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Prevalence and Determinants of Vitamin D Deficiency among Children Aged 0-14 Years in Lahore and Rawalpindi Pakistan

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ABSTRACT

Vitamin D isn't merely a vital portion of a nutritious diet but also acknowledged as the hormone benefited from sun. It takes responsibility as the intake of phosphorus and calcium also helps to support making bones and teeth stronger. The term deficit of Vitamin D means that a person does not have sufficient vitamin D in body. It mostly causes problems with bones and physique. Vitamin D deficiency can cause bones disorder, like rickets and osteomalacia also responsible for different metabolic syndromes, diabetes, autoimmune diseases, and heart diseases. A 42 percent of Americans have lack of vitamin D, and 15 percent of children between the ages of 1 and 11 are thought to be deficit in vitamin D. Additionally, researchers found that 32 young adults and 17 adolescents both had vitamin D deficiencies. The ambition of this study is to evaluate correlation of Vitamin D deficit among both genders of children, how Vitamin D deficiency is associated to different age groups of children from 0-14 years and to evaluate connection of Vitamin D deficiency with youngsters from different regions of Pakistan such as Rawalpindi and Lahore. The data in the form of reports was collected from the respective laboratories and then analyzed on the SPSS 22.0 to assess the relationship of Vitamin D deficit with children of different age groups belonging to different regions. A total of 1000 volunteers from various regions of Pakistan, aged 0 to 14, participated in the 01-year research on vitamin D deficiency. Our data demonstrate that 517 subjects were male out of 1000, and 483 were female also we found out that 43% of children were vitamin D deficit (<25 nmol/L), and out of 1000 participants 35.8% were inadequate to vitamin D (25-55 nmol/L). Only 15.9% of children observed as Vitamin D sufficient (55-100 nmol/L). We also noticed that kids aged 0 to 4 lacked vitamin D (22.2 %). The research demonstrated the deficit and inadequacy of Vitamin D in youngsters and teen-agers pose serious health complications in the pediatric population of Pakistan. The distribution of deficiencies was the same despite their age or gender. This form of vitamin D inadequacy in males most probably caused by various reasons, including low early-life consumption of vitamin D supplements.

Keywords: **rickets, osteomalacia, metabolic syndromes, diabetes, autoimmune diseases, heart diseases, chemiluminescence, public awareness campaigns**

INTRODUCTION

It is well known as fat-soluble which plays a pivotal part in the Body. It's essential for healthy bones, Teeth, and muscles, as well as for overall good. The sun's ultraviolet rays is first source of vitamin d, Skin use these rays and convert into vitamin D3. Still, vitamin D can be taken from many supplements and meals. Calcium and phosphorus is regulated by vitamin D from food, which is necessary for bone growth and development.

Without sufficient Vitamin D, the body cannot form and maintain strong. Vitamin D may help reduce the threat of infections, including respiratory tract infections and autoimmune conditions like multiple sclerosis and rheumatoid arthritis.

Production of D3 Vitamin by skin is first step and it is non enzymatic system. 7- dehydrocholesterol is responsible for making D3(cholecalciferol) with dual step process that the B ring is disintegrated through UV radiation (UVB 280 – 320 diapason) of the sun to produce vitamin D3, isomerization of D3 in a heat sensitive, however a non-catalytic phenomenon. Both the UVB intensity plus the location for saturation of skin contributing in the rate of this Vitamin's formation.(Holick et al., 1980)

Fatty fish food usually has amount of vitamin D. The vitamin D from fish meat has D3, and that utilize for bastion has contained D2 (ergocalciferol). UVB rays helps to generate D2 from ergosterol into few fungi and plants. It is different from D3 to have purely a dual bond between Carbon 22 and Carbon 23 and Carbon 24 has a methyl group inside the chain. First analog of Vitamin D is D2.(Houghton & Vieth, 2006)

DBP affinity is reduced by side chain variation from D3, allowing it to circulate more quickly, limit its transformation to 25OHD through at least few of the 25- hydroxylases to be defined, and change catabolism through the 24- hydroxylase (CYP24A1). As a result, if not taken daily, supplementation of D2 does not raise 25OHD levels in the blood to the same extent as equivalent amounts of D3. The VDR, however, is a favourite of 25(OH) 2D2 and 25(OH) 2D3, respectively.(Hollis, 1984)

It is initial step of vitamin D synthesis, which takes place by non enzymatic process in the epidermal skin. D3 vitamin is responsible for its common vitamin D in the body. 90% – 95% of D3 Vitamin within the human body is made up in the skin with the help of effect of sun light. thus, the sun light is the common source of vitamin D formation, also if it has adequate sun exposure, it will no requirement to obtain fresh vitamin D.(Acar & Özkan, 2021)

Activation of vitamin D deprived of enzymes is under the process of ultraviolet B rays having wavelengths of 280 – 310 nm in range which is responsible for bond making in the B ring of pro-D3 vitamin also 7- dehydrocholesterol, during D3 Vitamin formation into the epidermal skin. Latterly, there are distinct double bonds are produced between the broken domains of carbon within the B ring by heat sensitive by enzymatic phenomenon, and the production of D3 vitamin from pre-D3 vitamin has completed.

Sometimes too much exposure of sunlight is responsible for, pre-D3 vitamin to make them converts to a number of inactive by products because of photolysis, similar to unrecoverable lumisterol or tachysterol (able to reverse back to pre-vitamin D).

Vitamin D is not considered as proper Vitamin, because persons having exposure to sun are not able to bear salutary supplementation. Also, there are some other Vitamin D sources that are salutary, can be egg, fish, yolk, oil painting oil and many shops. (Jäpelt & Jakobsen, 2013)

Self-made or regular made form of vitamin D at home is known ergosterol or D2. Generally, natural intakes do not comprise respectable Vitamin D levels plus sun exposure or consuming food sources persistently have Vitamin D supplementation are needed to help scarcities.(Acar & Özkan, 2021) Vitamin D, even D2 or D3, doesn't have considerable natural exertion.

cholecalciferal is hydroxylized into 25-hydroxycholecalciferol via 25-hydroxylase enzyme. The biologically active form of 25-hydroxycholecalciferol is 1,25-dihydroxycholecalciferol, is produced by 1-a-hydroxylase, using it as a substrate.

There are three processes to metabolize vitamin D are: first is 25- hydroxylation, second is 1α- hydroxylation and third one is 24- hydroxylation, these all are handled by the cytochrome P450 (CYPs) and its chief purpose is oxidation.(Christakos, Dhawan, Verstuyf, Verlinden, & Carmeliet, 2016)

The endoplasmic reticulum (ER) has various enzymes like (CYP2R1), as far as mitochondria is concerned containing enzymes like (CYP24A1, CYP27AI, and CYP27BI. The reduced P450 reductase depends on NADPH serves as the patron of electron for enzymes of ER.(Bikle & Christakos, 2020)

The chain of electron patronfor the enzymes of mitochondria consist of ferredoxin reductase and ferredoxin. Metabolism of vitamin D needs CYPs to work, and responsible for formation of CYP24A1 and CYP2RI, all enzymes consist of features that are structurally same. These consist of 12 helices (A to L), circles, and a prosthetic group that is common, viz., protoporphyrin IX (brim), which contains iron and is connected to cysteine thiolate. Above the brim, in the region of the enzyme where I helix runs, a thr (ser) and asp (glu) brace is necessary for catalytic activity. CYP2R1 has two redundant helices that seem to form a substrate passage in the ER bilayer, same as other CYPs microsomes. As a gate, the B helix closes on the substrate list. It is unclear whether the CYPs of mitochondria have a substrate passage that is similar to this one.(Wills & Savory, 1984)

One or two cytochrome P450 enzymes carry out the 25-hydroxylation of D3 Vitamin and these enzymes are installed inside the portions of microsomes or mitochondria of the liver.(Hosseinpour, Ibranovic, Tang, & Wikvall, 2003) Currently, only the gormandizer has had both the (CYP27A1) 25-hydroxylase of mitochondria and (CYP2D25) the 25-hydroxylase of microsomes replicated and studied. In this study, the two enzymes' roles in the 25-hydroxylation of D3 Vitamin in primary hepatocyte cultures are studied. 7-nascence-hydroxy-4-cholesten-3-one and tolterodine were both shown to be selective inhibitors of the CYP27A- and CYP2D25- intermediated 25-hydroxylation of D3 Vitamin in inhibition studies. Each resource added, reduced the overall 25-hydroxylation of D3 Vitamin by the same amount in primary liver cells. Another hydroxylase conditioning tests weren't set up with any inhibition. Phorbol 12-myristate-13-acetate decreased the expression of CYP27A1

and CYP2D25 as well as the activity of the hepatocytes' 25-hydroxylase. The findings show that CYP27A1 and CYP2D25 are crucial for the 25-hydroxylation of D3 Vitamin in hepatocytes and for its bioactivation.(Wu et al., 2020)

In the kidneys concentration of 1, 25(OH) 2D circulates. Unlikely 25-hydroxylation, only one enzyme, CYP27B1, is known having activity of 25OHD 1-hydroxylase. Vitamin D3 must undergo both a 25- and a 1 alpha-hydroxylation in order to become the dihydroxyvitamin D3 hormone, which adjusts calcium.(Kim et al., 2007) The liver's mitochondrial CYP27A and CYP2D25 in microsomes are suitable for producing the earlier bio activation footstep. Significant 25-hydroxylase activity has also been shown to be compulsory. The enzyme isolated from the gormandizer order and generation of enzyme in COS cells by recombinant phenomenon catalyzing the 25- and 1alpha-hydroxylation of D3 Vitamin as well as the 1alpha-hydroxylation of D3 Vitamin itself.(Eghlidi, Garyfallou, Kohama, & Urbanski, 2017)

cDNA consists of 500 amino acids. The renal enzyme's DNA sequence and the resultant sequence of peptide are identical to those of vitamin D3 25-hydroxylase CYP2D25 of liver cells. CYP2D25 was found to be encoded by a single gene in gormandizer, according to genomic southern spot analysis. Immunohistochemistry experiments revealed that cortical proximal tubule cells express CYP2D25 almost exclusively.(Miller & Portale, 2000)

The Vitamin D3 25-hydroxyl epimerization of (25(OH) D3) in the form of (3- epi- 25(OH) D3) is triggered by the act of the enzyme 3- epimerase. In serum, 3-Epi-25(OH)D3 is a significant vitamin D source. It can be transformed to 3-epi-dihydroxyvitamin D3 by the enzyme CYP27B1, which typically exerts less natural energy than Vitamin D3 dihyroxyl (1,25(OH) 2D3.(Yang et al., 2022) The 25(OH) D3 3- epimerase has been inadequately characterized to date and the gene garbling it has not been linked. The 3- epimerase has been reported to be present in the microsomal bit of cells, including liver cells, and to use NADPH as cofactor.

The 3- epimers are formed by its action on 24, 25(OH) 2D3 and 1, 25(OH) 2D3 and. Utilizing 25(OH) D3 as a substrate also HPLC to examine conformation of product, we have characterised 25(OH) D3 3- epimerase activity in mouse and mortal microsomal liver in this study.

Conclusively, vitamin D3 plus 3- epi- 25(OH) D3 are interconverted reversibly by both human and parasite D3 Vitamin 3- epimerase, with NADH acting as the preferred cofactor. It is possible that enzyme possesses a firm linked NAD at its active site that is only released following reduction, as suggested by lack of requirement for exogenous NAD.(Tang et al., 2022)

The family member of cytochrome P450, CYP11A1 has a number of important roles to perform in the human body. With the formation of pregnenolone from cholesterol, catalyzing the first and charge-limiting stage in the steroid genesis process. In addition to the placenta, gonads, and other traditional steroid-producing organs, the establishment of CYPIIAI in brain, GIT, susceptible systems, and eventually skin. As a result of cholesterol conditions in mitochondria, StAR protein controls CYP11A1 activity primarily in skin.

Through interactions with a variety of receptors, the byproducts of these passageways control the protective hedge plus skin vulnerable processes in an environment-dependent manner. Skin pathology may result from instabilities in CYP11A1 activity.(Maharaj et al., 2019)

In the small intestine, calcitriol reacting vitamin D receptors to raise the efficiency of absorption of phosphorus and calcium in intestine from around 10- 15 till 30- 40 and 60 to 80, respectively. Similar to this, calcitriol interacts to vitamin D receptors to excite RANKL inside osteoblasts, which then stimulates immature preosteoclasts to develop into mature osteoclasts (bone resorbing). In order to maintain blood levels of phosphorus plus calcium, comparable osteoclasts (mature) remove phosphorus plus calcium from bone. Calcitriol also promotes reabsorption of calcium from glomerular filtrate in feathers.(Wang et al., 2022)

In accordance to studies, many kids aren't getting plenty of vital vitamin, which is critical for supporting children's healthy growth. About 42 percent of Americans are vitamin D deficit, which can be attributed to change in life and usage of sunscreen. Approximately 15 youngsters aged 1 to 11 had inadequate Vitamin D status. Additionally, researchers found that 32 young adults and 17 adolescents had vitamin D deficiencies.(Portal)

In addition to being an important component of a balanced diet, vitamin D is sometimes referred to as the "sun hormone." It is in charge of ensuring that phosphorus and calcium are consumed, which supports the development and strengthening of teeth and bones.(Moorani, Mustufa, Hasan, & Kubar, 2019) Children in Pakistan who are vitamin D deficit have weak bony mass, osteoporosis at an early age, also osteomalacia. In addition, it results in enhanced teeth depressions, damaging alveolar bone surrounding teeth, plus other issues related to complaints.(Fakhar-Ul-Zaman, Akram, Khan, & Rafique, 2016) It may result in melancholy, exhaustion, and decreased appetite. This study intends to track vitamin D inadequacy, and sufficiency, in Pakistani children, adults, adolescents, and elderly.(Bughio, Ahmed, Ashfaq, Nisa, & Hussain, 2020)

A sufficient intake of vitamin D is required to combat osteomalacia and rickets. Regarding the role of vitamin D in preventing fractures and osteoporosis, there is still debate.(Charoenngam, Shirvani, & Holick, 2019)

Vitamin D seems to have both direct plus indirect effects on bone remodeling and growth, which is crucial clinically to prevent rickets in young children also fracture plus osteoporosis in older people. What dosage of vitamin D is appropriate in relation to other situations, such as osteoporosis and osteomalacia, is the main point of contention regarding the final

decision.(Polly & Tan, 2014)

In overweight people who are more susceptible to advance diabetes mellitus also metabolic syndrome, vitamin D status is reduced.(Moukayed & Grant, 2019) The VDR is present in adipocytes, also 1,25(OH)2D boosted lipogenesis while reducing lipolysis. The VDR is visible in the pancreatic beta cells, and 1, 25(OH) 2D increases insulin production.(Wimalawansa, 2018) Vitamin D supplementation may be beneficial for people having diabetes mellitus or those with pre-diabetes, according to clinical trials, in terms of preventing or improving diabetes (Theik et al., 2021)

Vitamin D deficit can lead to cancer since when it is present in appropriate amounts in the body, it inhibits cell growth by preventing critical steps in cell cycle or by blocking growth factor signaling, promotes DNA repair, prevents angiogenesis of tumor, and lowers metastasis. The creation of an analogue that, when it collapses, can result in a tumor in the body because of its specific tissues with regard to impacts on bone reabsorption and calcium absorption.(Chandler et al., 2020)

Both myocytes and fibroblasts in heart have CYP27B1 and VDR articulations. In addition, vitamin D deficit in humans has been linked to cardiomyopathy, and a large count of studies for epidemiology have found an association between higher risk of cardiovascular disease and decreases in calcifediol status. Vitamin D plus its analogues contain cardiac hypertrophy markers, and elimination of VDR, especially from heart, resulting hypertrophy.(Cosentino et al., 2021) However, no significant clinical randomised trials have been conducted to date specifically to test the Vitamin D role or any analogues in the avoidance or cure from CVD, also the findings from crack studies having CVD as consequence haven't been convincing.(Hiemstra, Lim, Thadhani, & Manson, 2019; Purow & Sokol, 2019)

Reactions of innate immunity, involving the stimulation of Toll-like receptors, result in the production of antimicrobial peptides like reactive oxygen species and cathelicidin which cause the organism to perish. Vitamin D is necessary for cathelicidin to develop, thus if there is deficit of Vitamin D, the immune system is negatively impacted and many infectious disorders, such as atherosclerosis and allergies, are caused. (Maruotti & Cantatore, 2010).

A lipopeptide from contagious element, such as M. tuberculosis, that stimulates TLR2 in macrophages causes an enhancing expression of VDR CYP27BI, that in the existence of enough vitamin D, leads to production of cathelicidin.(Bui, Zhu, Hawkins, Cortez-Resendiz, & Bellon, 2021) Resulting, vitamin D levels that are appropriate encouraging the reaction from innate immunity. Generally speaking, vitamin D inhibits the acquired immunity. Vitamin D slows down maturation of DCs, affecting their capacity to deliver antigen and subsequently trigger T cells.(Chun, Liu, Modlin, Adams, & Hewison, 2014) Additionally, 1,25(OH)2D prevents the development of the Th1 cells, capable of producing IL-2, IFN-γ also Th17 cells capable in production of IL-17 by inhibiting the formation of IL-12, which is crucial for Th1 progress, also IL-6 and IL-23, which are crucial for Th17 growth and functioning.(Ao, Kikuta, & Ishii, 2021).

Present study was conducted with objectives 1) to evaluate the connection between young children's gender and vitamin D deficiency, 2) to evaluate the connection between different child age groups (0–14 years) and vitamin D deficiency and 3), to evaluate the connection between Rawalpindi and Lahore children's vitamin D deficiencies.

MATERIALS AND METHODS

A single point in time was used to collect the data for this cross-sectional investigation. The study included children aged 0–14 years. This age range was selected based on previous literature indicating a high prevalence (approximately 50%) of vitamin D deficiency among children within this group.

Sample Size

The University of Lahore conducted the study utilizing 1,000 vitamin D lab data from kids ages 0–14. Reports from several diagnostic labs in Rawalpindi and Lahore were acquired.

Sample Collection

After a 12-hour overnight fast, venous blood samples were collected aseptically. After being shielded from the sun, the samples were centrifuged to separate the serum. Since serum was needed for analysis, blood was drawn in red-top tubes or serum separator tubes (SST) without additions.

Laboratory Analysis

Venous blood samples were taken aseptically following a 12-hour overnight fast. After being shielded from the sun, the samples were centrifuged to separate the serum. Since serum was needed for analysis, blood was drawn in red-top tubes or serum separator tubes (SST) without additions. Serum 25(OH)D concentrations were measured to determine vitamin D status. Deficient levels were defined as less than 25 ng/mL, insufficient levels as 25–55 ng/mL, sufficient levels as 55–100 ng/mL, and excess levels as greater than 100 ng/mL

Inclusion Criteria

The study included children ages 0–14 whose blood vitamin D levels were less than 55 ng/mL. The study excluded children with underlying medical illnesses that are known to impact vitamin D metabolism, such as eating disorders, multisystem disorders, and chronic gastrointestinal ailments.

Laboratory Methods

A biochemical method for identifying tiny biological molecules is chemiluminescence immunoassay. Its foundation is antigen-antibody reactions, in which antibodies tagged with enzymes react with substrates to emit light. The intensity of light emitted is directly proportional to the sample's 25(OH)D content.

Vitamin D metabolites were separated, identified, and quantified using HPLC. Metabolites are extracted, separated using a stationary phase column, and then detected using a mass spectrometer or UV-visible detector. Accurate quantification of 25(OH)D2 and 25(OH)D3, including their epimers, is made possible by this method.

Instruments Used

CLIA technology is used by the DiaSorin Liaison system to quantitatively determine 25(OH)D. Acetonitrile is used to extract vitamin D, followed by phase separation, incubation with certain antibodies, and detection of the chemiluminescent signal that is released in proportion to the analyte concentration.

The PerkinElmer kit uses protein precipitation and solvent extraction techniques, which are then followed by HPLC-MS/MS analysis. To quantify 25(OH)D2 and 25(OH)D3, 150 µL of serum samples were combined with precipitating solution, centrifuged, dried under nitrogen gas, reconstituted with reagent, and then injected into the HPLC-MS/MS system.

Statistical Analysis

For statistical analysis, SPSS version 22.0 for Windows was used. Pearson's chi-square test was used to compare categorical variables. Pearson's correlation coefficient was used to evaluate correlations between binary variables. The mean ± standard deviation was used to express continuous variables. P-values less than 0.05 were regarded as statistically significant.

RESULTS

A total of 1,000 children aged 0–14 years were included in the study. Vitamin D levels were categorized as deficiency (<25 nmol/L), insufficiency (25–55 nmol/L), sufficiency (55–100 nmol/L), and excess (>100 nmol/L). Data are presented as frequency (n) and percentage (%) (Table 1). Associations were assessed using the Chi-square test, and $p < 0.05$ was considered statistically significant. Gender distribution according to age group is presented in table 2.

Table 1. Age distribution of study participants (n=1000).

Age range (years)	Frequency (n)	Percentage (%)
0–4	222	22.2
5–9	185	18.5
10–14	593	59.3
Total	1000	100.0

Table 2. Gender distribution according to age group.

Age group (years)	Males (n)	Females (n)	Total (n)
0–4	131	91	222
5–9	73	112	185
10–14	313	280	593
Total	517	483	1000

Table 3. Distribution of participants by region and age group.

Region	0–4	5–9	10–14	Total
Rawalpindi	65	0	361	426
Lahore	157	185	232	574
Total	222	185	593	1000

Table 4. Overall Vitamin D status among children (n=1000).

Vitamin D level (nmol/L)	Frequency (n)	Percentage (%)
Deficiency (<25)	430	43.0
Insufficiency (25–55)	358	35.8
Sufficiency (55–100)	159	15.9
Excess (>100)	53	5.3
Total	1000	100.0

Table 5. Association between Vitamin D status and age group.

Age group	Deficient	Insufficient	Sufficient	Excess	Total
0–4	97	75	36	14	222
5–9	125	47	12	1	185
10–14	208	236	111	38	593
Total	430	358	159	53	1000

Chi-square = 66.126; df = 6; p < 0.001.

Table 6. Association between Vitamin D status and gender.

Gender	Deficient	Insufficient	Sufficient	Excess	Total
Male	200	204	87	26	517
Female	230	154	72	27	483
Total	430	358	159	53	1000

Chi-square = 9.365; df = 3; p = 0.025.

Table 7. Association between Vitamin D status and region of residence.

Region	Deficient	Insufficient	Sufficient	Excess	Total
Rawalpindi	70	202	104	50	426
Lahore	360	156	55	3	574
Total	430	358	159	53	1000

Chi-square = 241.661; df = 3; p < 0.001.

The total of 1000 participants involved in the Vitamin D deficient study were from different regions of Pakistan such as Rawalpindi and Lahore (Table 3). Out of these 1000 participants, 426 from Rawalpindi and 574 from Lahore aging 0-14 years. 65 of the total 426 participants from Rawalpindi aging 0-4 years, there is no participant between 5-9 years and 361 between the ages of 10-14 years.

Out of the total 574 participants involved in this study from Lahore, 157 participants fall under the category of 0-4 years of age, 185 of them fall under the category of 5-9 years and 232 under the category of 10-14 years. It means that the greatest number of participants (574) belonged to Lahore and most of them aging 10-14 years.

Vitamin D status amongst 1000 children of 0-14 years old is characterized into: Insufficient (25 nmol/L), Excess (>100 nmol/L), Sufficient (55–100 nmol/L), and Deficient (25 nmol/L) (Table 4). Out of total 1000 (100%) participants, 430 (43.0%) participants involved in this research study are Vitamin D deficient (<25 nmol/L), 358 (35.8%) are Insufficient (25-55 nmol/L) of Vitamin D, 159 (15.9%) of the total participants have sufficient (55-100 nmol/L) levels of Vitamin D and 53 (5.3%) have excess (>100 nmol/L) Vitamin D levels.

In demand to calculate association between status of Vitamin D and the age of the 1000 children ranging from 0-14 years, we have applied Chi-square test (Table 5-7). The age of the children in years is characterized into descending from 0-4, 5-9, 10-14, along with characterization of Vitamin D levels into: Insufficient (25 nmol/L), Excess (>100 nmol/L), Sufficient (55–100 nmol/L), and Deficient (25 nmol/L). Out of total 1000 participants involved in this study, 222 fall under the age category of 0-4 years, most of the number about 97 are more susceptible to deficit of Vitamin D (<25 nmol/L), 75 children have insufficient (25-55 nmol/L) Vitamin D levels, participants having sufficient (55-100 nmol/L) levels are 36 and only 14 children have excess (>100 nmol/L) Vitamin D levels. Under the age category of 5-9 years, 185 of the total participants fall, mostly about 125 are deficit to Vitamin D (<25 nmol/L), 47 have inadequate (25-55 nmol/L) Vitamin D status, children having sufficient (55-100 nmol/L) Vitamin D level are 12 and only 1 participant has excess (>100 nmol/L) level of Vitamin D. Children aging 10-14 years are 593 out of the total 1000 involved participants in the study, 208 are insufficient to Vitamin D (<25 nmol/L), mostly about 236 children have insufficient (25-55 nmol/L) Vitamin D level, participants having sufficient (55-100 nmol/L) level of Vitamin D are 111 and 38 children are in excess (>100 nmol/L) of Vitamin D level. The p-value (.000) that we get after applying Chi-square test in order to calculate relationship of Vitamin D status with children's age is considered

statistically significant as it is < 0.05.

DISCUSSION

The current study examines the vitamin D levels among the pediatric population in two of Pakistan's biggest cities, Lahore and Rawalpindi. We observed the vitamin D deficiency and insufficiency according to the age, gender, region they live, socioeconomic status, and cultural practices they practice. Vitamin D is a fat-soluble vitamin vital to overall general well-being. It is primarily known for promoting bone health by helping the body absorb calcium and phosphorus from food.(Oden Akman, Tumer, Hasanoglu, Ilhan, & Caycı, 2011; Olmez, Bober, Buyukgebiz, & Cimrin, 2006) However, recent research has shown that vitamin D also plays a crucial role in many other physiological processes, including regulating the immune system, reducing inflammation, and supporting cardiovascular and respiratory health. Vitamin D can be obtained from exposure to sunlight, dietary sources such as fatty fish and fortified foods, and supplements. However, many people worldwide, including in Pakistan, are deficient in vitamin D, which can lead to various health problems.

Research on vitamin D levels in the Pakistani population has already been discussed in many kinds of research, but we did it on a specific age group. A total of 1000 volunteers from various regions of Pakistan, aged 0 to 14, participated in the 01-year research on vitamin D deficiency. Our data demonstrate that 43% of children had vitamin D deficiency (<25 nmol/L), and 35.8 % out of 1000 participants had Insufficiency of vitamin D (25-55 nmol/L). Only 15.9% of children found had a sufficiency of vitamin D (55-100 nmol/L). Multiple aspects affect the level of vitamin D in children, like childhood obesity, less sunlight exposure, and malnutrition. We contrasted our findings with earlier research on the Chinese pediatric population. We discovered the same findings: As children matured, their vitamin D deficiency increased.(Pediatrics, 2009)

We noticed that kids aged 0 to 4 lacked vitamin D (22.2 %). Lack of adequate nutrition in newborns may be the cause of deficiencies. To address their nutritional needs, newborns may require supplements. They will be vitamin D deficient if they do not take supplements with extra vitamin D. This condition can result in several illnesses, including rickets. In this condition, the bones become thin, weak, and malformed.(Ac, Manson, Abrams, & Aloia, 2011)

Our findings reveal that as children aged, their blood levels of the vitamin D metabolite 25- Hydroxy vitamin D declined, and vitamin D insufficiency became more common. Infants had a mean 25(OH) D level significantly greater than any other developmental stage. This is mostly due to the pediatric healthcare professionals' advice that every kid obtains 400 IU of vitamin D daily from two weeks after birth until they are two years old. In Pakistan, pharmacists sell a variety of oral supplements for babies that are produced in the form of oil or aqua drops that include vitamins D and A. Therefore, it is simple for parents to obtain these dietary supplements for their infants. According to the feeding guidelines for babies and toddlers, parents may also give their young children vitamin D-fortified formula, especially those under two.(Chung et al., 2009)

This duration of exposure is recommended twice a week, per the European Society for Pediatric Endocrinology (ESPE) Bone Club, which also draws attention to the fact that this time can be spent wearing full garments but not a cap. Teenage girls in Muslim communities dress in sun- protective gear and spend most of their time indoors. The female youngsters who participated in our study wore traditional Muslim head coverings. Children who spent more than 30 minutes in the sun each day in the male and female groups had vitamin D concentrations that were noticeably greater than those of children who spent less time in the sun.(GÜLLÜ et al., 1998; Hochberg et al., 2002)

In the current research, we took 1000 participants of the pediatric population from two different regions of Pakistan. Their lifestyle, environmental condition, and latitude are different from each other. 42.6% of children were affected with vitamin D deficiency in Rawalpindi, and 57.4% faced the problem of vitamin D deficiency. Many studies are discussed on children who had vitamin D deficiency based on region latitude and people's lifestyle. Our outcomes are compatible with previous research conducted in Turkey, America, France, Saudi Arabia, and China. Low vitamin D consumption, little sun exposure, and high-latitude living are the main contributors to a high prevalence of vitamin D deficiency in a certain area. Those who reside below the latitude of 35 degrees receive beyond sunshine exposure as opposed to those who reside at higher latitudes.(Guillemant et al., 1999; Lehtonen-Veromaa et al., 1999)

Additionally, this might not ensure optimal vitamin D levels. Research on teenagers in Sao Paulo, Brazil, a city close to the equator at latitude 23o, is the best example of this, showing a 60% prevalence of vitamin D insufficiency. An analysis of adolescent data from the National Health and Nutrition Examination Survey (NHANES III) revealed that vitamin D deficiency throughout the winter in the United States was 25% for boys and 47% for girls at low latitudes. In comparison, these values were 28% in females and 21% in males at high latitudes in summer.(Looker, Dawson-Hughes, Calvo, Gunter, & Sahyoun, 2002; Peters, Dos Santos, Fisberg, Wood, & Martini, 2009)

Low socioeconomic status and adults' inadequacy in vitamin D are related. The vitamin D intake for children whose moms have poor levels of education is also low during infancy and adolescence. Research on participants from diverse socioeconomic backgrounds in Rawalpindi and Lahore, Pakistan, showed that adolescents with low socioeconomic status and children whose mothers had poor educational levels had lower vitamin D levels. Furthermore, no vitamin D supplements were given to these kids.(Semba, Garrett, Johnson, Guralnik, & Fried, 2000)

The country's economic situation can greatly affect the availability of vitamin D in Pakistan. Generally, those with lower

socioeconomic levels or poverty are more likely to be vitamin D deficient than those with greater earnings. Pakistan belongs to the group of developing nations where the average person's income is insufficient to meet their basic needs. A substantial segment of the population lives in poverty, a significant obstacle to getting the essential nutrients for the body. Either they do not consume a vitamin D-rich diet, or they cannot pay for the supplements. The low level of literacy and lack of fundamental understanding of the essential nutrients needed by the body in Pakistan contribute to the country's population's vitamin D deficit.

The adolescents suffered from reduced vitamin D levels as well.they discovered that 35% of British kids from 4 to 18 have low vitamin D levels. These authors figured out The risk factors include being a teenager, watching TV for at least 2.5 hours a day, being obese, exercising for less than half an hour, and spending less time outside in the sun. Also, extremely low vitamin D levels can cause rickets complaints in children. In such instances, it is feasible to see widespread discomfort in load-bearing joints such as the vertebrae, femur, and calf, difficulties walking, climbing stairs, and running, alongside muscular cramps, facial twitches, overall weakness, deformity in the lower limbs, and carpopedal spasms. Extremity aches were brought up in a number of our situations. The group with regular extremities pain had lower winter vitamin D levels than those without discomfort. Physical examination, however, did not reveal any signs of active rickets.(Absoud, Cummins, Lim, Wassmer, & Shaw, 2011; Soliman, Adel, Wagdy, AlAli, & Aziz Bedair, 2011).

CONCLUSIONS

In conclusion, this study showed that vitamin D insufficiency and deficiency in children and adolescents are major health issues in Pakistan's pediatric population A troubling identity was that 43% of the study participants had Vitamin D3 levels deemed inadequate—less than 25 nmol/L. Only 15.9% had appropriate Vitamin D3 ranges (55-100 nmol/L), while 35.8% had insufficient amounts (25–55 nmol/L). The distribution of deficiencies was the same despite their age or gender. Children aged 0 to 14 have a prevalence of vitamin D deficiency of 59.3%. Remarkably, 51.7% of male adolescents were vitamin D deficient, compared to 48.3% of female kids. This form of vitamin D insufficiency in males may be caused by various reasons, including low early-life consumption of vitamin D supplements.

LIMITATIONS

The current study still has a few limitations. The participants weren't selected from the entire pediatric population of Rawalpindi and Lahore, Pakistan, and other potential correlating factors for vitamin D status, comprised of the investigation of chronic hepatitis beyond anti-HCV or HbsAg status and the inability to order a 75 g oral glucose tolerance test for patients with diabetes. We weren't monitoring serum calcium, phosphate, alkaline phosphatase, or parathyroid hormone levels. The probable influencing variables of vitamin D status should be studied in another research project that uses those who participated drawn from an ethnic sample.(Ecemis & Atmaca, 2013).

RECOMMENDATIONS

The seriousness of this issue necessitates quick action on the part of the Government and health care professionals to schedule public awareness campaigns promoting the value of sun exposure, which is the most readily available and affordable source of vitamin D. Furthermore, probable recommendations for vitamin D replacement in environments with limited resources like ours must be established. Additionally, the Government of Pakistan needs to ensure that certain foods, like ghee and bread, have been enriched with vitamin D.(Weaver & Fleet, 2004)

Supplementary materials

Not applicable

Author contributions

MSA & NA wrote the manuscript; AR conceived the idea for manuscript and checked the manuscript for grammatical mistakes before final submission.

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Conflict of interest
The authors declare no conflict of interest.

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