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**PREVALENCE OF VITAMIN D DEFICIENCY IN SCHOOL
CHILDREN**



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PREVALENCE OF VITAMIN D DEFICIENCY IN SCHOOL CHILDREN

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May 2022

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ABSTRACT

PREVALENCE OF VITAMIN D DEFICIENCY IN SCHOOL CHILDREN

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Master of Science in Chemistry

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Because of its role in promoting calcium and phosphate absorption and metabolism, Vitamin D is critical for strong bones and healthy muscles. A case-control study involved 140 volunteers from both genders. Their ages ranged between 6-12 years old. Oral consent was obtained from volunteers, school management, and their parents' consent. There were substantial discrepancies identified in the levels of vitamin D₂ (the inactive form) and vitamin D in the patient and control groups when comparing the levels of vitamin D, indicating that these two vitamins are depleting at different rates (active form). The serum level of Alkaline phosphatase was significant between the patient groups ($p=0.0001$) and we also found a decrease in the level of calcium compared to the normal group. When untreated, vitamin D if the low levels may leading to a other of health cases, not just in the elderly and housebound but also in people of all ages.

2022, 28 pages

Keywords: School Children, Prevalance, Vitamin D Defficiency, 1.25-dihydroxyvitamin D

ÖZET

OKUL ÇOCUKLARINDA D VİTAMİNİ EKSİKLİĞİ PREVALANSI

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Kalsiyum ve fosfat emilimini ve metabolizmasını desteklemedeki rolü nedeniyle D vitamini, güçlü kemikler ve sağlıklı kaslar için kritik öneme sahiptir. Bir vaka kontrol çalışması, her iki cinsiyetten 140 gönüllüyü içeriyordu. Yaşları 6-12 arasında değişiyordu. Gönüllülerden, okul yönetiminden ve velilerinden sözlü onam alındı. Hasta ve kontrol gruplarında D vitamini düzeyleri karşılaştırıldığında, D2 vitamini (inaktif form) ve D vitamini düzeylerinde, bu iki vitaminin farklı oranlarda (aktif form) tükendiğini gösteren önemli farklılıklar tespit edildi. Hasta grupları arasında serum Alkalın fosfataz düzeyi anlamlıydı ($p=0.0001$) ve biz de normal gruba göre kalsiyum düzeyinde bir düşüş saptadık. Tedavi edilmediğinde, düşük D vitamini seviyeleri sadece yaşlılarda ve eve bağlı kişilerde değil, her yaşta insanda çeşitli sağlık sorunlarına yol açabileceği sonucuna varılmıştır.

2022, 28 sayfa

Anahtar Kelimeler: Okul Çocukları, Prevalans, D Vitamini Eksikliği, 1.25-dihidroksi vitamin

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LIST OF SYMBOLS

%	Percent
/	Divide
+	Plus
>	Large Than
μL	Microliter
dL	Deciliter
L	Liter
mg	Milligram
mL	Milliliter
ng	Nanogram
nm	Nanometer
nmol	Nanomole
O ₂	Oxygen
°C	Degrees Celsius
Pg	Picogram
U	Unit
α	Alpha
β	Beta

LIST OF ABBREVIATIONS

AA	Amino Acid
ALP	Alkaline Phosphatase
AMR	Analytic Measurement Range
Ca	Calcium
C-BP	Calcium-Binding Proteins
DBP	D Binding Protein
DM	Diabetes Mellitus
HPLC-UV	High-Performance Liquid Chromatography With UV
ID-LC	Isotope Dilution-Liquid Chromatography
LBD	Ligand-Binding Domain
LSCa	Lower Serum Calcium
MS	Multiple Sclerosis
RA	Rheumatoid Arthritis
RBCs	Red Blood Cells
RXR	Retinoid X Receptor
SCD	Sickle Cell Disease
T1D	Type 1 Diabetes
TFIIB	Transcription Factor IIB
TMS	Tandem Mass Spectrometry
TSS	Transcription Start Site
UVB	Ultraviolet B
VD	Vitamin D
VDB	Vitamin D Binds
VDD	Vitamin D Deficiency
VDR	Vitamin D Response
VDRE	Vitamin D Response Element

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1. INTRODUCTION

Because of its role in promoting calcium and phosphate absorption and metabolism, Vitamin D is critical for strong bones and healthy muscles (Pedersen 2008). Sources food such as eggs, fatty, fish, and fortified cereals are also available. The human body utilizes ultraviolet B in milk and cod liver oil to synthesize a considerable amount of (UVB) rays from the sun. Vitamin D deficiency accounts for a small percentage of total vitamin D requirements (Mosekilde 2008). ergocalciferol and cholecalciferol are two different forms of vitamin D. (cholecalciferol). After sun exposure, the skin produces vitamin D₃ and stores it. Vitamin D₁ is obtained from animal sources, whereas vitamin D₂ is obtained from the sun. The synthetic form present in many fortified foods and is generated from vegetation.

Vitamin D's major function has been thought to be calcium absorption from the intestine is important for skeletal health [Calcium homeostasis in the human body, for example] (bone mineralization, remodelling, and maintenance (Figure 1.1). In addition to its long-established involvement in bone health, vitamin D has recently been demonstrated to have a significant influence on cell proliferation and differentiation. Vitamin D (Mehta *et al.* 2012).

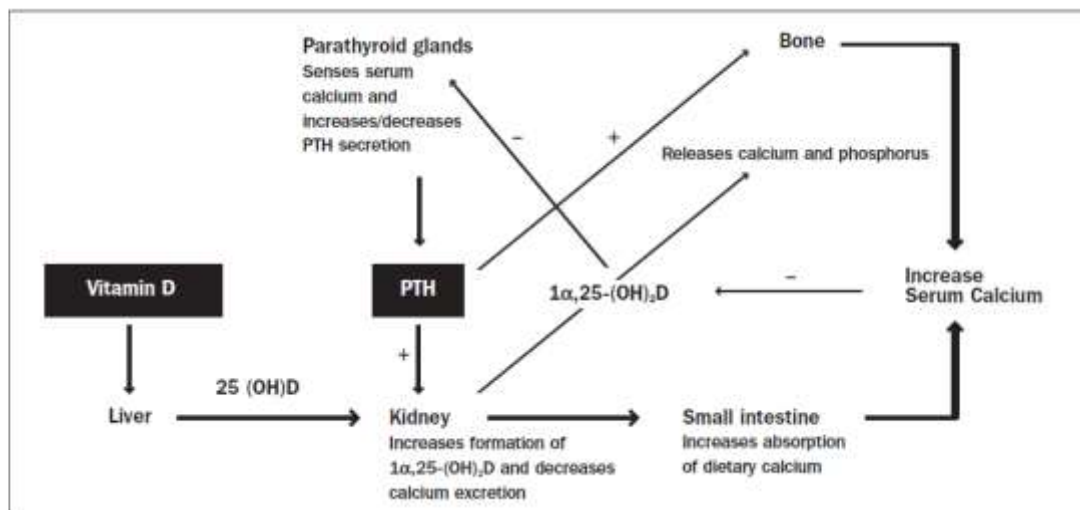


Figure. 1.1 PTH's physiological role in serum calcium level maintenance

involved vary greatly from person to person. More than 100 times the daily dietary intake of heme iron is found in a single unit of red blood cells (RBCs). Two RBC units a month for four months would be enough for a patient with transplant anemia who needs 24 units of RBCs a year. After four years, this results in an iron buildup of 20 g, which is seven times the average total amount of iron in the human body. As a result, the number of RBC transfusions predicts iron overload (Cid 2014).

Iron is a necessary element of biological processes and metabolic, but excessive amounts can harm the body due to oxidative stress. Patients with thalassemia and certain SCD patients need monthly blood transfusions to survive into their 30s. Chronically giving large amounts of blood, along with extravascular hemolysis and an increase in intestinal iron absorption, leads to severe hemosiderosis in all organs, even with chelation treatment, despite the fact that blood transfusion promotes life and development. Excess iron in the body can build up in the heart, pituitary, bone marrow, central nervous system, pancreas, spleen, and liver causing damage to these organs (Fahmy *et al.* 2015).

Vitamin D regulates gene expression and signal transmission in almost all tissues. To maintain epithelial cell quiescence and differentiation, VDB to and activates the VDR and activates pathways that protect cells from both internal and external stresses (Welsh 2012). Vitamin D synthesis begins for conversion of cholesterol to 7-DHC. 7-dehydrocholesterol is subsequently transferred to the skin's epidermis and stored in the membranes of fibroblasts and keratinocytes. Skin 7-DHC is transformed to previtamin D by UVB photolysis (280–320 nm), which is then turned into vitamin D by photolysis in the skin. Before VD-mediated thermo-isomerization is physiologically active, a series of enzyme changes must take place in the liver and kidney. Vitamin D gets to the liver with the help of a protein called vitamin D binding protein (DBP), which is very similar to albumin. DBP is responsible for transporting 85–88 percent of VD metabolites, with albumin accounting for 12–15 percent of the total (Cooke and Haddad 1989). CYP2R1 enzyme 25-hydroxylase (CYP2R1) converts VD to 25-hydroxyvitamin D [Calcidiol], the primary form of VD that circulates in the bloodstream (Bikle *et al.* 1984). Vitamin D2 and D3 hydroxylation is possible at position 25 of CYP2R1. A CYP2R1 gene

mutation is to blame for rickets. It has been crystallised with the VD substrate in the active site of the CYP2R1 enzyme (Bikle *et al.* 1985).

Objectives of Study

- 1- The goals of this study are to find out how common vitamin D deficiency is and to find out why people have it.
- 2- Vitamin D's influence on schoolchildren.



2. LITERATURE REVIEW

2.1 Mechanism of Genomic Action of Vitamin D

1 α .25-(OH)₂D The active form of vitamin D, 2D, has both calcaemic and non-calcaemic properties. Calcaemic functions include regulating the levels of phosphate and calcium in the blood. This is done by the kidney, the parathyroid, the bone, and the intestine. In bone marrow, non-calcaemic functions include differentiation and stopping the growth of new cells (lymphocytes and osteoclast precursors), the breast, epithelial cells, prostate, skin, and immune system. (Strushkevich and colleagues 2008). Non-genomic and genomic pathways have a role in the biological effects of 1.25-(OH)₂D in a variety of cells. Co-activator proteins and transcription integrators such as C-BP are all involved in the process of initiation of genomic activity once 1.25-(OH)₂D is linked to VDRs. Changes in gene transcription are the result of this genomic process. A receptor on the cell surface and second messengers work together to speed up the response time of vitamin D (Figure 2.1) (Cheng *et al.* 2004). In a nutshell, the VDR-mediated transcriptional activation of 1.25-(OH)₂D pathway is responsible for the above-mentioned tasks. When 1 α .25-(OH)₂D reaches a target tissue, it VDR is a protein that binds to VDR and regulates vitamin transcription. D genes that are involved in physiological processes 1 α .25-(OH)₂D's actions. VDR attaches to as a VDR/VDR, DNA. To control gene expression, VDR / RXR heterodimers or homo-dimers are used. (St-Arnaud *et al.* 1997).

The dimers interact with transcription factor IIB to turn off or turn on vitamin D response elements (VDREs) in the promoter region of target genes (TFIIB). 1.25- is the only connection that VDR has (OH). 2D needs a partner called RXR and a number of other trans-activators to expose DNA and effectively transcribe target genes (Zehnder *et al.* 2001).

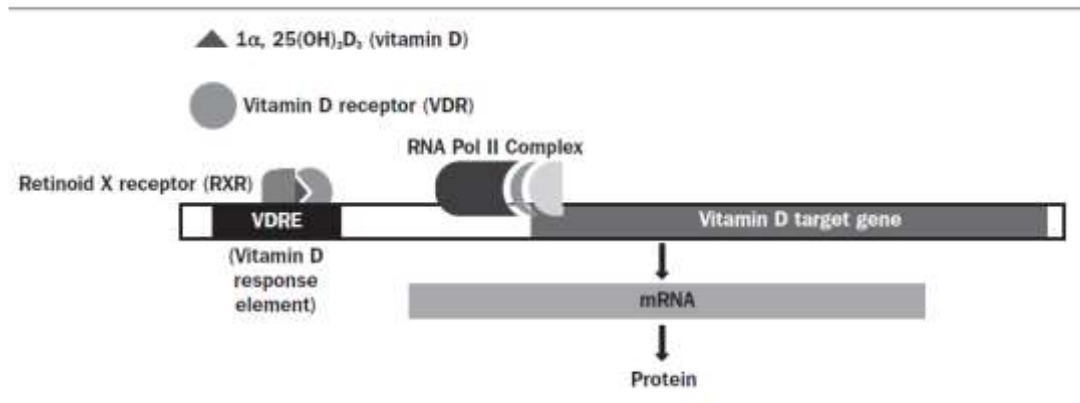


Figure 2.1 vitamin D's genomic action is represented in a diagram

1.25-(OH)₂D is thought to control 300–800 genes. The 1.25-(OH)₂D hormone encourages 25-hydroxyvitamin D-24-hydroxylase in target cells, which is the main enzyme that breaks it down, among other genes (CYP24A1) (Flanagan *et al.* 2003). This helps regulate vitamin D homeostasis by attenuating the 1.25-(OH)₂D biological signal inside target cells. The precise process behind vitamin D's physiological effects is given further down. VDR, the only nuclear protein with a high affinity for vitamin D₃, mediates vitamin D's genomic effects. The VDR has two zinc-finger isoforms that come together to make a 66-AA domain that binds to DNA (Wang *et al.* 2003).

The VDR protein has a 300-amino-acid ligand-binding domain (LBD) with 12 α-helices at its carboxy-terminus. Members in the superfamily nuclear receptor, co-repressor proteins and CoA all interact with the LBD in the nucleus (Fritsche *et al.* 2003). In the absence of ligand DNA-bound VDR, co-repressor proteins bind VDR to enzymes that deacetylate histones, encouraging chromatin condensation (e.g., NCoR, SMRT, and Alien). As a consequence, VDR has the same effect on cells as retinoic acid and thyroid hormone receptors, which are to shut them down. When 1.25-(OH)₂D binds to VDR's LBD. Co-repressor molecules are replaced by CoA proteins like CREB binding protein (CBP). This causes a change in the shape of the protein (DeLuca 2004). These CoA synthesis have activity for acetyltransferase histone, which causing chromatin to loosen up.

Thyroid hormone receptor associated mediator complexes, for example, interact with ligand-activated VDR. The protein TRAP220 is discovered in the human body. Build a protein-rich diet with 15–20 proteins by combining mediator complexes. a link to the transcription mechanism at its most fundamental level (Kogawa *et al.* 2010). As a result, VDR that has been activated by ligand has two functions. A need for 1.α25-mediated direct transcriptional regulation (OH). Two-dimensional means that at least one active VDR protein is close to the main transcription start site of a gene (TSS). This is done with the help of Vitamin D response elements (VDRs) in the promoter regions of target genes (VDREs). Within the first 1000 bp of the upstream promoter sequence of the TSS, RGKTSAA is a common VDR core binding motif, where the majority of natural VDREs are located. Because of the Pol II complex's unique three-dimensional architecture, individual promoter regions can communicate with it at the same time. Through the promoter, a massive protein mediator complex This method allows people in different locations to communicate with one another.

2.2 Vitamin D Deficiency

Those at greater risk of vitamin D insufficiency include those who avoid contact to the sun, reside at high latitudes, have dark skin, are obese, or have chronic renal disease. Recent studies have shown serum (OH)D values as low as 25 nmol/L (less than 20 ng/mL). A study has found that some people don't get enough vitamin D (Panda *et al.* 2001). At 25 nmol/L (10 ng/ml), a person has a severe lack of vitamin D. Vitamin D levels between 10 and 19 ng/mL (25 to 49 nmol/L) show a lack of vitamin D. Those unfavorable effects are recorded below 25 nmol/L (10 ng/ml) at a high dosage level. Bone loss goes up in both children and adults, and the number of fractures goes up. Secondary hyperthyroidism is more likely to happen at a concentration of 10–19 ng/mL than at a concentration of 25–49 nmol/L.

A 25-hydroxyvitamin D threshold of 30 ng/mL (75 nmol) is necessary to get adequate bone mineral density at a youths (19–49 year olds) and middle-aged (>50 year olds). Studies are finding that 25-hydroxyvitamin D levels of 30–44 ng/ml (75–110 nmol/L)

may lower the risk of common cancers, autoimmune diseases, type 2 diabetes, heart disease, and infectious diseases (Dardenne *et al.* 2001).

2.3 Vitamin D Deficiency Prevalence

The median 25-hydroxyvitamin D content in German children (1–17 years old) is 44 nmol/L, according to studies of the German population. Blood levels of 25(OH)D were below 25 nmol/L in 30–50% of the patients and below 48 nmol/L in more than 90% of the cases, according to another study of 199 12.5-year-old children from four Northern European countries (Kawashima *et al.* 1981). In a Swiss study of 3276 people (aged 25 to 75), the median blood 25-OHD concentration was 46 nmol/L, with 34% having values below 38 nmol/L and almost having 70% concentration under 30 ng/mL (Zierold *et al.* 1994). Similar information has been made available in a number of other nations. In their study, 36% of men and 47% of women had less than 30 nmol/L of 25-(OH)D in their blood, according to van der Wielen and coworkers' findings. The elderly in the United States are notoriously susceptible to vitamin D deficiency (Zehnder *et al.* 1999).

A recent study found that the vitamin D levels of 96.5 percent of Qatari health care workers were below the detectable limit of 3 ng/mL, and 20 percent had levels below the detectable limit of 30 ng/mL. Vitamin D deficiency is very common in young people who can walk but don't have any problems with their bones or muscles.

2.4 Vitamin D Deficiency's Consequences

Vitamin D's primary role is to ensure that the body has enough calcium and phosphorus to support proper metabolic activity. There is evidence of a vitamin D deficit. It has been connected to a wide range of ailments. The link between poor income and rickets and osteoporosis are both caused by a lack of vitamin D is widely recognized (Makin *et al.* 1989). Vitamin D insufficiency also affects the immune system success in reproduction as well as the ability to fight diseases, such as TB, viral infections, and influenza. It has the potential to trigger or exacerbate autoimmune diseases and raise the

risk of death from heart disease, stroke caused by hypertension, Irritable bowel syndrome, muscular weakness, falls, fractures, and malignancies of the breast, colon, and prostate (Reddy And Tserng 1989).

Many studies have shown that VD may make it less likely that people will get colorectal cancer. Breast, lung, ovarian, and prostate cancers are among the several types of cancer that may be helped by supplementing with vitamin D. Researchers are looking into the role of vitamin D in autoimmune diseases like MS, T1D, and RA. Also extensively researched is the effect of vitamin D in asthma development and control (St-Arnaud 1999).

2.5 Vitamin D Measurement

The majority of genetic investigations on vitamin D metabolism have focused on the $1\alpha,25\text{-(OH)}_2\text{D}$ and 25-(OH)D are two types of vitamin D metabolites that are often measured in serum. On the other hand, the 25-(OH)D serum concentration is often thought to be the most accurate way to measure a person's vitamin D status in vivo. It contains both endocrine and paracrine biological mechanisms for vitamin D production, but does not include the active hormone $1\alpha,25\text{-(OH)}_2\text{D}$.

When secondary hyperthyroidism is combined with vitamin D deficiency, the vitamin D status information is either normal or high. 25-(OH)D is more stable in the blood than $1\alpha,25\text{-(OH)}_2\text{D}$ because it has a longer half-life (20 days) and more of it. So, the best way to find out how much vitamin D you have is to look at your 25-(OH)D levels in the blood (Holick 2007)

Several immunological and mass spectrometry-based tests were used to measure the amount of 25-hydroxyvitamin D in the serum, and spectrophotometric approaches are being used. But, a number of studies have shown substantial variability in the outcomes of 25-hydroxyvitamin D tests among analytical approaches. Techniques used in radio immunochemistry include HPLC-UV and ID-LC/tandem MS, both of which

use isotope dilution-liquid chromatography (ID-LC/tandem MS). There has been a considerable difference between the methodologies for many years, which can lead to patient misdiagnosis and misunderstanding of population data (Jenster *et al.* 1997).



3. MATERIALS AND METHODS

3.1 Selection of Patients

A case-control study involved 140 volunteers from both genders. Their ages ranged between 6-12 years old. Oral consent was obtained from volunteers, school management and their parents' consent. This field survey was conducted in primary schools in Samaraa District / Salah Al-Din Governorate for 7 schools, and we took random samples from each teacher of 20 students. Then we took samples from the volunteers and transferred them to the laboratory (chemistry department) of Samaraa General Hospital / Salah Al-Din Governorate. The results were 100 volunteers suffer from vitamin D deficiency and 40 volunteers healthy.

3.2 Sample Collection

The blood sample is intended for biochemical markers analysis. 5 ml was the amount of blood to be taken for the study. After taking blood samples serums were separated from the blood by centrifugation. The samples were centrifuged as much as possible for 10 min at 3000 rpm. After completing the required number of samples, the samples were taken out of the refrigerator and allowed to reach room temperature. Then the specific analyzes of the samples were performed.

3.3 Laboratory Equipment and Tools

The devices and equipment used in this study are given in Table 3.1. They were used of these devices and tools to assist the researcher in obtaining accurate results, as well as shortening the time if it works automatically.

Table 3.1 laboratory equipment and tools

Equipments and tools	Made	Note
Cobas e411 65G6-11	Germany	It was used for laboratory diagnosis. This device was used to detect the levels of the following tests: Vitamin D (total) and 1.25-dihydroxy vitamin
Cobas c311 04530152001	Germany	It was used clinical chemistry tester yields diagnostic accuracy data. It was used for the following analyses Calcium and alkaline phosphatase
Freezer	Germany	It was used to save as well as store such as biochemicals, blood, serum, and plasma.
Incubator	Germany	It was used to save blood, serum and plasma during work because the tests need to be serum at a temperature of 37 °C.
Syringe	China	It was used to draw blood from patients' hands and transfer it to a test tube in order to do tests on the blood drawn.
Pipettes	Germany	It was used as a scientific instrument used to measure or transport small amounts of liquid such as serum and blood in milliliters (ml) or microliters (µL). It was used in a wide variety of experimental processes in medical research, and biochemistry.
Test Tubes	China	It was used to save as well as store such as blood, serum, and plasma.

3.4 Chemicals and Test Kits Used in Study

All the biochemical reagents and kits shown in Table 3.2.

Table 3.2 chemicals and test kits used in study

Vitamin D (total) reagent	05894913190
1.25-dihydroxy vitamin D Reagent	05894913191
Calcium Reagent	8C87
Alkaline phosphatase Reagent	8L92

3.5 Method

3.5.1 Vitamin D (total) and 1.25-dihydroxy vitamin D level in serum by cobas e411

. **Principle:** The assay took a total of 27 minutes to complete.

- 1st incubation: The sample (20 L) is incubated with pretreatment reagents 1 and 2 to release bound 25hydroxyvitamin D from the VDBP.
- When the pretreatment sample is mixed with the ruthenium-labeled vitamin D binding protein, the 25-hydroxyvitamin D and the ruthenylated VDBP join together to form a complex. To avoid cross-reactivity, the material is tested using an unlabeled antibody that binds to the vitamin D metabolism 24-hydroxyvitamin D.
- Vitamin D binding proteins that had been unbound by streptavidincoated microparticles and biotin-labeled 25-hydroxyvitamin D became occupied during the third incubation step. Biotin and streptavidin combine with the ruthenylated vitamin D binding protein and the biotinylated 25-hydroxyvitamin D to make a complex that binds to the solid phase.
- Upon inhalation into the measuring cell, the reaction mixture collects the magnetically attracted microparticles on the electrode's surface. ProCell/ProCell M is then used to get rid of the chemicals that aren't bound. When a voltage is applied to an electrode, which is picked up by a photomultiplier, this is called chemiluminescent emission.
- Reagent barcodes or ebarcodes are used to build a calibration curve for the instrument and a master curve for comparison.

. Procedure

Only the following specimens were tested and found to be acceptable. To get serum, regular sampling tubes or tubes with separating gel were used. Plasma with K2 and K3 EDTA, Li heparin, and K2. It is possible to employ plasma tubes with separating gel. Slope, intercept, and correlation coefficient are all within three nanograms per milliliter of serum in comparison to plasma in the experiment described above

25-hydroxyvitamin D is stable in plasma, serum, Li heparin, and K2 and K3 EDTA for 8 hours at 20-25 °C, 4 days at 2-8 °C, and 24 weeks at 20 °C (5 °C). One time is all it takes to become frozen. The Elecsys Vitamin D total II test indicated that 25

hydroxyvitamin D is stable, which agrees with earlier studies that used a vitamin D binding protein assay and mass spectrometry (Lewis and Elder 2008).

There were only a few commercially available sample collection tubes available for testing at the time of this research, therefore not all tubes from every manufacturer were evaluated. Some test findings may be affected by the use of various sample collecting procedures by different manufacturers. For processing samples in these tubes, only use tubes made by the manufacturer the company recommends (sample collection systems). Before performing the assay, centrifuge any samples that contain precipitates. Heat-inactivated samples should not be used. Do not utilize azide-stabilized samples or controls. Make certain that the samples, calibrators, and controls are all between 20 and 25 degrees Celsius prior to doing any measurements. Due to the possibility of evaporation, sample analyzers, calibrators, and controls should be measured or examined within two hours.

3.5.2 Measurement calcium level in serum by cobas c311

. Principle

- 1- This is an in vitro test that uses Roche/Hitachi COBAS C311 systems to measure the amount of calcium in human blood, plasma, and urine.
- 2- Under alkaline circumstances, calcium ions form a compound with 5-nitro-5'-methyl-BAPTA (NM-BAPTA). In the second stage, this complex reacts with EDTA.
- 3- Photometry records the change in absorbance, which is directly related to the amount of calcium.

. Procedure: Universal Precautions apply. Serum, Li-heparin and Urine are acceptable.

Serum or plasma should be withdrawn from blood cells as quickly as possible since prolonged contact with the clot may produce lower calcium readings. Because EDTA chelates calcium, it prevents the NM-BAPTA reaction from taking place in sera from

patients receiving EDTA therapy for hypercalcemia. Calcium precipitates with fibrin, lipids, and denatured protein during storage and freezing.

3.5.3 Measurement alkaline phosphatase level in serum by cobas c311

. Principle

The cobas C311 performs a colorimetric assay using a standardized procedure. At the presence of magnesium and zinc ions, phosphatases cleave p-nitrophenyl phosphate into phosphate and p-nitrophenol.

The catalytic ALP activity is related to the amount of p-nitrophenol generated. It is determined by the increase in absorbance.

. Procedure

AMR (Analytic Measurement Range): 5-1200 U/L Extended Range: 5-6000 U/L with automatic re-run (1:5 dilution) Report values less than 5 as “6000 U/L” If the provider requires further dilution on a sample reporting >6000 U/L, use 0.9% NaCl as the diluent. Diluted value must fall between 5-1200 U/L. Using the rerun function, find samples with greater activity levels. The rerun function dilution of samples is a 1:5 dilution. When utilizing the repeat function to dilute samples, the results are automatically multiplied by a factor of 5.

3.6 Statistics Analysis

Statistical Analysis System –(SAS) 2019 program is utilized to decide the impact of diverse variables on inquire about parameters. The t-test is utilized to essentially compare implies. In this study, the estimation of the correlation coefficient between the variables.

4. RESULTS AND DISCUSSION

One of the most common disorders in the world is vitamin D insufficiency, and research into the connection between this case and vitamin D deficiency is ongoing. The study included two groups of colleges.

This case-control study was conducted from December 2021 and March 2022 on 140 patients and healthy individuals with vitamin D deficiency, the first group patients consist of 100 patients (female and male). The diseased group was compared with a healthy group of 40 (male and male) people as a control group.

4.1 The Results of the Patient and Control in the Group Study

4.1.1 Age

In this study, it has been founded significant defferenses in age as in mean between control and patient with deficiency in level of vitamin D, this study helping to match age with deferences in level of vitamin D due to the variation in age, many studied depened on principle of age matching. As showing in the table 4.1 and figure 4.1.

Parameters	Mean \pm SD
	Age (year)
Patients group	9.54 \pm 1.90
Control group	8.60 \pm 1.85
T-test	2.660
P-value	0.009
($P \leq 0.05$), ($P \leq 0.01$).	

Table 4.1 comparison of the age distribution between the patients and the control group

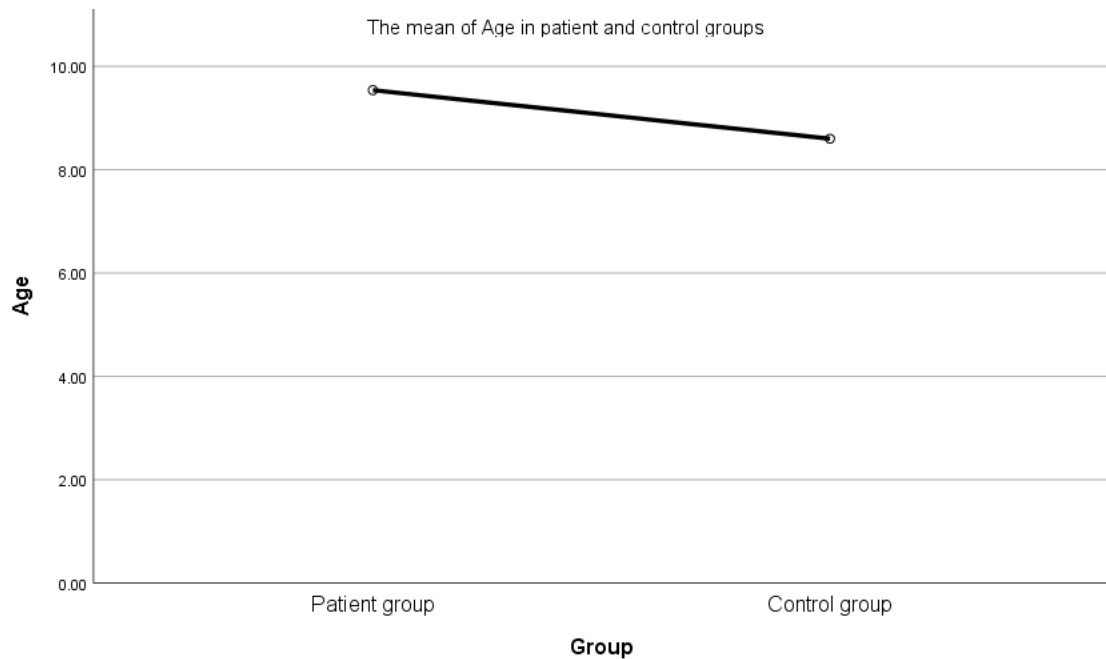


Figure 4.1 comparison of mean age in the two groups

4.1.2 Vitamin D (total)

In this table 4.2 and figure 4.2, it has been compare between level of vitamin D in patient and control group, it has been founded significant defferenses in level between patient and healthy children. This mean decrease level in vitamin d2 (non active forme) and vitamin D (active form), it has been founded many causes for this state, may be age, or one complication disease or not expoler to sun light, this study agreement with many study and conformed this state (Burnand *et al.* 1992). The mean of vitamin D was (20.03 ± 4.27) and the normal value (30-100 ng/mL), while the mean of control group was (59.37 ± 15.97). Perhaps the reason for the lack of vitamin D in children is due to the environment in which they live, as they live in the city, unlike children in the countryside and villages. People need natural nutritional supplements in order to get adequate amounts of vitamins and calcium. Note that the volunteers will not suffer from any health problems or other diseases

Parameters	Mean \pm SD	
	Vitamin D (total) (30-100 ng/mL)	1.25-dihydroxyvitamin D (15-60 pg/mL)
Patients Group	20.03 \pm 4.27	10.40 \pm 1.92
Control Group	59.37 \pm 15.97	34.82 \pm 10.85
T-test	22.778	21.778
P-value	0.0001	0.0001
(P \leq 0.05), (P \leq 0.01).		

Table 4.2 comparison between patient and control in the vitamin d (total) and 1.25-dihydroxyvitamin D

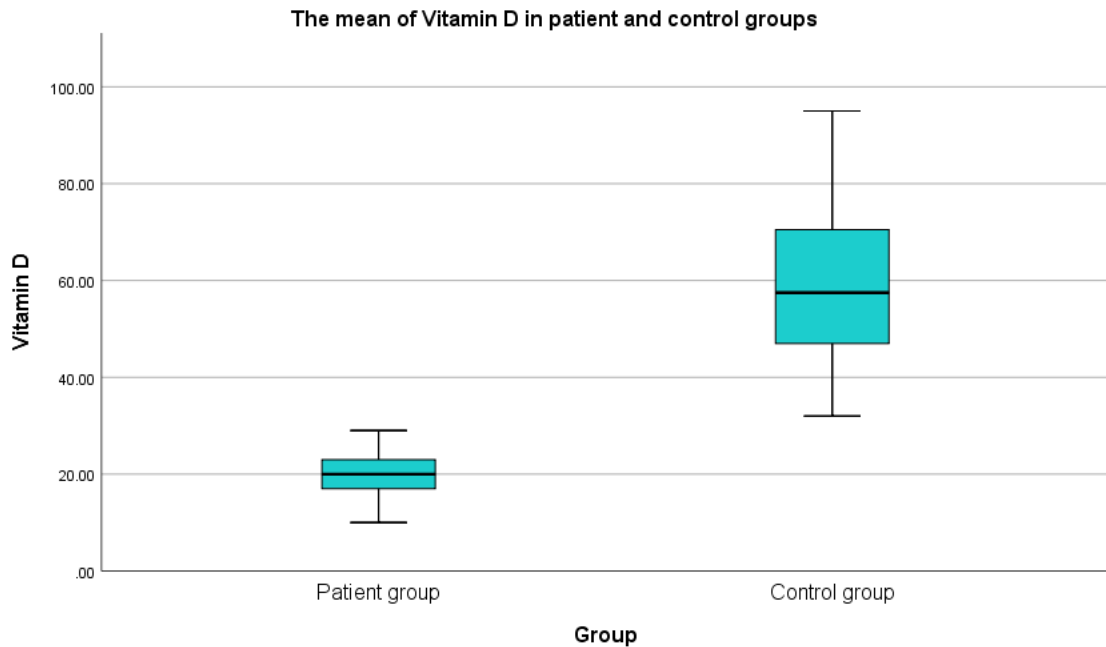


Figure 4.2 the mean of vitamin d in patient and control group

4.1.3 1.25-Dihydroxy vitamin D

Table 4.2 and Figure 4.3 reveal that the blood level of 1.25-dihydroxyvitamin D substantially differed between the two groups of patients ($p=0.0001$), and that the study similiar of vitamin d3 deficiency had a low level (Looker *et al.* 2008).

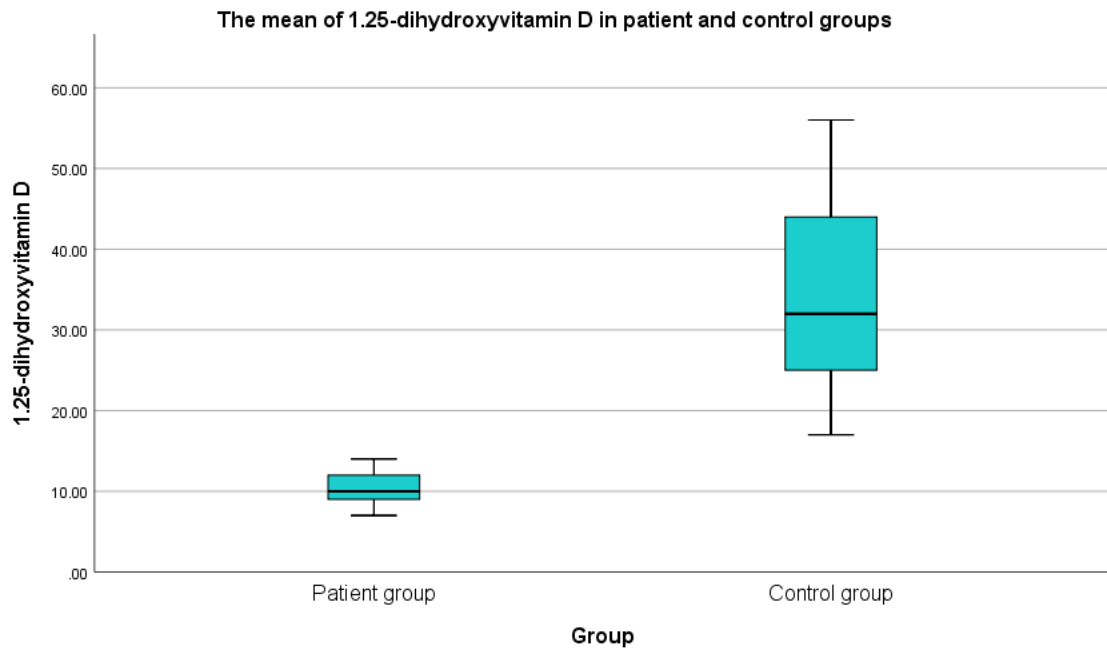


Figure 4.3 1.25-dihydroxyvitamin D serum levels in patients and control group

There are both non-genomic and genomic routes by which 1.25-(OH)₂D affects the body. Once VDRs and 1.25-(OH)₂D connect, the genomic activity is kicked off. VDRs then engage with other factors transcription including transcription integrators and coactivator proteins like C-BP. Changes in gene transcription are the result of this genomic process. Faster responses are the result of Vitamin D working with a cell flat receiver and other minor messengers. In a nutshell, the VDR-mediated transcriptional activation of 1.25-(OH)₂D pathway is responsible for the above-mentioned tasks. 1.25-(OH)₂D is a protein that interacts to VDR and modulates vitamin transcription when it reaches a target tissue. D genes that are involved in physiological processes 1.25-(OH)₂D's actions.

4.1.4 Calcium

One of the most important previous annotations displayed that risk of development, noticed in "vitamin D" deficiency and polymorphisms in in genes of enzymes envelopment in vitamin D metabolism, is calcium, in this study significant defferenses in the level of Ca between patient control group (Van and Lips 2011). May be also these factors, influence of disease recurrence, and increase susceptibility of cardiovascular

diseases. Another researcher was submitted genetic factor for pathogenesis in presence of VDR FokI F allele. Equally vitamin D₃ had an reverse correspondence with activity of RA. From these records, evidence indicated vitamin D₃ level is concomitant with the development and activity of deficiency Vitamin D. Table 4.3 and Figure 4.3 demonstrate that the mean blood Vitamin D level of patients was substantially lower than that of the normal donors in their research.

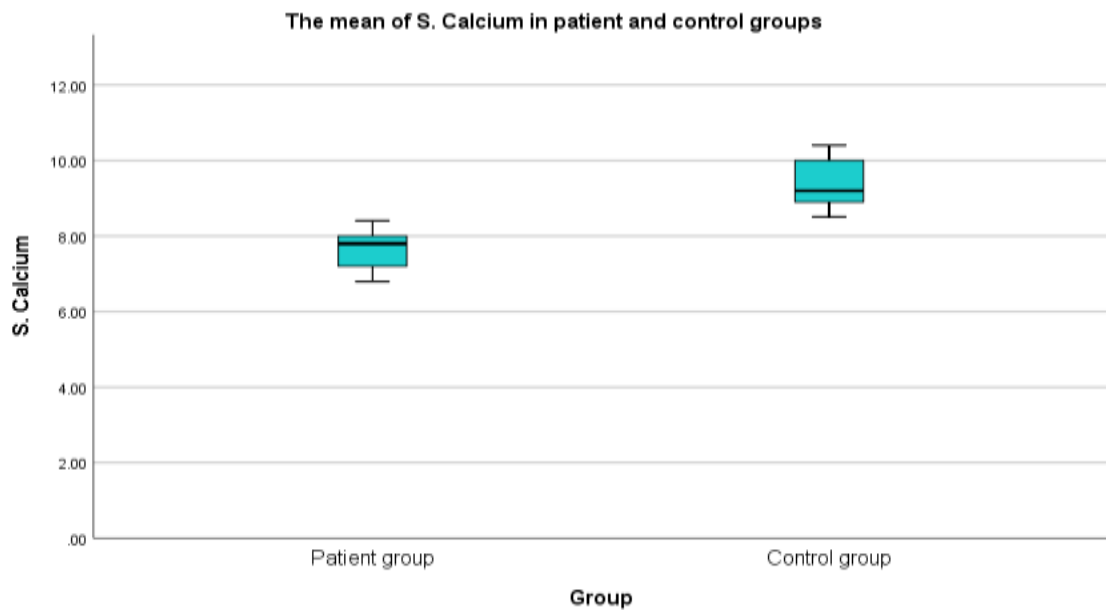


Figure 4.4 serum level of calcium in patient and control group

4.1.5 Alkaline phosphatase

The serum level of alkaline phosphatase has been significantly between pateint group ($p=0.0001$) as shown in figure 4.4 and table 4.3. Elevated level in pateint with difecincy of vitamin D, that the study simaliar of (Ginde *et al.* 2009).

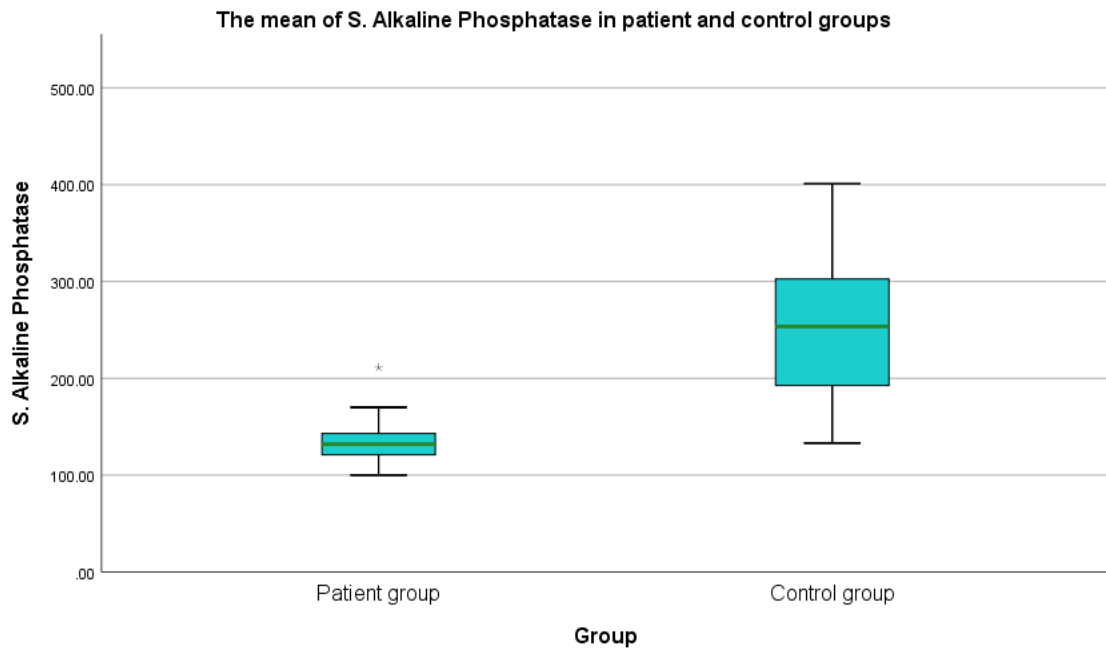


Figure 4.5 serum level of alkaline phosphatase in pateint and control group

In this study, has been founded significantly in level of serum calcium and alkaline phosphatase between pateint group and control, this study agreement with study (Mahdy *et al.* 2010).

Parameters	Mean \pm SD	
	S. Calcium (8.5-10.5 mg/dL)	S.Alk. Phosphatase (129-417 U/L)
Patients Group	7.65 \pm 0.47	134.64 \pm 19.21
Control Group	9.38 \pm 0.57	250.42 \pm 72.44
T-test	14.802	18.263
P-value	0.001	0.0001
(P \leq 0.05), (P \leq 0.01).		

Table 4.3 comparasion between patient and control in the calcium and alkaline phosphatase

Vitamin D insufficiency is a serious health issue that affects people all over the world. Many people are worried about not getting enough vitamin D, and it has now been related to a variety of ailments. Serum levels of 25-hydroxyvitamin D are linked to metabolic syndrome in a bad way, stroke, plasma glucose concentrations, systolic blood

pressure and abdominal obesity. Hyperparathyroidism secondary to VDD has been related to higher systolic blood pressure, LSCa, lower HDL cholesterol, and an increased risk of insulin resistance. Serum 25(OH)D levels are also linked to higher rates of disease, such as most-case illness, cardiac arrest, and diabetes.



5. CONCLUSION AND RECOMMENDATIONS

1- Vitamin-D deficiency/insufficiency, which affects not just the children, but the general population as a whole. Deficiency in vitamin D is widespread, and this research shows the need for a population-wide remedy. The results of large-scale genomic and functional investigations must be confirmed in order to verify past findings.

2- Over the course of human development, sunlight has provided all of the vitamin D we've needed. Melanin pigmentation may have evolved to enable individuals to roam about and produce enough vitamin D in their skin in order to meet their needs north and south of the equator. As an example, consider Sun exposure has had a negative impact on the world's population. Low levels of vitamin D in the population. A significant rise in skin cancer has been documented as a result of this. As a consequence of these high rates, the notion of never exposing the skin to the sun without any type of sun protection, such as a hat or sunscreen, was advocated. "Sun-safe" messages may have caused people at risk of vitamin deficiency to spend more time in the sun.

3- A person's 25(OH)D level can be used to figure out their vitamin D status. An individual's vitamin D status may be assessed using the vast majority of commercially available tests. The best method is called "liquid chromatography–tandem mass spectrometry", however other radioimmunoassays are available. Vitamin D2 is only 30–50% as good as vitamin D3 at keeping 25(OH)D levels in the blood at a healthy level. When vitamin D2 was transformed to 1.25(OH)2D2, it didn't make vitamin D3 less useful.

4- When the level of 25(OH)D in the blood was higher than 75 nmol/L or 30 ng/mL, more vitamin D2 was needed. Since vitamin D2 was just as good as vitamin D3 at increasing the amount of 25(OH)D in the blood, we wonder whether this is really essential.

5- The recommended levels of vitamin D in a child's and adult's diet need to be looked at again. Many studies published in the last 10 years show that the 1997 Institute of Medicine recommendations aren't enough, and some experts, including us, say that children and adults should take 800–1000 IU of vitamin D from food and supplements when they can't get enough sunlight.

Recommendations

1- Increasing the number of volunteer and turn towards remote areas as they have more immunity compared to city areas.

2- Include ages up to 15 years in order to look at these ages, they also suffer from vitamin D deficiency or not.

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