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PLASMA VITAMIN D TRENDS ACROSS VARIOUS AGE GROUPS IN THE COSMOPOLITAN CITY OF PAKISTAN.

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ABSTRACT

BACKGROUND: Vitamin D belongs to a group of fat-soluble pro-hormones that can be generated from a precursor underneath the skin, 7-dehydrocholesterol, which yields vitamin D3 (cholecalciferol). **OBJECTIVES:** Measure the plasma vitamin D level trends among various age groups. The study further identified the age group with the highest number of optimum plasma vitamin D levels. **METHODOLOGY:** Cross-sectional study was completed from June 2020 through August 2022. All samples were obtained from the population of various areas within Lahore, one of the cosmopolitan cities of Pakistan. A 5 ml venous blood from 2661 participants was drawn to measure plasma vitamin D levels. The participants' age was divided into seven groups and was further stratified into male and female groups. Vitamin D levels were categorized on standard criteria as optimum, mildly deficient, severely deficient, excess, and toxic. The blood samples were analyzed in an ISO-certified laboratory. **RESULTS:** We enrolled 2661 participants. The highest percentage of vitamin D deficiency was reported in group-3 (21-30 years; 39.3%). With regards to gender, among males, group one (1-10 years; 24.2%) was found to be most deficient in vitamin D. Further, the optimum plasma levels of vitamin D among females with the highest percentage of vitamin D was in group seven (above 60 years; 25.7%) and group one (1-10 years; 18.9%) among males. **CONCLUSION:** Our results revealed that the most vulnerable age group with severe vitamin D deficiency was the young age group females (39.3%), and the optimum level was reported in older females (25%), and among males, vitamin D deficiency was detected among older age groups (13.7%) and the optimum level was noticed in younger males. Further studies are required to elucidate the probable causes of severe vitamin D deficiency.

KEY WORDS: Vitamin D, Cosmopolitan city, Vitamin D Deficiency

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INTRODUCTION

Vitamin D belongs to a group of fat-soluble pro-hormones that can be generated from a precursor underneath the skin, 7-dehydrocholesterol, which yields vitamin D3 (cholecalciferol).[1] Some dietary components also serve as a good source of vitamin D, such as beef liver, salmon & tuna fish, cheese, mushrooms, egg yolk, and fortified cereals. Ergocalciferol, or vitamin D2, on the other hand, is derived from plants that are formed as a result of irradiation of ergosterol and is obtained by humans through diet.[2] Irrespective of its source, vitamin D has to be converted into its active form in our body by a couple of hydroxylation reactions taking place in the liver and kidney, finally yielding 1,25 dihydroxycholecalciferols, the active form of vitamin D.[1] Vitamin D is a unique chemical substance because it not only acts as a vitamin but also sub-serves the function of a hormone. It has far-reaching effects throughout the body, to the extent that researchers now believe it can affect almost 3% of the human genome. Vitamin D has receptors distributed throughout the body, including smooth muscle cells and vascular endothelium.[3] Therefore, it positively affects bone health and reduces the risk of many autoimmune disorders, type II diabetes mellitus, multiple cancers, infectious diseases, and cardiovascular disorders. More than a vitamin, or a hormone, now vitamin D is considered to be an immunomodulating agent.[1] Despite its overall health benefits and metabolic actions, there are nations in the world, like ours, who suffer from a deficiency of vitamin D. This deficiency of vitamin D has now become a pervasive health disorder, affecting all age groups worldwide.[4] Pakistan, being a developing Asian country, is among the top nations to face this immense public health concern. Vitamin D deficiency in Pakistan can be attributed to several reasons, like lack of awareness and poor access to rich dietary sources of $25(OH)D_3$, which provide around 10-20% of the total vitamin D requirement.[5] Regarding de novo synthesis of vitamin D, it is essential to note that only ultraviolet B radiations (UVB) of the sun are helpful, which depends upon the season and latitude of a particular geographic area. The UVB spectrum ranges from 280-320 nm.[4] Other contributing factors in vitamin deficiency include altitude, weather conditions, and cultural and social factors, such as outdoor behavior, clothing styles, sunscreens use, and skin color, which also affect the synthesis of vitamin D from ultraviolet B radiations. Research has proven that dark skin types produce up to six times less vitamin D than pale skin. These facts make us believe that the Pakistani population, primarily brown and dark complexion, will likely suffer from vitamin D deficiency.[6] Considering the significant impact of vitamin D on various organs throughout the human body, finding the truth about its plasma levels in our population residing in Lahore is imperative.

Lahore is a densely populated metro city with an estimated population of 13.54 million in the year 2021-22, showing a 3.41% increase from 2020-21. It is the 2nd most populous city in Pakistan after Karachi and the 26th most populous city globally. As the population shows a rapid upwards trend, it is utmost necessary to identify the age group(s) most vulnerable to vitamin D deficiency and consequently exposed to complications such as rickets and osteomalacia. The current study measures plasma vitamin D levels in different age groups residing in Lahore and identifies the age group most vulnerable to vitamin D deficiency.

METHODOLOGY:

A cross-sectional study was done from June 2020 through August 2022. The blood samples were obtained from the 2661 population of various areas within Lahore, one of the cosmopolitan cities of Pakistan. The sample size was calculated using Openepi software version 3. The participants were categorized into various age groups, from one year to above 60 years of age. Gender-wise stratification was done to address bias. The results obtained highlighted the most vulnerable group with vitamin D deficiency.

Approximately 5 ml of venous blood was obtained from the recruited individuals while adhering strictly to aseptic measures. 0.2 ml of dipotassium EDTA was added to each sample. Samples were then thawed, using a low-speed vortex, then carefully transferred to centrifuge tubes for centrifugation at a maximum of 25000-30000 g/minute for 15 minutes duration. Vitamin D levels were estimated quantitatively by utilizing a single-step immunoassay using CIMA technology called Chemiflex. This technique utilizes paramagnetic anti-vitamin D-coated microparticles mixed with measured sample voluandith assay diluent. $25(OH)D_3$ present in the sample is dislodged from vitamin-binding proteins, bind with coated microparticles, forming large antigen-antibody complexes. After 10 minutes of incubation, a conjugate composed of acridinium-

labeled vitamin D is mixed with the reaction solution, binding with the anti-vitamin D coated D-coated tiles. Further incubation for 15 minutes is done, followed by washing with a given buffer solution. Given that trigger and pre-trigger solution are added to the mixture, the resulting chemiluminescent reaction is estimated and detected by ARCHITECT *i*system optics. The ARCHITECT *i*system employs a four-parameter logistic curve fit data reduction method to generate a calibration curve, with measuring intervals ranging between 3.4 nmol/L to 155.9 nmol/L.

All samples were stored, processed, and analyzed in Arif Clinical Research Laboratory in Lahore. The results of plasma estimation of vitamin D are listed in Table – 3, given below.

RESULTS

The seven major categories of various age groups are listed below in Table 1 according to manual kit criteria[7]. Plasma Vitamin D estimations were performed in 2661 individuals (n=2661). These individuals were categorized by age groups (Table-1), between males and females within each category. Vitamin D deficiency was graded as 'normal, mild deficiency, severe deficiency, excess and toxicity' (Table-2).

TABLE-1: Categories of various age groups

Category number	Age group (in years)
1	1-10
2	11-20
3	21-30
4	31-40
5	41-50
6	51-60
7	Above 60

Each age group is further split into males and females separately, and according to the kit manual, The highest percentage of vitamin D deficiency was reported in group-3 (21-30 years; 39.3%). With regards to gender, among males, group one (1-10 years; 24.2%) was found to be most deficient in vitamin D. Further, the optimum plasma levels of vitamin D among females with the highest percentage of vitamin D was in group seven (above 60 years; 25.7%) and group one (1-10 years; 18.9%) among males—further, the severity of vitamin D deficiency presented in Table 3.

Table-2: Plasma vitamin D levels estimated in different age groups.

Age Group	Optimum level		Mild deficiency		Severe deficiency		Excess		Toxicity		
	30-100 ng/ml		**20-30 ng/ml**		**Less than 20 ng/ml**		**100-150 ng/ml**		**>150 ng/ml**		**Total**
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	
0 - 10	39	21	23	16	50	42	6	2	3	4	206
11 - 20	16	25	11	14	28	56	3	1	1	0	155
21 -30	48	73	20	39	101	192	3	6	4	2	488
31 - 40	73	109	38	58	118	199	1	1	1	0	598
41 - 50	40	102	37	54	98	105	2	2	1	1	422
51 - 60	42	92	31	63	55	102	6	3	3	2	399
Above 60	45	96	31	69	64	57	4	4	2	1	373

Figure: 1 Plasma Vitamin D Levels estimated in different age groups

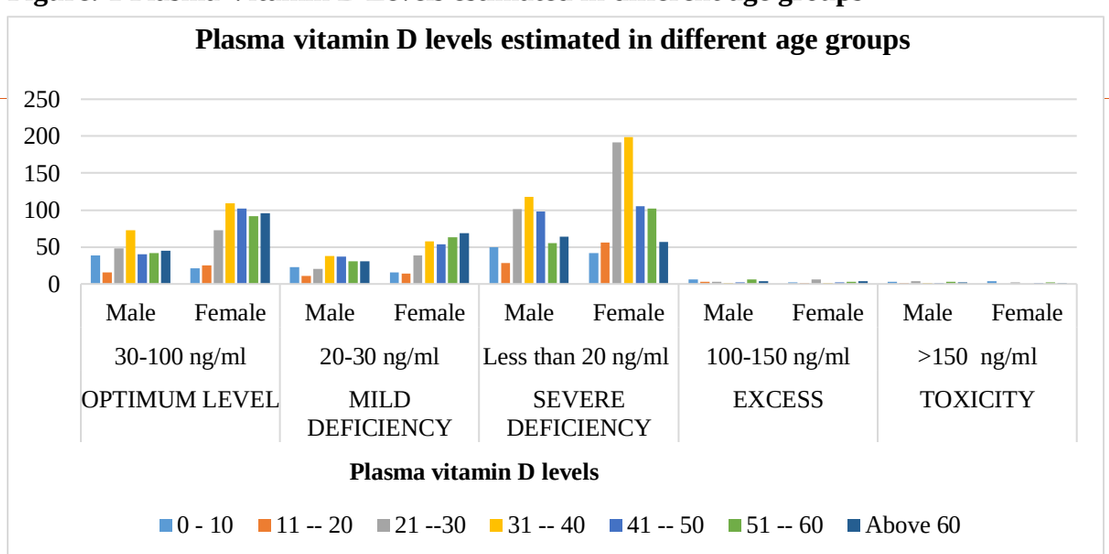

Table-3: Vitamin D levels expressed in percentages.

Age group	Optimum level		Mild deficiency		Severe deficiency		Excess		Toxicity	
	30-100 ng/ml		20-30 ng/ml		Less than 20 ng/ml		100-150 ng/ml		>150 ng/ml	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
0 - 10	18.90 %	10.10 %	11.10 %	7.70%	24.20 %	20.30 %	2.90 %	0.90%	1.40 %	1.90%
11 -- 20	10.30 %	16.10 %	7.00%	7.70%	18.60 %	36.10 %	1.90 %	0.60%	0.60 %	0.00%
21 --30	9.80%	14.90 %	4.00%	7.90%	20.60 %	39.30 %	0.61 %	1.22%	0.81 %	0.40%
31 -- 40	12.20 %	18.20 %	6.30%	9.60%	19.70 %	33.20 %	0.16 %	0.16%	0.16 %	0.00%
41 -- 50	9.04%	23.00 %	8.30%	12.20 %	22.17 %	23.75 %	0.45 %	0.45%	0.22 %	0.22%
51 -- 60	10.02 %	25.50 %	7.70%	15.70 %	13.70 %	25.50 %	1.50 %	0.75%	0.75 %	0.50%
Above 60	12.06 %	25.70 %	8.31%	18.40 %	17.10 %	15.20 %	1.07 %	1.00%	0.50 %	0.26%

The vitamin D level was classified according to kit manual criteria. The results show (Figure 2) that females have a severe vitamin D deficiency at ages 21-30 and mild deficiency at ages 11-20. Contrary, their male counterpart has severe vitamin D deficiency in a middle age group (41-50 years) and mild deficiency in age group from 31-40 years of age. Additionally, older age group females (51-60 and >60 years) were found to have an optimal level of vitamin D. However, in males, the children group (0-10 years age) was reported to have an optimal level of vitamin D as shown in Figure 2 and Table 4.

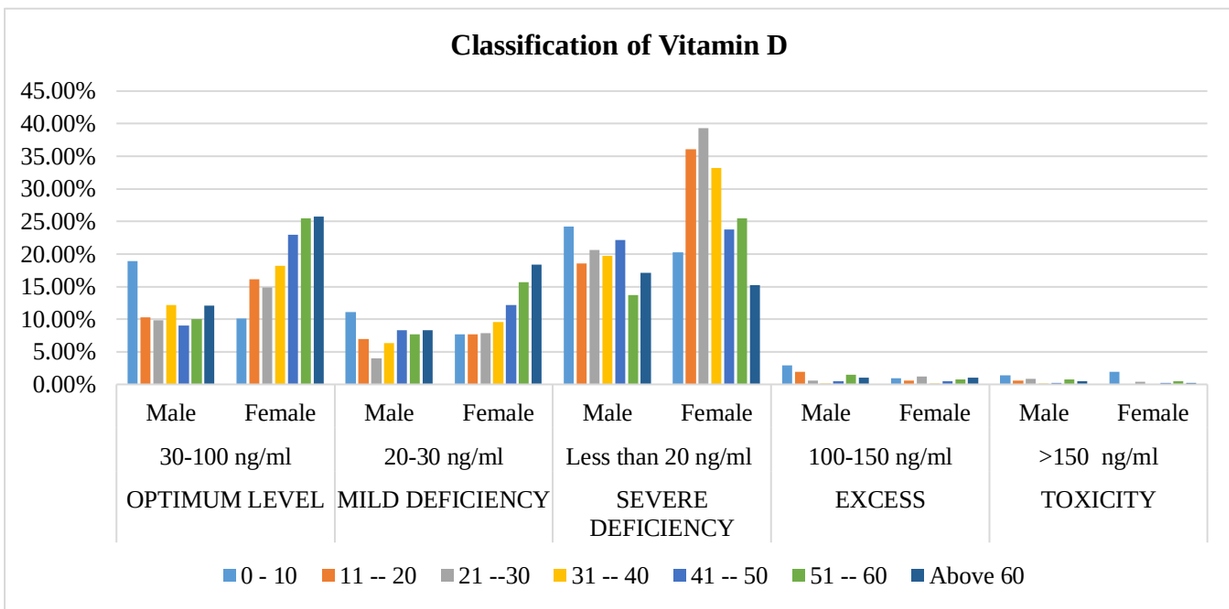

Figure 2: Percentage of Vitamin D levels by gender and severity

Table 4: Highest and Lowest Percentage Age in each group

SR NO	Vitamin D levels	Highest %age	Lowest %age
1	Optimum levels	Males: age group 1 (1-10 yrs): 18.9% Females: age group 7 (above 60 yrs) 25.7%	Males: age group 5 (41-50 yrs): 9.04% Females: age group 1 (1-10 yrs) 10.1%
2	Mild deficiency	Males: age group 1 (1-10 yrs) 11.1% Females: age group 7 (above 60 yrs) 18.4%	Males: age group 3 (21-30 yrs) 4.0% Females: age group 3 (21-30 yrs) 7.9%

3	Severe Deficiency	Males: age group 1 (1-10 yrs): 24.2% Females: age group 3 (21-30 years): 39.3%	Males: age group 4 (51-60 years) 13.7% Females: age group 7 (Above 60 years): 15.2%
4	Excess	Males: age group 1 (1-10 years): 2.9% Females: age group 3 (21-30 years): 1.2%	Males in age group 4 (31-40 years): 0.16% Females: age group 3 (21-30 years): 0.16%
5	Toxicity	Males: age group1 (1-10 yrs): 1.4% Females: age group 1 (1-10 yrs): 1.9%	Males: group 4 (31-40 yrs); 0.1% Females in age group 2 (11-20 years): 0%

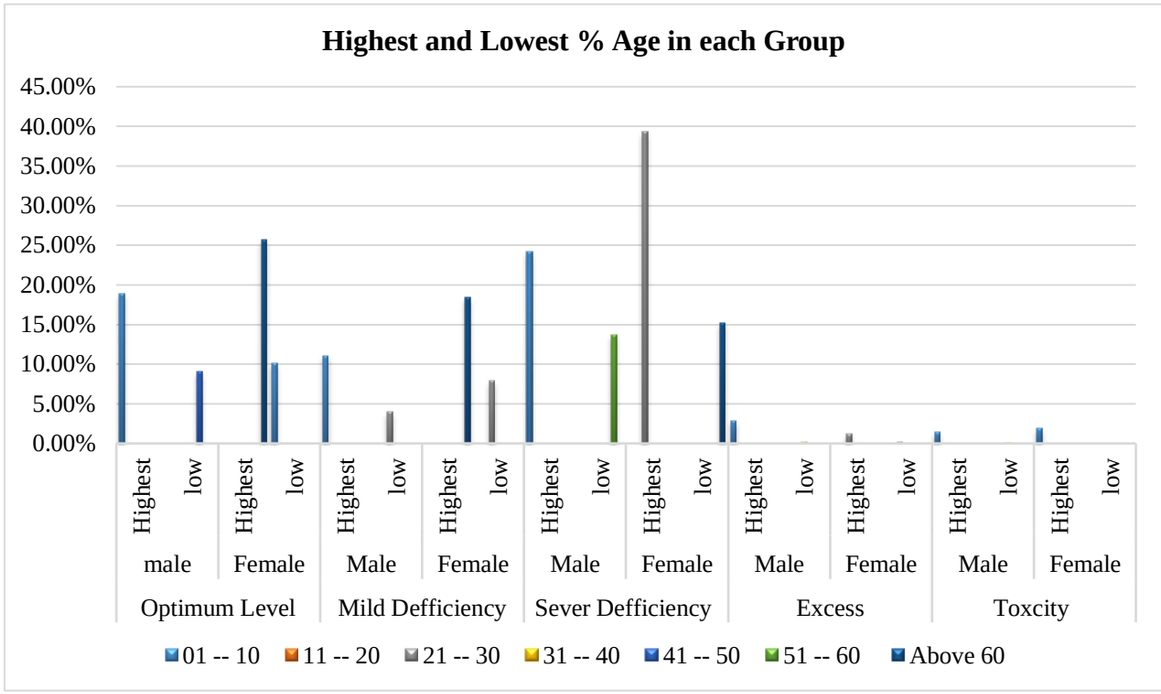

Figure 3: Highest and lowest percentage of Vitamin D by each group

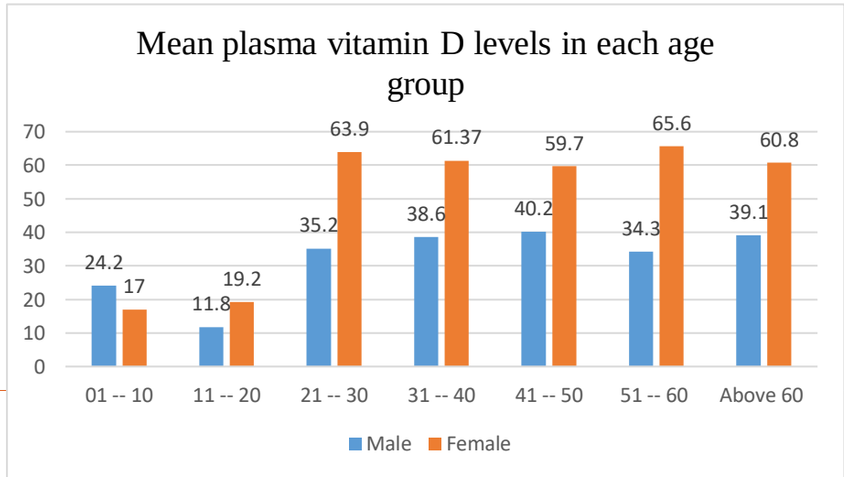

Figure 4: Mean Plasma Vitamin D level by each group

The mean vitamin D level varies significantly in males and females among all age groups, as shown in Figure 4. The obtained results show that the age group among females with the highest percentage of severe vitamin D deficiency is group-3 (21-30 years; 39.3%). In comparison, the age group among males with the highest severe vitamin D deficiency rate is group-1 (1-10 years; 24.2%). Regarding toxicity, among females, it was age group-1 (1-10 years; 1.9%), whereas, among males, most cases with toxicity belonged to age group-1 (1-10 years; 1.4%).

Table 5: Mean Plasma Vitamin D Levels in Each Age Group

Age	Male	Female	Total
01 -- 10	24.2	17	206
11 -- 20	11.8	19.2	155
21 -- 30	35.2	63.9	488
31 -- 40	38.6	61.37	598
41 -- 50	40.2	59.7	442
51 -- 60	34.3	65.6	399
Above 60	39.1	60.8	373

DISCUSSION

According to the Endocrine Society's Practice Guidelines on Vitamin D, a level of 25-hydroxyvitamin D < 20ng/ml is considered to be deficient, a level between 21-29ng/ml is insufficient, and a level of 30-100ng/ml falls within an acceptable range.[1] Having identified the innumerable health benefits of vitamin D, researchers have now explored the existing vitamin D levels in their population to realize the magnitude of this emerging issue of public health concern. In our study, vitamin D deficiency was observed in all groups, with more severe at ages 21-30 years and mild deficiency at ages 11-20. Contrary, their male counterpart has severe vitamin D deficiency in the middle age group (41-50 years) and mild deficiency in the age group from 31-40 years of age. Additionally, older age group females (51-60 and >60 years) were found to have an optimal level of vitamin D. However, in males, the children group (0-10 years) was reported to have an optimal level of vitamin D. Our study results are consistent with many regional studies. According to a study conducted in 2008, over a billion people worldwide were estimated to be either vitamin D deficient or insufficient.[3] A study conducted among healthy young people residing in eastern Saudi Arabia showed around 28%-37% prevalence of vitamin D deficiency.[8-9.]Our results showed variations with regard to studies done in the West. The variations are more likely due to geographic and climatic changes apart from nutritional and economic issues. The studies in Western settings showed that the public health issue is not only affecting the Asian population. A study showed that 40% European population is deficient in vitamin D, with around 13% population suffering from severe Vitamin D deficiency (<12ng/ml).[10] Similarly, another study conducted in the United States of America revealed severe vitamin D deficiency in around 5.9% population, along with an overall prevalence of vitamin D deficiency of 24%.[10-11] Saraffin et al. in 2015 conducted research on the Canadian population and came up with the finding that 7.4% population had severe vitamin D deficiency, while overall, 37% population was identified to be suffering from vitamin D deficiency.[12] Despite all these findings, some countries still have an even higher prevalence of low vitamin D levels. Notable among these are India, Tunisia, Pakistan, and Afghanistan. Some studies even reveal that around 490 million people in India are deficient in vitamin D.[13-14]

Our studies are consistent with a few studies in Pakistan. Pakistani citizens showed overall vitamin D deficiency in 53.5% of the population, vitamin D insufficiency in 31.2%, and only 15.3% had normal vitamin D levels.[15] In a similar study conducted in Karachi in 2012, the median vitamin D level of 18.4ng/ml, with 84.3% of participants suffering from deficient/insufficient vitamin D levels.[12] In the province of Sindh, a cross-sectional study conducted in 2018 recruited 100 apparently healthy volunteers and reported vitamin D_3 deficiency in 77% of these volunteer subjects.[15] Along with the same line, many other researchers have necessitated the need to generate data in our country's other regions to identify the vulnerable age groups suffering from vitamin D deficiency. Unlike our study, most of the research studies have highlighted the overall percentage of patients deficient in vitamin D. Our study has bridged significant research gaps by providing data regarding vitamin D deficiency in specific age groups.

CONCLUSION

The most vulnerable age group with severe vitamin D deficiency was group-3 females (39.3%), whereas severe vitamin D deficiency was detected least in group-6 males (13.7%). Further studies are required to elucidate the probable causes of severe vitamin D deficiency in group-3 females (21-30 years of age). The age group with the highest percentage of optimum vitamin D levels was group-7 females (25.7%),

whereas optimum levels were least observed in age group-5 males (9.04%). The study will help to adopt public measures for the prevention of vitamin D deficiency in the most susceptible age group and to formulate policies at the government level for prompt access to better healthcare facilities for these patients. This will considerably help to reduce the incidence of vitamin D deficiency in the most vulnerable group by providing better healthcare facilities for these individuals.

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