Lampyrid 2023: Volume 13, 204–213
ISSN: 2041-4900
https://lampyridjournal.com

Molecular Study of VDR Gene in Women with Osteoporosis in Mosul City

Maryam M. Alani, Haytham L. ALheyali

Mosul University \ Collage of Science | Biology Department

Email: alanimaryam272@gmail.com

Abstract

Osteoporosis is one of the most common diseases in the world, and it is identified by the changes that occur in bone mineral density according to international health standards, and the disease is diagnosed by dual-energy X-ray DEXA. Osteoporosis is divided into two types (primary and secondary), where the primary type is in elderly men and women due to aging and in women in menopause. As for secondary osteoporosis, it is the result of different diseases and treatments, or because of tumors, cancerous diseases, systemic diseases, and endocrine diseases. Different diets, wrong diets, and lack of exercise are all causes of osteoporosis [1]

The current study included (96) women, ages ranging from (45-35) years, from the reviews to the private pathological analyzes laboratories in the city of Mosul, in a period of time ranging from September to November of the year 2021, and the clinical cases of the disease were relied upon in selecting samples. The samples were divided into two groups depending on the biochemical results: The first group: This group included 25 women who did not suffer from any problems and was considered as a control group, The second group: This group included 71 women with osteoporosis based on biochemical results. The biochemical indicators associated with osteoporosis were measured (Osteocalcin, Pyridinoline, Hydroxyprolin), and the level of a number of biochemical variables (V.D, Uric acid, Ca) was determined, and the genetic variation of a number of genes associated with osteoporosis was determined (COL1A1gene, COL1A2). gene, VDR gene), the VDR gene at the locus (rs7975232), the results for women with osteoporosis showed that the recurrence value of the CC mutant genotype in the group of women with osteoporosis was 18%, compared to 15% for the mutant genotype in the control group. 15%, while the value of the normal (normal) genotype, AA, was 37% among women with osteoporosis, compared to the healthy group, in which the percentage of the normal (normal) genotype was as high as 40%. As for the heterogeneous genotype, AC, it was 45% in the group of afflicted women. The percentage of observations was equal to the control group 45%, As for the allelic frequency, the results showed that the incidence of the mutant allele C was high in the patients group, 40% compared to the control group, 38%. As for the natural allele, the percentage was 60% in the patients, compared to 62% in the control group. And after conducting a sequence test and matching the VDR gene in the study samples with the control sample and matching it with the gene sequence on the NCBI website, it turns out that we have many different genetic variations, which are divided into two basic types, either transition variations or (Transversion) variations and their locations depending on the type of bases heterogeneous. The results of the study also showed that there is a genetic relationship between variations and different genotypes with biochemical indicators of osteoporosis.

Keywords: T-ARMS-PCR, VDR gene, polymorphism, osteoporosis

Introduction:

It is a bone condition that causes fractures in any portion of the body's bones due to a lack of bone strength or weak bone structure and minerals. Until a fracture in the leg, wrist, or spine occurs, this illness frequently goes undiagnosed [2]According to worldwide health standards, osteoporosis is one of the most prevalent diseases in the world. The disease is detected by changes in bone mineral density and is diagnosed using dual-energy X-ray DEXA technology. [3]Osteoporosis is split into two types (primary and secondary), with the primary kind affecting older people due to aging and menopause in women. When it comes to secondary osteoporosis, it is brought on by many conditions and therapies, as well as by tumors, malignant conditions, systemic illnesses, and endocrine disorders. Osteoporosis can

be brought on by a variety of dietary factors, including poor diets and inactivity [3](The World Health Organization defined osteoporosis as (a progressive systemic skeletal disease characterized by a decrease in bone mass and a subtle structural deterioration of bone tissue, which results in osteoporosis and then fracture) and stated that the disease is caused by an imbalance between bone resorption and bone formation. Osteoporosis is a common bone illness that affects 200 million women and men worldwide, the majority of them are over 60 [4] Osteoporosis is a health issue that impairs sufferers' lives and can occasionally result in their death. drugs called bisphosphonates that stop bone resorption It is one of the most often prescribed medications for osteoporosis. [5]

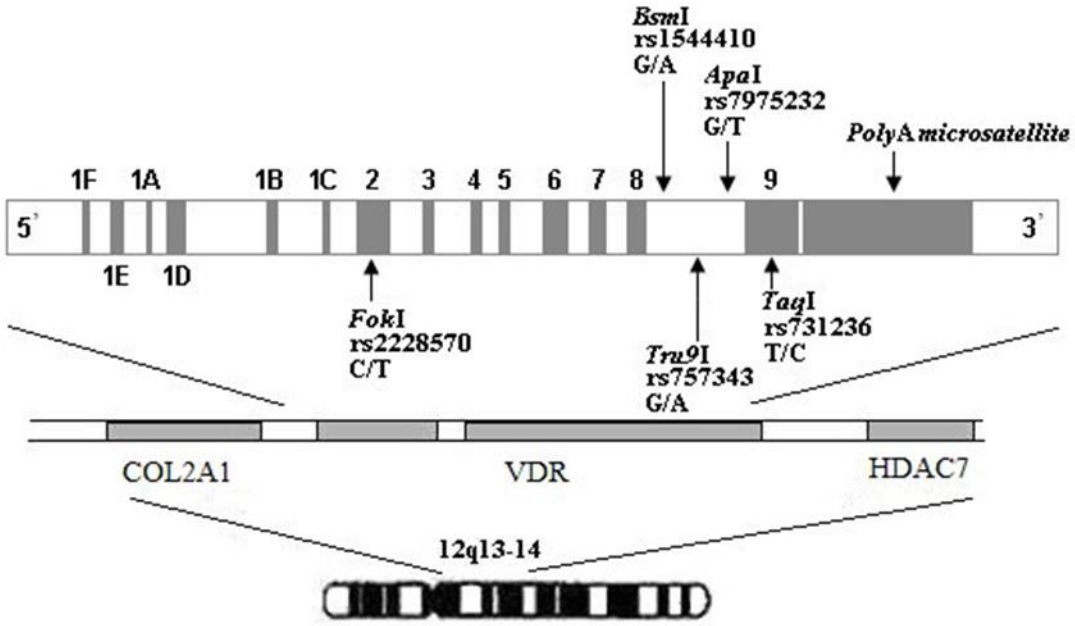
According to the WHO diagnostic criteria utilizing standard deviation scores for bone density scores related to maximum bone mass in healthy young women, osteoporosis is defined as having a bone mineral density (BMD) score of 2.5 or less. Low bone mass is defined as having a bone density T score between 1 and 2.5. The majority of fragility fractures, however, occur in people with a T score value higher than 2.5, indicating that BMD is a restricted indicator of fragility within diagnostic clinics. [6]Yang However, loss of bone density is a risk factor for fracture.

This condition is a hereditary disorder, and there are numerous genes that prevent harm from developing and genetic mutations that result in a flaw in the produced protein. Osteoporosis is characterized by a high genetic component because genetic factors influence bone tissue, mineral density, and other risk factors for fractures. Source Osteoporosis is a polygenic condition influenced by COL1A1, COL1A2, TGFB1, TGFB3, and VDR genes,

VDR is the gene responsible for encoding the vitamin D receptor protein on the membranes, as this receptor is linked to the active form of vitamin D, which is 1,25-dihydroxyvitamin D3 (hydroxyvitamin D), and it has an important role in regulating bone growth. This gene, which is found on chromosome 12q12.14, has 12 exons and approximately 63 different genetic variations. Among these variations are rs10735810, rs1544410, rs731236(4), rs7975232(5), and rs757343, which can be found in the exon region, intron, or catalyst of the gene. In relation to bone mineral mass or markers of bone turnover and fracture risk, these variations play a significant role in the occurrence and progression of osteoporosis. In addition to the binding of the VDR protein to the active form of vitamin D known as calcitriol, there are six SNPs unique to this gene. It can join forces with the retinoid X receptor (RXR) protein thanks to this interaction. The resultant complex binds to particular DNA sequences that are the genes for By activating or deactivating these genes, the VDR gene encodes and controls the activity of vitamin D-responsive genes. This substance aids in regulating the intake of calcium and phosphate by osteoblasts [7]

This gene, a nuclear transcription factor that regulates vitamin D function, has two promoter regions, eight exons that code for proteins (2–9), six untranslated exons (1a–1f), and two promoter regions. Through copying or post-copying processes, the 1,25(OH)D3 VDR complex can regulate how the body reacts to vitamin D. The risk of developing osteoporosis and osteoporosis may be correlated with genetic variations in the VDR gene that influence the function of the VDR protein [7] Vitamin D-resistant rickets, a recessive syndrome characterized by hypocalcemia and hypophosphatemia and resistant to therapy with vitamin D and its active receptors, is one of the prevalent bone illnesses caused by the gene mutation

This gene was the first to be studied with association with osteoporosis and the focus was on gene polymorphisms near the 3′ end of the VDR gene that were characterized by the restriction genes BsmI, ApaI and TaqI. [8]



Materials and method

Case Study: The current study included (96) women, ages ranging from (45-35) years, from the reviews to the private pathological analyzes laboratories in the city of Mosul, in a period of time ranging from September to November of the year 2021, and the clinical cases of the disease were relied upon in selecting samples. The samples were divided into two groups based on the biochemical results:

The first group: This group included 25 women who did not suffer from any problems and was considered as a control group.

The second group: This group included 71 women with osteoporosis based on biochemical results .

Collection of Blood sample

5 ml of venous blood was withdrawn from these patient and divided into groups, the first part was placed in tubes containing EDTA anticoagulant to extract DNA, and the second group was placed in tubes free of any anticoagulant. The tubes were left for one hour until the blood clotted, after which a centrifugation was carried out for a period of (10) ten minutes at a speed of (3000) cycles/minute to obtain the blood serum on which the biochemical tests were conducted.

DNA extraction:

DNA was extracted from the blood of (71) patient with control groups who were subjected to this study, using the modified method presented by (Iranpur and Esmailizadeh., 2010).

Genotyping:

Tetra-ARMS-PCR Reactions:

The DNA concentration in all study samples is adjusted after being measured by biodrop by diluting them with TE buffer solution to obtain the required concentration for performing PCR reactions and was (25) ng/microliter for each sample. Four primers are added for each primer reaction (F-outer and R-outer) for the whole gene, forward outerreverse inner for the normal allele, forward outer-reverse inner) for the mutant allele. The PCR reaction mixture is prepared by mixing the nucleic acid of each

sample and the primer designated for the mutations under study with the components of the master-mix in a 0.2-ml PCR-tube produced by the English by Biolaps Company. Mix in the Microfuge for a period between (5-3) seconds to ensure that the reaction components are mixed, Then, the PCR tubes were inserted into the thermocycler within the special program for each mutation, then the reaction product is injected into the pits of the prepared agarose gel, at a concentration of 2%, with the addition the Ladder DNA prepared by Biolaps Company, in one of the first holes, after which the samples are migrated Running the electrophoresis device for a period of 45 minutes, after which the bands are imaged using a gel-documentation device.

Determination of genetic variation of the VDR gene at locus ((rs7975232) in using a technique Tetra-ARMS-PCR

Methods :

Detection of ((rs7975232) polymorphism by ARMS-PCR:

The presence of the mutation A C was detected in the site ((rs7975232) as 4 µl was added (100 nanograms) of template DNA and 1 µl (10 picompl) of each mutation-specific primer ((rs7975232) which was designed by the researcher using the Pimer 3 program and used for the first time on this gene and was prepared by the Korean company Macrogen and added to Contents The master mix and the final reaction volume was 20 µl. (Rizk et al., 2019).

Table (1): shows the primers used to determine the genetic variation at locus (rs7975232) using the PCR technique

Primer	Sequence	Band size	Annealing
F-outer	CAAACACTTCGAGCACAAGGGGCGT	514bp	67
R-outer	AGGGATGGACAGAGCATGGACAGGG		
F-inner	AGGCACAGGAGCTCTCAGCTGGACC	317 Bp	
R-inner	GGGGTGGTGGGATTGAGCAGTGAAGT	248 bp	

Then the PCR tubes were placed in a thermocycler to conduct the polymerase chain reaction, depending on the special program for the reaction, as shown in Table (2):

No.	Stage	Temperature	Time	Cycle number
1.	Initial denaturation.	95.0	5.0 min.	1
2.	Denaturation.	95.0	45.0 sec.	35
3.	Annealing.	67.0	1.0 min.	
4.	Extension.	72.0	1.0 min.	
5.	Final extension.	72.0	7.0 min.	1
6.	Stop reaction.	4.0	5.0 min	1

Table (3): shows the approved program in the ARMS-PCR technique to determine the mutation (rs7975232)

Determine the nucleotide sequence of the amplified pieces based on the DNA Sequencing technique

The sequence of the nitrogenous bases of the gene was determined for the VDR samples that were included in the study for the purpose of verifying the validity of the designed primer that was used in the ARMS-PCR technique and for the purpose of detecting the presence of any additional variations of the gene. These genes were analyzed using the 3130 Genetic Analyzer from Hitachi, Japan

These gene sequences were matched with the documented gene sequences at the National Center for Biotechnology Information (NCBI), and the results were analyzed based on BLAST software.

3-6) Shows the primers of the VDR gene that were tested by DNA sequencing.

	Primer	Sequence
VDBP	Forward	CAAACACTTCGAGCACAAGGGGCGT
	Reverse	AGGGATGGACAGAGCATGGACAGGG

Result and discussion

The results showed, as in Figure (1), the existence of a relationship between women who suffer from osteoporosis and the genetic variation of the VDR gene at the site (rs7975232), as it is clear from the result of the PCR reaction that the genetic variation of the gene appears in the three genotypes CC, AC, AA And with proportions Different percentages as shown in Table 4)

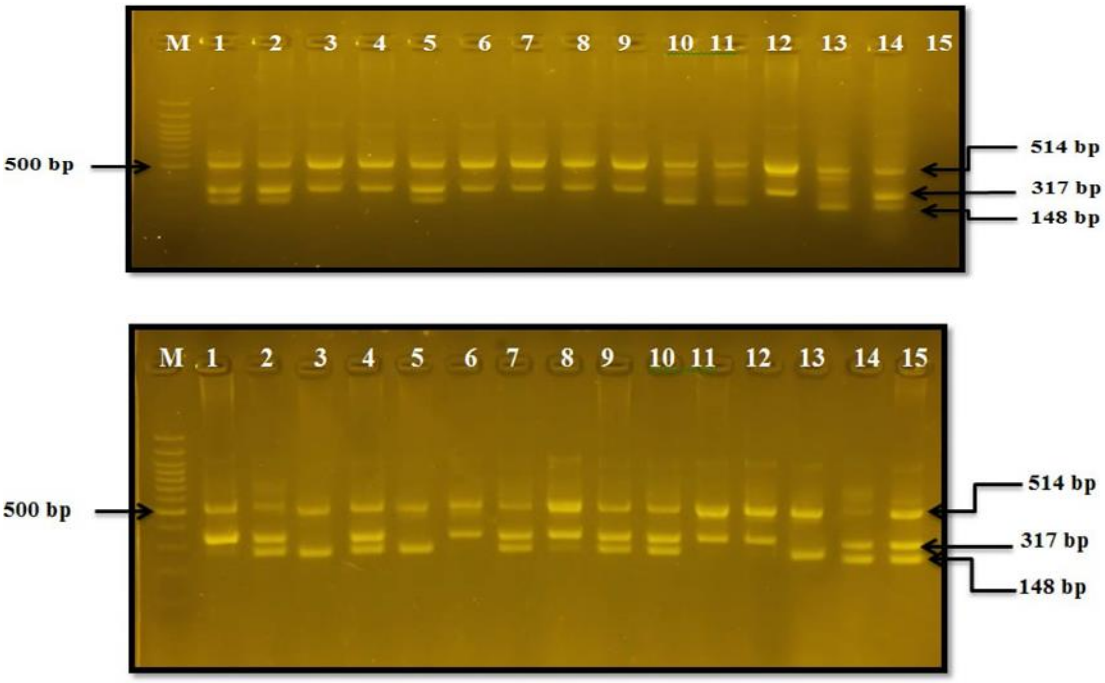


Figure (4-3): The product of the PCR reaction for the genetic variation (rs7975232) of the VDR gene shows that the result was a reaction containing 3 bundles, the first of 248 bp for the main gene, the second of 317 bp for the normal allele, and the third bundle of 514 bp for the mutant allele, M is Volumetric guide of 100 bp, prepared by Biolabs, and separated by 2% acarose gel.

Table (5) Distribution of allele incidence and genotype of the VDR gene at locus (rs7975232) between a group of healthy women and women suffering from osteoporosis, noting that the A allele is the natural allele and the C allele is the mutant allele

Genotypes	**Patients**		**Control**		**P Value**	**OR**
	NO.	**%**	**NO.**	**%**		
AA	26	37	8	40	P = 0.5329	1.2973
AC	32	45	9	45		
CC	12	18	3	15		
Alleles	**NO.**	**%**	**NO.**	**%**	**P Value**	**OR**
A	84	60	25	62	P = 0.7719	1.0877
C	56	40	15	38		

Table (6) shows the allelic incidence and frequency of the different genotypes of the VDR gene at the site (rs7975232), as the results showed for women with osteoporosis that the frequency value of the CC mutant genotype in a group of women with osteoporosis was 18% compared to the mutant genotype of group control by 15% While the value of the normal (normal) genotype AA was 37% for women with osteoporosis compared to the healthy group, in which the percentage of the normal (normal) genotype was 40%, while for the heterogeneous genotype AC in the group of women with osteoporosis it was 45%. Observations equal to the control group 45%. As for the allelic frequency, the results showed that the incidence of the mutant allele C was high in the patients group, 40% compared to the control group, 38%. As for the natural allele, the percentage was 60% in the patients, compared to 62% in the control group. The results of the study also showed that the odds ratio for the unhealthy genotype (mutant) AA was O.R = 1.29 at the probability level P = 0.0278, and for the unhealthy allele (mutant) the odds level was O.R = 1.08 at the probability level P = 0.0150 and this is considered a risk factor because it is above (1)

In recent years, the role of vitamin D in calcium and bone tissue mineralization and remodeling has expanded through the VDR gene. Previous studies have shown that this gene regulates the expression of many genes involved in calcium and phosphate homeostasis, cellular proliferation, differentiation, and immune response[10]. It is the gene Responsible for encoding the vitamin D receptor protein on the membranes, as this receptor binds to the active form of vitamin D, which is 1,25-dihydroxyvitamin D. The VDR gene contains many single nucleotide polymorphisms (SNPs) and has a direct association with many diseases, most notably osteoporosis [11]

Individual zygotes in recent studies of the C allele of the Teql polymorphism have shown a higher risk of osteoporosis in Saudi and Caucasian populations [12]Conflicting results have been reported for the Cdex polymorphism, which has been described as playing a dual role in both protective and risk factors. It causes osteoporosis. The AA/cdx2 genotype was associated with increased BMD in postmenopausal women, the A allele positively modulated bone mineral density in postmenopausal Japanese women, and the presence of the secondary A allele for the cdx2 form was associated with decreased bone mass in the spine and the genotype AA shows lower expression of the VDR gene than genotypes AG and GG [13]

Studies have shown that a deficiency in the VDR gene inside the body leads to the formation of several diseases, disorders, and tumors, the most important of which is the formation of mouth cancers [14] and the formation of osteoporosis, as this gene has an important role in bone metabolism and its balance, as the largest concentration of the BSML form and its direct effect To form fragility and that

the deficiency in this gene form leads to the formation of osteoporosis at menopause coinciding with environmental factors such as lack of sun exposure, calcium deficiency, gender, age and race [15]

Analyze the results of the DNA Sequence test

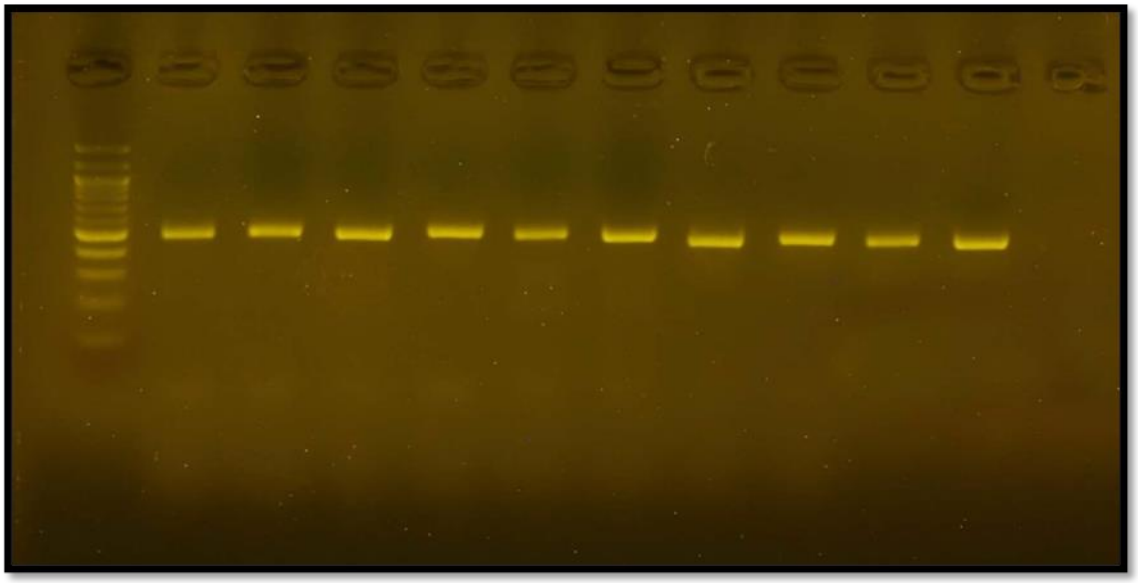


Figure (1): shows the product of the PCR reaction of the VDR gene, with a reaction product of 514 bp, M is a size guide of 100 BP, prepared by Biolabs, and separated by a 2% acarose gel.

The results of matching the nucleotide sequence of the VDR gene for the samples included in the study showed that it was 100% identical to the nucleotide sequences of the NCBI site, and this indicates the accuracy of the primer designed for the first time and that was used in this study.

The aim of conducting a nucleotide sequence determination test is to confirm conclusively that the primers used in this study are affiliated with the VDR gene, in addition to identifying new mutations of the gene that may directly or indirectly affect the activity of the gene, which is one of the main reasons for the development of the disease. Sequencing test for VDR gene amplification The presence of unregistered variations in the sequence of a number of nucleotides, and these variations are shown in the following figures:

The aim of conducting a nucleotide sequence determination test is to confirm definitively that the primers used in this study belong to the VDR gene, in addition to identifying new mutations of the gene that may directly or indirectly affect the activity of the gene, which is one of the main reasons for the development of the disease. Sequencing test for VDR gene amplification The presence of unregistered variations in the sequence of a number of nucleotides, and these variations are shown in the following figures

Figure (2): Matching the nucleotides of the VDR gene to the nucleotide sequences of the NCBI site [15]

Discussion ;

protein on the membranes, as this receptor binds to the active form of vitamin D, which is 1,25-dihydroxyvitamin D 3-hydroxyvitamin D. Numerous single nucleotide polymorphisms (SNPs) in the VDR gene have been found to be associated with a variety of illnesses, most notably osteoporosis [16]

Recent research on the Teql polymorphism's C allele found that single zygotes had a higher risk of osteoporosis in Saudi and Caucasian populations The Cdex polymorphism, which has been identified as playing a dual function in both protective factors and risk factors for osteoporosis, has reportedly produced conflicting results. In postmenopausal women, the AA/cdx2 genotype has been linked to higher BMD. The A allele positively modulates mineral density in postmenopausal Japanese women, and the presence of the secondary A allele for the CDX2 form was associated with a decrease in bone mass in the spine, and the AA genotype shows lower expression of the VDR gene than the AG and GG genotypes.

[17] Studies have demonstrated that a VDR gene shortage inside the body results in the emergence of a number of illnesses, ailments,[18] and malignancies, the most significant of which is the development of oral cancers. [19]

And the development of osteoporosis, since this gene plays a significant role in bone metabolism and its balance, as the BSML form with the highest concentration has a direct impact on the development of fragility.[20]

Along with environmental factors like lack of sun exposure, calcium shortage, gender, age, and race, the lack of this hereditary type causes osteoporosis to develop at menopause. [21]

References :

1-Muñoz, J., Akhavan, N. S., Mullins, A. P., & Arjmandi, B. H. (2020). Macrophage polarization and osteoporosis: a review. Nutrients, 12(10), 2999.

2-Song, S., Guo, Y., Yang, Y., & Fu, D. (2022). Advances in pathogenesis and therapeutic strategies for osteoporosis. Pharmacology & Therapeutics, 108168.

3-Salari, N., Ghasemi, H., Mohammadi, L., Rabieenia, E., Shohaimi, S., & Mohammadi, M. (2021). The global prevalence of osteoporosis in the world: a comprehensive systematic review and meta-analysis. Journal of orthopaedic surgery and research, 16(1), 1-20.

4-Anam, A. K., & Insogna, K. (2021). Update on osteoporosis screening and management. Medical Clinics, 105(6), 1117-1134.

5-Noh, J. Y., Yang, Y., & Jung, H. (2020). Molecular mechanisms and emerging therapeutics for osteoporosis. International journal of molecular sciences, 21(20), 7623.

6-Yong, E. L., & Logan, S. (2021). Menopausal osteoporosis: screening, prevention and treatment. Singapore Medical Journal, 62(4), 159.

7-Malloy, P. J., & Feldman, D. (2011). The role of vitamin D receptor mutations in the development of alopecia. Molecular and cellular endocrinology, 347(1-2), 90-96.

8-Abbassy, H. A., Elwafa, R. A. A., & Omar, O. M. (2019). Bone mineral density and vitamin D receptor genetic variants in egyptian children with beta thalassemia major on vitamin D supplementation. Mediterranean journal of hematology and infectious diseases, 11(1). (Muhammad Afzal et al., 2020)

9-Wang, Y., Zhu, J., & DeLuca, H. F. (2012). Where is the vitamin D receptor?. Archives of biochemistry and biophysics, 523(1), 123-133.

10-Dobrijevic, Z., Robajac, D., Gligorijevic, N., Šunderic, M., Penezic, A., Miljuš, G., & Nedic, O. (2022). The association of ACE1, ACE2, TMPRSS2, IFITM3 and VDR polymorphisms with COVID-19 severity: A systematic review and meta-analysis. EXCLI journal, 21, 818.

11-Bollen, S. E., Bass, J. J., Fujita, S., Wilkinson, D., Hewison, M., & Atherton, P. J. (2022). The Vitamin D/Vitamin D receptor (VDR) axis in muscle atrophy and sarcopenia. Cellular signalling, 96, 110355.

12-Bollen, S. E., Bass, J. J., Fujita, S., Wilkinson, D., Hewison, M., & Atherton, P. J. (2022). The Vitamin D/Vitamin D receptor (VDR) axis in muscle atrophy and sarcopenia. Cellular signalling, 96, 110355. (Jiang et al.,2020)

13-Jiang, L.L.; Zhang, C.; Zhang, Y.; Ma, F.; Guan, Y. Associations between Polymorphisms in VDR Gene and the Risk of Osteoporosis: A Meta-Analysis. Arch. Physiol. Biochem. 2022, 128, 1637–1644.

14-Aziz, A., Shah, M., Siraj, S., Iqbal, W., Jan, A., Khan, I., ... & Angioni, S. (2023). Association of vitamin D deficiency and vitamin D receptor (VDR) gene single-nucleotide polymorphism (rs7975232) with risk of preeclampsia. Gynecological Endocrinology, 39(1), 2146089.

15-Banjabi, A. A., Al-Ghafari, A. B., Kumosani, T. A., Kannan, K., & Fallatah, S. M. (2020). Genetic influence of vitamin D receptor gene polymorphisms on osteoporosis risk. International journal of health sciences, 14(4), 22

16-Voutsadakis, I. A. (2020). Vitamin D receptor (VDR) and metabolizing enzymes CYP27B1 and CYP24A1 in breast cancer. Molecular biology reports, 47(12), 9821-9830.

17- Bass, J. J., Nakhuda, A., Deane, C. S., Brook, M. S., Wilkinson, D. J., Phillips, B. E., ... & Atherton, P. J. (2020). Overexpression of the vitamin D receptor (VDR) induces skeletal muscle hypertrophy. Molecular metabolism, 42, 101059.

18- Kise, S., Iijima, A., Nagao, C., Okada, T., Mano, H., Nishikawa, M., ... & Sakaki, T. (2023). Functional analysis of vitamin D receptor (VDR) using adenovirus vector. The Journal of Steroid Biochemistry and Molecular Biology, 230, 106275.

19- Banjabi, A. A., Al-Ghafari, A. B., Kumosani, T. A., Kannan, K., & Fallatah, S. M. (2020). Genetic influence of vitamin D receptor gene polymorphisms on osteoporosis risk. International journal of health sciences, 14(4), 22.
20-Chatterjee, I., Lu, R., Zhang, Y., Zhang, J., Dai, Y., Xia, Y., & Sun, J. (2020). Vitamin D receptor promotes healthy microbial metabolites and microbiome. Scientific Reports, 10(1), 1-18.
21-Chatterjee, I., Lu, R., Zhang, Y., Zhang, J., Dai, Y., Xia, Y., & Sun, J. (2020). Vitamin D receptor promotes healthy microbial metabolites and microbiome. Scientific Reports, 10(1), 1-18.