Association of Anti-Mullerian Hormone and 25-Hydroxyvitamin D in women with low bone mineral density in Babylon, Iraq

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Abstract

**Background:** Decrease Anti-mullerian hormone and vitamin D was linked to increased risks of osteopenia and osteoporosis.

**Aim of the study:** To find any association of Anti-Mullerian hormone and 25-hydroxyvitamin D serum levels with bone minerals density test results in women.

**Material and Methods:** The study was design as a cross-sectional; it includes one hundred forty five females, their age range from 23-68 years. These females attended Rheumatology department of Merjan Medical City in Babylon /Iraq through the period from September 2017 till March 2018. Hormonal profile (anti-mullarian hormone, and vitamin D) were assessed; and minerals density of the bone was measured by dualenergy x-ray absorptiometry for all participants.

**Results:** The incidence of osteoporosis and osteopenia in our studied population was (31.72%, 23.45%) respectively and (44.83%) were with normal result of bone mineral density study. Significant positive correlation (p<0.05) was found between Anti-Mullerian Hormone and Vitamin D serum concentrations with bone minerals density study results.

**Conclusions:** Vitamin D serum levels significantly correlates with Anti-mullerian serum levels in patients with abnormal bone mineral density.

**Key Words:** Ovarian reserve, Anti-Mullerian Hormone, Vitamin D, Bone Mineral Density

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1. Introduction

The ovary is a dynamic organ that has most dramatic changes in structure and function of human tissue[1]. The biological and chronological age of the ovary is not constantly corresponding to each other, so it's vitally important to assess female reproduction potential [2, 3].Ovarian reserve (OR) is estimation of the ovarian follicles pool size and the oocyte quality, decrease of ovarian reserve with aging, leading to a decline of the reproductive function of women[4,5]. The commonly used biomarkers to assess OR is measurement of Follicular stimulating hormone (FSH), Estradiol hormone (E2) on day 3 of the cycle, Anti-mullarian hormone (AMH) and Inhibin B are other biomarkers of OR, in addition to Antral Follicle count (AFC) [6,7]. AMH is a glycoprotein and one of the transforming growth factors beta group [8], formed via granulosa cell (GC) of preantral and early antral follicles [9]. This hormone has an essential function in the follicle growth regulations [10]. AMH level and primordial follicles decrease with age [11-13].

Vitamin D is fat-soluble secosteroid-cholecalciferol[14]; it is called (sunshine-vitamin), because of its production in skin by the ultraviolet-B energy [15]. It is important for bone tissue mineralization: either directly or indirectly [16, 17]. Vitamin D biological actions are mediated by its receptor (VDR) [18], VDR is expressed in GCs and cumulus oophorus cells of the ovary[19]. Appearance of (VDR) in GCs indicates that vitamin D controlling the expressions and the action of main enzymes included steroid-genesis of females sex hormones [20].

Bone minerals density is a way for measuring calculating bone strength and what number of grams of calcium and minerals are packed into a bone section, and is supposed to be the typical measurement for the identification of osteopenia and osteoporosis [21], its best calculated by Dual energy x-ray beam absorptiometry (DEXA) at the lumbar spines and proximal femurs [22].

2. Aim of the study

To find any association of Anti-Mullerian hormone and 25-hydroxyvitamin D serum levels with bone minerals density test results in women bring up from Rheumatology department for DEXA unit in Babylon province.

3. Materials and methods

The study design was a cross-sectional study, accepted by the research ethical Team of University of Babylon, College of Medicine. Before enrollment, every participant signed an informed written consent about agreement to be enrolled in our study. One hundred forty five females were selected from 400 females who were referred to DEXA unit from the Rheumatology department of Merjan Medical city in Babylon city in Iraq; from September 2017–March 2018 of whom 60 females were menopause and 85 females were premenopausal.

3.1. Exclusion criteria:

1. Females without medical illnesses such as diabetes mellitus, hypertension, renal failure, and thyroid disease.
2. Females who had history of taking steroid, antihypertensive treatment and oral contraceptive pills.
3. Pregnant women.
4. Females with history of cancer.
5. Females with gastrointestinal diseases which restrict the absorptions of nutrients such as celiac and crohn's diseases.
6. Females with inflammatory or connective tissue diseases such as Rheumatoid arthritis and Systemic Lupus Erythematosus.

A questionnaire was used for accumulating demographic characteristics (age, residences, education, occupation, nutrition, smoking, drinks, sun exposure, parity, and age of menopause); Anthropometric measures including (weight, height for measuring BMI) were obtained.

3.2. Hormonal and biochemical analysis:

Blood samples were collected between 08:30-11:00 a.m. by venipuncture for every participant into (10) milliliter (ml) gel tubes; these samples were left for at least (15) minutes at temperature of the room prior to centrifugations at (3000) revolutions per minute (rpm) for (10) minutes for separating the serum. Two serum aliquots were stored at -20˚C for later measurements of AMH, and vitamin D levels. All serum was tested by Sensitive Enzyme Linked Immunosorbant Assay method (ELISA) in one run for each test within about three months of collection. The AMH kit used was from Ansh Lap; U.S.A. The minimal limit of AMH (ng/ml) is 1 and the maximum is 4. The vitamin D kit used was from CALBIOTECH; U.S.A. The minimal limit of vitamin D(ng/ml) is 30 and the maximum 100.

3.3. Bone Mineral Density Measurement:

Minerals density of the bone was assessed at the lumbar spine with (DEXA) by a technician according to the manufacturer's instructions. The patient should be wearing comfortable, loose clothing and should be avoiding garments that have belts, metal zippers, removing any things such as wallets or keys in the area of the scanning. The spine and hip which measured by the central DEXA analysis, the subject lies on an expanded bench, an x-ray producer is situated under the subject and an imaging device, is located above the subject. For spinal assessment, the subject's legs are held on an expanded box to flatten the pelvis and lower spine. For hip assessment, the subject's feet is located in a brace which rotated the hip inward, in the two circumstances, the detector is sluggishly passing above the region, images were generated on a monitor of a computer. The bone minerals density measuring test is typically finished from 10 to 30 minutes. A BMD measurement can be reported as a T-score which is a measure of the subject's BMD compared to healthy controls who are at the peak of their bone mass. Any score upward of (-1) is considered normal. Scores between (-1 and -2.5) denote osteopenia. Results less than (-2.5) suggest osteoporosis.

3.4. Statistical analysis

Statistical examinations supported by using SPSS version 20. Categorical variables expressed as frequencies and percentages. Continuous variables expressed as (Means ± SE). ANOVA test was used for...
compares means among three groups. Persian correlation was used for assessing the relation between two continuous variables. A p-value of $\leq 0.05$ considered as significant.

### 4. Results

#### 4.1. The distribution of study subjects with normal and abnormal bone mineral density according to DEXA results:

Majority of these females with abnormal bone mineral density (55.17%), while (44.83%) of study females were diagnosed as normal bone mineral density.

#### 4.2. Mean Differences of Study Variables According to DEXA Results:

Table (4-1) shows mean differences of study variables including (age of women, and BMI) according to DEXA results including (osteoporosis, osteopenia and normal bone mineral density). There was significant differences between age (Mean $\pm$SE) of the three groups ($p<0.05$). While there was no significant variances between (body mass index) of the three groups.

Table (4-1): Comparison of demographic data between subjects with bone minerals density results. Values are (Mean $\pm$SE). No. = 145.

<table>
<thead>
<tr>
<th>Study variables</th>
<th>Normal No.=65</th>
<th>Osteopenia No.=34</th>
<th>Osteoporosis No.=46</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>43.19 ± 1.51</td>
<td>45.35 ± 1.83</td>
<td>54.82 ± 1.55</td>
<td>$&lt;0.05^*$</td>
</tr>
<tr>
<td>BMI (Kg/m$^2$)</td>
<td>30.75 ± 0.62</td>
<td>30.67 ± 0.88</td>
<td>30.84 ± 0.78</td>
<td>$&gt;0.05$</td>
</tr>
</tbody>
</table>

#### 4.3. Comparison of hormonal levels between study groups according to bone mineral density results:

Table (4-2) shows mean differences of study variables including (AMH, and vitamin D) between study groups including (patients with osteoporosis, osteopenia and normal bone minerals density) according to bone minerals density results. There were significant differences between these study groups regarding to AMH, and Vitamin D serum levels ($p<0.05$).

Table (4-2): Comparison of hormonal serum levels (AMH and vitamin D) between study groups. Values are (Mean $\pm$ SE). Total no. =145.

<table>
<thead>
<tr>
<th>Study variables</th>
<th>Normal No.=65</th>
<th>Osteopenia No.=34</th>
<th>Osteoporosis No.=46</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMH (ng/ml)</td>
<td>00.53 ± 00.09</td>
<td>00.77 ± 00.17</td>
<td>00.26 ± 00.06</td>
<td>$&lt;0.05^*$</td>
</tr>
<tr>
<td>Vitamin D level (ng/ml)</td>
<td>34.23 ± 2.06</td>
<td>17.60 ± 1.30</td>
<td>10.77 ± 0.80</td>
<td>$&lt;0.05^*$</td>
</tr>
</tbody>
</table>

$\leq 0.05$, significant difference from corresponding values. AMH: Anti-mullerian hormone.

#### 4.4. The Correlations between Vitamin D with (AMH) in female patients with abnormal bone mineral density.
*P value ≤0.05, significant difference from corresponding values. (AMH: antimullerian hormone)

Figure (4-1): The Correlation between (Vitamin D and AMH) in patients with abnormal bone mineral density results. Values are Mean ± SE. (No. = 80).

Figure (4-1) shows the correlation between (Vitamin D and AMH) in females with abnormal bone mineral density. In our result, there was significant positive correlation between (Vitamin D with AMH) in females with abnormal bone mineral density (p<0.05).

4.5. Comparison of Anti-mullerian hormone and vitamin D serum levels between premenopausal and menopausal females with normal bone mineral density.

Table (4-3) shows the comparison of Anti-mullerian hormone and vitamin D serum levels between premenopausal and menopausal females with normal bone mineral density results. There were significant differences regarding Anti-mullerian hormone and vitamin D serum levels between premenopausal and menopausal females with normal bone mineral density results (p<0.05).

Table (4-3): Comparison of Anti-mullerian hormone and vitamin D serum levels between premenopausal and menopausal females with normal bone mineral density results. Values are Mean ± SE. (No. = 65).

<table>
<thead>
<tr>
<th>Study variables</th>
<th>Pre-menopause (no.=52)</th>
<th>Post-menopause (no.=13)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin D (ng/ml)</td>
<td>44.58±5.949</td>
<td>31.64±1.991</td>
<td>&lt;0.05*</td>
</tr>
<tr>
<td>AMH (ng/ml)</td>
<td>0.64±0.114</td>
<td>0.107±0.013</td>
<td>&lt;0.05*</td>
</tr>
</tbody>
</table>

*P value ≤0.05, significant difference from corresponding values. (AMH: antimullerian hormone).

4.6. Comparison of Anti-mullerian hormone and vitamin D serum levels between premenopausal and menopausal females with abnormal bone mineral density.

Table (4-4) shows the comparison of Anti-mullerian hormone and vitamin D serum levels between premenopausal and menopausal females with abnormal bone mineral density results. There were significant differences in Anti-mullerian hormone while no significant differences in vitamin D serum levels between premenopausal and menopausal females with abnormal bone mineral density results (p<0.05).
Table (4-4): Comparison of Anti-mullerian hormone and vitamin D serum levels between premenopausal and menopausal females with abnormal bone mineral density results. Values are Mean ± SE. (No. = 80).

<table>
<thead>
<tr>
<th>Study variables</th>
<th>Pre-menopause (no.=37)</th>
<th>Post-menopause (no.=43)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin D (ng/ml)</td>
<td>14.92±4±1.322</td>
<td>12.60±0.974</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>AMH (ng/ml)</td>
<td>0.835±0.149</td>
<td>0.184±0.069</td>
<td>&lt;0.05*</td>
</tr>
</tbody>
</table>

*P value ≤0.05, significant difference from corresponding values, AMH: anti-mullerian hormone.

5. Discussion

In our results, 145 females were examined by DEXA scan, of whom (55.17%) were diagnosed as abnormal bone mineral density and (44.83%) were with normal bone mineral density results. This is in accordance with other studies in Saudi, Syrian and Iranian women, [23-26]. While this is in discordance with Brazilian study, 36.5% of women had normal BMD, and 63.5% were diagnosed with abnormal BMD [27]. These variances can be explained by differences in populations studied (sample sizes, genetic background, age categories).

In our study, the females age were ranging from (23-68) years, this coincides with Mishra et al. study[28] as they included females at reproductive, and menopausal age. There was significant variances among age of the three groups (p<0.05) as illustrated in table (4-1). Montazerifar and his team found that there was a significant difference in the age of abnormal BMD and normal BMD subjects[29]. Oldness is a mainfactor that influences the OR and produces aging of the ovary; age reduces fertility level [3]. Bone mineral density (BMD) decreases with age, especially in women after menopause because of changes in sex hormone levels [30]. Otherwise Chawla and his team concluded that, depleted BMD is not a condition restricted to menopausal females only. It is broadly predominant in females over 40 years old in the lack of risk features[31]. Although BMI was not found to be significant in this result, table (4-1), this is in accordance with Unni and his team [32]. Possibly a small sample size clarifies why this results not noticed in our and their reassessment. While Kim et al., and Naz et al. from Korea and Iran have emphasized the relationship concerning BMI and BMD mostly recognized as the increasing capacity of additional weightiness on bones, leading to elevated bone creation and decreased bone loss.[33, 34]

There was significant differences between study groups regarding Vitamin D serum levels (p<0.05) as shown in table (4-2). Many studies have shown the same result with ours [35-37]. Vitamin D serum concentrations found to be significantly related with (BMD) results [31]. Through its direct and indirect effect, indirect influences, by controlling the intestinal calcium and phosphate absorptions and calcium reabsorptions in the kidney[38, 39]. Direct influences are directed to osteoblasts to increase differentiation and mineralization [40-42]. Other studies demonstrated that serum vitaminD had no significant relation to BMD status [43-48]. These variances might be due to differences in population, age group and the vitamin D levels used to define deficiency and insufficiency in different studies.

There was significant differences between study groups regarding to AMH serum levels (p<0.05) in this study as shown in table (4-2). Karlamangla and his team agree with this result [49]. BMD loss is normal with increasing age in women but the degrees of deterioration differ between females. (AMH) is usually assessed in fecundity health centres to measure OR[50], (AMH) concentrations also drop with aging [51].

Significant correlation was found between (Vitamin D and AMH) in patients with abnormal bone mineral density (p<0.05) as shown in figure (4-1). This relationship bases on the occurrence of each vitamin D receptor and 1α-hydroxylase enzyme in female reproductive organs[52]. A number of analyses demonstrated a positive correlation between vitamin D concentrations with anti-Müllerian hormone (AMH). As Vitamin D supplements prevented the periodic variations in AMH concentrations, concluding that AMH productions may be controlled by vitamin D.[53-55]. Other study found no periodic variation in AMH [56]. Some evidence that little 25(OH)D (<30ng/mL) accompanied with low AMH [57]. Although preceding analysis reported an influence of vitamin D on AMH at each cellular and serum concentrations[53, 58, 55], at the levels of cell, Malloy et al. found that the promoter region for the AMH gene has a domain for vitamin D response element (VDRE). Vitamin D, by this VDRE, directly modifies AMH expressions[59]. The gene for AMH has a VDRE, which suggests that vitamin D might control AMH expressions [58]. In other result, a reverse relation occurred in follicular fluid 25(OH)D concentrations and AMH receptor II mRNA gene expressions. Its deficit must be measured as soon as using AMH concentrations for medical analysis[53]. Human studies of AMH and vitamin D concentrations also measured significant differences [50].
have shown no correlation[60-64]. These studies with no association sampled women from fertility clinic populations only. The results from medical analysis demonstrate the requirement for other research to estimate the influence of vitamin D on AMH concentrations. In our result there was significant difference in vitamin D serum levels between premenopausal and menopausal females with normal bone mineral density results (p<0.05) as shown in table (4-3). This estimated that post-menopausal females are a major concern for vitamin D assessment, as they are predisposed to decrease bone density associated with reducing levels of estrogen [65]. While there was no significant difference in vitamin D serum levels between premenopausal and menopausal females with abnormal bone mineral density results (p<0.05) as shown in table (4-4) which might be due to small sample size. In this study there was significant difference regarding Anti-mullerian hormone serum levels between premenopausal and menopausal females with normal bone mineral density results (p<0.05) as shown in table (4-3), and there was also significant difference regarding Anti-mullerian hormone serum levels between premenopausal and menopausal females with abnormal bone mineral density results (p<0.05) as shown in table (4-4). Other studies found same result that AMH levels Predicted menopause which could emphasize the need for prevention of bone demineralization [66, 67].

6. Conclusion

Vitamin D serum levels significantly correlated with AMH serum levels in patients with abnormal bone mineral density.

Suggestions

1. Multicenter, bigger sampling analysis is needed to approve these initial outcomes.

2. Checkup the patients each 6 months for two years to estimate the alterations which will be happen afterward proper management.

Limitations:

A numerous limits were found in the present work (cost, sample size, and time were limited), which could cause inadequate statistical rule for analysis of some variables and limited reproducibility of the results.

Acknowledgements

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Author's contribution:

HAJ. did the study design, revision of manuscript and data analysis. NAK. did the patients consultation, hormonal analysis and the manuscript preparation.

Conflict of Interest: The authors declare that there are no conflicts of interest

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