INTRODUCTION

Vitamin D3 is found in several types of foods, and most of it is made from dehydrocholesterol-7 in the dermis of the skin when exposed to ultraviolet (UVB) sunlight, then the enzyme 25-hydroxylase (CYP2R1) located in the liver convert vitamin D to 25-hydroxycholecalciferol (25-OH D3). Vitamin D has a role in bone mineralization and calcium hemostasis by mineralization of the collagen matrix and many effects outside the skeleton 1. Autoimmune diseases arise from the abnormal activation of the immune system by losing immune tolerance and producing autoantibodies such as anti-glutamic acid decarboxylase autoantibody in diabetes mellitus type 1 2,3. Vitamin D plays in regulating several immune activities, such as anti-oxidant, anti-inflammatory, and anti-fibrosis, many studies in the last decade have increased interest in finding a relationship between the level of vitamin D and the emergence of autoimmune diseases. Studies have found an inverse relationship between vitamin D levels and the extent of development of these diseases in several countries around the world, such as lupus erythematosus, multiple sclerosis, ulcerative colitis, common psoriasis, and RA 4,5,6. The RA is considering an autoimmune disease with an age-related incidence that affects 1% of adults and the prevalence increasing with age to reach 2% of the population characterized by a chronic systemic disease involving joints and other vital organ morbidity and mortality significantly reported 5,7.

Vitamin D or 25 (OH) D may have an immunological role in the development or emergence of RA from its effect on the gene expression process of pro-inflammatory cytokines 8,9.

Although several studies confirm the role of vitamin D as a factor in the emergence of autoimmune diseases, additional data is needed to support these findings 2, so the present study aimed to partially fill the gap of data related to vitamin D abnormality (either deficiency or insufficiency) in RA individuals in Babylon province.

MATERIAL AND METHODS

STUDY DESIGN AND POPULATION

All standard cases had been studied in the center in a private OZONE medical center of rheumatology and physical treatment, Babylon province, Iraq. The ethical approval of all patients was obtained to use their demographic and clinical properties data in the present study. According to inclusion/exclusion criteria of this study only 30 RA patients (19 females, 11 males) matched demographically with 30 healthy individuals, all of them were subjected to the study between December 2018 and March 2019. The venous blood samples were obtained for all participants in the present study and centrifuged serum was collected and stored at -20°C for the immunological detection of vitamin D (25OH D) analyses.

IMMUNOLOGICAL DETECTION OF VITAMIN D

The serum samples of all study groups were subjected to an automated quantitative test kit (60 test) for determination of 25-hydroxyvitamin D Total from VIDAS® (bio Mérieux, French) depending on the principle of ELFA (Enzyme-Linked Fluorescent Assay) technique 10. All study groups, were categorized according to age into three subgroups 16-35, 36-55, 56-75; and categorized according to vitamin D
serum level; deficiency (<20 ng/mL), insufficiency (20–30 ng/mL), and normal (>30 ng/mL) 11.

**STATISTICAL ANALYSIS**

The statistical analyses had been carrying out by using SPSS Software for Windows version 16.0 (SPSS Inc., Chicago USA, IL, USA). The associations between RA patients and control according to demographic data and four categories of vitamin D serum levels were calculated and the differences were considered significant when p-value <0.5.

**THE RESULTS**

### Table (1): The demographic characteristics RA patients and control

<table>
<thead>
<tr>
<th>Item</th>
<th>RA patients n=30</th>
<th>Control n=30</th>
<th>Chi-square</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years, mean ±SD (range)</td>
<td>47.7±12.06 (52)</td>
<td>47.03±12.39 (44)</td>
<td>--</td>
<td>0.83</td>
</tr>
<tr>
<td>Sex, Male (%) / Female (%)</td>
<td>11(36.6)/19(63.4)</td>
<td>12(40)/18(60)</td>
<td>0.0686</td>
<td>0.7934</td>
</tr>
<tr>
<td>BMI, kg/m² mean ±SD</td>
<td>37.08±4.6714</td>
<td>33.506±4.6059</td>
<td>--</td>
<td>0.004</td>
</tr>
</tbody>
</table>

**VITAMIN D STATUS IN THE STUDY POPULATION**

The serum levels of vitamin D were evaluated for RA and control, the level of vitamin D means ±SD for the age group (16-35) was 9.25±1.89, 22.83±9.78 for RA patients and control group respectively, and show significant differences (P=0.014) between both study groups. For the age group (36-55) The serum level of vitamin D mean ±SD was 19.77±11.98, 35.5±13.67 for RA patients and control group respectively, it revealed highly significance (P<0.0016) between both study groups. In the age group (56-75) The serum level of vitamin D mean ±SD was 17.63±8, 36.5±10.34 for RA patients and control group respectively, there was high significance (P<0.001) between both study groups. The present study does not reveal any significances between RA patients and control according to mean ±SD of serum level vitamin D in three categories deficiency; insufficiency, and normal level as reported in (table.2). The number frequency of the two study groups was appeared significant (P< 0.0001, P=0.0003) in both categories; deficiency and normal vitamin D respectively, while insufficient vitamin D did not reveal significant differences between both RA and control. The overall serum level means ±SD was 17.63±10.61, 33.3±12.73 for RA patient (n=30) and healthy control (n=30) respectively, and appeared high significance (P=0.0001).

**CHARACTERISTICS OF THE STUDY POPULATION**

The demographic properties (age, sex, and BMI) of the study population (RA patients and control) are shown in table (1). The age (years) mean ±SD were 47.7±12.06, 47.03±12.39 for RA patients and control respectively. The sex of most RA patients was female 19(63.4%). In this study, age and sex had been matched between two study groups, and no significant differences (P=0.83). The BMI (kg/m²) mean ±SD was 37.08±4.6714, 33.506±4.6059 for RA and control respectively. The RA patients were highly obese and different significantly from the control group (P=0.004).

### Table (2): Evaluation of vitamin D in RA and control according to three categories of serum level value; Deficiency, Insufficient and Normal

<table>
<thead>
<tr>
<th>Age group (year)</th>
<th>Study group</th>
<th>Deficiency of Vitamin D ng/mL</th>
<th>Insufficient of Vitamin D ng/mL</th>
<th>Normal of Vitamin D ng/ml</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>16-35 N(mean±SD)</td>
<td>RA</td>
<td>4(9.25±1.89)</td>
<td>--</td>
<td>--</td>
<td>0.014</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>2(11.5±4.94)</td>
<td>2(25±4.24)</td>
<td>2(32±1.41)</td>
<td></td>
</tr>
<tr>
<td>P-value</td>
<td></td>
<td>0.43</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>36-55 N(mean±SD)</td>
<td>RA</td>
<td>10(11.1±3.24)</td>
<td>4(23.7±2.36)</td>
<td>4(37.5±9.25)</td>
<td>0.0016</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>2(15.5±4.94)</td>
<td>2(27.5±2.12)</td>
<td>1(41.1±11.35)</td>
<td></td>
</tr>
<tr>
<td>P-value</td>
<td></td>
<td>0.13</td>
<td>0.13</td>
<td>0.585</td>
<td></td>
</tr>
<tr>
<td>56-75 N(mean±SD)</td>
<td>RA</td>
<td>6(12.83±2.48)</td>
<td>--</td>
<td>--</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>--</td>
<td>3(26±3)</td>
<td>7(41±8.86)</td>
<td></td>
</tr>
<tr>
<td>P-value*</td>
<td></td>
<td>&lt;0.0001</td>
<td>0.5196</td>
<td>0.0003</td>
<td></td>
</tr>
<tr>
<td>Total N=60(%)</td>
<td>RA</td>
<td>20(66.6)</td>
<td>5(16.6)</td>
<td>5(16.6)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>4(13.3)</td>
<td>7(23.3)</td>
<td>19(63.3)</td>
<td></td>
</tr>
</tbody>
</table>
DISCUSSION

The current study showed that patients with rheumatoid arthritis had been lower vitamin D serum values than healthy controls, and indicated a negative correlation between vitamin D serum values and activity of RA disease, this evidence supported by many meta-analyses studies worldwide. The anti-inflammatory action for the vitamin D in synovial fluid and reverse serum level with C-reactive proteins may explain the importance of this vitamin in the progress of RA disease in those that suffering deficiency in vitamin D. The demographic characteristic; age, sex, and BMI RA and control matched between two study groups according to inclusion to minimize the differences (no significance) between RA patients and healthy control and reveal accepted results. It was found a positive relationship between age and onset of RA disease which improved with previous studies. It was reported in (table.1) the mean age of 47.7 of all RA participants and the onset of disease activity for most RA patients were over 40 years. The change related to disease activity registered in Twenty-eight joints DAS28 over age may return to different activation of markers, CD25, CD69, CD95, and HLA-DR, on the CD4 T cell surface. The sex ratio (2:1) between female and male explain the activity of RA in female (63.3%) more than male (36.6) as shown in table (table.1), this fact variable between many studies but most of them insure the high prevalence of female other than male and some studies include only female sex, this difference may return to activity of sex hormones such as estrogen, progesterone, and androgens which influencing the preclinical phases and onset of RA in the female by affecting on induction or subversion of many immune cells. The BMI of RA patients in the current study (table.1) A positive association has been demonstrated between obesity and most rheumatoid arthritis cases as many studies mentioned and explained this state which affects not only RA disease onset, also effect on the activity of biological therapy (antitumor necrosis factor alfa Anti-TNF) of RA patients. The previous worldwide studies by, in addition to recent local studies in Iraq by reported the association between BMI and female sex in decreasing vitamin D value in the onset of many autoimmune diseases which support findings of the present study. The current study found out significant differences between patients who are suffering from RA and healthy control number frequency according to deficiency of vitamin D serum level (table.2), also, low serum level of RA significantly suggests the potential role for the vitamin D in the maintenance of immune homeostasis according to the expression of vitamin D receptors on immune cells. Also, the Utilization of vitamin D supplements appeared in the improvement of rheumatic therapy. Although several studies confirm the present lack of vitamin D in certain patients who suffered from rheumatoid arthritis, this deficiency may vary according to seasons.

CONCLUSIONS

The present study concluded that lack of vitamin D is one of the predisposing factors related to increasing RA incidence especially in female forty-year age and obesity BMI and many studies required to understand vitamin D mechanisms on genetically, demographically, and environmentally onset of RA.

ACKNOWLEDGMENTS

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REFERENCES

Evaluation Of Vitamin D Level In Serum Blood Of Rheumatoid Arthritis Patients In Babylon Province


