ISSN 0975-2366
DOI:https://doi.org/10.31838/ijpr/2020.12.04.238

**Research Article**

# Correlation of VitaminD level with AMH and HMGB1 in pre and post-menopausal women in Babylon province

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Received: 04.04.20, Revised: 04.05.20, Accepted: 04.06.20

**ABSTRACT**
From a group of pre and post-menopausal women, respectively (34 and 46) were selected as test samples as well as 24 healthy women as control samples. All the test and control samples evaluated to measure the Total Vitamin level, Anti Mullerian hormone, and HMGB1 level. The study was planned to assess the relationship between Vit. D. level, AMH, and HMGB1 at the Babylon women at age < 45 years as (Pre – Menopausal) and > 45 years as Post-menopausal. The time between sample collection and measure of the studied parameters was in between (Jullay 2019 – Until January 2020) in Merjan medical city, GIT, and Live Center laboratory. Pre-menopausal women show a low level of AMH in comparison with postmenopausal and control samples. postmenopausal women show a low level of HMGB1 in comparison with the premenopausal and the control samples, While the result of premenopausal show that increased level of HMGB1 in comparison with the control sample. Highly significant decrease of VitaminD level of pre- and post-menopausal women in comparison with healthy women. While no significant result of AMH level and significant increase of HMGB1 level. A significant negative correlation of Total vitaminD level with HMGB1 level at both pre and post-menopausal women, on the other hand, insignificant correlation has been found between Vit. D and other parameters.

**Keywords:** vitamin, HMGB1, AMH, postmenopausal

## INTRODUCTION

Menopause is a condition in which an adult female is characterized by the absence of menstrual bleeding mainly caused by a loss of ovarian activity, as well as a decrease in estrogen, and it has been found that 80% of women at this stage suffer from physical, hormonal and psychological changes [1, 2]. It often occurs in ages ranging from 45-55 years [3]. During menopause, women experience recurrent vitaminD deficiency caused by a decrease in the 7-dehydrocholesterol levels in the skin; expansion of fatty tissue in the body; decreased bioavailability of vitaminD , which is one of the fat-soluble vitamins and, decrease in the activity of 1-α-hydroxylase in the kidneys [4]. Androgen deficiency can worsen sexual function disorders which are common in postmenopausal women [5]. VitaminD plays a role in several cellular functions, including cell differentiation, apoptosis, decreased proliferation, immune-suppression, and reduced inflammation [6]. VitaminD performs its biological role through association with its own receptors, the latter of which are found in reproductive vital organs such as the ovaries, placenta and uterus [7]. Several studies have emphasized the effective role of vitaminD in the reproductive function and the potential role of vitaminD in the ovarian reserve [8]. According to several studies, it is found that the acute deficiency of vitaminD and abnormal anti-Müllerian hormone (AMH) levels, both of which are responsible for different abnormalities observed in PCOS patients [9]. HMGB1 is a highly widespread and highly conserved nuclear protein that is released upon stimulation by inflammatory factors in the cells of the pituitary gland, monocytes, and macrophage [10]. It was found to stimulate the immune system and lead to an inflammatory response to certain types of stress caused by a variety of cytokines and chemotaxis of pro-inflammatory cells. HMGB1 is an internal immune auxiliary substance that was involved in diseases mediated by TLR2 / 4 causing an immune response [11]. Recent studies have confirmed that the binding of HMGB1 with

its receptor (TLR4) causes NF-κB activation and then induced the release of downstream inflammatory mediators [12]. The expression of HMGB1 demonstrated no significant changes with vitaminD state and cell type [13].Accordingly, this study aimed to evaluate the role of vitaminD in productive effectiveness in women by comparing the level of AMH and HMGB1 in pre- and post-menstrual.

## MATERIALS AND METHODS

From a group of pre and post-menopausal women, respectively (34 and 46) were selected as test samples as well as 24 healthy women as control samples. All the test and control samples evaluated to measure the Total Vitamin level, Anti Mullerian hormone, and HMGB1 level. The time between sample collection, and measure of the studied parameters was in between (July 2019 – January 2020) in Merjan medical city, GIT and Live Center laboratory. The method used in the estimation of VitaminD level is done according to the manual procedure of Biomeriox Company, by using the Vidas technique, while the other parameters (AMH and HMGB1) were done by using Elabscience protocol of ELISA technique. The statistical analysis was done by using the SPSS program in data analysis of Chi-Square, ANOVA, and Bivariate Correlation.

## RESULTS

The result of table 1 shows that all women were in low VitaminD level at both pre and post-menopausal age, but the lowest level was seen at post-menopausal women at age 55 -65 years old rather than other in comparison with the control. Pre-menopausal women show a low level of AMH in comparison with postmenopausal and control samples, this result might be showing that the women at age > 45 years have normal hormonal levels rather than low hormones at age < 45, the lowest level at age range 15 – 24 years old. The HMGB1 level showed that low level at postmenopausal women in comparison with premenopausal and control samples, While the result of pre-menopausal show that increased level of HMGB1 in comparison with the control sample. Table (2) shows the highly significant decrease of VitaminD levels of pre- and post-menopausal women in comparison with healthy women. While no significant result of AMH level and significant increase of HMGB1 level. The result of the table (3) shows that a significant negative correlation of Total vitaminD level with HMGB1 level at both pre and post-menopausal women, while no significant with other parameters.

**Table 1: The Vitamin D level among Pre and Post -Menopausal women.**

| Patient Groups | 25 (OH) Vit D (ng/ml) Total | | |
|---|---|---|---|
| | Category (years) | Mean ±SD | P. value |
| Pre-Menopausal | 15 - 24 y (n=15) | 13.28 ± 7.96 | 0.000 |
| | 25 -34 y (n=17) | 12.59 ± 7.34 | |
| | 35 -45y (n=14) | 11.24 ± 7.48 | |
| | Control (n=24) | 29.07 ± 11.95 | |
| Post-Menopausal | 45 -54 y (n=27) | 18.45 ± 14.61 | 0.000 |
| | 55 - 64y (n=4) | 8.95 ± 4.68 | |
| | >65y (n= 3) | 15.46 ± 5.65 | |
| | Control (n= 24) | 29.07 ± 11.95 | |

SD: Slandered deviation

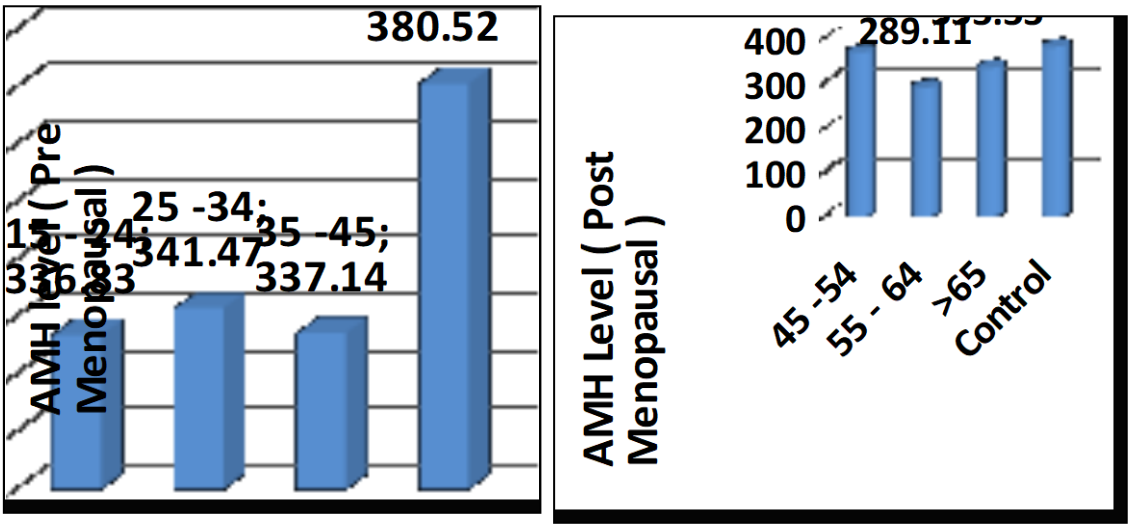


**Fig.1: The AMH level among Pre and Post -Menopausal women**

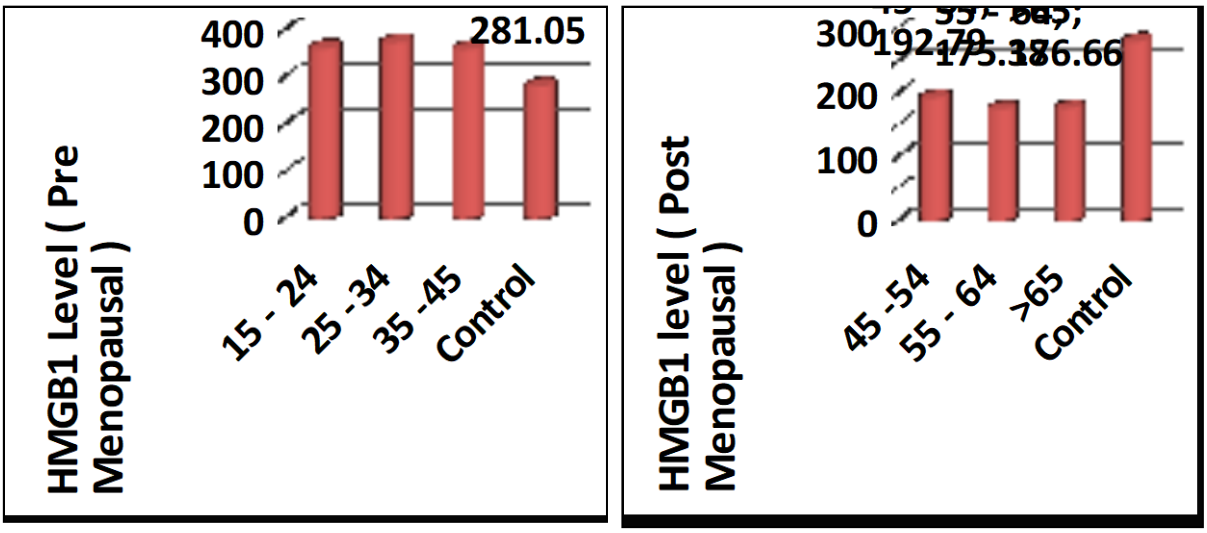


**Fig.2: The HMGB1 level among Pre and Post -Menopausal women.**

**Table 2: Result of total VitaminD level in association with AMH and HMGB1 at pre, Post-menopausal women, and control.**

| Groups<br>Parameters | Pre Menopausal n=34 Mean ±SD | Post Menopausal n=46 Mean ±SD | Control n=24 Mean ±SD | P. Value |
|---|---|---|---|---|
| 25(OH) vit D (ng/ml) | 11.84 ± 7.01 | 12.41 ± 7.46 | 24.47 ± 15.55 | 0.000 * |
| AMH | 345.29 ± 42.56 | 338.64 ± 40.04 | 358.33 ± 121.86 | 0.52 |
| HMGB1 | 357.66 ± 71.24 | 365.46 ± 66.15 | 310.57 ± 111.93 | 0.02 * |

SD: Slandered deviation

**Table 3: Pearson Correlation analysis of all studied parameters at pre, Post-menopausal women.**

| Correlations of Pre-menopausal | | Vit D | AMH | HMGB1 |
|---|---|---|---|---|
| Vit D | Pearson Correlation | 1 | | |
| | Sig. (2-tailed) | | | |
| AMH | Pearson Correlation | .173 | 1 | |
| | Sig. (2-tailed) | .080 | | |
| HMGB1 | Pearson Correlation | -.507-** | -.319-** | 1 |
| | Sig. (2-tailed) | .000 | .001 | |
| **Correlations** of Post - menopausal | | Vit D | AMH | HMGB1 |
| Vit. D | Pearson Correlation | 1 | | |
| | Sig. (2-tailed) | | | |
| AMH | Pearson Correlation | .223 | 1 | |
| | Sig. (2-tailed) | .064 | | |
| HMGB1 | Pearson Correlation | -.583-** | -.419-** | 1 |
| | Sig. (2-tailed) | .000 | .000 | |

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

## DISCUSSION

Menopause means the interruption of menstruation and is characterized by decreased estrogen and progesterone secretions, [3]. Estrogen increases the activity of the enzyme 1-$\alpha$-hydroxylase and is responsible for activating vitaminD and regulating its receptors. (VDR) during the menopausal stages [14] , That the weak ovaries cause a gradual decrease in the estrogen production [15]. This is reinforced by a deficiency in vitaminD [16]. Our study shows that all women were in low VitaminD level at both pre and post-menopausal ages, but the lowest level was seen at postmenopausal women at age 55 -65 years old rather than other in comparison with control. Also, the AMH level displayed that low level at premenopausal women in comparison with postmenopausal and control samples, this result might be shown that the women at age > 45 years have normal hormonal level rather than low hormone at age < 45, the lowest level at age range 15–24 years old. While The HMGB1 level was show that low level at postmenopausal women in comparison with premenopausal and control samples, While the result of premenopausal show that increased level in comparison with the control sample. The connection between vitaminD and the AMH is described by being a mind boggling process and in a way that accomplishes organic parity in the levels ofthose markers. Naderi and collaborators proposed that the expansion in vitaminD. levels prompts an expansion in the AMH serum level. [17]. Irani and collaborators show somewhat various outcomes, the organization of nutrient D in ladies with PCOS may diminish the AMH levels [18]. However, many studies have shown no association between AMH levels and vitaminD [19]. The research paper shows that a significant negative correlation of Total vitaminD level with HMGB1 level at both pre and postmenopausal women , while insignificant with other parameters. In human ovarian cells, studies have shown that vitaminD increases the production of ovarian hormones such as progesterone, estradiol, and estrone [20]. Besides, the recognition of the functional VDR element in the promoter region of the anti-mullerian hormone (AMH) gene suggests that vitaminD modulates AMH secretion.[21] AMH is secreted by granulosa cells of primary, preantral, and small antral follicles undergoing gonadotropin-independent development. It inhibits the recruitment of primordial follicles into folliculogenesis [22]. In female reproductive physiology, AMH is a reliable marker of ovarian reserve. Several studies have shown an association between serum vitaminD and markers of ovarian reserve. A study by Merhi *et al*. reported a positive correlation between serum vitaminD and AMH in women aged 40 years or older.[23] Furthermore, Dennis *et al.,* showed that the seasonal changes in AMH level correlated with the vitaminD level in women and that cholecalciferol supplementation prevented the seasonal AMH change.[24] Makwe, and Aliyu, in 2019 reveals insignificant differences in the AMH mean serum level between deficient, insufficient, and sufficient vitaminD patients groups, suggesting that there was no correlation between serum 25 (OH) D, and serum AMH [25]. study for Merhi, *et al* in 2012 showed that

after evaluating the participants by age group (<35; 35-39; and ≥40 years old), a multivariate logistic regression analysis detected a significant positive correlation between serum AMH and serum 25OH- D levels in late reproductive-age women (≥40 years old) suggesting that vitaminD deficiency might be associated with a low ovarian reserve in women of this age group [23]. On the other hand, a cross-sectional study conducted on 73 healthy normal-weight women with no history of infertility showed that serum 25OH-D is positively correlated with total testosterone and free androgen index but not with serum AMH levels [26]. Furthermore, serum AMH levels show a seasonal variation that is positively correlated with the seasonal changes in serum 25OH-D in premenopausal women, also Serum AMH levels exhibit a significant 18% decrease in winter compared to summer, and the extent of seasonal changes in serum AMH correlated with the change in serum 25OH-D. More importantly, 1,25 dihydroxyvitaminD 3 supplementation blocked the seasonal variation of both AMH and 25OH-D levels [24]. The data of Nguyen *et al* in 2016 suggest that vitaminD doesn't have a direct impact on the levels of HMGB1 and Rage receptors [13].

## CONCLUSION

All of the women included in the study showed a low level of vitaminD and the lowest level appeared in the group between the ages of 55-65 in the postmenopausal stage. Besides, there may be a functional correlation between the level of vitaminD and the level of an HMGB1 protein that has emerged through the negative correlation between these two indicators, regardless of age.

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