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

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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-α-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

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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 protection 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 α and β 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-ß in groups 1–3 were significantly greater than group 4 (p 0.05; Table 2). The levels of IL-4 and TGF-ß 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.

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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	
	Total	**50**	**198.3333±43.55304**	
Post-Menopausal	50 - 59	17	205.4901±25.49831	0.002
	60 - 69	8	197.3007±12.42200	
	>70	5	199.7235±25.74265	
	Total	**30**	**200.3443±20.78728**	

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).

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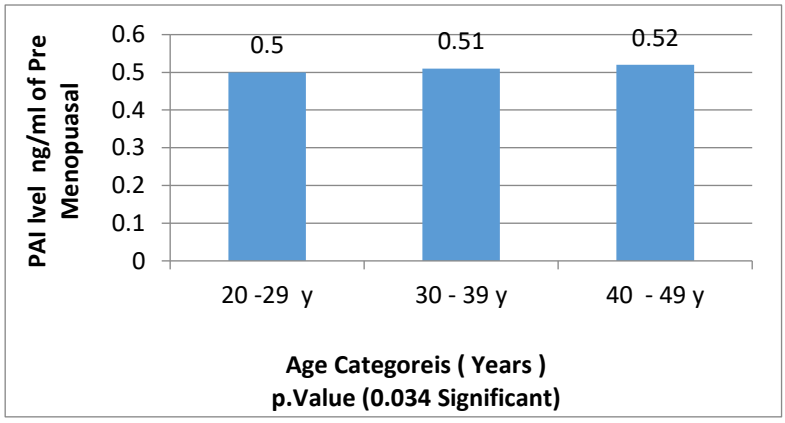
The PAI-1 level was show that low level at, this result might women lowest level at age range 20 – 29 years old in comparison within age categories other (Fig. 1).

A)



B)

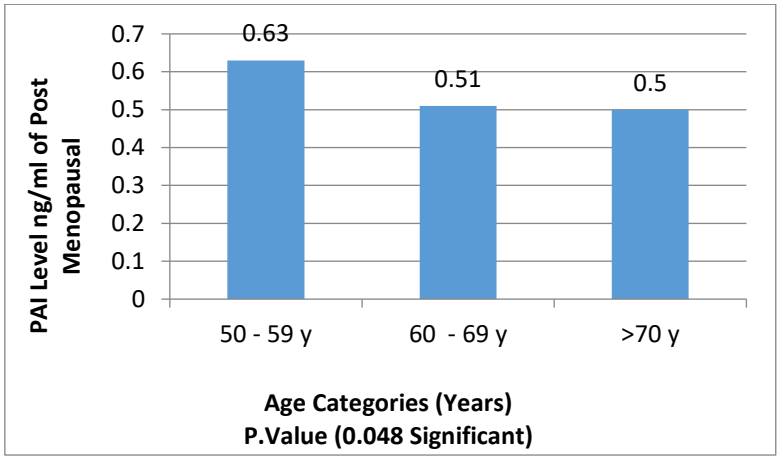
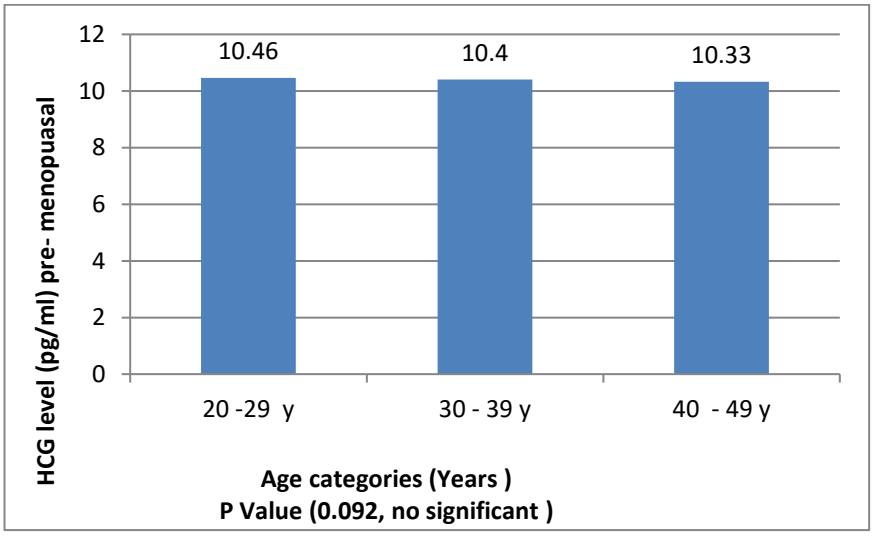


Fig. 1. The PAI-1 level A) of pre-Menopausal and B) of post-Menopausal.

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)



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B)

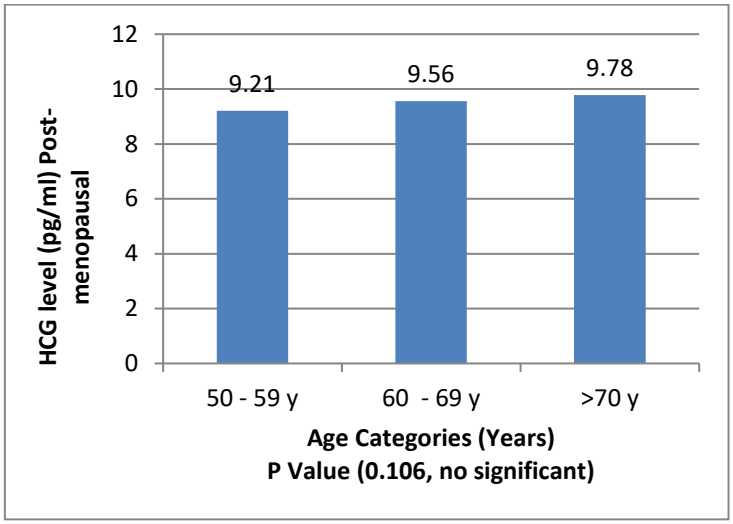

Fig. 2. The HCG level A) of pre-Menopausal and B) of post-Menopausal.

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 pre-menopausal 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

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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 cholectalciferol, 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 theendothelium) (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

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