

# Correlation of Vitamin D3, PAI-1, and HCG Hormone in Pre- and Post-Menopausal in Babylon Province

Thanaa Abdulmahdi Mokif<sup>1</sup>, Zena Abdul-Ameer Mahdi<sup>2</sup>,  
Raheem Tuama Obayes Al- Mammori<sup>3</sup>,  
Hussein Oleiwi Muttaleb Al-Dahmoshi\*<sup>4</sup>,  
Noor Salman Kadhim Al-Khafaji<sup>5</sup>

## Abstract

**Background:** Menopause is a unique event in women's life it usually occurs naturally, most often after age 50 when woman has not menstruated in 12 consecutive months. This study was planned to assess the relationship between Vitamin D3 level, PAI-1 and HCG in Babylon women at age <50 years as pre-menopausal and > 50 years as post-menopausal.

**Methods:** The sample were selected from a group of pre- and post-menopausal women, 30 and 50 respectively. All the tests were evaluated to measure Vitamin D3 level, PAI-1 and HCG level. The sample was collected between July 2019 and January 2020 at Merjan medical city GIT and Liver Center, Babylon province, Iraq.

**Results:** The result of current study revealed that there are significant differences in vitamin D3 level in various age categories within postmenopausal women ( $p=0.02$ ) also there is no significant differences in PAI-1 and HCG with in these two groups,  $p=0.08$  and  $0.07$ , respectively. Also, there is significant negative correlation between vitamin D3 and PAI-1 in postmenopausal women ( $p$  value is  $0.01$ ).

**Conclusions:** Indeed, postmenopausal women regarded as elderly, but they have sufficient vitamin D3 and normal PAI-I levels as markers for normal non fibrosis status.

**Keywords:** And PAI-1, HCG, Menopause, Vitamin D3.

## Introduction

The deficiency in vitamin D is more commonly noted after menopause, mainly attributed to an increase in body fat levels, decrease of 7-dehydrocholesterol (7-DHC) levels in skin, reduction in the bioavailability of vitamin D, which is a fat-soluble vitamin, and reduction in kidney 1- $\alpha$ -hydroxylase activity (1). 7-DHC produces vitamin D3 (cholecalciferol), the natural form of vitamin D. When 7-DHC is exposed to irradiation, it

forms pre-vitamin D3, which submits to “a temperature-sensitive rearrangement of three double bonds” to become vitamin D3 that is biologically inactive (2). The vitamin D binding protein (DBP); which binds to vitamin D and its metabolites in serum, transports vitamin D to the liver through the bloodstream. Vitamin D is converted to 25-hydroxyvitamin D3 (25(OH)D3) in the liver when it is hydroxylated at C-25. Vitamin D in the form

1: Babylon Technical Institute, Al-Furat Al-Awsat Technical University, 51015 Babylon, Iraq.

2: college of pharmacy, AhlAlbayt university, Karbala, Iraq.

3: Babylon GIT and Liver Center, Babylon health directorate, Hilla, Iraq.

4: Department of Biology, college of science, university of Babylon, Hilla, Iraq.

5: Department of Biology, college of science, university of Babylon, Hilla, Iraq.

\*Corresponding author: Hussein Oleiwi Muttaleb Al-Dahmoshi; Tel: +96 47807771; E-mail: dr.dahmoshi83@gmail.com.

Received: 22 Aug, 2021; Accepted: 29 Nov, 2021

of 25(OH)D3 is the most widely distributed type. Its level in the bloodstream has been used as a biomarker for vitamin status (3). There has not been any evidence that 25(OH)D3 synthesis is well controlled (4). Different cytochrome P-450 enzymes (CYPs), like CYP27A1, CYP2R1, and CYP2D25, have been proposed as potential alternatives for the enzyme that converts D to 25 (OH)D3 (5). The decrease in androgens during the post-menopausal period can exacerbate sexual function issues in women (6). Vitamin D plays a role in several cellular functions, including cell differentiation, apoptosis, decreased proliferation, immune-suppression, and reduced inflammation (7).

Plasminogen activator inhibitor-1 (PAI- 1) is a serpin protease that acts as a prominent inhibitor of endogenous fibrinolysis. Endothelial cells, adipocytes, and platelets are the main producers of it (8). PAI- 1 can bind with tissue-type (t-PA) and urokinase-type (u-PA) plasminogen activators and inhibits them. This means that PAI-1 diminishes plasminogen conversion into plasmin, the major fibrinolysis enzyme (9). PAI-1 belongs to the superfamily of serine protease inhibitors to one chain of glycoprotein (or serpins). It consists of 379 amino acids apparently weighing 48 kDa molecules (10). Plasminogen is found predominantly in the plasma, and the liver is its major synthetic source. In various mice tissues, including brain, adrenal, kidney, heart, testis, lung, uterus, spleen, gut, and thymus, plasminogenic mRNA was identified, however, which suggest an extensive functionality of the plasminogen-activator system (11). Whereas the glycoprotein hormone which represents the human chorionic gonadotropin (hCG) has two subunits  $\alpha$  and  $\beta$  are connected non-covalently. It belongs to the glycoprotein hormones group, which also includes LH, TSH, and FSH (12). The steady-state levels of hCG are determined by its metabolism in the placenta, liver, blood, and kidney (13). Serum and urine hCG levels give essential details in a variety of clinical conditions including the pregnancy identification and monitoring, pre-natal

testing, gynecological cancer, and pregnancy-related diseases (14).

## Materials and Methods

### *Ethical approval*

The research related to human use has been complied with all the relevant national regulations, institutional policies, and in accordance the tenets of the Helsinki Declaration and has been approved by the authors' institutional review board in March 2019, project No. 87.

### *Patients*

Between (July 2019 – Until January 2020), from a group of pre- and post-menopausal (PPM) women, 30 and 50, respectively, blood was collected in Merjan medical city hospital, GIT and Liver Center laboratory. Informed consent has been obtained from all individuals included in this study.

### *ELISA*

The method used in estimation of Vitamin D3 level and other parameters (PAI-1 and HCG hormone) were done by using Elabscience ELISA Kits (Elabscience/China) according to manufacturer instructions.

### *Statistical analysis*

The statistical analysis was done by using SPSS program in data analysis of Chi –Square, ANOVA and Bivariate Correlation.

## Results

The results show that, all women were in low Vitamin D3 level at both pre- and post-menopausal age, but lowest level was seen at post-menopausal women at age 60 -69 years in comparison within age categories other (Table 1).

### *anti-inflammatory cytokines*

The levels of both IL-4 and TGF- $\beta$  in groups 1–3 were significantly greater than group 4 (p 0.05; Table 2). The levels of IL-4 and TGF- $\beta$  in group 3 were also significantly greater than all other groups (p< 0.05; Table 2). The serum levels of anti-inflammatory cytokines among healthy mice are also shown in Table 2.

**Table 1.** Vitamin D3 level in association with age groups PPM women.

Vitamin D3	Age (year)	Number	Mean±SD	p Value
Pre-Menopausal	20 - 29	15	201.8818±27.45288	0.074
	30 - 39	17	189.9625±13.38645	
	40 - 49	18	205.3432±25.33070	
	<b>Total</b>	<b>50</b>	<b>198.3333±43.55304</b>	
Post-Menopausal	50 - 59	17	205.4901±25.49831	0.002
	60 - 69	8	197.3007±12.42200	
	>70	5	199.7235±25.74265	
	<b>Total</b>	<b>30</b>	<b>200.3443±20.78728</b>	

Vitamin D3 level was lower in the premenopausal than post- menopausal but no

significant differences of PAI-1 and HCG levels between two groups were not found (Table 2).

**Table 2.** Vitamin D3 level in association with PAI-1 and HCG at pre, post-menopausal women.

Groups Parameters	Pre-Menopausal n= 30 Mean ±SD	Post-Menopausal n= 50 Mean±SD	p Value
Vit. D 3 (ng/ml)	204.02±23.98	384.99±105.31	0.00
PAI-1 (ng/ml)	0.52±0.05	0.50±0.05	0.08
HCG (pg/ml)	10.47±1.55	10.35±1.38	0.07

There was not found any correlation between HCG with PA1 and Vitamin D3 at PPM women.

However, a positive correlation between PAI-1 and Vitamin D3 level we detected (Table 3).

**Table 3.** Pearson correlation of all studied parameters among pre-, post-menopausal women.

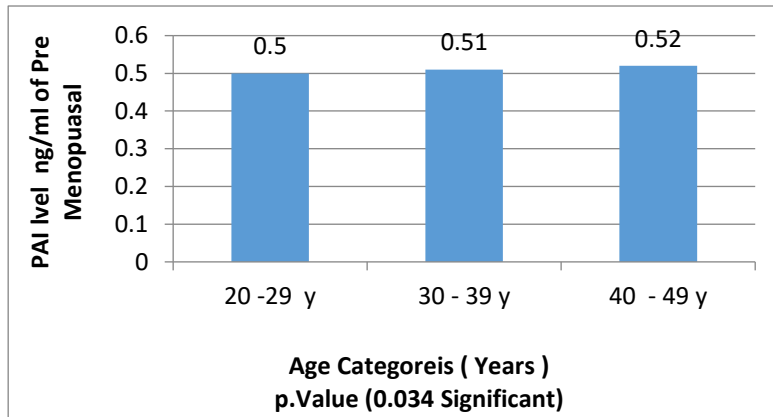
Correlations of pre-menopausal		HCG	PAI-1	Vitamin D3
HCG	Pearson Correlation	1		
	Sig. (2-tailed)			
PAI-1	Pearson Correlation	-.077-	1	
	Sig. (2-tailed)	0.439		
Vit. D3	Pearson Correlation	-0.133-	0.016	1
	Sig. (2-tailed)	0.179	0.870	
Correlations of post-Menopausal		HCG	PAI-1	Vitamin D3
HCG	Pearson Correlation	1		
	Sig. (2-tailed)			
PAI-1	Pearson Correlation	-0.130-	1	
	Sig. (2-tailed)	0.283		
Vit. D3	Pearson Correlation	-0.193-	0.294*	1
	Sig. (2-tailed)	0.109	0.013	

\*\*Correlation is significant at the 0.01 level (2-tailed).

\*Correlation is significant at the 0.05 level (2-tailed).

The PAI-1 level was show that low level at, this result might women lowest level at age A)

range 20 – 29 years old in comparison within age categories other (Fig. 1).



B)

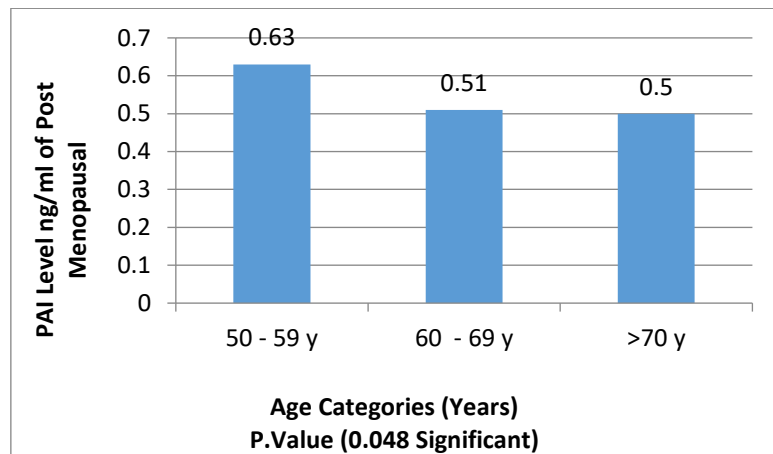
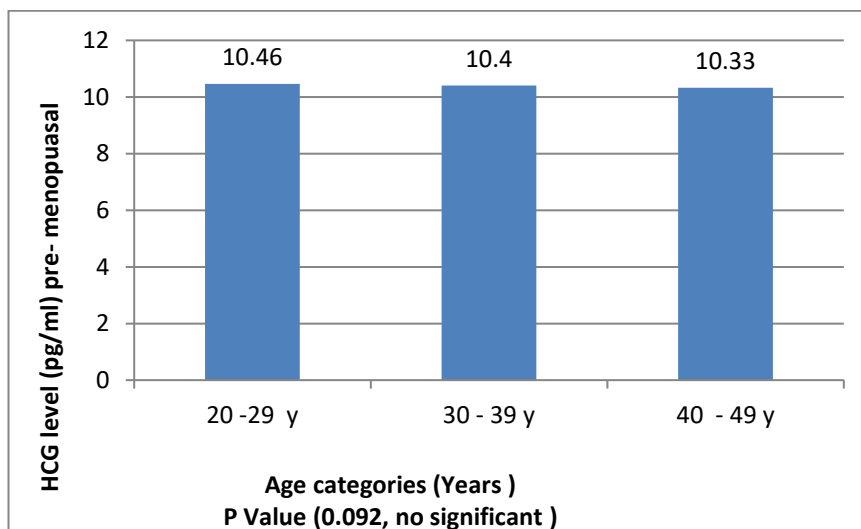


Fig. 1. The PAI-1 level A) of pre-Menopausal and B) of post-Menopausal.

while post-Menopausal, this result might be show that the women at age > 70 years have lowest level

rather than women at age 50-59 years in comparison within age categories other (Fig. 2).

A)



B)

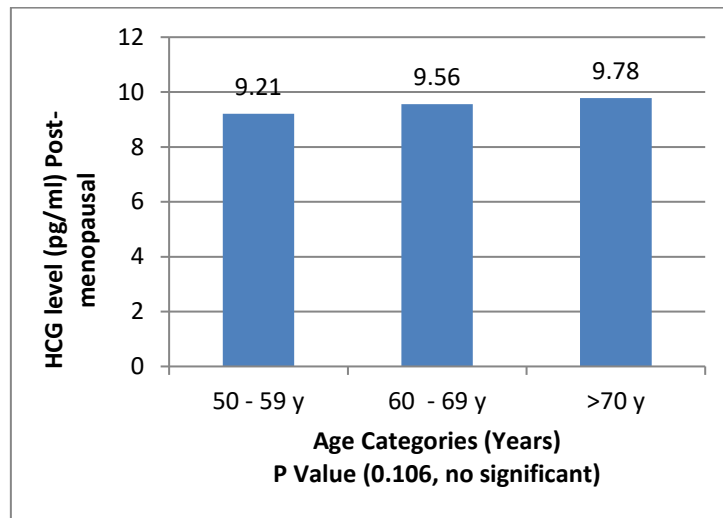


Fig. 2. The HCG level A) of pre-Menopausal and B) of post-Menopausal.

The results showed that the HCG level was the highest in the categories 20-29 age group compared to the other groups (30- 39) year, (40- 49) year.

The HCG level was show that higher level at pre- menopausal women in comparison with Post-Menopausal, While the result of age range 50-59 years at post-menopausal show that lowest level in comparison with other age groups (Fig. 2).

## Discussion

The menopause is a hormonal instability phase in women which poses the risk of insufficiency of essential micronutrients, such as magnesium and vitamin D, in addition to other physiopathological effects (15). Menopause-related symptoms affect around 80% of menopausal women (16). Our research demonstrated all women was in low Vitamin D3 level at both pre and post-menopausal age, but lowest level was seen at post-menopausal women at age 60 -69 years old, while the result PAI-1 level was show that low at premenopausal women in comparison with post – Menopausal, this result might be show that the women at age > 70 years have lowest level rather than women at age 50-59, also The present study demonstrate that significant differences in Vitamin D3 level the premenopausal women revealed that lower level than post-menopausal. In counter with no

significant differences of PAI-1 and HCG levels after comparison between pre- and post-menopausal, a positive correlation of PAI-1 with Vitamin D3 level was found (Table 3).

Against the positive association between PAI-1 and level Vitamin D3 insufficiency is common in pre- and postmenopausal T2DM; however, it is more prevalent in postmenopausal women (17). Other findings of previous studies suggest that oestrogen enhances the activity of 1-hydroxylase (expressed in the kidneys), which is necessary for vitamin D stimulation and modulates the vitamin D receptor (VDR) (18). The amount of oestrogen generated by the ovaries gradually decreases during menopausal stages (19). This reduction in the synthesis of oestrogen is intended to encourage the deficit in vitamin D. The subsequent challenge is the reduction of the number of vitamin D receptors (20). For 463 postmenopausal women, levels of 25 OH have been investigated and noticed that just 32 % of them had adequate levels (21). vitamin D shortness among the postmenopausal groups is therefore commonly seen. It has been found that the drop in oestrogen related with postmenopausal women reduces the efficiency of 1-alpha hydroxylase vitamin D, which is necessary to activate vitamin D and its receptors (VDRs) (22).

Vitamin D is essential for female reproductive health. Vitamin D insufficiency

was shown to be very common over the world, affecting roughly half of the population. Several health issues in women such as polycystic ovarian syndrome, endometriosis, Infertility, and pregnancy related concerns including caesarian and section preeclampsia have been associated with hypovitaminosis D (23). It is worth mentioning that female reproduction has not been fully investigated in terms of the effects of vitamin D. Recent reports from fundamental studies however clearly supports the possible function of vitamin D in human reproduction, especially Women (24,25). It turns out that the effects of a lack of Vitamin D3 on pregnant women expose them to the problems of spontaneous abortion or premature birth due to failure of the placenta function, pre-eclampsia, gestational diabetes, bacterial vaginitis, and poor growth and development of the fetus and childhood, as preserving must be maintained at sufficient levels throughout the period pregnancy (25,26).

There are two main forms of vitamin D; D2 and D3 which are usually known as ergocalciferol or cholecalciferol, respectively. Each of these forms attaches to DBP and are delivered to all the body's critical organs, where they act as a natural ligand for vitamin D receptors, allowing them to perform their biological functions. In clinical studies, D deficiency has been linked to an increase in thrombotic occurrence, suggesting that D along with its companion molecule play a role in the control of thrombosis-related pathways (26). In a human uterine fibroid cell line, it has been found that 10 nM 1,25(OH)2D3 functioned by binding with VDR and then lowering PAI-1 protein expression (27). Another research (28) found that 1,25 (OH) 2D3 decreased strong PAI- 1 reactivity in fibroblasts. Vitamin D, either directly or indirectly, regulates the expression of many genes involved in the differentiation,

regulation of cellular proliferation, angiogenesis, and apoptosis. All these processes have the potential to be relevant to thrombotic diseases (29). Several studies have shown that abnormal fibrin clot structure, particularly higher fibrin fiber concentration and resistance to fibrinolysis, is associated with CVD (30). Furthermore, various modulators, including blood flow conditions, blood cell interactions, and endothelial cells, can alter the fibrin clot structure *in vivo* (31). Ex-vivo studies may not be able to detect these effects (for example, vitamin D's effects on the endothelium) (32,33). Both PAI-1 and VDR have been demonstrated to play key roles in the development of tissue fibrosis, making them possible susceptibility genes for Keloid disease. The physiological and hormonal status of postmenopausal different totally from premenopausal women (34,35). Where other data in 2019 showed PAI-1 has been implicated in a variety of disorders, including cardiovascular disease, obesity, and cancer, and is thus a promising therapeutic target. Moreover, due to various pathophysiological functions of PAI-1 and the difficulty in determining their relative impact in any given illness setting, developing therapeutically useful compounds is challenging (36). Indeed, postmenopausal women regarded as elderly, but they have sufficient vitamin D3 and normal PAI-I levels as markers for normal non fibrosis status

### Acknowledgements

We thank the Iraqi ministry of higher education the University of Babylon, Al-Furat Al-Awsat Technical University, Ahlalbait university, Babylon GIT and Liver Center for facilities needed to carry out this study. This work was personally funded by authors

The authors declare they have no conflict of interest.

## References

- Caruso S, Rapisarda AMC, Cianci S. Sexuality in menopausal women. *Curr Opin Psychiatry*. 2016;29(6):323-30.
- Holick MF. Resurrection of vitamin D deficiency and rickets. *J Clin Invest*. 2006;116(8):2062-72.
- Bikle DD. Vitamin D: Production, Metabolism and Mechanisms of Action. In: Feingold KR, Anawalt B, Boyce A, et al., editors. *Endotext*. South Dartmouth (MA): MDText.com, Inc.; 2000.
- DeLuca HF. Evolution of our understanding of vitamin D. *Nutr Rev*. 2008;66(10 Suppl 2):S73-87.
- Zhu J, DeLuca HF. Vitamin D 25-hydroxylase - Four decades of searching, are we there yet?. *Arch Biochem Biophys*. 2012;523(1):30-6.
- Snijder MB, van Dam RM, Visser M, Deeg DJ, Dekker JM, Bouter LM, et al. Adiposity in relation to vitamin D status and parathyroid hormone levels: A population-based study in older men and women. *J Clin Endocrinol Metab*. 2005;90(7):4119-23.
- Pfotenhauer KM, Shubrook JH. Vitamin D deficiency, its role in health and disease, and current supplementation recommendations. *J Am Osteopath Assoc*. 2017;117(5):301-305.
- De Taeye B, Smith LH, Vaughan DE. Plasminogen activator inhibitor-1: a common denominator in obesity, diabetes and cardiovascular disease. *Curr Opin Pharmacol*. 2005;5(2):149-54.
- Fortenberry YM. Plasminogen activator inhibitor-1 inhibitors: a patent review (2006-present). *Expert Opin Ther Pat*. 2013;23(7):801-15.
- Blasi F, Carmeliet P. uPAR: a versatile signalling orchestrator. *Nat Rev Mol Cell Biol*. 2002;3(12):932-43.
- Zhang L, Seiffert D, Fowler BJ, Jenkins GR, Thinnis TC, Loskutoff DJ, et al. Plasminogen has a broad extrahepatic distribution. *Thromb Haemost*. 2002;87(3):493-501.
- Lapthorn AJ, Harris DC, Littlejohn A, Lustbader JW, Canfield RE, Machin KJ, et al. Crystal structure of human chorionic gonadotropin. *Nature*. 1994;369(6480):455-61.
- Cole LA. Immunoassay of human chorionic gonadotropin, its free subunits, and metabolites. *Clin Chem*. 1997;43(12):2233-43.
- Stenman UH, Tiitinen A, Alfthan H, Valmu L. The classification, functions and clinical use of different isoforms of HCG. *Hum Reprod Update*. 2006;12(6):769-84.
- Vázquez-Lorente H, Herrera-Quintana L, Molina-López J, Gamarra-Morales Y, López-González B, Miralles-Adell C, et al. Response of vitamin D after magnesium intervention in a postmenopausal population from the province of Granada, Spain. *Nutrients*. 2020;12(8):2283.
- Roberts H, Hickey M. Managing the menopause: an update. *Maturitas*. 2016;86:53-8.
- Fondjo LA, Sakyi SA, Owiredu WKBA, Laing EF, Owiredu E-W, Awusi Er K, et al. Evaluating Vitamin D Status in Pre- and Postmenopausal Type 2 Diabetics and Its Association with Glucose Homeostasis. *Biomed Res Int*. 2018;9369282.
- Kanwar SNG, Shekhawat M, Sharma P, Hada R. Comparison of vitamin D levels in Pre and PostMenopausal Type 2 diabetic females. *IOSR JDMS*. 2015;14(8):70-73.
- Harlow SD, Gass M, Hall JE, Lobo R, Maki P, Rebar RW, et al. Executive summary of the Stages of Reproductive Aging Workshop + 10: addressing the unfinished agenda of staging reproductive aging. *Menopause*. 2012;19(4):387-395.
- Bischoff-Ferrari HA, Borchers M, Gudat F, Dürmüller U, Stähelin HB, Dick W. Vitamin D receptor expression in human muscle tissue decreases with age. *J Bone Miner Res*. 2004;19(2):265-9.
- Schmitt EB, Nahas-Neto J, Bueloni-Dias F, Poloni PF, Orsatti CL, Petri Nahas EA. Vitamin D deficiency is associated with metabolic syndrome in postmenopausal women. *Maturitas*. 2018;107:97-102.
- Fondjo LA, Owiredu WKBA, Sakyi SA, Laing EF, Adotey-Kwofie MA, Antoh EO, Detoh E. Vitamin D status and its association with insulin resistance among type 2 diabetics:

A case-control study in Ghana. *PLOS One*. 2017;12(4):e0175388.

23. Hantoosh HA, Mahdi MH, Imran BW, Yahya AA. Prevalence of vitamin D deficiency in Iraqi female at reproductive age. *Med J Babylon*. 2019;16(2):119-122.

24. Amini S, Jafarirad S, Amani R. Postpartum depression and vitamin D: A systematic review. *Crit Rev Food Sci Nutr*. 2019;59(9):1514-1520.

25. Cermisoni GC, Alteri A, Corti L, Rabellotti E, Papaleo E, Viganò P, et al. Vitamin D and endometrium: A systematic review of a neglected area of research. *Int J Mol Sci*. 2018;19(8):2320.

26. Al-Msaid HLF, AL-Sallami ASM. Study of Catsper1 protein levels in unexplained and idiopathic infertile men. The role of some biochemical markers and hormonal evidence in predisposition to osteoporosis in postmenopausal women. 2018;9(2):195-8.

27. Holick MF. The vitamin D deficiency pandemic: approaches for diagnosis, treatment and prevention. *Rev Endocr Metab Disord*. 2017;18(2):153-65.

28. Mohammad S, Mishra A, Ashraf MZ. Emerging role of vitamin D and its associated molecules in pathways related to pathogenesis of thrombosis. *Biomolecules*. 2019;9(11):649.

29. Halder SK, Osteen KG, Al-Hendy A. 1,25-Dihydroxyvitamin d3 reduces extracellular matrix-associated protein expression in human uterine fibroid cells. *Biol Reprod*. 2013;89(6):150.

30. Barbosa EM, Nonogaki S, Katayama ML, Folgueira MA, Alves VF, Brentani MM. Vitamin D3 modulation of plasminogen activator inhibitor type-1 in human breast carcinomas under organ culture. *Virchows Arch*. 2004;444(2):175-82.

31. Undas A, Ariens RA. Fibrin clot structure and function: a role in the pathophysiology of arterial and venous thromboembolic diseases. *Arterioscler Thromb Vasc Biol*. 2011;31(12):e88-99.

32. Byrnes JR, Wolberg AS. Red blood cells in thrombosis. *Blood*. 2017;130(16):1795-1799.

33. Hammer Y, Soudry A, Levi A, Talmor-Barkan Y, Leshem-Lev D, Singer J, et al. Effect of vitamin D on endothelial progenitor cells function. *PLOS One*. 2017;12(5):e0178057.

34. Gong ZH, Ji JF, Yang J, Xiang T, Zhou CK, Pan XL, et al. Association of plasminogen activator inhibitor-1 and vitamin D receptor expression with the risk of keloid disease in a Chinese population. *Kaohsiung J Med Sci*. 2017;33(1):24-29.

35. Badr Roomi A, Nori W, Mokram Hamed R. Lower Serum Irisin Levels Are Associated with Increased Osteoporosis and Oxidative Stress in Postmenopausal. *Rep Biochem Mol Biol*. 2021;10(1):13-19.

36. Rashid FA, Mahdi S, Mahdy SA, Salim AT. Effect of Obesity on Plasma Alkaline Phosphatase Activity in Breast Cancer. *Rep Biochem Mol Biol*. 2021;10(2):307-313.