mizoram. Keeping this in view the aim of our study is to find out the deficiency of Vitamin D in our region, Mizoram (NE India). Our study population included patients attending Genesis Laboratory, Aizawl, Mizoram (November 2021 – November 2022) for various ailments who were advised serum Vitamin D level estimation. The study population was grouped according to gender and age in decades. Blood was collected in plain vials and serum obtained was used for vitamin D estimation. Out of 480 patients, 180 were male and 300 were female. Age of the study population ranged from 11 – 79 years. 17% of the total study population had normal values, 62.7% was vitamin D Deficient and 20.3 % showed Vitamin D insufficiency. The prevalence of Vitamin D Deficiency was high in all age groups, there were a higher percentage of females in the vitamin D insufficient and deficient groups. Maximum numbers of patients with vitamin D deficiency were in the age group of 60-69 years with a female predominance. Prevalence of Vitamin D deficiency is very high in our region that is in Mizoram, as is reflected from our study. This pattern is seen in other parts of our country too. Also, the deficiency is high in the age groups N – N years and females outnumbered male.

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Introduction

Vitamin D is a fat soluble vitamin involved in the calcium and phosphorus metabolism. The synthesis of vitamin in the skin depends on various factors such as latitude, clothing, skin colour, sunscreen usage, duration and time of exposure to sunlight. In spite of abundant sun exposure Vitamin D deficiency is common in all age groups of India [1-4]. As per the FAO/WHO expert Consultation report, thirty minutes of exposure of skin to sunlight without the application of sunscreen is optimum for the synthesis of vitamin in the subcutaneous fat [5]. Vitamin D is crucial for maintain bone mineralization, prevention of Rickets in children and osteoporosis and osteomalacia in adults [6,7]. In human, the vitamin D related compounds include D2 (ergocalciferol) and vitamin D3 (cholecalciferol). While D2 is mainly found in plant kingdom, synthesized in mushrooms and yeast, D3 is synthesized in the skin when exposed to ultraviolet rays. Hence, the name “sunshine vitamin” [8,9]. Vitamin D is obtained from sunlight or from food rich in vitamin D such as mushrooms, dairy products and fish. In the body vitamin D is metabolized in the liver to 25 hydroxy vitamin D [25(OH)D] or calcidiol. This is found in the blood circulation and is measured in the serum to access deficiency states. The renal enzymes metabolize calcidiol to calcitriol, the physiologically active form of vitamin D called 1,25-dihydroxy vitamin D. This form regulates the calcium and phosphorus

metabolism [9]. Other than skeletal system, vitamin D is associated with cardiovascular disease, type 2 diabetes mellitus, multiple sclerosis, preeclampsia, tuberculosis, cancer [9-11]. Vitamin D deficiency causes demineralization of bones, rickets in children and osteomalacia in adults. Hence, vitamin D is usually prescribed in both children and adults. Excessive intake of the vitamin causes hypervitaminosis and hypercalcemia, leading to loss of appetite, irritability, over calcification of bones, soft tissues, renal stones and even induce hypertension [12,13]. The recommended daily dietary allowances of vitamin D are as follows: upto 1 year of age- 400 IU, 1 year to 70 years- 600 IU and >70 years – 800 IU [14-16]. This dietary allowances complements the naturally synthesized vitamin D on exposure to sunlight [14-17].

Biological balance of Vitamin D is done by human skin. A total of 20,000 units of vitamin D can be made in skin every day, and the sunlight that makes Vitamin D begins to break it down. After skin turns dark (tan), because of heavy and prolonged exposure to sunlight, the dark skin synthesizes less amount of Vitamin D which may be up to 10,000 units. Thus, melanin content of skin may regulate cholecalciferol production [18]. Humans have a natural system in the skin that prevents Vitamin D toxicity. All sunscreens block the Vitamin D production [19]. Vitamin D is believed to act as a potent antioxidant protecting against free radical damage and an inducer of cellular differentiation, protecting against

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carcinogenesis [20, 21]. It also has a role to fight against serious infections, by controlling T Cell Antigen Receptor (TCR) signaling and activation of human T cells [22]. VDD can therefore be associated with an increase in risk for certain diseases. However, since these diseases are multifactorial, optimum Vitamin D levels may not prevent these diseases, but their risk is lowered.

Materials and Methods

This retrospective study was done for a period of one year (1st November 2021 –30th November 2022) in patients who attended Genesis Laboratory, the first ISO accredited laboratory in Mizoram. Patients from all age group, religion, gender, and occupation who attended OPD were eligible to participate.

Age and gender were the only two variables derived from the dataset and investigated as predictors of vitamin D status. The study population was thus grouped according to gender and age in decades, the minimum range being 10-19 years and the maximum being 70-79 years.

Blood samples (5 ml) were collected in plain vacutainers with draw volume 1-10 ml, allowed to clot and centrifuged at 3500 rpm for 10 min to separate serum. All serum samples were analyzed for vitamin D as soon as they were received in the lab, there were no stored samples. The compound measured for vitamin D activity in the serum was 25 hydroxy D. Serum 25 hydroxy D was determined.

To facilitate interpretation of results, the study population was divided into three groups based on their serum 25 hydroxy D status:

Vitamin D Deficiency (<20 ng/ml).

Vitamin D Insufficiency (20-29 ng/ml).

Sufficient Vitamin D (30- 100 ng/ml).

Potential toxicity (>100 ng/ml)

Quantitative determination of 25-OH- vitamin D was performed by Automated Vidas System Biomerieux, France by the principle of Enzyme Linked Fluorescent Assay (ELFA) Technology.

Statistical Analysis

Data were analyzed using MS Office Excel 2007 and results were expressed as percentage of the sub-population in question.

Results

A total of 480 patients who underwent vitamin D estimation were included in our study with 180 male subjects and 300 female subjects.

The age group of our subjects ranged from 11- 79 years. We had 21 subjects in age group 10-19 years. In the age group 20-29 years there were 11 male and 18 female subjects. Out of a total of 480, there were 180 male subjects (N) and 300 female subjects (N) as shown in Table 1. As it is clear from the table below that in age group <10 year there were no subjects, in 30-39 years there were 91 subjects, in 40-49 years there were 43 subjects, in 50-59 years there were 99 subjects, in the age group 60-69 years, there were 168 subjects whereas in the age group more than 70 years there were 29 subjects.

Age group (years)	Overall (n=480)			Males (n=180)			Females (n=300)		
	NVD %	VDI %	VDD %	NVD %	VDI %	VDD %	NVD %	VDI %	VDD %
10-19 (n=21)	13.95	20.93	65.12	20.00	20.00	60.00	8.70	21.74	69.57
20-29 (n=29)	5.63	11.97	82.39	9.09	20.45	70.45	4.08	8.16	87.76
30-39 (n=91)	10.76	15.19	74.05	26.47	20.59	52.94	6.45	13.71	79.84
40-49 (n=43)	10.39	19.05	70.56	20.00	21.43	58.57	6.21	18.01	75.78
50-59 (n=99)	12.78	17.62	69.60	15.87	22.22	61.90	11.59	15.85	72.56
60-69 (n=168)	20.48	16.67	62.86	25.88	20.00	54.12	16.80	14.40	68.80
70-79 (n=29)	20.62	20.62	58.76	21.57	21.57	56.86	19.57	19.57	60.87

NVD: Normal Vitamin D Status (>30 ng/ml); **VDI:** Vitamin D Insufficiency (20-30 ng/ml); **VDD:** Vitamin D Deficiency (<20 ng/ml)

Out of 301 patients who had frank deficiency (vitamin D levels <20ng/dl),112 were male and 189 were female, whereas out of 99 patients who had insufficient vitamin D levels (21 to 29ng/dl), 38 were male and 61 were female. Eighty subjects had normal vitamin D levels out of which 44 were male and 36 were female subjects. Vitamin D deficiency was seen in 400 subjects out of which 301 had frank vitamin D deficiency (<20ng/dl) whereas 99 had insufficient vitamin D levels (21-29 ng/dl). About 16.7% percent of the study population had normal vitamin D levels (>30 ng/dl). Out of 301 subjects who had frank vitamin D deficiency, 112 were male and 189 were female, whereas out of 99 subjects

who had insufficient vitamin D levels 38 were male and 61 were female. A total of 80 had normal vitamin D levels out of which 44 were male and 36 were female.

Out of the total study population of 480, 62.7% had frank deficiency of vitamin D, 20.6% had insufficient vitamin D levels and 16.7% had normal vitamin D levels. In the age group 60-69 years, this had maximum number of subjects (168) as many as 62.8% had frank vitamin D deficiency with two third female majority. In this age group, vitamin D insufficiency was found in 16.7% subjects again with nearly two third female majority. A meagre 20% subjects had normal vitamin D levels.

In the next age category (50-59 years) which had 99 subjects, more than half were vitamin D deficient with female preponderance here too with 61 female subjects out of the total of 99. Vitamin D insufficient was 69.6% in this group with 32 male and 38 female subjects. 12.7% subjects had normal vitamin D levels.

In the age group 30-39 years, there were 91 subjects. 74% had frank vitamin D deficiency with 47 female and 44 male subjects whereas 17.6% had insufficient levels with equal sex distribution. 12.7% patients had normal vitamin D levels with male preponderance. Out of the 29 septuagenarian, 10 were female and 10 males. 16 had frank vitamin D deficiency and 6 had insufficient and 7 had normal levels.

Discussion

Earlier studies in India report vitamin deficiency in both genders and all age groups, each of those studies dealt with one specific age group [5-7]. This study, however, deals with vitamin D status in all age groups from infancy to old age among both genders. Rucker et al. note that although frank vitamin D deficiency causes rickets and osteomalacia, identification of vitamin D insufficiency, which affects bone health, is likely of greater clinical importance. Although in the present study, the prevalence of vitamin D insufficiency was lower compared to that of vitamin D deficiency in the total study population, at all age groups and in both genders, this prevalence cannot be underestimated since the percentage of individuals in this category was higher than those with normal vitamin D levels in almost all subgroups. Vitamin D insufficiency is known to cause myopathy, osteopenia, secondary hyperparathyroidism and osteomalacia [2]. Patients with osteomalacia often complain of isolated or global bone discomfort along with aches and pains in their joints and muscles and these patients may be misdiagnosed with fibromyalgia [19]. In the background of these reports, the observed high prevalence of vitamin D insufficiency needs to be kept in mind considering the fact that currently there are increasing reports of aches and pains in joints and muscles in our hospitals with frequent referrals to a neurologist.

The prevalence of vitamin D deficiency is reported to be high during all three trimesters in pregnant women in India and this could manifest in infants at birth and beyond [8-10]. Some of the previous authors maintain that vitamin D deficiency in maternal blood correlated with that in cord blood [8]. It has been discussed by previous authors that vitamin D deficiency manifests with compromised length from birth itself without any potential for catch up growth in view of the fact that vitamin D deficiency continues to afflict even toddlers and school children in India [24]. The small sample size is a limitation of this study, hence further studies with larger cohorts may be conducted to arrive at assertive conclusions regarding vitamin D status in infants and childhood of both genders in the Mizo population.

Ford et al. have reported widespread vitamin D deficiency in a UK-inner city population, more so in women. The results of the present study support these reports in that females showed a higher prevalence of vitamin D deficiency than males, in the total study population as well as in all age groups. This is an important finding that needs attention particularly in the reproductive age group, bearing in mind the fact that low maternal vitamin D can

result in low vitamin D in new born and have serious pediatric implications. Ford et al. attribute the higher prevalence of vitamin D deficiency in Asian women over Asian men to the type of clothing worn by the former. Asian women tend to wear clothes that cover most of the skin and studies have shown that fully covered dressing style contributes to vitamin D deficiency. In the present study, the higher prevalence of the vitamin deficiency could be due to the type of clothing or the increased use of sunscreens particularly by the female fraternity, because it has been reported that wearing a sunscreen with an SPF of 15 reduces vitamin D synthesis in the skin by 99% [19]. However, the authors also wish to draw one's attention to the role of changing dietary habits in determining the vitamin D status in the current population.

For further research that looks into not just sun exposure as a causative factor but also changing dietary habits as additional causative factors responsible for the increasing vitamin D deficiency in the community. An in-depth research into those components of the diet that have a direct bearing on serum total cholesterol for example the type of cooking oil used, could provide vital clues to the role of changing dietary habits in determining vitamin D status in the present population. Bogh also mentions that vitamin D synthesis begins in the bowel epithelium with an oxidation of cholesterol to 7 dehydro-cholesterol. Studies on possible alterations of the gut flora, due to changing dietary habits and its effect on the availability of 7 dehydrocholesterol for vitamin D synthesis could throw more light on the cause of the increasing prevalence of vitamin D deficiency as only ten percent of vitamin D is obtained from the diet in humans and the rest of it comes from solar UV-induced biosynthesis in the skin [9].

Conclusion

To conclude, vitamin D deficiency was seen in 62.7% of our study population with a mean value of 16.6 ng/ml. This further reiterates the fact that hypovitaminosis D is a common problem in India and our region is no exception.

Vitamin D deficiency is regarded as a pandemic by several researchers.

Although this problem does not spare any age group as seen in our study, the most affected age groups were those in the 60-69 years and, with a female predominance. Hence, medically monitored supplementation of vitamin D on a regular basis in this age group along with lifestyle modifications may have a positive long term impact and perhaps act as preventive measure to prevent the diseases that are presently plaguing not only the Mizo population, but the human civilization at large.

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Compliance with Ethical Standards

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