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Establishment of Vitamin D Reference Values in the Palestinian Society And Investigation of Cultural, Behavioral, and Socioeconomic Effects

Mohammed Ibrahim Issa Al-mahariq

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Establishment of Vitamin D Reference Values in the Palestinian Society And Investigation of Cultural, Behavioral, and Socioeconomic Effects

Prepared by:

Mohammed Ibrahim Issa Al-mahariq

B.Sc: Medical Technology, Al-Quds University- Jerusalem

Supervisor: Dr. Ahmad Younes M. Amor

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Prepared by: Mohammed Ibrahim Issa Al-mahariq

Registration number: 21710922.

Supervisor: Dr. Ahmad Younes M. Amor

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The names and signatures of the examining committee members are as Follows:

1- Head of Committee	Dr. Ahmad Amor	Signature:……
2- Internal Examiner	Dr. Fawaz Awad	Signature:……
3- External Examiner	Dr. Samir Al-khatib	Signature:……

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Dedication

To my mother and father

To my wife, Maram

To my sons, Ibrahim and Omer, and my daughter Ileana

To my sisters and brother

To my teachers.

I dedicate this work.

Mohammed Ibrahim Issa Al-mahariq

Declaration:

I certify that this thesis submitted for the degree of Master is the result of my research, except where otherwise acknowledged. This thesis (or any part of the same) has not been submitted for a higher degree to any other university or institution.

Name: Mohammed Ibrahim Issa Al-mahariq

Date: 21/8/2022

Signature: 

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First and foremost, I would like to express my gratitude to my great God, the most merciful, for the power and endurance he gave me throughout my life and studying period.

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Lastly, I would like to express my deep appreciation to my family for their endless support during my study, to whom I dedicate this thesis.

Abstract

This thesis documents several key contributions made to the vitamin D field. Vitamin D is recognized as a sunshine vitamin with regular sun exposure conditions. Unsurprisingly, vitamin D dietary intake is considered of minor importance in science. However, sun exposure reveals about 90% of vitamin D. In the last decade, vitamin D testing and the use of vitamin D supplements have increased significantly with neither clear nor agreement on blood level recommendation of vitamin D in healthy individuals. The recommended sufficient level of serum vitamin D is ≥30 ng/mL (≥75 nmol/L). In contrast, vitamin D deficiency is defined as 25(OH)D levels ≤ 20 ng/ml (≤ 50 nmol/L), and the serum level between 20 and 30 ng/ml (50-75 nmol/L) indicates insufficiency. The main objective of this study is to establish a population-based reference range of vitamin D in Palestinian society and investigate its associated cultural, socioeconomic, and human behavioural factors. We adopted a cross-sectional study that was conducted on 300 healthy Palestinian individuals from different cities and villages in the West Bank. Those individuals did not report a history of kidney, liver, or malabsorption disorders. The sample consisted of 123 males and 177 female adults aged between 19 and 65 years old. We used a structured questionnaire to obtain demographic and socioeconomic data, and information about health status and risk factors related to vitamin D insufficiency. Moreover, serum 25(OH)D was measured by the Abbott chemiluminescence immunoassay analyzer (ARCHITECT *i*1000SR) to establish and provide reference values. The mean 25(OH)D serum level was 13.4 ng/ml (5th; 95th percentile, 12.34; 14.5). Notably, 92% of the studied sample had 25(OH)D serum levels below 30 ng/mL, 79.3% below 20 ng/mL and 61% below 12 ng/ml. The younger group (18-28) years of age was reported to have a significantly lower serum 25(OH)D level compared to the other three different age groups (29-40),(41-50),(51-65) years old. Our

results also showed that serum 25(OH)D level was not significantly associated with gender, obesity (BMI), sun cream use, sun exposure, and wearing a hijab (head cover). However, no significant differences of serum 25(OH)D level were reported among individuals consuming cheese, yoghurt, egg, fish, milk, bread, and nuts meals intake.

In conclusion, according to the lack of correlated evidence of major skeletal and non-skeletal disease conditions with vitamin D levels that are recommended as optimal (≥20 ng/mL, or even ≥30 ng/mL), and in light of our study to establish vitamin D reference level in healthy adults, we recommend to consider levels below 20ng/ml and close to 14 ng/ml as sufficient serum 25(OH)D level for the general population in the West Bank society. However, our study sample is limited with the small number of participants, hence further investigation and research are needed to establish more comprehensive and inclusive conclusions regarding reference levels and related factors.

معرفة المعدل الطبيعي لفيتامين د في المجتمع الفلسطيني و مدى تأثره بالجوانب الثقافية و السلوكية و الإجتماعية و البيئية.

إسم الطالب: محمد إبراهيم عيسى المحاريق

إسم المشرف: الدكتور أحمد يونس عمرو

ملخص الدراسة

تساهم هذه الدراسة في مناقشة و توثيق نسبة فيتامين د والعوامل المرتبطة به، ويعتبر التعرض لأشعة الشمس هو المصدر الرئيسي لإنتاجه، حيث يوفر التعرض لاشعة الشمس بشكل يومي وكافي ما يقارب ال 90% من التركيز الكلي في الجسم، بينما يعتبر تناول الأطعمة الغذائية ذو مساهمة قليلة و ثانوية في زيادة نسبة الفيتامين في الدم . في العقد الأخير، هناك زيادة واضحة في تناول المكملات الغذائية وعمل الفحوصات المخبرية الخاصة بقياس نسبة فيتامين د عالميا، ويعزى هذا الإزدياد لعدم وجود مستويات مرجعية واضحة وثابتة خاصة بتركيز فيتامن د في أمصال دم الأشخاص الأصحاء. حيث تبلغ نسبة التركيز الكافي الموصى بها عالميا 30≤ نانوغرام / مل (75 ≤نانومول/ لتر)، ولكن يعتبر المستوى الدال على نقص التركيز 20 ≤ نانوغرام/مل (50 ≤ نانومول/لتر)، بينما يعتبر التركيز ما بين 20 و 30 نانوغرام/مل كمية غير كافية من الفيتامين في الدم.

تهدف هذه الدراسة المقطعية إلى تحديد المستوى الطبيعي لفيتامين د الخاص بسكان الضفة الغربية في دولة فلسطين، و تحديد الإعتبارات البيئية، والاجتماعية والاقتصادية والسلوك الشخصي ومدى تأثيرها على تركيز فيتامين د حيث تم دراسة 300 عينة من كلا الجنسين بالغين وذو صحة سليمة، و لايعانون من أي تاريخ مرضي خاص بالكلى و الكبد و سوء الإمتصاص في الأمعاء، و تتراوح أعمارالمشاركين بين 19 و 65 عام. حيث تم إختيار 123 ذكر و 177 أنثى بطريقة عشوائية من مدن وقرى ومخيمات الضفة الغربية. و كذلك تم إستخدام إستبانة خاصة مثبتة لجمع المعلومات الديمغرافية و العوامل الخطرة المتعلقة بفيتامين د حيث تم جمع عينة الدم و إستخلاص المصل لقياس نسبة التركيز بإستخدام جهاز chemiluminescence immunoassay analyzer (ARCHITECT *i*1000SR).

حيث كانت النتائج تشير الى أن نسبة تركيز فيتامين د في الدم تساوي 13.4 نانوغرام / مل ، مع ملاحظة أن 92% من الأشخاص هم دون مستوى ال 30 نانوغرام / مل، و 79.3 % أقل من 20 نانوغرام / مل، في حين أن 61% من الأشخاص المشاركين كانت نسبهم أقل من 12 نانوغرام/مل . كما تبين أن الأشخاص ذوو الفئات العمرية

الأصغر (18- 28عام) كانت لديهم نسبة تركيز فيتامين د أقل من الأشخاص في الفئات العمرية العليا مثل (29-40)،(41-50)،(51-65)عام. كما أوجدت الدراسة أنه لا يوجد إرتباط بين مستويات تركيز فيتامين د والجنس والسمنة وإستخدام كريم واقي الشمس و التعرض لأشعة الشمس و كذلك إرتداء الحجاب للنساء المشاركات في الدراسة. و بالإضافة لذلك فقد تم دراسة عادة تناول أنواع محددة من الوجبات الغذائية، و قد تبين أنه لا يوجد اختلاف في مستويات فيتامين د لدى الأشخاص الذين يتناولون وجبات غذائية تحتوي على كميات كافية من الجبنة و الزبادي و كذلك تناول كميات كافية من البيض والحليب والسمك والخبز من قبل الاشخاص المشاركين في الدراسة.

في الخلاصة، ووفقًا لعدم وجود أدلة تربط بين أمراض جهاز الهيكل العظمي وغيره من الأجهزة الرئيسية في الجسم مع المستويات الموصى بها لتركيز فيتامين د (≤ 20 نانوغرام / مل ، أو 30≤ نانوغرام / مل) ، وفي ضوء دراستنا لتحديد المستوى المرجعي لفيتامين د لدى البالغين الأصحاء ، نوصي بالنظر إلى المستويات التي تقل عن 20 نانوغرام / مل وقريبة من 14 نانوغرام / مل كمستوى مرجعي لفيتامين د لعامة السكان في مجتمع الضفة الغربية. ومع ذلك ، فإن عينة دراستنا محدودة بعدد صغير من المشاركين ، وبالتالي هناك حاجة إلى مزيد من التحقيق والبحث للتوصل إلى استنتاجات أكثر شمولية فيما يتعلق بالمستويات المرجعية والعوامل ذات الصلة بهذا الفيتامين.

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List of Abbreviations

1,25(OH)2D	1,25-dihydroxyvitamin D
µg	microgram
25(OH)D	25-hydroxyvitamin D
BMD	Bone Mineral Density
CLIA	Chemiluminescence Immunoassay
cm	Centimetre
CMIA	Chemiluminescent Microparticle Immunoassay
C^o	Celsius Degree
CYP24A1	Cytochrome P450 Family 24 Subfamily A Member 1
CYP27A1	Cytochrome P450 Family 27 Subfamily A Member 1
CYP27B1	Cytochrome P450 Family 27 Subfamily B Member 1
CYP3A4	Cytochrome P450 Family 3 Subfamily A Member 4
D2	Ergocalciferol
D3	Cholecalciferol
DHCR7	7-Dehydrocholesterol Reductase
FGF23	Fibroblast Growth Factor 23

GI	Gastrointestinal Tract
HPLC	High-Performance Liquid Chromatography
ID-LC-MS/MS	Isotope Dilution Liquid Chromatography-Tandem Mass Spectrometry
IL-12	Interleukin 12
IOM	Institute of Medicine
IU	International Units
kg	kilogram
L	Liter
LC-MS/MS	Liquid chromatography tandem mass spectrophotometry assay
LRP2	LDL Receptor Related Protein 2
mL	millilitre
NF-κB	Nuclear Factor kappa B
nmol	nanomole
PBMC	Peripheral Blood Mononuclear Cells (PBMC)
PMCA1b	Plasma Membrane Calcium ATPase 1b
PTH	Parathyroid Hormone

RANKL	Receptor Activator of Nuclear Factor kappa Beta (NFkB ligand)
RIA	Radioimmunoassay
RLUs	Relative Light Units
RXRA	Retinoid X Receptor Alpha
SNP	Single Nucleotide Polymorphisms
SPSS	Statistical Package of Social Science software
TB	Tuberculosis
TRPV6	Transient Receptor Potential Vanilloid subfamily member 6
U.S	United States of America
UVB	Ultra-violet B radiation
VDBP	Vitamin D Binding Protein
VDDR	Vitamin D-Dependent Rickets Type I
VDR	Vitamin D Receptor
VDREs	Vitamin D-Responsive Elements

Chapter One

1.1 Introduction

In the last decade, vitamin D had special importance due to its consideration in many biological events in the human body. Recent reports state that the low-level effects will contribute to many functional disorders and physiological problems. Bone haemostasis was the central core function in classical consideration; however, the new vitamin has become more critical in many human system regulations and progression (Muscogiuri 2020). Recent statistics show that about 40% of the European population is vitamin D deficient, and 13% are severely insufficient (Amrein *et al.* 2020). Moreover, in the United States, about 35% of the adult population is vitamin D deficient, whereas, in Bangladesh, India, and Pakistan, over 80% of adults are considered vitamin D deficient (Sizar *et al.* 2021).

In 1913, previous theoretical developments found that McCollum was the first scientist to contribute to discovering vitamin D. In addition, McCollum was the first scholar to describe vitamin D. Notably, he rejected the hypothesis that vitamin A is essential in the treatment of rickets patients in Scotland. Expressively, McCollum destroyed the vitamin A in the cod liver oil by bubbled oxygen. And he noted that brake in the healing of xerophthalmia and vitamin A deficient, while the rickets treatment and progression were still achieved.

McCollum discovered a new vitamin called vitamin D. This has been widely adopted in the field. Vitamin D is considered a prohormone and not a vitamin after the second half of the 20th century due to its endogenous synthesis and abundance in many food sources and other food substances such as vegetables, fruits, and grains (DeLuca 2014).

The contributions made by McCollum have broad applicability. The clinical testing of vitamin D has recently increased. The results of these tests have advanced consideration in healthcare. Despite the traditional association with bone health benefits, vitamin D is becoming more reliable for other conditions. Significantly, the average serum level is essential for preventing chronic diseases like cardiovascular disease, diabetes, cancers, and immune system disorders (Mehta 2018). Thereupon, an adequate amount is recommended and must be in beneficial status. The contribution of dietary intake is considered poor and leaves a gap, but the endogenous source comes from ultraviolet-B radiation (UVB-light), making the concentration more adequate. The median concentration of vitamin D intake in German society achieved by the National Nutrition Survey II was 2–4 g per day for an adult. The international average reference value of healthy bone mass and intermediate calcium level was more than 50 nmol/l (20 ng/ml) of 25(OH)D serum concentration. However, while considering the US Institute of Medicine, the value was more than 40 nmol/l (Society 2012). The cut-off value of the insufficient amount of vitamin D is reported in the range of 30-50 nmol/l (12-20 ng/ml) (Elrayah *et al.* 2020). This value may cause unfavourable bone-health outcomes and skeletal disorders, including fracture and bone loss. Moreover, the increased risk of mortality and infections may occur with continuously decreased levels and is considered as a low amount of vitamin D with a value below 30 nmol/L (or 12ng/ml) (Amrein *et al*. 2020). Based on the preceding, the estimated average amount of vitamin D intake without exposure to sunlight is 400 IU per day to maintain bone health and the highest biological needs (LeFevre & LeFevre 2018).

The first main focus in this field is the level of vitamin D serum level. The determination of vitamin D serum level is variable according to the different factors that affect its concentration and classification of its deficiency or insufficiency in diagnosis

groups. Since vitamin D depends on UVB radiation in its biosynthesis, the environmental factor such as exposure time to the sunlight will affect its concentration level and becomes variable seasonally at a latitude distant or near the equator. Summer and autumn have higher concentration levels, while spring and winter are lower. Furthermore, the outdoor worker comparable with the indoor is variable. On the other hand, the food intake has attention to the variable that affects the vitamin D level. Several studies show that the populations that depend on food habits like meat and seafood meals have a higher concentration level than vegetarians and vegans(Wang *et al.* 2020).

The contributions made should be of broad interest. The genetic variable also affects the vitamin D concentration and also its functions which mainly represent single nucleotide polymorphisms (SNP) in the responsible genes of vitamin D pathways like (DHCR7, CYP2R1, CYP3A4, CYP27A1, VDBP, LRP2, CYP27B1, CYP24A1, VDR, and RXRA). On the other hand, age and gender have little effect on vitamin D serum level and are considered negligible (Ferrari *et al.* 2017). Moreover, the inverse relationship between BMI and vitamin D concentration was reported, especially for obese people who have a vitamin D deficiency as a result of sequesters of 25(OH)D substance in adipose tissue (Kumaratne *et al.* 2017). In addition, skin color, ethnicity, lifestyle, and physical activity show a variation in the vitamin D level. The dark skin participants are more likely to be hypovitaminosis D (deficiency/insufficiency) (Cork 2017).

1.2 Structure of vitamin D:

Vitamin D has a similar chemical structure in its two isoforms, vitamin D3 (cholecalciferol) and vitamin D2 (ergocalciferol). Still, they differ from each other in the side chains. Also, the binding will vary with the vitamin D binding protein (VDBP) and its metabolism (Bokhari & Albaik 2019).

Vitamin D2 has a double bond between C22 and C23. Another difference in the methyl group attached at C24 in the side chain (Figure 1) is that this difference in D2 will lower its binding affinity to DBP, limiting its conversion to 25 hydroxyvitamin D (25OHD) and become easier in clearance from circulation (Bikle 2014).

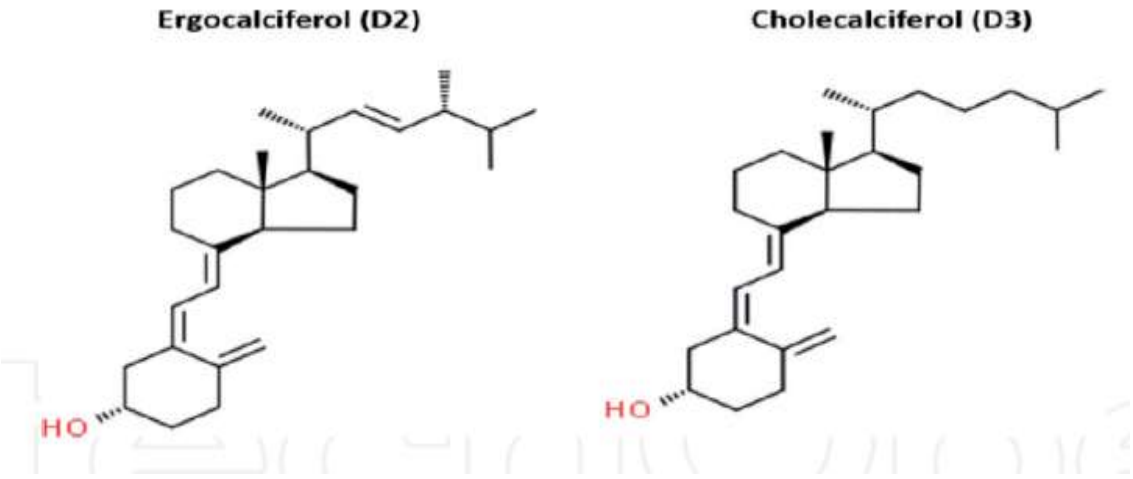


Figure (1): Chemical structure of vitamin D isoforms (D2, D3) (Bokhari & Albaik 2019).

1.3 Source of vitamin D:

Many natural substances provide the body with the essential quantity of vitamin D. The main two isoforms are vitamin D3 (cholecalciferol), an animal-derived form, and an endogenous (human) source. The other one is vitamin D2 (ergocalciferol), a plant-derived form produced after exposure to ultraviolet radiation (Pilz *et al.* 2018).

The essential primary source of vitamin D is photosynthesis derived of 7-dehydrocholesterol (7-DHC) substance into vitamin D molecule. It depends on ultraviolet radiation intensity and duration (Christakos *et al.* 2016). Also, the endogenous state production in the skin allows generating a sufficient concentration level of vitamin D in the body (Roth *et al.* 2018).In the middle east and north Africa, the duration of sun exposure reach 300/360 days and spans region latitudes from 15 to 39°

N (Chakhtoura *et al.* 2018). The individual habits in the exposure of hand, face, and arms to the sunlight enable the production of vitamin D. Thus, the exposure for 10-15 minutes will achieve one erythema dose (erythema is the amount of the sun that produce faint redness color of the skin) which is responsible for providing 500µg of vitamin D3. The excess exposure will produce an inactive form of vitamin D such as tachysterol and lumisterol which are considered a safe way to prevent toxicity dose of vitamin D. Having this in mind, the use of sunscreen, skin pigment, clothing wear habits, and lifestyles avoids sunlight has an essential state in vitamin D production (Laird *et al.* 2010).

Nutritional source of vitamin D is found naturally in some foods (Table1), such as cod liver, cheese, beef liver, tuna fish, salmon, oily fish, mackerel, and egg yolks)(Roth *et al.* 2018). It also can be found in fortified food (dairy products, orange juice, margarine, and breakfast cereals).The level of vitamin D circulating in the body from our diet will have about 10-20% of total vitamin D in circulation (Bokhari & Albaik 2019).

Table (1): Natural sources of vitamin D and the concentration level intake.

Food	IU/serving	µg/serving	Percent DV (daily value, based on 800 IU/day for adults
Salmon (sockeye) cooked, 3 ounces	570	14.2	71
Swordfish, cooked, 3 ounces	566	14.1	71
Cod liver oil, 1 teaspoon	450	11.2	56
Sardines, canned in oil, drained,1 cup	288	7.2	36
Tuna (white), canned in water, drained, 3ounces	68	1.7	9
Egg, 1 large hard cooked (50 g)	44	1.1	5
Beef liver, cooked (pan fried), 1 slice (81 g)	40	1.0	5
Cheese, Swiss, 1 cup (diced)	25	0.7	3

1.4 Synthesis and metabolism of Vitamin D:

The primary two precursors of vitamin D are the ergosterol substance (previtaminD2) and 7-dehydrocholesterol (previtaminD3). It is stored and accumulated in the human skin layer, especially dermis and epidermis bilayers (Acar & Özkan 2021).

In a nonenzymatic step formation, the exposure of the skin to the sunlight will develop vitamin D3 (cholecalciferol) and vitamin D2 (ergocalciferol), which are thermally converted to vitamin D3 (Tsiaras & Weinstock 2011).

The breakdown and cleavage of the B ring in (7-DHC) produce the pre-vitamin D3 molecule within the 280–320 nm UV radiation spectrum. Then, it is followed by thermal isomerization to become a vitamin D3 (cholecalciferol) molecule, Figure(2).The skin melanin pigment and sunlight intensity are the main factors responsible for vitamin D production (Bikle 2014).

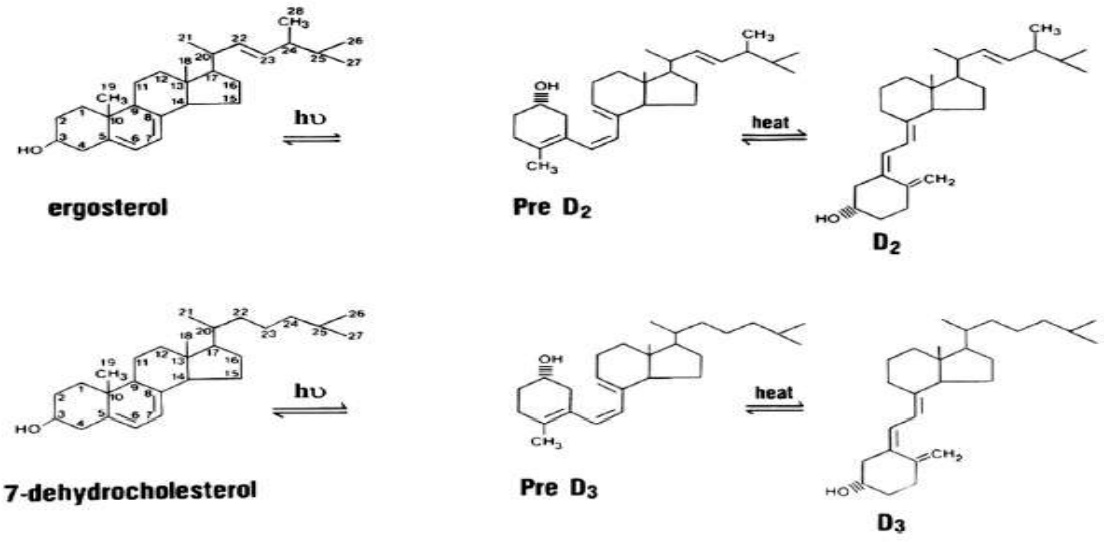


Figure (2): The production and metabolism of vitamin D3 and D2 (Bikle 2014).

Vitamin D circulates within the blood stream in non-active form and needs the specific vitamin D binding protein (VDBP) to be transported and activated in the liver by 25-

hydroxyls enzyme, the 25-hydroxylase enzyme, which hydroxylates the vitamin D3 in C25 to produce 25- hydroxylase vitamin D3 [25(OH)D3]. The primary circulating form of vitamin D (25(OH)D3) in serum is considered the vitamin D biomarker concentration status because it is circulating in the blood stream, and its half-life is about two weeks. To this end, the excess non- hydroxylated amount will accumulate in the adipose tissue, liver, and skeletal muscle (Laird *et al.* 2010).The transported amount of vitamin D via the VDBP reaches 99%. Furthermore, the remnant is transported by the albumin protein (Close 2015). Cytochromes P450 (CYPs) will perform the hydroxylation reaction in the liver cells. The main enzymes that contribute to the [25(OH)D3] hydroxylation process are CYP27A1 (mitochondrial) and CYP2R1 (microsomal) in the liver cells (Christakos *et al.* 2016).

The CYP2R1 has the highest affinity for attachment to 25(OH)D3 or D2 approximate to sub-micro molar concentration level. It reveals the highest specificity for vitamin D. This genetic defect shows the hereditary type of rickets disease-associated directly with a mutation in CYP2R1. In addition, the CYP3A4 isoform secreted from the (microsomal) liver and intestine cells which have a function in the drug-metabolizing effect and plays a key role in the 25-hydroxylase activity of 25(OH)D3 and 25(OH)D2 forms (Dong *et al.* 2021). The $1\alpha,25(OH)_2D3$ is the further hydroxylation form of vitamin D catalyzed by the mitochondrial CYP27B1 in the kidney. The $1\alpha,25(OH)_2D3$ is the main dynamic and potent form of vitamin D, which is hormonally bound with the VDBP (Kumar *et al.* 2012), and has a 1000-fold lower concentration than [25(OH)D3] with a shorter half-life (Dong *et al.* 2021).

The insufficient amount in the production of $1\alpha,25(OH)_2D3$ due to a mutation in the CYP27B1 enzyme will accumulate the substrate molecule 25(OH)D3 in vitamin D-dependent rickets type I (VDDR) condition (Cheng *et al.* 2004). The renal tubules are

the main source of secretion of CYP27B1 enzyme, which is stimulated by parathyroid hormone (PTH), calcitonin, and insulin-like growth factor 1, while inhibited by fibroblast growth factor (FGF-23), phosphate, and calcium (Bouillon *et al.* 1998).Therefore, the CYP2R1, CYP27A1, and CYP27B1 are the main three CYP450 enzymes in the liver and kidney that produce the active form of vitamin D. In conclusion, the conversion of 7-DHC to the active form 1α,25$(OH)_2$D3 by UV light exposure and heat (Δ) isomerization in the skin, the CYP27A1 in the liver and CYP27B1 in the kidney act as the main production stage of vitamin D, Figure(3) (Sakaki *et al.* 2005a).

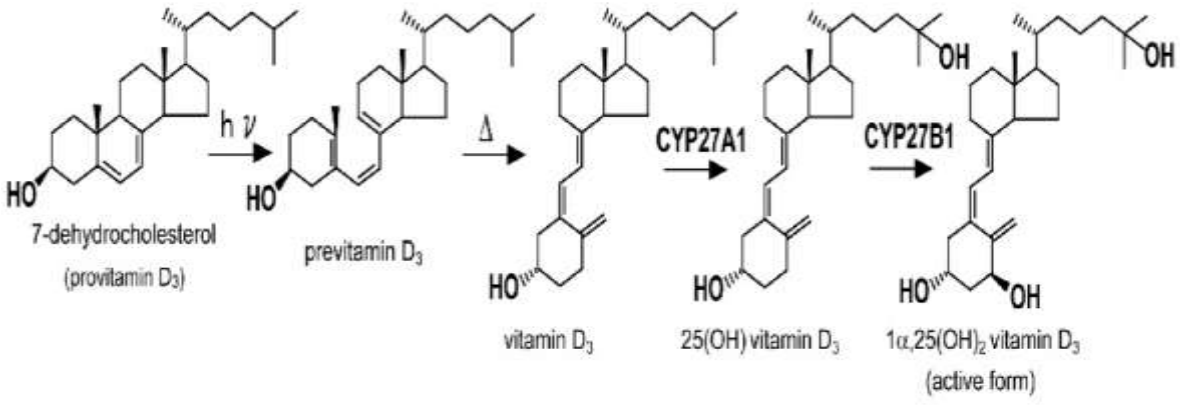


Figure (3): The schematic diagram of vitamin D active form production 1α,25(OH)2D3 (Sakaki *et al.* 2005b)

The most imbalance of vitamin D in serum concentration and dysregulation function in interior human cells appeared as a consequence of the change of the extracellular form [25(OH)D3], not in the active hormone level (1,25$(OH)_2$D3) (Adams *et al.* 2014). Then the vitamin D is produced and becomes functional in the steroidal hormone form by attachment to the VDR (nuclear vitamin D receptor) to regulate the gene transcription function (Hewison 2012a).

The feedback loop that regulates the vitamin D concentration is controlled by the level of the two main molecules, 25(OH)D and 1,25$(OH)_2$D, that are adjusted by the 25(OH)D-24-hydroxylase CYP24A1 enzyme (Theobald *et al.* 2019).The CYP enzyme that contributes to vitamin D regulation process is CYP24A1 (known as 24-hydroxylase), which catalyzes the inactivation reaction of 25(OH)D3 and 1,25$(OH)_2$D3 and activates the production of calcitroic acid molecule (water-soluble) by C-24 – oxidation reaction of 1,25$(OH)_2$D3. In the same manner, it will promote the conversion of 25(OH)D3 substances into inactive forms 24,25$(OH)_2$D3, and 25(OH)D3 – 26,23-lactone (Figure 4) (Jones 2012).

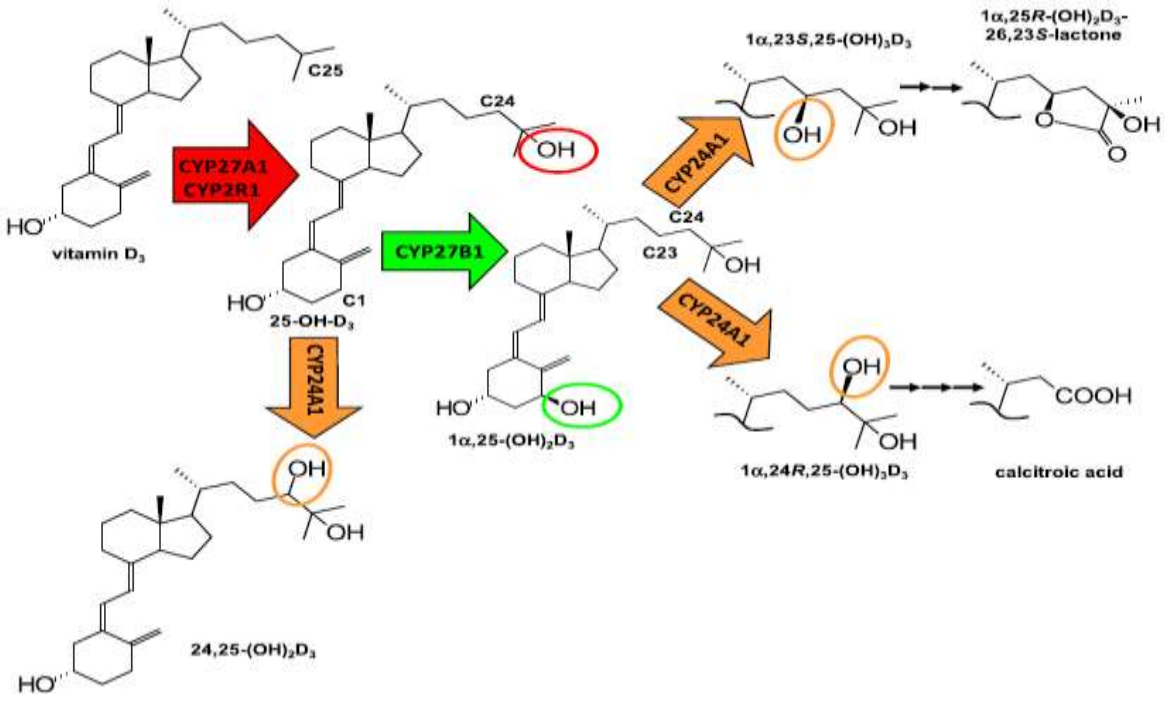


Figure (4): Catabolic products catalyzed by CYP24A1 in the regulation process of vitamin D (Jones 2012).

1.4.1 25-hydroxyvitamin D 1-alpha-hydroxylase:

The 25-hydroxyvitamin D 1-alpha-hydroxylase, also known as CYP27B1 or 1-alpha-hydroxylase enzyme is encoded by the *CYP27B1* gene (Monkawa *et al.* 1997). The main source discovered and reported was in kidney epithelial tissue in 1971. At the same time, with more reliable work on vitamin D, the secretion of this enzyme will be noticed from tissues other than kidney such as placenta, pulmonary alveolar macrophages, bone cells, melanocytes, epidermal keratinocytes, where the epithelial cells are considered the most tissue cells involved in the secretion of 1-alpha-hydroxylase. In the skin, the $1,25(OH)_2D$ regulates the differentiation and proliferation of the epidermal cell layer, and decreased production will constrict this function. Subsequently, the expression will occur in tissue epithelial cells like prostate, colonic mucosa, mammary epithelium, cervical epithelium, endometrium, various ocular barrier cells, and sinonasal epithelial cells. In addition, the secretion of CYP27B1 triggers an increase in the pathological condition in the epithelial tissue, such as skin inflammation, Crohn's Disease, inflammation in bowel tissue, and so on. The increased level of colitis is related to over expression of CYP27B1 by inflammatory cytokines, which are essential in improving and healing of inflammation conditions (Bikle *et al.* 2018).

The expression of the 1-alpha hydroxylase enzyme is important in the implantation and placentation of fetus cells. It was increased in the human placenta trophoblasts and decidua but not in the endometrium stromal cells (Bikle *et al.* 2018). The ectopic endometrium will involve an increased level of the 1alpha –OHase gene (Viganò *et al.* 2006). In addition, the preeclampsia condition has a higher level of the 1alpha –OHase enzyme production (Zehnder *et al.* 2002).

The CYP27B1expression were noted in other tissue types, such as bone tissue and the expression occurred within the total different cells type, in mesenchymal stem cells

(MSC), osteoblasts, osteoclasts, and osteocytes. However, the local synthesis and production of 1,25D in osteoclast cells was noticed in osteoclastogenesis regulation and maintaining the reabsorption function. The differentiation process of osteoclast cells from peripheral blood mononuclear cells (PBMC) will be stimulated by 25OHD by reducing its resorptive activity (Reinke *et al.* 2016). Taken together, VDR in bone cells contributes to CYP27B1 in bone homeostasis via calcium and phosphorus balance. Furthermore, vitamin D deficiency or VDR mutation results in bone defect and rickets disease (Holick 1996). The specific deletion mutation in CYP27B1 in chondrocyte cells will show a reduction in the osteoclastogenesis process emphasized by an increase in trabecular bone volume in neonatal long bones and a low level of angiogenesis to produce the widened zone of the bone plate that grows on an embryonic day 15.5 of mice modelled (Naja *et al.* 2009)

Vitamin D production is not limited to a particular tissue. In addition, it is not only secreted from the local site but possibly correlated in response to immune system cell differentiation and proliferation. The most form cells, like in macrophage cells in sarcoidosis and tuberculosis pathological conditions, were promoting the expression CYP27B1 to encourage the use of 25(OH)D as a substrate to produce a $1,25(OH)_2D$ intracellularly. The concentration of 25(OH)D in the serum enhances macrophage response to autophagy in the *M.tuberculosis*. Even the low level will inhibit the production of CYP27B1 and reduce the $1,25(OH)_2D$ production, which will not be enough for binding with VDR in that cell to enable the expression of vitamin D-dependent antimicrobial genes (Prietl *et al.* 2013). Extrarenal expression of CYP27B1 has also been discovered in patients with tuberculosis. It was mainly discovered in pulmonary alveolar macrophages, coccidioidomycosis, and cryptococcosis in the peritoneum of peritoneal dialysis patients. Other possible sources were peritonitis,

synovium of patients with inflammatory arthritis, and granulomata in the colon of patients with Crohn's disease. In addition, the secretion from subcutaneous fat necrosis of the new-born, granulomata within lymph nodes, circulating monocytes, and from patients with chronic renal failure, Dendritic cells (DC), and Th-B lymphocytes (Bikle *et al.* 2018).

Table (2): External overproduction of CYB27B1 in different granulomata forming diseases.

Non-infectious	**Infectious**	**Neoplastic**
Sarcoidosis	Tuberculosis	B- cell lymphoma
Crohns disease	Leprosy	Hodgkins disease
Silicone granulomata	Candidiasis	Lymphomatoid granulomata
Paraffin granulomata	Histoplasmosis	Dysgerminoma
Berylliosis	Coccidiomycosis	Seminoma
Wegeners	Cat scratch fever	Mesothelioma
Infantile fat necrosis	mMar. infection	
Slack skin disease		

Moreover, the production of CYP27B1 is expressed in pancreatic and parathyroid endocrine glands. The secretion of CYP27B1 will interfere with the PTH gene expression or secretion, and local synthesis will contribute to classical tumour suppressor gene function (Lauter & Arnold 2009). In ovary and ovarian cancer tissue, the over expression of CYP27B1 was determined and noticed. In addition, in malignant tissues, including lung, colon, and breast cancers, the production of CYP27B1 will be enhanced (Brożyna *et al.* 2015). In the male reproductive system, the vitamin D expression pattern CYB27B1 and VDR are secreted in the epididymis, prostate, spermatozoa, Leydig, and Sertoli cells of the testes and have an essential role in sperm maturation (Blomberg Jensen *et al.* 2010). Secretion of CYP27B1 is noticed in brain tissue, especially in the cerebellum and cerebral cortex. The secretion occurred in the umbilical vein within endothelial cells and contributed to the adhesion operation strategy (Bikle 2014).

1.5 Vitamin D Function:

A fat-soluble substance has many different biological functions and systemic regulation pathways. The main classical and first identified function for vitamin D was in calcium and phosphorus homeostasis for bone mineralization and composition. However, the contribution role of vitamin D in nonclassical function has been improved in immune system regulation and enhancement, cancer cell proliferation, keratinocyte differentiation, and muscle strength (Capuano *et al.* 2021).

1.5.1 Vitamin D and bone:

The active form of vitamin D, 1,25$(OH)_2$D3 is produced in the proximal renal tubule part due to the presence of (CYP27B1). The improper production of (CYP27B1) that could accur regarding the deletion, or nonfunctional mutation will enhance the vitamin

D dependency rickets type 1, which characterized by hyperparathyroidism and hypocalcaemia. It also reduces bone mineralization, which makes clear the responsibility of (CYP27B1) in calcium homeostasis and regulation. On the other hand, the (CYP24A1) is the enzyme responsible for the breakdown of $25(OH)_2D3$ into an inactive form of vitamin D (24,25-dihydroxy vitamin D3). Thereupon, on degrading the 1,25-dihydroxyvitamin D3 into calcitroic acid, the mutation in (CYP24A1) will interfere with the catabolism process. It also will emphasize the idiopathic immature hypercalcemia in humans. The (CYP27B1) and (CYP24A1) are regulated and controlled together with parathyroid hormone, serum calcium concentration, and 1,25-dihydroxyvitamin D3 itself to maintain the function of reabsorbing calcium ions or excreted within renal tubules (Cheng *et al.* 2004). In the hypocalcaemia condition, the parathyroid hormone will be increased to stimulate the production of $1,25(OH)_2D3$ by increasing the concentration of (CYP27B1) enzyme and inhibiting the (CYP24A1) secretion. The 1,25-dihydroxyvitamin D3 accumulation inhibits the parathyroid hormone production, decreasing the production of (CYP27B1) and stimulating (CYP24A1) secretion. Under those circumstances, intestinal calcium absorption is highly regulated through the VDR and $1,25(OH)_2D3$ to maintain the bone mineralization strategy. The expression of VDR appeared through all small and large intestinal segments, and calcium absorption was facilitated in proximal and distal tubules loops in the kidney. In Figure (5), the $1,25(OH)_2D3$ and parathyroid hormone control the calcium homeostasis with VDR binding.

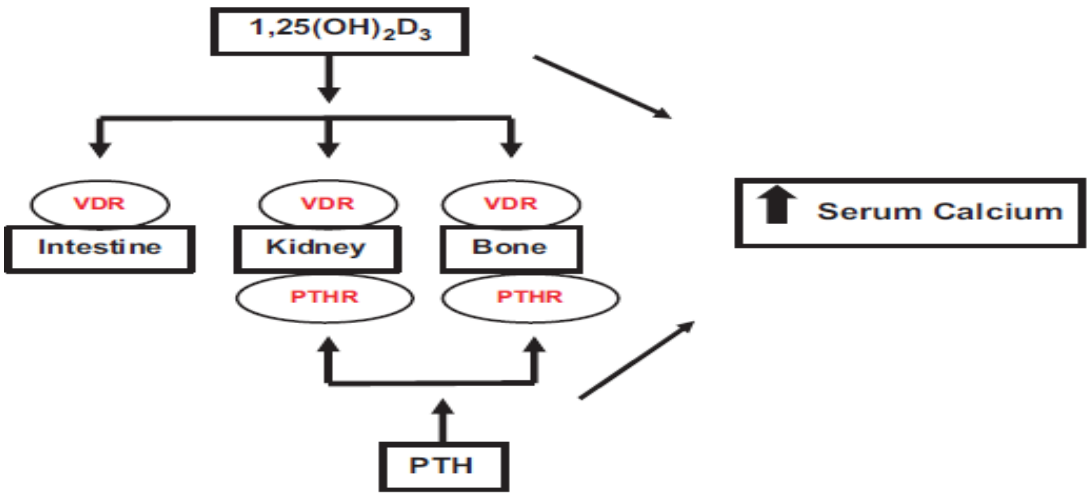


Figure (5): Calcium homeostasis and regulation of 1,25(OH)2D3 and parathyroid hormone . (Veldurthy et al. 2016).

In a low level of calcium serum concentration, the $1,25(OH)_2D3$ increases and stimulates the absorption through the act of VDR in the intestine. In contrast, if the concentration is still low, the action of 1,25(OH)2D3 and parathyroid hormone will trigger the release of calcium from its stores in bone and reabsorbed in the kidney tubules (Veldurthy *et al.* 2016). Furthermore, in the insufficient calcium level, the vitamin D will achieve homeostasis via the complexes with VDR in the osteoblast to trigger the NF-κB ligand (RANKL) activator receptor in the plasma membrane, on the preosteoblasts, the binding of plasma membrane RANK its ligand (RANKL), will induce the development of preosteoclast to osteoclast cells. These fine cells facilitate the release of collagenases and hydrochloric acid to liquefy bone and free its unique calcium and phosphorus stores into circulation (Holick 2006). The $1,25(OH)_2D3$ improved to stimulate osteoclastogenesis by both activating the expression of RANKL factor (receptor activator of nuclear factor-1-B ligand), which increases the differentiation of osteoclast and reduces the antagonist osteoprotegerin (OPG) expression. The $1,25(OH)_2D3$ will raise the expression of the intercellular adhesion

molecule-1, which enables the adhesion between osteoclast precursors and stromal osteoblasts (Kogawa *et al.* 2010).

In bone mineralization, the calcium and phosphate have an important role in control and regulation through the consequence of fibroblast growth factor 23 (FGF23) hormone and α-Klotho receptor on vitamin D. The FGF23 is a phosphaturia hormone that is released in osteocytes and osteoblasts which enable excretion of phosphate within the kidney and reduce the production of 1α-hydroxylase enzyme, so the 1α,25-dihydroxyvitamin D3 concentration will be lowered. Also calcium reabsorption increased in distal tubules (Andrukhova *et al.* 2017). The existence of FGF23 in many tissues such as bone, kidney, heart, parathyroid gland, and blood vessels will promote calcium and phosphate homeostasis. This promotion contributes to many stabilization conditions such as bone mineralization, cardiovascular pathophysiology, and chronic kidney disease (Erben 2016).

1.5.1.1 Regulation pathway and absorption of intestinal calcium

The ($1,25(OH)_2D3$) is considered the most important vitamin D form in controlling and regulating the absorption of calcium ions from the intestine. Even confirmed, the calcium will begin to be absorbed after ($1,25(OH)_2D3$) treatment of deficient or insufficient conditions within 2-4 hours (Figurer 6).

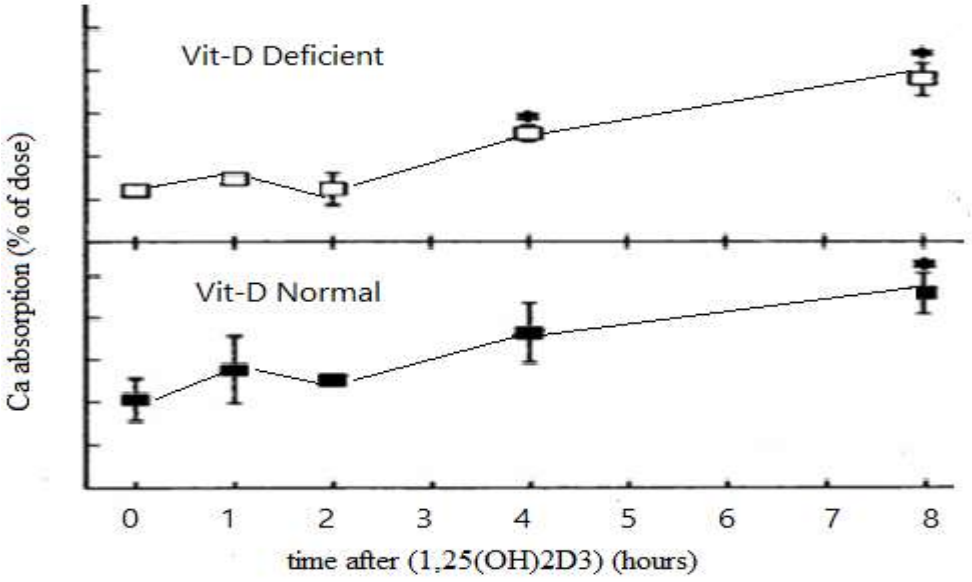


Figure (6): The time duration of the increased level of calcium absorption after(1,25(OH)2D3) intake (Christakos 2012).

The epithelial calcium transporter channels TRPV6 facilitate calcium absorption in the intestine and promote transcellular uptake and influx. The TRPV6 contributes to the calcium-binding protein as calbindin-D9k to maintain the calcium level under the influence of ($1,25(OH)_2D3$). Furthermore, the calbindin-D9k prevents calcium accumulation and toxicity within the intestinal cells to its buffering mechanism. The increased calcium level initiates the influx of calcium in the endoplasmic reticulum. It reaches the basolateral membrane, extruded by a special calcium pump called intestinal plasma membrane ATPase (PMCA1b). In addition, the sodium-calcium channel fixed in the basolateral membrane contributes to reabsorbing the calcium and maintaining its concentration. With transcellular uptake, the calcium reabsorption process may be accomplished via the paracellular pathway in-between the intestinal cells Figure (7) (Christakos 2012).

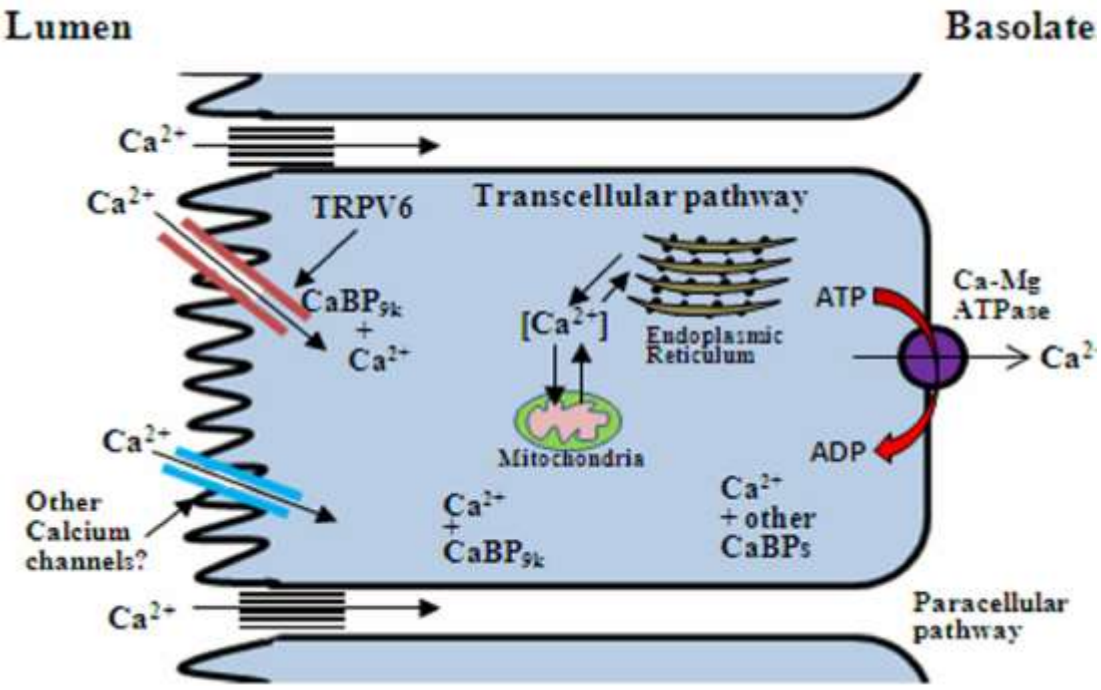


Figure (7): The reabsorption process of calcium ion within the intestine via the transcellular and paracellular pathway (Christakos 2012).

1.5.1.2 Bone Pathophysiological defect and vitamin D

The skeletal deformities and complications mainly appear with vitamin D insufficiency. Identically, the inadequate amount of vitamin D combined with improper calcium and phosphorus homeostasis and the dietary calcium-phosphorus absorption in the intestine will decrease to 10-15% and 50-60%, respectively. In infants, rickets disease develops with a decreased calcium level, marked by bone deformities of both upper and lower limbs. The low calcium level will trigger (PTH) to maintain serum calcium level by increased reabsorption in the distal and proximal convoluted tubules. Thus, PTH induces phosphorus excretion within the urine and decreases reabsorption in the kidney tubules. In the same fashion, rickets infants have an average value of calcium and a low level of phosphors and 25(OH)D. On the other hand, insufficient calcium levels may

lead to further skeletal abnormalities such as tetany, hypocalcaemia myocardiopathy, seizures, laryngospasm, and death (Holick 2006).

Vitamin D reduction with advanced age has a critical indication regarding variability in the lifestyle of old people. The decrease in the epidermis layer means a reduction of 7-dehydrocholesterol concentration in the skin. By the same token, the decrease in physical activity and the changes in different biological processes will lower the concentration of vitamin D by about 25% in 65 years elderly people compared with 20-30 years adults. Similarly, the impaired calcium absorption within the intestine bowel in elderly people due to reduced hydrolysis of 25(OH)D to (1,25(OH)2D3) will appear in kidney failure patients, decreased concentration of estrogen level in women as well as increased resistance of bowel mucosa layer to absorption (Hill & Aspray 2017). All of these will promote osteoporosis condition in elderly individuals and become more exposed to the risk of bone fracture, especially in the hip and non-vertebral bone. Osteoporosis patients have a low bone mineral density (BMD) that causes the fragility to increase and increases the risk of bone fracture in those 65 and older. Osteoporosis patients have 80% prevalence of vitamin D deficiency worldwide (Navarro Mendoza *et al.* 2016).

1.5.2 Vitamin D and gene expression:

Vitamin D plays an important role further than mineral homeostasis. The gene expression function has recently become one of the most significant functions enhanced by vitamin D. The hereditary defect in metabolic pathways and functions of the hormonal form, 1,25-dihydroxyvitamin D3 ($1,25(OH)_2D_3$), was emphasized. Vitamin D combined with VDR (nuclear vitamin D receptor) enables its gene expression function in target cells. The VDR is a phosphoprotein receptor with a high affinity for binding with the 1,25-dihydroxyvitamin D3. It also guides its action to regulate gene expression

in specific cells via combination with the vitamin D-responsive elements (VDREs) as specific DNA sequence and form the heterodimer association with a nuclear steroid receptor called retinoid X receptors (RXRs). The structural composition of VDR enables the ligand for binding function. The VDR has two domains, the N-terminal, which has a zinc finger domain, and the hormone-binding domain on C-terminal. The structure domains will determine the DNA binding of a "hinge" segment in N-terminal and the conserved region in the C terminal (VDREs). The influence of 1,25-dihydroxyvitamin D3 form in the hormonal binding region of VDR will promote a strong dimerization with RXR. It will facilitate a strong ligand binding with VDREs, although the 1,25-dihydroxyvitamin D3 will stimulate transcription in a specific gene (Haussler *et al.* 1995). The heterodimerization complex between (VDR)/(RXR) and binding with VDREs will encourage the conformational changes via recruitment of the transcriptional factor. This enhances the gene expression function (Beckett 2020). On the other hand, in numerous genes, the binding of 1,25-dihydroxyvitamin D3 with VDR will inhibit the transcriptional expression. In Figure(8) thus the suppression of CYP27B1 form in renal expression that activates the synthesis of 1,25-dihydroxyvitamin D3 and activates the CYP24B1which facilitates the degradation to calcitroic acid, will be regulated and also controlled via the VDR cellular concentration (Pike & Meyer 2012).

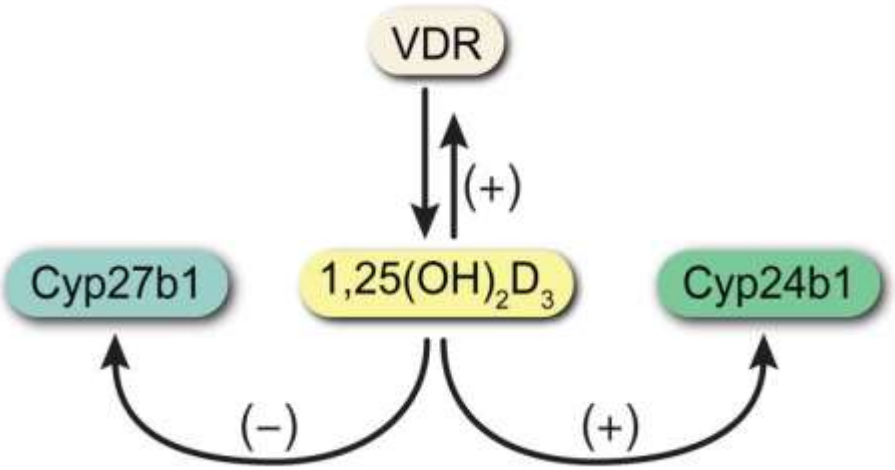


Figure (8): VDR functional activity mediated by CYP27B1, CYP24B1, 1,25-dihydroxyvitamin D3 concentration (Pike & Meyer 2012).

The gene network regulated by the vitamin D and its receptor prolonged in transcriptional effects requested in different cells and tissue. The bone regulation process in addition to the mineralization, calcium, and phosphorus gene homeostasis, the regulation of bone lifespan to osteoblast differentiation and osteoclasts resorbing activity which determined by the receptor activator called nuclear factor kappa-*B* ligand (RANKL) (Kogawa *et al.* 2010). Other regulated gene expression pathways of the hormonal form of vitamin D improved within bile acid metabolism in the colon, adaptive immune system, hair follicles developing, keratinocytes cell differentiation, and xenobiotic compounds degradation in different tissue (Pike & Meyer 2012).

1.5.3 Vitamin D and muscle strength:

The contribution of vitamin D in muscle growth and strengthening advanced recently. The association between the insufficient level of vitamin D and muscle weakness with falling conditions have a critical association. Such association is explained by the presence of VDR in many tissues and the myoblast cells, so vitamin D enhances the synthesis of protein in muscle cells, which contributes to the growth of

type 2 muscle fiber in size and number (Laird et al. 2010). The attachment of 1,25-dihydroxyvitamin D3 will trigger the gene expression and protein synthesis with the contribution of the retinoic receptor (RXR) and vitamin D response element (VDRE). The effect of gene coding encourages the calcium-binding protein called (calbindin-D9K) which increases calcium influx levels that facilitates muscle contraction. Another contribution of the genomic impact of VDR in skeletal muscle will be emphasized in the expression of insulin growth factor(IGF) that is responsible for the differentiation and proliferation of cells (Figure 9) (Latham *et al.* 2021)

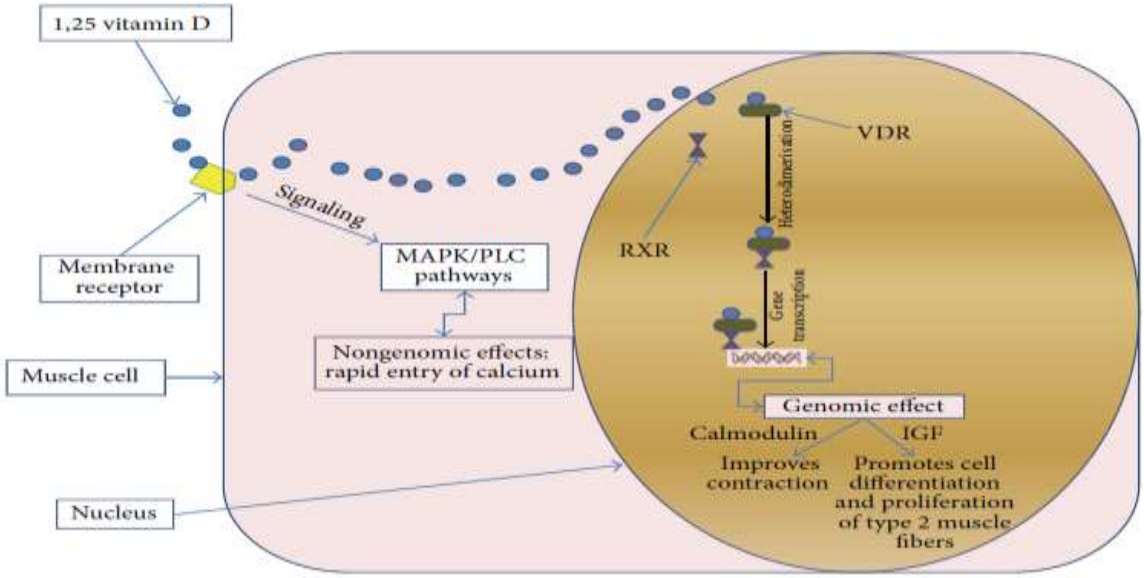


Figure (9): Vitamin D contribution and skeletal muscle, the genomic and nongenomic pathway promote calcium influx in cells and prevent weakness and illness (Halfon et al. 2015).

The increased level of vitamin D by supplementation will contribute to the healing of muscle dystrophy and weakness. The (4000 IU/day) is a recommended dose achieved during four months with a 30% prognosis with VDR contribution (Halfon *et al.* 2015).

1.5.4 Vitamin D and the immune system

Many functions reported for vitamin D concentrated on skeletal and calcium homeostasis. The additional notable role is vitamin D's ability to regulate and adjust the immune system mechanisms within the innate and adaptive immunomodulatory action. The considerable observation was noticed by Finsen, who won the Nobel Prize for Medicine and Physiology in 1903. He treated lupus vulgaris and tuberculosis (TB) with irradiated ultra-violet light. A consecutive study shows the oral supplement of vitamin D's success in treating and monitoring TB infection (Chun *et al.* 2014). The association of the immune system and the active form of vitamin D is achieved and regulated with VDR in the different immune cells, such as in neutrophils, macrophages, and monocytes. Therefore, the activation of immune cells will increase the finding of VDR, which complexes with ($1,25(OH)_2D3$)/RXR and lead to regulating the expression of antimicrobial proteins like cathelicidin (CAMP) and defensin β2 (DEFB) peptides. In addition, the presence of antimicrobial pathogens will force the secretion of 1α-hydroxylase CYP27B1 and promote the production of cytokines protein such as interferon-γ or IL-4. The excess antimicrobial protein production will destabilize the plasma membrane within bacterial, viral, and fungal cells. Moreover, the low level of the active vitamin D is correlated with the end stage of renal disease and other septic infections such as upper respiratory infection, influenza, pulmonary disease, infection in allergic patients, and asthma (Martens *et al.* 2020).

The expression of nuclear VDR in the adaptive immune cells is another vitamin D regulation pathway condition. The activation of B and T immune cells will encourage the release of nuclear receptors and promote the gene expression up to regulate about five hundred responsive element genes. These are required for the proliferation and differentiation of adaptive immune cells. These regulations cleared the B cells'

homeostasis in autoimmune diseases via inhibiting memory and plasma B cell generation (Prietl *et al.* 2013). The regulation of the adaptive immune system correlates to nuclear receptor VDR expression will be achieved by vitamin D by mediating the Th1 and Th2 cells, the decreased production of proinflammatory type-1 cytokines like IFN-γ, TNF-α,IL-8, IL-6, IL-9, and IL-12. While on the other hand, vitamin D will trigger the secretion anti-inflammatory cytokines type -2, such as IL-4, IL-5, and IL-10 and prevent the activation of NF-kB p65 (Bui *et al.* 2021). Recently, the association between vitamin D and the disease correlated to B and T lymphocytes has become more apparent. The correlation of vitamin D with autoimmune disease as in diabetes type 1, while the oral supplement will decrease the illness, and other conditions like multiple sclerosis and Crohn's disease correlate with an insufficient vitamin D level (Hewison 2012b).

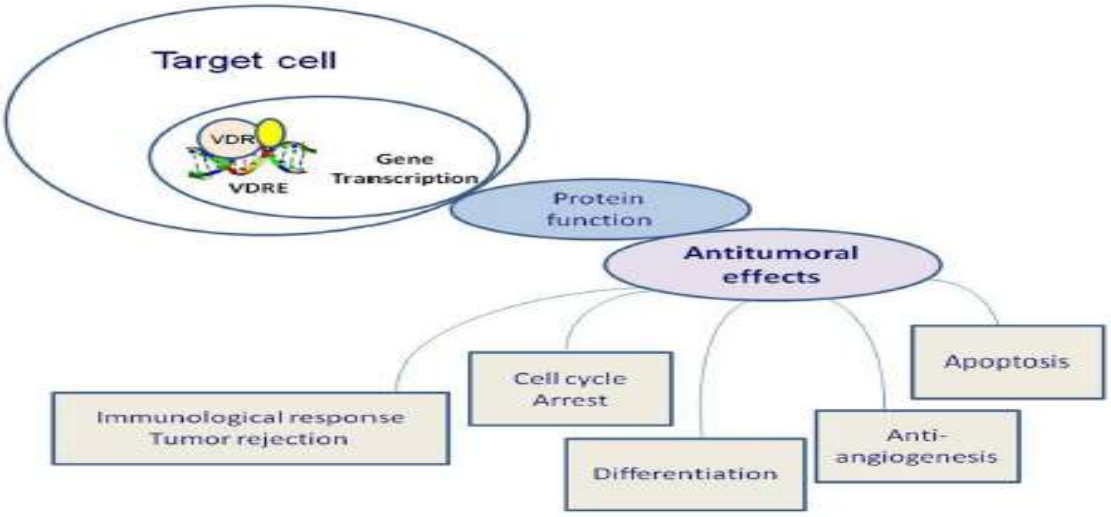
1.5.5 Vitamin D and cancer:

The recently published data investigate the association of vitamin D with a newly critical function other than classical calcium and bone mineralization. The nature of the vitamin forming and metabolites process from beginning in the skin to creating a hormonally active form through its precursors, enzymes, and receptors will encourage the many complex pathways to activation the functions. The role of vitamin D in cell proliferation, differentiation, cell growth, and apoptosis enable it to be a regulator in the body against cancer disease and metastasis. To this end, the activity of vitamin D in transcription factors will modulate the mutagenesis cells action.

The significant presence and distribution of VDR in cells type and tissue-like in the cardiovascular system, endocrine system, epidermis, gastrointestinal system, immune system, bone marrow, reproductive system, central nervous system, renal system, and other tissues and its binding with $1,25(OH)_2D$ will activate over 60 genes in deferent

cell lines. So, the formation of heterodimers with (RXR) retinoid X receptor enables this complex to occupy a specific sequence in the VDRE site and enhance the gene transcription in the vitamin D-specific responsive genes (Figure10) (Vuolo *et al.* 2012b).

Several VDR polymorphisms and genotypes variant is associated with cancer cell development. The one with a high associated risk with cancer cells is Bsm I, which has BB, Bb, and bb variants, men with colon cancer have a higher incidence twice when having a bb genotype than BB. Men who have an insufficient level of vitamin D and bb variant have a higher twice incidence of prostate cancer other than the BB variant. At the same time, in women, the incidence is also doubled with the bb variant in breast cancer and four times in metastasis than in the BB variant type (Garland *et al.* 2006).



Figure(10): Different regulatory functions in response to activation of VDR (Vuolo et al. 2012a).

Vitamin D role in the cell proliferation and apoptosis process will improve its relationship with the cell growth regulation process. Many studies that reflect this association have supported the evidence of these mechanisms. On the other hand, the mechanism of vitamin D function in decreasing and reducing cancer risk and

progressing cell proliferation within cancer cells is still not clearly explained. The gap in understanding how vitamin D prevents cell cancer proliferation and biological regulation function relevant to cancer cells inhalation and treatment is not clear. In addition, many hypotheses studied and analyzed this function in one cell type have not been generalized yet (Fleet *et al.* 2012).

1.6 Vitamin D blood level

The vitamin D metabolites available and concentrated in the human blood are deferring throw the development mechanism of its precursor (cholecalciferol D3, ergocalciferol D2) in the skin layer by UVB, successive creation with the hydroxylation process in the liver and kidney to form the 25(OH)D (nonactive form) and 1,25$(OH)_2$D (active form) respectively. The detection of vitamin D in serum and plasma depends on the availability of the nonactive form, considered the primary and best indicator form for analysis. The 25(OH)D level reflects the assumption of the animal and plant precursors amount (25(OH)D3 and 25(OH)D2). At the same time, the other metabolites have a different concentration but are not included in the vitamin D serum level detection (Couchman & Moniz 2017).

The total vitamin D concentration is mainly acquired by sunlight exposure, the rich source of animal or plant meal intake, or pharmaceutical supplementation. Thereupon, the total level will be affected by personal habits like sunlight exposure time and food diversity source, biological factors such as skin colour and body mass index, and genetic factors. According to these, the concentration varied between the high level of toxic attention and severe deficiency. A sufficient amount of vitamin D intake is achieved within the 30-100 ng/ml range, and the insufficient level has the 20-30 ng/ml range. Above all, less than 20ng/ml is considered a deficiency level, see Table (3) (Abdulrahman *et al.* 2022). However, since the variation in adequate level of 25(OH)D,

the concentration was determined as the function of PTH levels indicates a sufficient amount of 25(OH)D serum level. In Figure (11), the level of PTH started to become plateaus with the 30-40 ng/ml of 25(OH)D serum level (Holick 2009).

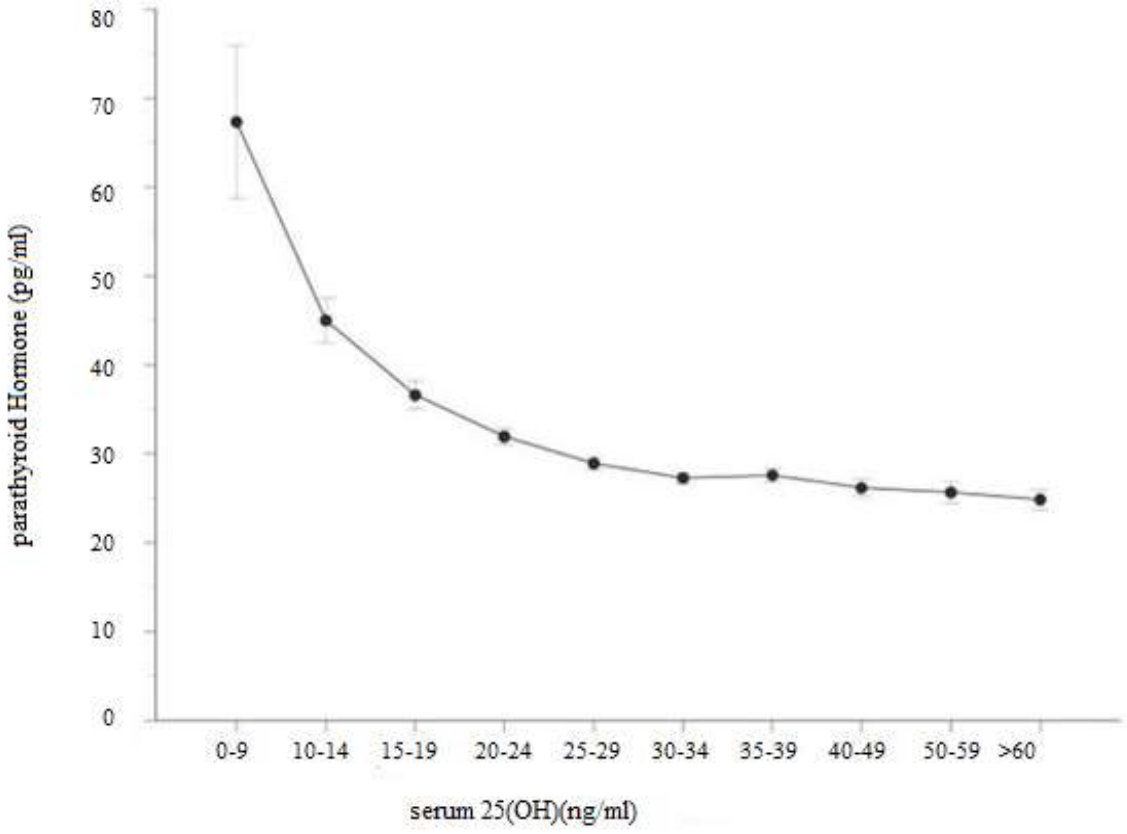


Figure (11): Adequacy analyses level of 25(OH)D in accordance with the function of (PTH) serum level (Holick 2009).

In addition, the unit used for the determination of blood vitamin D concentration is nanograms per millilitre (ng/mL) in USA standardization, and measurement by nmol/L can be calculated by multiplying the factor 2.5 with the (ng/mL) result(Ferrari *et al.* 2017).

Table (3): Concentration of vitamin D and its categories.

Classification	**25(OH)D ng/ml (in adults)**	**25(OH)D nmol/l (in adults)**
Toxic level	>100	> 250
Sufficiency or optimal level	30-100	75-250
Insufficiency	20-30	50-75
Deficiency	10-20	25-50
Severe deficiency	<10	<25

1.7 Problem statement

Several studies investigated the definition of sufficient vitamin D in the form of a plasma 25(OH)D concentration and concluded that there are different values. These values are as >50 nmol/L (>20 ng/mL) (Lips 2001), >75 nmol/L (>30 ng/mL) (Bischoff-Ferrari *et al.* 2006), >80 nmo/L (>32 ng/mL) (Hollis & Wagner 2006), and >100 nmol/L (>40 ng/mL) (Ashwell *et al.* 2010). On the other hand, other studies revealed different values in different societies and populations. A study defining 50 nmol/L (20 ng/mL) as serum 25(OH) D threshold found that 50% of the Western-European population is below this level. At the same time, a higher percentage was reported in South America and a lower percentage in North America (van Schoor & Lips 2018). These differences suggest ethnic and genetic differences in the

metabolism of vitamin D and its requirements, in addition to possible lifestyle habits, dietary habits, and human behaviour.

We doubt that a single reference value is sufficient in a global medical system for all these reasons. We think that a population-based reference value for vitamin D is needed for each country or ethnic group in the country. Furthermore, these values should be compared with different groups to understand the effect of factors associated with these values.

To the researcher's knowledge, no studies on vitamin D reference values nor the factors affecting these values are available in Palestine. Most of the diagnostic laboratories in Palestine often depend on western textbooks or standard commercial kits for reporting vitamin D reference ranges. In this thesis, we will establish reference ranges of vitamin D among healthy Palestinians. In addition, we will investigate possible cultural, behavioural, and socioeconomic factors associated with fluctuations in these ranges. The results will be compared with other countries vitamin D reference ranges. Moreover, all related factors will be investigated and compared accordingly.

1.8 Study Objectives:

The main objectives of this thesis are:

- To establish a population-based reference range of vitamin D in the Palestinian society.
- To investigate socioeconomic, cultural, and human behavioural factors associated with fluctuations of vitamin D blood level.
- To compare these factors with other communities and countries with similar conditions.
- To provide evidence-based national guidelines for diagnosing vitamin D deficiency in Palestine.

Chapter Two

2.1 Methodology

We present in this section an overview of the methodology adopted in this research. A clear description of the study population, sample, data collection procedures, tools, and statistical analysis can be found as follows.

The 25-hydroxyvitamin D (25(OH)D) was considered many years ago as the core substance of the principle method in detecting serum levels of vitamin D. Also, it is evaluated as the best biomarker substance in the human blood for analysis and determination of vitamin D level. Therefore, the detected serum level includes the two forms of vitamin D, 25-hydroxyvitamin D3 (25(OH)D3) and 25-hydroxyvitamin D2 (25(OH)D2), for endogenous skin sources and other plant food intake sources, respectively. Furthermore, vitamin D2 and D3 may be present from supplementation and medication sources (Wallace *et al.* 2010). Vitamin D3 has more bioactivity than vitamin D2 in the blood (Hollis 2010). The estimated half-life of (25OHD2) in blood circulation is 2-3 weeks. In fact, this existence is not influenced by the calcium and parathyroid hormone concentration (Hutchinson *et al.* 2017). Vitamin D has a specific binding protein that transports it within the circulation, called vitamin D binding protein (VDBP) which makes one of the discrepancies and difficulties for the determination, and causes variability in the results of different methods for vitamin D analysis (Jukic *et al.* 2018).

The other metabolites of vitamin D such as $1,25(OH)_2D$ can be detected in the blood and analyzed according to the clinical requirement. However, this active form is

problematic to assess concerning the low level of picomol concentration. Also, it is hard to detect with the UV or mass spectrometry methods (Hollis 2010).

Various methods and techniques can detect the level of vitamin D, which firstly occurred in the 1970s via high-performance liquid chromatography (HPLC). It is based on the VDBP protein detection principle, then the approach advanced to demand with the antibody against the 25(OH)D2 molecules via the radioimmunoassay (RIA) method. A further expansion is approved by the enzymes (EIA) or chemiluminescent substances (CLIA) and chemiluminescent microparticle immunoassay (CMIA) that become more specific and precise in the determination of vitamin D serum levels. Recently, mass spectrometry was operated and submitted in 2004 to operate the liquid chromatography combined with mass spectrometry LC-MS method for determining vitamin D metabolites (Wallace *et al.* 2010). It can determine the concentration of vitamin D2 separately from vitamin D3.

Moreover, the isotope dilution liquid chromatography-tandem mass spectrometry (ID-LC-MS/MS) was applied and considered a reference detection procedure for vitamin D analysis concerning its high specificity, sensitivity, and precision in the detection of analytes in the same run (Hutchinson *et al.* 2017).With this in mind, discrepancies in the immunoassay determination of vitamin D appeared firstly, the most potent attraction of the hydrophobic form of 25(OH)D2 with vitamin D binding protein(VDP). It makes assay challenging to assess the competition between the 25(OH)D capture antibody and VDBP in patient samples. Secondly, the presence of some metabolites like vitamin D epimers (epimer is a substance have a similar structure to vitamin D except for a stereochemical difference at one site). It is found in human sera with forms like 3-epi-25OH-D3 and 3-epi-25OH-D2, which is another form of difficulty in determining serum vitamin D concentration, particularly the epimers in the past was measured only

in children under one year old. Nevertheless, recently it was determined in the adult serum with 17% of total vitamin D measurement (Farrell *et al.* 2012).

2.2 Stability of 25(OH)D in serum:

The preanalytical stability condition is a critical point in the chemical determination level. The stability performance of metabolites must be known to be more reliable. The 25(OH)D and 1,25$(OH)_2$D metabolites have extreme stability and low variations in their concentration under different conditions. The temperature variation does not affect the stability. In addition, the exposure to light and freezing-thawing cycle status does not influence its stability. The 25(OH)D is stable for seven days at 4C^o and still has two months and 3-months stability at -20C^o and -40C^o ,respectively. Moreover, the vitamin D tolerates the change up to 4 times of the freezing-thawing cycle (successive thawing and freezing process) (Bozkurt et al. 2018).

The high stability property of 25(OH)D and 1,25$(OH)_2$D metabolites with variability or change in light exposure and temperature variance is related to the complex attractive nature of vitamin D molecule with the binding protein. It makes it more resistant to the variability condition (Hollis 2010).

2.3 Chemiluminescence microparticle immunoassay(CMIA) principle in vitamin D detection

The detection of 25(OH)D in serum was accomplished via the chemiluminescence microparticle immunoassay(CMIA) method by the Abbott Diagnostic machine/analyser (ARCHITECT i1000SR), with the 5P02-25 ARCHITECT 25-OH Vitamin D Reagent Kit (Lot 33590UD03). It mainly involved a pre-treatment step with (8-anilo-1-naphalensulfonic acid) to free the vitamin D from (VDBP) complex. The method occurred in one competitive immunoassay Chemiflex step, via the incubation step

involved with the paramagnetic anti-vitamin D coated microparticles (monoclonal rabbit anti-vitamin D IgG antibody). The competition attachment of Acridinium-labelled substance (biotinylated Vitamin D anti-biotin IgG acridinium-labelled) with free vitamin D molecule will accomplish. Hence, the amount of vitamin D in the mixture was bound to the Acridinium-labelled conjugate complex produce the chemiluminescence reaction (light yielded by a chemical reaction) which estimated as a relative light units (RLUs) and detected by the machine optics, Figure(12) (Hutchinson et al. 2017).

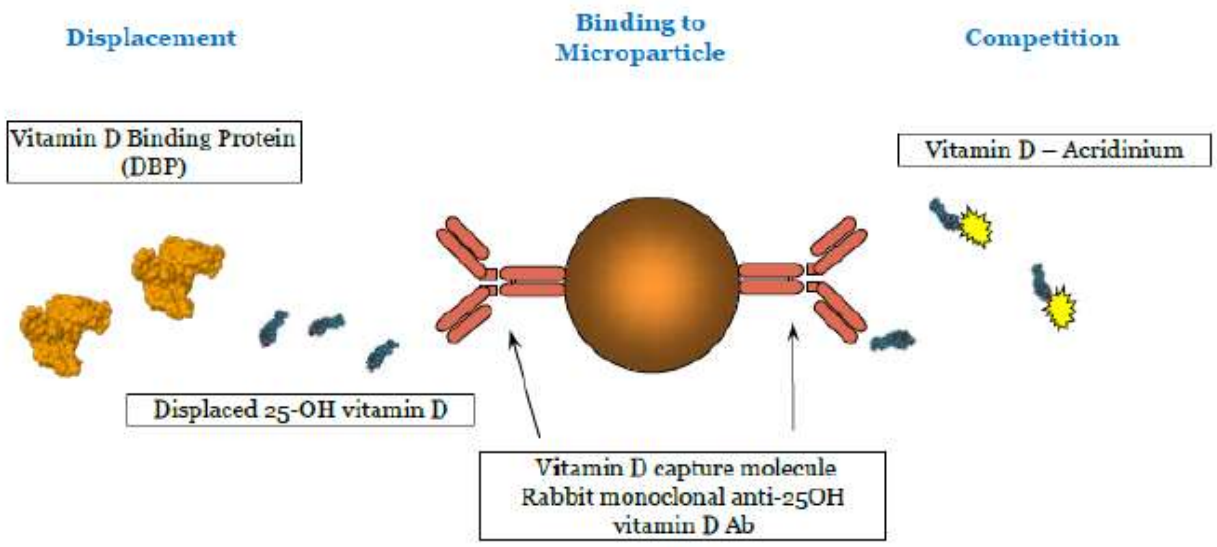


Figure (12): Architect 25-OH vitamin D detection assay (Hutchinson 2017).

2.4 Study population

The study population consisted of healthy Palestinian adults of which we selected a sample collected with the nominative randomization technique in the West Bank in winter season between January and March 2022. The apparently healthy participants included in the study have not reported any disorder of bone mineralization, kidney functions, or liver disease and were free from cardiovascular problems and immune system complications. In addition, the participants were not influenced by cancer or malignant abnormalities. Therefore, the precaution included the medication intake, which affects the level of vitamin D and make serum level variation such as the antiepileptic medication as phenobarbital, carbamazepine, phenytoin, and anti-inflammatory drugs such as corticosteroids, antifungal agents as clotrimazole, and ketoconazole, Rifampin and isoniazid, such as only healthy people were included with the exclusion of those who have suffered medical conditions. The included participants were adults between 19 to 65 years old within the male and female gender.

2.5 Sample collection and processing

For analyzing serum vitamin D levels, venous blood samples were collected from 300 healthy Palestinian participants. The sample was selected randomly for participants who were free of any apparent medical complication or illness and referred to the laboratory for follow-up or to check their health conditions. The included laboratories were in Ramallah (Arabcare Specialized Laboratory), in Hebron (Palestinian Medical Laboratory-Hebron city, Palestinian Red Cresent laboratory-Hebron city, Hebron Charitable Medical Center- Hebron city, AL-Rahma medical laboratory- Souref, Yatta central laboratory- Yatta, Al Samou Specialized Medical Center- AL- Samou), in Bethlehem (Yamamah Hospital Laboratory, Top-Lab laboratory), in Qalqelia (Health Work committees laboratory, B-care laboratory). Table 4 represents the distribution of

the collected samples. The 5 ml blood sample was drawn in an evacuated plain tube and allowed to clot within 15-30 minutes at room temperature. After that, the serum was collected after a 4500 rpm centrifugal force process. The haemolysed, icteric, lipemic samples were avoided and rejected, and the serum samples after collection were stored at -20C^{o} for up to five days. The samples were transported under special conditions (in icebox and isolation package) to be analyzed at the Arabcare Specialized laboratory within 48h. (Appendix I)

The serum vitamin D test was performed and measured by the Abbott chemiluminescence immunoassay analyzer (ARCHITECT *i*1000SR) with the 5P02-25 ARCHITECT 25-OH Vitamin D Reagent Kit (Lot 33590UD03). All samples collected throughout the West Bank were analyzed using one analyser at Arabcare Specialized laboratory.

Table (4): Distribution of study subjects according to location.

City	Sample size
Ramallah	65
Hebron	90
Bethlehem	29
Qalqelia	65
Nablus	36
East Jerusalem	15

2.6 Data collection

A questionnaire to collect socio-demographic variables affecting vitamin D was designed and distributed to each participant, including questions that are suspected to affect vitamin D levels. Initially, the questionnaire was developed in English (Appendix II) by consulting several references and then translated to Arabic (Appendix III). Each questionnaire data has a match code with its related sample collecting tube to facilitate data entry.

The questionnaire included governorate, age, gender, wearing hijab (head cover) for females, living location, educational level, marital status, and working status. In the second part, the health and medical condition assessment questions were included such as height, weight and BMI calculation, smoking habits, sun exposure time per day, use of sunblock cream, physical exercises, having GI problems, having kidney disease, taking calcium supplemented food, ingested fast-food meals, and taking anti-vitaminD medication such as antiepileptic (phenobarbital, carbamazepine, phenytoin), anti-inflammatory drugs (corticosteroids), antifungal agents (clotrimazole and ketoconazole rifampin and isoniazid). Finally, the nutritional variable and the number of meals per day were documented for different types of food such as milk, nuts, cheese, yogurt, eggs, bread, and salmon.

2.7 Ethical approval

Since this thesis deals with human participants, ethical approval was obtained according to the Research Ethics Committee (REC) at Al-Quds University (Appendix IV). The researcher explained to all participants the aims of this thesis. The researcher also signed consent approval for participation from the targeted participants to approve the agreement to participate in this study. (Appendix V)

2.8 Statistical analysis

The statistical package for social science (SPSS) version 23.0 was used to enter and analyze the data. Demographic characteristics, health, and medical information delineated and classified the data. The frequency and percentile were calculated for the categorical data, and continuous variables were described using median, mean, and standard deviation.

Groups were compared using t-tests and variance for continuous variables, while chi-square tests were used for categorical variables. In addition, the association between the health data and 25(OH)D levels was assessed by a general linear model (ANOVA type I) test. For 25(OH)D level and age of participants, the Kolmogorov–Smirnov test confirmed the normality distribution, and the mean, median, and standard deviation were calculated.

Chapter Three

Results

3.1 Study Samples:

This cross-sectional study was carried out to evaluate 25(OH)D levels among a healthy population in the West Bank. The participants were chosen randomly from cities, towns, villages, and campuses to achieve this goal. The 25(OH)D serum levels were measured in the collected blood samples. The results were correlated with various factors associated with vitamin D levels provided by the subjects included in the study.

3.2 Socio-demographic and characteristics of the distribution of the subjects.

According to Table (5), the female participants occupied 59% (177) of the sample study, with a mean age of 36.56 years (12.9 standard deviation), and 123 (41%) were males (mean age was 39.46 years with 13.7standard deviation).The minimum age was 19 years and the maximum age was 65 years for both. The governorates of the included individuals were divided into the north (Qalqilyah city and Nablus), middle (Ramallah and East Jerusalem), and south (Bethlehem and Hebron) governorates with the distribution (33.7%), (26.7%), (39.7%), respectively. According to the residency classification, the residents in the cities were 178 (59.3%), and in the towns were 65 (21.7%), in the villages 50 (16.7%), and in the refugee camps 7 (2.3%). Additionally, the table shows the distribution of subjects according to the educational level of which 49.7% of participants had a university education compared to primary, secondary, and college levels of education which represented 51.3%.

Table(5): Distribution of the study subjects according to the demographic variable.

Variable		Frequency	Percent (%)
Gender	Male	123	41%
	Female	177	59%
Age (years)	18-28	97	32.3%
	29-40	83	27.7%
	41-50	61	20.3%
	51-65	59	19.7%
Governorate (G)	North-G (Qalqelia, Nablus)	101	33.7%
	Middle-G (Ramallah,East Jerusalem)	80	26.7%
	South-G (Hebron, Bethlehem)	119	39.7%
Educational levels	Primary school	46	15.3%
	Secondary school	80	26.7%
	College education	25	8.3%
	University education	149	49.7%
Living /location	City	178	59.3%
	Town	65	21.7%
	Village	50	16.7%
	Refugee camp	7	2.3%

3.3 The physique properties and lifestyle behaviour distribution of the study participants.

The distribution according to physique properties and lifestyle behaviour is described in Table (6). The mean weight of the individuals was 73.44Kg with a standard deviation of 15.6 (the minimum weight was 41Kg, and the maximum weight was 120Kg). Nearly 25.7% of participants' weight was between 61-70 Kg. On the other hand, the participants' weights over 100 Kg and below 50 Kg had 4.7% and 4% frequencies for each. The height range was between 140 cm and 189 cm, with a height mean equal to 165 cm and 9.2 standard deviations. Moreover, the mean BMI for the participants, was 26.8 Kg/m^2 with a 5.0 standard deviation (the minimum value of BMI was 17.3 Kg/m^2, and the maximum was 44.1 Kg/m^2). Nearly 41.3% of participants lie within the normal BMI range (18.5-24.9 Kg/m^2) and 35% of individuals distributed within over weight values (25-29.9 Kg/m^2).

Participants' mean sun exposure time behaviour was 2 hours within the day (the minimum time was zero or no exposure, and the maximum time was 8 hours per day). In the physical activity distribution, 66.3% of participants spent less than half an hour within one week in exercise activity, and the mean time was 1.5 hours/week with a 0.82 standard deviation. Moreover, according to the individuals, the use of sunblock cream was accomplished by 29.7% of the total

participants. In addition, the distribution of female participants according to wearing hejab (head cover) and the number of children also explained. Around 93.2% of females wear hejab, and 32% with no child. On the other hand, 41% of female participants have 1-4 children. While for the total participants, marital status distribution was as follows: single 90 (30%), married 196 (65.3%), divorced 6 (2%), and widowed 8 (2.7%).

Table (6): Distribution of the study subjects according to physiques lifestyle behaviour properties.

Variable		Frequency	Percent
Weight (Kg)	40-50	12	4.0%
	51-60	59	19.7%
	61-70	77	25.7%
	71-80	60	20.0%
	81-90	49	16.3%
	91-100	29	9.7%
	>100	14	4.7%
Height (cm)	140-150	7	2.3%
	151-160	68	22.7%
	161-170	123	41.0%
	171-180	79	26.3%
	181-190	23	7.7%
BMI(Kg/m^2)	<18.50 (Underweight)	2	0.7%
	18.51-24.99 (Normal weight)	124	41.3%
	25.00-29.99 (Over weight)	105	35.0%
	>30 (Obesity classes)	69	23.0%
Sun exposure (Minutes/day)	<1 (No exposure)	26	8.7%
	1-10	10	3.3%
	11-30	59	19.7%
	31-60	88	29.3%
	61-120	42	14.0%
	>120	75	25.0%
Physical exercise (Hour/week)	<0.5	199	66.3%
	0.5-2	64	21.3%
	2.1-4	27	9.0%
	4.1-7	8	2.7%
	>7	2	0.7%
Using Sun-block creams	Yes	89	29.7%
	NO	211	70.3%
Females wearing hijab	Yes	165	93.2%

Variable		Frequency	Percent
	NO	12	6.8%
Number of children	None	57	32.2%
	1-4	73	41.3%
	5- 8	37	20.9%
	8 -13	6	3.4%
Marital status	Single	90	30%
	Married	196	65.3%
	Divorced	6	2%
	Widowed	8	2.7%

3.4 Medical history of participants

The distribution of the participants according to their health status is represented in table (7). The medical and health condition included gastrointestinal (GI) problems, kidney disease, taking calcium supplements, and taking anti-vitamin D medication like antiepileptics (phenobarbital, carbamazepine, and phenytoin), anti-inflammatory drugs (corticosteroids), antifungal agents (clotrimazole and ketoconazole rifampin and isoniazid). Around 3.3% of participants consumed calcium supplements, whereas about 4.3% of participants were using anti-vitamin D medication, and no one suffered from kidney or GI disease problems.

Table (7): Distribution of the participants according to special health characteristics.

Variable		Frequency	Percent %
Taking calcium supplements	Yes	10	3.3%
	NO	290	96.7%
Taking anti-D medication	Yes	13	4.3%
	NO	287	95.7%

3.5 Nutritional and eating behaviour characteristics

Referring to Table (8), participants differed in meal types and amounts during the week. The recommended amounts for serving meals for dairy products were established with one cup of milk (250ml), three-quarters of a cup (200g) for yogurt, and two slices (40g) of white cheese. Therefore, 2.5 meals per day are recommended for the adult population according to the Australian Dietary Guidelines. At the same time, the number of meals for fish, nuts, bread, and an egg was recommended three meals from different total intakes per day, with an average amount of 100 g for fish, 120 gm (2 eggs) of egg, and 30gm of nuts (Australia 2013). Accordingly, the results indicated that 36.7% of the participants did not consume milk, and 34.3% took one to two meals a week, whereas 37.7% of participants consumed 1 to 2 meals of nuts per week and 31.3% took 2-5 meals within the week. For the yogurt meal, 43.3% of participants took 1-2 meals, and 28% took 3-5 meals throughout the week. While 36.7% of the participants consumed 1-2 eggs, 25.7% had 2-5 eggs weekly, and 24.7% didn't consume them. Moreover, about 46% of participants took cheese meals one to two times weekly, 23% had 3-5 meals per week, and 20% did not. Bread meal consumption was 77% of total participants with more than five times per week, while 5% did not consume it. On the other hand, 61.3% of participants took one meal to two meals of fish type, 6.3% consumed 2-5 fish meals, and 31% did not consume it.

Table (8): Distribution of different meal intake and consumption types by subjects.

Variable (meal/week)		Frequency	Percent %
Milk (Meal/week)	NONE	110	36.7%
	1-2	103	34.3%
	3-5	42	14.0%
	>5	45	15.0%
Nuts (Meal/week)	NONE	49	16.3%
	1-2	113	37.7%
	3-5	94	31.3%
	>5	44	14.7%
Yogurt (Meal/week)	NONE	58	19.3%
	1-2	130	43.3%
	3-5	84	28.0%
	>5	28	9.3%
Eggs (Meal/week)	NONE	74	24.7%
	1-2	110	36.7%
	3-5	77	25.7%
	>5	39	13.0%
Cheese (Meal/week)	NONE	60	20.0%
	1-2	138	46.0%
	3-5	69	23.0%
	>5	33	11.0%
Bread (Meal/week)	NONE	13	4.3%
	1-2	13	4.3%
	3-5	43	14.3%
	>5	231	77.0%
Fish (Meal/week)	NONE	93	31.0%
	1-2	184	61.3%
	3-5	19	6.3%
	>5	4	1.3%

3.6 Biochemical measurements of vitamin D

The descriptive analysis of vitamin D serum levels was done for the participating subjects and summarized in Table (9). The mean vitamin D serum level in the total analyzed samples was 13.43 ng/ml, with a standard deviation of 9.5, and the minimum was 1.9 ng/ml. And the maximum was 47.7 ng/ml. The mode value was 6.6 ng/ml and the median reported for the cases was 9.9 ng/ml. Moreover, the lower and upper difference within 95% confidence interval was 12.34 and 14.5, respectively.

Table (9): Serum vitamin D levels according to the total participants.

Vitamin D result (ng/ml)	
Mean	13.4 ng/ml
Std. Deviation	9.5
Median	9.9 ng/ml
Mode	6.6 ng/ml
Minimum	1.90 ng/ml
Maximum	47.70 ng/ml
95% Confidence Interval of the Difference (lower)	12.34 ng/ml
95% Confidence Interval of the Difference (upper)	14.50 ng/ml

The distribution of participants with corresponding vitamin D serum levels is shown in Table (10). Around 61.0% of participants had a vitamin D serum level below 12ng/ml, and 50.3% of tested participants' results were within the 5-12 ng/ml range. The level of vitamin D below 5ng/ml occurred in nearly 11% of participants. The histogram chart in Figure (13) shows these distributions.

Table (10): Distribution of participants according to the serum vitamin D levels in ng/ml.

Vitamin D (ng/ml)	Frequency	Percent (%)	Cumulative Percent(%)
<5	32	10.7%	10.7%
5-12	151	50.3%	61.0%
13-20	55	18.3%	79.3%
21-30	38	12.7%	92.0%
>30	24	8.0%	100.0%

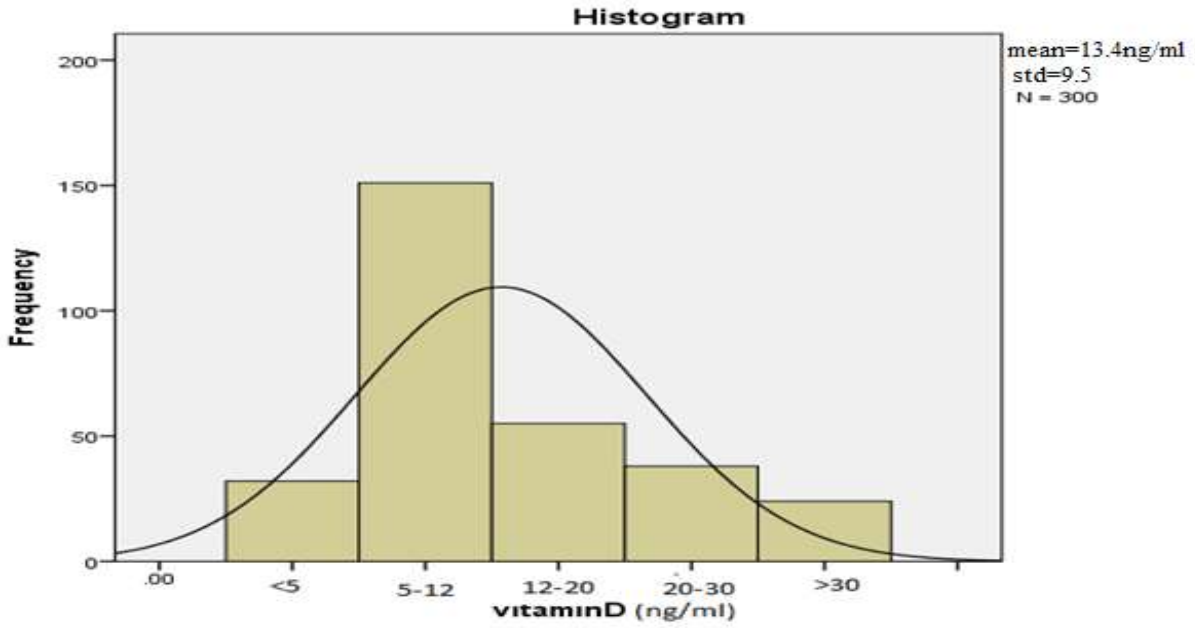


Figure (13): The distribution of participants according to their vitamin D serum levels in ng/ml.

3.7 Effect of physique properties on vitamin D level

Referring to Table (11), which represents the comparison of vitamin D level between age groups, gender, and BMI factors, there was no significant difference (p=0.67) in vitamin D level between males (Mean = 13.2 ng/ml, SD=7.45) and females (mean = 13.61 ng/ml, SD=10.69). The influence of body mass index variation shows no significant difference in vitamin D serum levels between the four groups (P = 0.263), were the underweight group (BMI <18.5) has the lowest mean serum level of vitamin D (mean=9 ng/ml). In contrast, the highest mean (15.3 ng/ml) was noticed and reported for the obese group (BMI>30). Moreover, Table (11) represents the comparison effect of four different age groups the vitamin D levels. Some results displayed a statistically significant difference (p=0.001) in the mean concentration of serum vitamin D, mainly related to the differences between the first group and the three other groups. The multiple comparisons found that the mean value of vitamin D was significantly different between age group 1 (18-28) and the other groups (2, 3, 4) p= (0.005,0.019,0.003), respectively. The (18-28) age group had the minimum level (mean=10.2ng/ml), while the (51-65) age group had the maximum level (mean =15.5ng/ml).

Table (11): The association of vitamin D levels with gender, age, and BMI variant groups.

Variable	Categories	N	Mean ± SD	T	P- value
Gender	Male	123	13.2 ± 7.5	0.41	0.67
	Female	177	13.6 ± 10.7		
Age	19-28	97	10.2 ± 7.3	6.4	0.001
	29-40	83	14.7± 9.8		
	41-50	61	14.9 ± 10.7		
	51-65	59	15.5 ± 9.7		
BMI (Kg/m^2)	<18.50 (Underweight)	2	9 ± 4.9	1.8	0.263
	18.51-24.99 (Normal weight)	124	12.6 ± 9.8		
	25.00-29.99 (Over weight)	105	13.3 ± 8.2		
	>30 (Obesity classes)	69	15.3 ± 10.5		

Level of significance is <0.05

3.8 Lifestyle and levels of vitamin D

According to Table (12), independent sample t-test was conducted to compare the mean values of vitamin D levels according to the use of sun blocks. No significant difference (p= 0.89) in vitamin D levels was reported for participants who used sun-block cream (Mean = 13.5 ng/ml, SD=10.8) compared to participants who did not use cream (mean = 13.4ng/ml, SD=8.9). In addition, the sun exposure habits did not show any significant differences(p=0.249) in the mean vitamin D level among different exposure groups. The mean time of exposure for the respondents was 2 hours per week. On the other hand, there was a significant difference (p=0.03) in vitamin D serum level between participants who used calcium supplements (Mean = 20.74 ng/ml, SD=13.9) and those who are not using calcium supplements (mean = 13.17 ng/ml, SD=9.2).

Table (12): The effects of using sun-block cream, calcium supplement consumption, and sun exposure time on serum vitamin D level.

Variable	Categories	N	Mean ± SD	T	P- value
Using of sunblock Cream	Yes	89	13.5 ±10.8	0.13	0.893
	NO	211	13.4 ± 8.9		
Calcium supplement Food	Yes	10	20.7±13.9	2.49	0.013
	NO	290	13.17±9.2		
Sun exposure time (minutes/day)	<1 No exposure	26	16.0 ±10.8	1.16	0.249
	1-10	10	9.8 ±5.2		
	11-30	59	13.5 ±11.0		
	31-60	88	13.2 ± 9.8		
	61-120	42	12.4 ± 8.6		
	>121	75	13.7 ± 8.5		

Level of significance < 0.05

3.9 Effect of intake of Vitamin D-containing food

The differences in vitamin D concentration according to the types and number of meals intake are explained in the Table (13).

Seven types of meals were examined (milk, nuts, yogurts, eggs, cheese, bread, and fish). The number of meals was categorized into four different groups (no meal

intake or none, 1-2, 3-5, and more than 5). The mean concentration of vitamin D did not have a significant different value in all types of meals intakes (cheese $p = 0.30$, yogurt $p = 0.70$, milk $p = 0.55$, nuts $p = 0.68$, egg $p = 0.69$, bread $p = 0.61$, fish $p = 0.21$). In addition, the effects within the same groups did not affect the level of vitamin D in the participants.

Table (13): The difference in vitamin D concentration according to the types and number of meal.

Type of meal	Groups	N	Mean ± SD	T	P- value
Milk (Meal/week)	NONE	110	12.4 ± 9.2	1.324	0.556
	1-2	103	14.1 ± 8.6		
	3-5	42	14.3 ± 10.7		
	>5	45	13.3 ± 10.6		
Nuts (Meal/week)	NONE	49	13.3 ±9.9	2.062	0.685
	1-2	113	13.9 ± 9.7		
	3-5	94	13.6 ± 9.7		
	>5	44	11.9 ± 7.8		
Yogurts (Meal/week)	NONE	58	12.2 ± 7.4	6.353	0.706
	1-2	130	13.7 ± 9.2		
	3-5	84	13.9 ± 11.6		
	>5	28	13.0 ± 6.7		
Eggs (Meal/week)	NONE	74	12.5 ± 8.4	2.424	0.690
	1-2	110	13.3 ± 8.9		
	3-5	77	14.3 ± 11.1		
	>5	39	13.3 ± 9.8		
Cheese (Meal/week)	NONE	60	11.7 ± 6.9	3.228	0.304
	1-2	138	14.3 ± 10.2		
	3-5	69	13.2 ± 9.9		
	>5	33	13.3 ± 9.7		
Bread (Meal/week)	NONE	13	13.6 ± 11.1	0.496	0.616
	1-2	13	12.3 ± 9.7		
	3-5	43	15.2 ± 9.4		
	>5	231	13.2 ± 9.4		
Fish (Meal/week)	NONE	93	11.7 ± 8.5	2.561	0.211
	1-2	184	14.0 ± 9.8		
	3-5	19	15.4 ± 11.3		
	>5	4	15.9 ± 4.7		

Level of significance < 0.05

Chapter Four

4.1 Discussion

In this study, we established reference ranges of vitamin D among healthy adults in Palestine and investigated possible cultural, behavioural, and socioeconomic factors associated with fluctuations in these ranges.

Recently, the results indicate that the information on the biological and clinical significance of 1,25-dihydroxyvitamin D3 (1,25$(OH)_2$D3) (active form) has critical values in healthy bone development and other health conditions. The decreased serum vitamin D value will contribute to bone fracture and tooth attachment problems. Moreover, the insufficiency levels will promote the weakness of muscles and increase the risk of colorectal cancer and other major cancer types. Even though knowledge about vitamin D production and its metabolism has developed and advanced, variations still appear according to the deficiency and insufficiency levels of vitamin D. As a consequence, the serum 25(OH)D (inactive form) concentration that reflects the guideline intake for vitamin D must be re-evaluated at a national level to be more beneficial in the diagnosis and help in public health (Norman 2008).

To overcome this challenge, the concentration of vitamin D among the population with special demographic (age, gender, ethnicity, place of residency) and special health factors such as BMI, physical exercise, and sun exposure should be evaluated. The analyzed results under these conditions will determine the normal vitamin D level related to the particular community with a healthy population (García-Dorta *et al.* 2021). Moreover, defining national-based vitamin D levels for a healthy population will improve the health system, including costs, safety, avoidance of misdiagnosis, and

unnecessary use of vitamin D supplements. This will decrease the excessive requests for vitamin D tests in the laboratories. Recently, 31.3% of vitamin D tests in the UK were requested without a clear clinical situation or inappropriate symptoms (fatigue, exhaustion). Hence, there was no improvement in these cases by vitamin D supplementation (Woodford *et al.* 2018).

In the synthesis pathway of vitamin D sufficient sun exposure is critical and reliable to achieve an appropriate amount of vitamin D (which may reach 90% of total vitamin-D synthesized) through the endogenous synthesis pathway in the skin (Mendes *et al.* 2018). In Palestine, the cities in the West Bank lie in the high solar radiation area within 34,33E and 30,33N locations that facilitate sun exposure for nearly all days of the year. Hence, the sun exposure level and quality in these cities should not be suggestive for vitamin D insufficiency.

4.2 Vitamin D status

To evaluate the vitamin D 25(OH) D level, the serum level between 20-30 ng/ml concentrations is considered insufficient. In contrast, a value below 20 ng/ml (<50 nmol/litter) is reported as a vitamin D deficiency level (Holick *et al.* 2011). Whereas the level below 12ng/ml (< 30 nmol/ml) is defined as a severe deficiency, and it should be prevented (Amrein *et al.* 2020).

To achieve our goal, our cross sectional study was performed among healthy participants in the West Bank with the same ethnic group located in a high solar radiation area with no vitamin D supplementation. The participants had a mean 25(OH)D serum level of 13.4 ng/ml. Based on the population distribution, 92% of the healthy individuals had vitamin D levels below 30 ng/mL, 79% below 20 ng/ml, and 61% of participants had a level below 12 ng/ml value.

The prevalence of 25(OH)D concentrations below 30 ng/ml were reported in the United States of America for 75% of the population (Del Valle *et al.* 2011). In France, there was 80.3% (Souberbielle *et al.* 2016). And in Germany, 88.1% (Rabenberg *et al.* 2015). To this end, the increased prevalence was reported for adjacent countries, such as the Syrian population, 92% (Sayed-Hassan *et al.* 2014), whereas in Jordan, 89.7% (El-Khateeb *et al.* 2019). In this study, the vitamin D level was measured for normal participants in the total population with an optimal health condition and who do not suffer from any health complications. In addition, for the level below 20 ng/ml, the total participants were free from any skeletal and non-skeletal pathological conditions such as infections, immune system disorders, kidney failure, cancers, and so forth. Moreover, the participants with vitamin D levels below 20 ng/ml which is close to 13.4 ng/ml value, we found an adequate amount (13.4 ng/ml) suitable for a normal healthy population with a particular lifestyle and demographic variable, with no need for supplementation or appearance of illness complication. So, we believe that the need for readjustment and optimization of the vitamin D serum values in a normal healthy adult population is crucial in the healthcare system to avoid misdiagnosis, unnecessary costs, safety, and over-treatment with vitamin D supplements.

4.3 Lifestyle behaviour

4.3.1 Sun exposure

In this study, the serum level of 25(OH) D was analysed for the participants living with high solar radiation (the West Bank). The mean time of sun exposure reported by the study participants was two hours per day. There were no significant variations in vitamin D serum levels among different time groups of sun exposure. The study also revealed that vitamin D status was not influenced by the hours spent in sunlight and vitamin D levels. Exposing the body for 3 to 8 minutes to the sun will facilitate

synthesizing about 400-1000 IU (10-25 μg) of vitamin D with consideration to the skin color and the seasonally month. Light skin is faster in synthesizing Vitamin D, and summer months have more sunlight (Terushkin *et al.* 2010). Sunlight exposure is important for vitamin D synthesis. A255–330 nm spectral range of UVB (optimal 295nm) is required with whole-body exposure for 15-20 minutes to maintain one erythema dose (light pink color of skin) that facilitates the production of nearly 250 μg vitamin D (10,000 IU) (Mostafa & Hegazy 2015). The cutaneous vitamin D synthesis is adequate enough when the skin exposure reaches 30 minutes twice a week (Aleksova *et al.* 2015).

The recommendation for sun exposure is reported for 5-15 minutes from 10 AM to 3 PM at least two to three times every week with coverless arms, face, and hands. This should be adequate to maintain sufficient vitamin D in people with different skin colors. This equals about 25% of what would cause a minimal erythemal response(a slight pinkness to the skin) (Holick 2004). In addition, the Australian study showed a sufficient amount of vitamin D obtained for a short time of face, arms and hands unprotected sun exposure outside with peak period of UV radiation from (10:00-15.00) for most times of the year (Samanek *et al.* 2006).

Moreover, to maintain the necessary serum amount of vitamin D (>30 ng/ml), the dietary intake of vitamin D for adults was recommended at 400 IU/day. It was established based on bone health as a biomarker. Then the level increased to be 600 IU/day later by the IOM committee (Institute of Medicine) for U.S. and Canadian populations(Cork 2017). Moreover, the required amount at least without adequate sun exposure equals 800-1000 IU/day of vitamin D supplements to maintain a target of 25(OH)D concentration of 20 ng/mL (50 nmol/L) (Holick & Chen 2008).

In such a manner, there were several studies on the effect of solar irradiation on 25(OH)D production in European countries. The solar irradiation in European cities, such as London has (2.88 kWh/m2/day), Rome (4.5 kWh/m2/day), and Paris (3.25 kWh/m2/day) (García-Dorta *et al.* 2021). On the other hand, the daily solar radiation level for the West Bank areas equals nearly 5.89-6.75 kWh/m2/day (Kanters *et al.* 2014). It is higher than that for European cities. The comparison between the concentration means of vitamin D shows that the mean values reported for the normal adult population in London, Paris, and Germany were (18.9 ng/ml), (24.0 ng/ml), (and 20.4 ng/ml) respectively.

In contrast, for the Middle East countries, the mean vitamin D level in Jordan was 11.0 ng/ml and 9.9 ng/ml in the Syrian population (Lips *et al.* 2019). In this thesis, it was 13.4 ng/ml. In relation to these data, the researcher finds that decreased mean levels of vitamin D were reported for the countries with high solar radiation and vice versa where in countries with low solar radiation a higher level of vitamin D mean value was achieved, this may be related to the food meals intake supplemented with vitamin D like milk and juice.

4.3.2 Use of sunscreen cream

The theoretical advice for using sun creams is to prevent sunburn by blocking UVB radiation and preventing skin cancer. Theoretically, the regular use of these creams may put the population, especially the elderly, at the risk of vitamin D deficiency. The Australian study was accomplished by marks *et al*, shows no differences in the level of vitamin D in participants before and after using sunscreen with sufficient sun exposure (Marks *et al.* 1995). This finding is supported by several studies explaining that frequent sunscreen use was not related to lower 25(OH)D levels, due to sunscreen application prior to intentional prolonged sun exposure (Linos *et al.* 2012). In this thesis, there were

similar results. First, total participants had no significant difference in the mean concentration of vitamin D with the sunblock use. Secondly, 89 participants used sunscreen (vitamin D mean 13.54 ng/ml), and 211 participants did not use it (vitamin D mean 13.38ng/ml).

4.4 Socio-demographic status of participants

4.4.1 Age and gender differences

Age is a risk factor for decreased vitamin D levels (25OHD). The kidney produces less 1, 25$(OH)_2$D and less VDR according to age development. In addition, the primary source of vitamin D in the skin is converting the 7-dihydrocholesterol, which decreases with age due to the reduced epidermis layer of the skin (Gallagher 2013). This thesis reported a significantly lower Vitamin D serum level among the younger group (18-28 years old) compared to the other three different groups (29-40),(41-50),(51-65)years.

Furthermore, opposing the expected, younger age participants (18-28years) have lower levels (mean = 10.2ng/ml) compared to the serum level of older (51-65years) participants (mean = 15.6ng/ml). It can be interpreted due to spending more time with a computer at home and at work and staying indoors longer. The increased level of hypovitaminosis in indoor workers was noticed within the Taiwan study population in two genders, male and female, and about 62% of total 2880 included participants within the range of 20-30ng/ml for younger groups (Wang *et al.* 2020).

Many studies that recruited the same age group showed similar results. Han Seok Cho *et. al.* In 2011 demonstrated the prevalence of vitamin D for 3,047 males and 3,878 females in South Korea. Serum was analyzed for vitamin D levels and supported the idea that younger participants have a lower level than the other old groups, the results

indicated the most prevalent of vitamin D insufficiency occurred in age within 20–29 years, 65.0% in males and 79.9% in females (Choi *et al.* 2011). Masoumpour *et al*. studded in 2008 the prevalence of vitamin D levels in 520 Iranian men and found no decline in the serum level of 25-hydroxyvitamin D based on the age of the participants (MASOUMPOUR *et al.* 2008).

This study reported no significant differences in vitamin D serum levels between males and females. This was in agreement with Ginde *et al* study on the US population in 2009, which found that the variation in vitamin D levels according to age and gender is not remarkable (Ginde *et al.* 2009), (Schleicher *et al.* 2016).However, the differences in gender in the Saudi Arabia population were significant and a decreased levels of vitamin D for women was reported. The study justifies this difference based on the sun exposure limitation due to the covering of the body and hormonal changes (Alghamdi *et al.* 2020). However, this was not in agreement with our study which found no significant differences in Vitim D levels based on sun exposure.

4.5 Obesity indicators (BMI)

Previous studies have illustrated the inverse association between the concentration level of serum vitamin D (25(OH)D) and BMI in adults, mainly with classification overweight (BMI =25- 30 Kg/m^2) and obese (BMI≥30 Kg/m^2) (Oliai Araghi *et al.* 2015; Khodabakhshi *et al.* 2022). In this study, there were no statistically significant differences between the mean levels of vitamin D according to the BMI. The BMI groups showed a low vitamin D level in participants in the underweight range. In contrast, the participants with BMI levels in the healthy, overweight, and obesity range showed an immediate increase in serum concentration of vitamin D. The association between vitamin D status and obesity was approved by many researchers in a healthy

and non healthy population (Pourshahidi 2015; Ren *et al.* 2021). On the other hand, other researchers have not confirmed this relationship (Sneve *et al.* 2008; Mansoor *et al.* 2020).This matches our outcomes showing no association between the vitamin D serum level and BMI (Jorde *et al.* 2010).

4.6 Vitamin D and nutrition effects

4.6.1 Vitamin D in Dairy Products

Milk is considered a complete consumer food source and provides many nutrients, especially carbohydrates, protein, minerals, fats, and vitamins. Consuming adequate amounts of dairy food is important to improve the quality of diet (Bowman & Vinyard 2004). Dairy meals are natural supplies of calcium and vitamin D, and 3 cups are the recommended daily intake from fat-free or low-fat milk and milk products for individuals older than nine years (Millen *et al.* 2016).The low level of calcium will trigger the elevation of parathyroid hormone. Subsequently, the vitamin D becomes more reproducible and converted into the active form (1,25-dihydroxyvitamin D). The decreased serum vitamin D level is also detected (measured by inactive form level), and the calcium absorption process becomes more effective within the intestinal tract. On the other side, the insufficient amount of vitamin D is associated with a decreased level of calcium absorption across the intestinal tract (Polzonetti *et al.* 2020).

In this study, the low calcium level was not noticed according to the health apparent , and none of all participants suffered from hypocalcaemia. The decreased mean vitamin D level was not related to decreased calcium level. In cow milk, the vitamin D level reaches up to 40 IU per liter (Reeve *et al.* 1982), whereas the daily requirement of vitamin D is 600 IU/day (Mandrioli *et al.* 2020). It concluded that milk does not

provide the dietary needs of vitamin D. Still, with the consumption of milk derivatives, cheese is considered as the best choice to represent the dietary intake of these nutrients.

The total milk and milk derivatives intake will provide 10% of the dietary intake of vitamin D (Jakobsen & Saxholt 2009). Polzonetti *et al.*,in 2020 detected the natural amount of vitamin D for whole milk and yogurt with about 0.1 µg (4 IU) in each 100g of food. In comparison, a higher amount was detected for cheese within the same amount of food (0.3-0.6 µg) (12-24IU), and 1.5 µg (6 IU) of vitamin D in Butter food (Polzonetti *et al.* 2020). In our study, the mean vitamin D concentration was not statistically different among participants who took milk meals and others who did not. moreover, no significant variants in serum vitamin D level was reported for participants who take yogurt and cheese. In addition, with the increased number of meals, the serum level of vitamin D differences was not reported.

4.6.2 Vitamin D status in fish and egg nutrients

Fish meals are considered the primary source of vitamin D dietary intake. Such foods, and eggs are not commonly consumed by the population, which provides different amounts of vitamin D to maintain requirements. In this study, 69%of participants consumed fish meals, while the egg meal intake occurred in nearly 75% of participants. The wild salmon fish amount of 3.5 oz (100 gm) delivered as one meal has near 988 IU an average vitamin D content, while for farmed type, the mean serum level of vitamin D decreased by 25% to become 240 IU. Other types like blue, white fish and, tuna have 280 IU, 104 IU, 404 IU, respectively (Lu *et al.* 2007).For egg meals, Schmid & Walther represent the amount of vitamin D in egg food and found it in whole egg and egg yolk particularly, the provided amount of vitamin D could be reached to 14.4 to 29.3µg/kg in an entire egg. Moreover, the egg yolk contains 32.5-55.8 µg/kg (Schmid & Walther 2013). Despite these, there were no significant differences in vitamin D levels based on

male and female dietary intake, which matched with the Hintzpeter.et al. In 2008 Survey study for a total of 1763 men and 2267 women, 18 to 79-year olds in the German National Health centers, and included no significant determinant of vitamin D level correlate with vitamin D intake from diet and supplements (Hintzpeter *et al.* 2008). The same results were confirmed by Thuesen *et al*, Jungert & Neuhäuser-Berthold (Thuesen *et al.* 2012; Jungert & Neuhäuser-Berthold 2013). On the other hand, the measurements of vitamin D were associated with the estimated dietary intake of vitamin D and modestly improved the plasma 25-OHD concentration (Zgaga *et al.* 2011).

4.6.3 Vitamin D status with bread intakes

The dietary intake contributes to the vitamin D level increment. The fibers containing food like bread play critical roles in the bioavailability of vitamin D and absorption across the gastrointestinal tract. Hence, the fibers impair the micelle formation and affect the release of the fat droplet in lipophilic food absorption. Thus, the reduced bioavailability of vitamin D was shown to cause a higher prevalence of rickets and osteomalacia for increased fiber intake in the Asian immigrant population (Borel *et al.* 2015). This study found no association between bread intake and serum vitamin D level. Moreover, the number of meal intakes did not influence the vitamin D status that matched the Maurya & Aggarwal study. When discussing the influence of fibers intake influence on two vitamin D participant groups, low fibers wheat bread (3 g/100 g) and other group (12 g/100 g), vitamin D level was not significantly different between the two groups (Maurya & Aggarwal 2017).

4.7 Conclusions and Recommendations

This thesis revealed the vitamin D status in adult individuals in the West Bank. In conclusion, participants' mean vitamin D level was 13.4 ng/ml, with 61% below the cut-off value (12ng/ml) of vitamin D status. It seems reasonable to consider 25(OH)D serum levels close to 14 ng/mL as an optimal value, at least for the general population of the West Bank. Because of that, the concentration level determined concerning the optimal condition among the participants included healthy skeletal and nonskeletal conditions, solar radiation, and dietary intake. Considering that younger people have a lower level, gender, body mass index, and nutritional habits were not significant factors.

This cross-sectional study focused on the 25(OH)D status among a healthy population throughout various variables examined. However, this study is limited to the small number of individuals who participated. On the other hand, the limitation of this study design does not permit examining more sample size of the adult population due to the financial restriction, specifically the high cost of biochemical tests for vitamin D determination. Moreover, the sample distributions were not enrolled to cover all regions in the various urban communities.

The optimal vitamin D level according to the adult population needs further research to identify the precise vitamin D level throughout the diagnosis of musculoskeletal and non-skeletal condition tests. Such knowledge will facilitate achieving and determining the optimal vitamin D status. Consequently, the determination of calcium, phosphorus, lipid profile and parathyroid hormone level is recommended to investigate the precise detected amount of vitamin D and confirm it. Moreover, the inflammation condition should be included and diagnosed via further biochemical tests such as C-reactive protein to ensure the health condition of the participants. Furthermore, the genetic factors area correlated variance of vitamin D status. In addition, the researcher

recommends more studies to determine the associated single nucleotide polymorphism in the related population study.

On the other hand, measuring 25(OH)D is the most common vitamin D metabolite in clinical practice. The immunochemical method used in the current detection procedure might not be reliably compared to the reference Liquid Chromatography with tandem mass spectrometry (LC-MS/MS) method. That method is more accurate and precise in validating the vitamin D status and confirms the limitation of the technique applied.

The PMOH (Palestinian ministry of health) is strongly recommended to re-evaluate the average natural levels of essential nutrients, vitamins and salts, such as Mg, Mn, Zn, Iron, A, B12, E, C, and vitamin D. Moreover, to invest more efforts in controlling vitamin D levels among citizens in the West Bank. This step will help in diagnosing patients accurately, saving their time, money, and unnecessary suggested treatments. Applying strict rules regarding issuing random laboratory checks for patients to reduce the estimated treatment expenses. In addition, the determination of the normal levels of all essential nutrients would encourage people to reduce using supplements randomly without a real need for them. Finally, stakeholders and decision-makers are advised to create long-term plans to watch the vitamin D levels among individuals and offer strategies to study all the related variables.

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Appendix I

Sample collection protocol

Participant and target population:

- Random healthy participant
- Adult age (18-64 years).
- Male and female.

Sample collection protocol:

1- Draw the blood sample into the evacuated plain tube. Fill up about 3 ml of venous blood in the collection tube.

2- Good labelling sheet obviously and clear label and sticker on the collection tube by Name, Date, and same number on tube and questionnaire sheet.

3- Allow the blood to clot for 15 to 30 min at room temperature (RT) (18-22°C).

4- Place tubes in the centrifuge and spin at 4500 rpm at RT (18-22°C) for 5 min.

5- Collect the serum by plastic / Pasteur pipette and transfer to new labelled plastic test tube, being careful not to disrupt the clot in the bottom of the tube, close carefully using a stopper or parafilm.

6- Avoid collection of haemolysed or lipaemic serum sample.

7- Transfer tubes to a freezer for storage (-20C^{o}).

8- The samples should be processed and reach the appropriate storage conditions as soon as possible.

9- Transfer the samples were collected in appropriate condition (with ice) to be analysed within 48h.

Questionnaire:

- Make sure the attached questionnaire be completed and participant information is correctly collect.
- Be sure the attached number between the questionnaire and corresponding tube is obvious and clear.

Ethical approval:

- Participants included in this study must sign an informed consent form.

Appendix II

Questionnaire

Establishment of Vitamin D Reference Values in the Palestinian Society

And Investigation of Cultural, Behavioral, and Socioeconomic Effects

File code: _____

Date: _____

- **Demographic data:**

1- Name: _____

2- Governorate: _____

3- Age: _____

4- Gender: Male / Female

If female,

Wearing hijab: Yes / No

Children no: ____ (or pregnant)

5- Where do you Live?

□ City □ Town □ Village □ Refugee camp

6- What is your educational level?

□ Primary school □ Secondary school □ College education

□ University education

7- Marital status:

 Single married divorced widowed

8- Religion :

 Muslim christian jew refused

9- do you work?

 Yes No

Profession:_____

➢ Health Information

10- Height:---------- (m)

11- Weight:----------(kg) BMI:----------

12- Smoking Habits :

 Yes No Passive smoking

If yes:

Cig/ day: ---------- years: ----------

Argileh/ day:---------- years:----------

13- Sun exposure time/day ----------(hours)

14- Using sunblock cream: Yes/ No

15- How many hours do you exercise / week?

 0-½, ½-2 2–4 4–7 Or more.

16- Having GI problem: Yes/ No

If yes, what: ----------

17- Having kidney disease: Yes/ No

If yes, what: ----------

18- Taking calcium supplemented food: Yes/ No

19- Taking food meals of:

Milk, frequency/week: ---------- Yogurt, frequency/week: ----------

Nuts, frequency /week: ---------- Eggs, frequency/week: ----------

White cheese, frequency/week: ---------- Bread, frequency/week: ----------

Salmon, sardine, tuna, frequency/week: ----------

20- How many times have you eaten fastfood (pizza, burger, sausages, or shawarma) in the last week?

□ 0-1 □ 2-3 □ 4-6 □ 7 or more.

21- Taking anti-vitamin D medication: Yes/No

Antiepileptic – Phenobarbital, carbamazepine, phenytoin

Anti-inflammatory drugs – corticosteroids

Antifungal agent – clotrimazole and ketoconazole

Rifampin and isoniazid.

Appendix III

إستبانة

معرفة المعدل الطبيعي لفيتامين د في المجتمع الفلسطيني و مدى تأثره بالجوانب الثقافية و السلوكية و الإجتماعية و البيئية.

رقم الملف:----------

التاريخ:----------

البيانات الديموغرافية :

1-المحافظه:----------

2- العمر:----------

3- الجنس: ذكر/ انثى

اذا كانت انثى:

هل ترتدي الحجاب : نعم / لا

4- عدد الاولاد: ---------- (او حامل)

5- المكان الذي تعيش فيه او الموقع:

□ مدينة □ بلدة □ قريه □ مخيم لاجئين

□ مستوى إبتدائي □ مستوى ثانوي □ مستوى كلية □ مستوى جامعي

6-ما هو المستوى التعليمي الذي وصلت إليه؟

7- الحالة الاجتماعية

□ اعزب / انسه □ متزوج / ه □ مطلق/ ه □ أرمل / ه

8- الديانة:

□ مسلم □ مسيحي □ يهودي □ لا ارغب

9- هل تمارس عملا ما ؟ نعم/ لا.

المهنة :----------

- **المعلومات الصحية :**

10- الطول:---------- (متر)

11- الوزن:---------- (كغم)

معامل ---------- BMI

12- هل انت مدخن؟

□ نعم □ لا □ مدخن سالب

اذا كانت الاجابة بنعم

كم عدد السجائر المستهلكة يوميا :---------- فترة تدخين السجائر بالسنوات :--------

كم عدد رؤووس الارجيله المستتهلكة يوميا:---------- فترة تدخين الارجيلة بالسنوات :--------

13- فترة التعرض للشمس لليوم الواحد بالساعات ---------- .

14- هل تستخدم كريمات واقية لاشعة الشمس: نعم / لا .

15- ما هي الفترة التي تقضيها في عمل التمارين الرياضية خلال الاسبوع الواحد ؟

□ 0-½, □ ½-2 □ 2–4 □ 4–7 □ اكثر من ذلك.

16- هل تعاني من مشاكل في الجهاز الهضمي؟ نعم / لا

اذا كانت الاجابة نعم ، ما هي المشكلة ؟----------

17- هل تعاني من اعراض مشاكل امراض الكلى ؟ نعم/ لا

اذا كانت الاجابة نعم ، ما هي المشكلة ؟----------

18- هل تتناول اي من مغذيات الكالسيوم ؟ نعم / لا

19- هل تتناول الوجبات الغذائية التالية و ما هو عدد تناولها خلال الاسبوع الواحد :

الحليب ، عدد الوجبات خلال الأسبوع ---------- الجبنة ، عدد الوجبات خلال الأسبوع----------

البيض ، عدد الوجبات خلال الأسبوع---------- الزبادي ، عدد الوجبات خلال الأسبوع----------

المكسرات ، عدد الوجبات خلال الأسبوع--------- الخبز ، عدد الوجبات خلال الأسبوع----------

سمك السلمون، الساردين، التونة ، عدد الوجبات خلال الأسبوع----------

20- ما هي عدد المرات التي تتناول فيها وجبات سريعة (بيتزا، هامبرجر، شاورما ،بروست ، الخ..)

□ 0-1 □ 2-3 □ 4-6 □ اكثر من ذلك

21- هل تتناول اي من الادوية التالية التي تثبط انتاج فيتامين د : نعم / لا

ادوية معالجة الصرع ـ فينوباربيتال ، كاربامازيبين ، فينيتوين

ادوية مضادة للالتهابات – الكورتيكوستيرويدات

ادوية معالجة الفطريات ، كلوتريمازول وكيتوكونازول

ادوية اخرى مثل ريفامبين وإيزونيازيد.

التوقيع :----------

Appendix IV

REC Approval Letter

Al-Quds University
Jerusalem
Deanship of Scientific Research

بسم الله الرحمن الرحيم

جامعة القدس
القدس
عمادة البحث العلمي

Research Ethics Committee
Committee's Decision Letter

Date: January 8, 2022
Ref No: 214/REC/2022

Dears Dr. Ahmad Amro, Mr. Mohamed Almahareq,

Thank you for submitting your application for research ethics approval. After reviewing your application entitled "Vitamin D status among Palestinian adults and establishment of reference rang in the West Bank in Palestine", the Research Ethics Committee confirms that your application is in accordance with the research ethics guidelines at Al-Quds University.
We would appreciate receiving a copy of your final research report/ publication.
Thank you again and wish you a productive research that serves the best interests of your subjects.

PS: This letter will be valid for two years.

Sincerely,

Suheir Ereqat, PhD
Associate Professor of Molecular Biology

Research Ethics Committee Chair

Cc. Prof. Imad Abu Kishek - President
Cc. Members of the committee
Cc. file

Abu-Dies, Jerusalem P.O.Box 20002
Tel-Fax: #970-02-2791293
research@admin.alquds.edu
أبوديس، القدس ص.ب. 20002
تلفاكس: #970-02-2791293

Appendix V

Consent form

نموذج موافقة للمشاركة في الدراسة البحثية

Establishment of Vitamin D Reference Values in the Palestinian Society

And Investigation of Cultural, Behavioral, and Socioeconomic Effects

معرفة المعدل الطبيعي لفيتامين د في المجتمع الفلسطيني و مدى تأثره بالجوانب الثقافية و السلوكية والإجتماعية و البيئية .

تقوم هذه الدراسة البحثية على تقييم الوضع الطبيعي لفيتامين د لدى الأشخاص الأصحاء في المجتمع الفلسطيني، بحيث يتم إنشاء المعدل الطبيعي الخاص بهم، ومدى تأثير السلوك الإجتماعي و الثقافي و الإقتصادي على القيم الطبيعية لدى هؤلاء الأشخاص، و مقارنة هذه النتائج بمجتمعات أخرى. بالإضافة لذلك، بحث إمكانية التأثير على تشخيص المرضى لدى الأطباء الفلسطينين و مدى إستهلاك المكملات الغذائية عشوائيا .

- سوف يتم جمع العينات الخاصة بهذه الدراسة (عينة دم فقط) وكذلك تعبئة الاستبانة بأشراف ذوي الأختصاص .
- في حال توقيعك على هذا المستند فأنت تقر بأنك توافق إختياريا على المشاركه في هذا البحث و أن المعلومات المذكوره قد شرحت لك بالكامل.

الباحث: محمد ابراهيم عيسى المحاريق / جامعة القدس
مشرف الدراسة : الدكتور أحمد عمرو/ كلية الصيدلة – جامعة القدس.
إسم المشترك :
التوقيع :
رقم الهاتف:
التاريخ: