serum 25-hydroxy vitamin D Correlation with the activity of disease and level of inflammatory cytokines in rheumatoid arthritis patients in the north of Iraq

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Abstract:

Objective This work aimed to show the relation vit.D with inflammatory cytokines in patients with rheumatoid erythrites in the north of Iraq.

Methods. ELISA was used to calculate the amount of vit.D IL-17, IL-10, IL-6, IL-4, IL-1, and TNF in the blood of 40 healthy patients and 100 rheumatoid arthritis patients. DAS was used to assess the activity of the disease.

Results. The differences in BMI, age, and gender were absent in the RA patients and these patients had lower levels of vit D than the control patients and had a positive correlation with anti-inflammatory cytokines ( IL-4, IL-10) and a negative correlation with inflammatory cytokines IL-17 IL-6, IL-1.

Conclusion. Patients with RA who had a low level of vit.D in serum are shown to be adversely correlated with pro-inflammatory cytokines which may suggest that patients with low vit.D levels are more likely to have RA.

Keywords: cytokines disease activity, 25-hydroxyvitamin D, arthritis, rheumatoid

Introduction

Between 0.5 to 1 % of the global population is affected by rheumatoid erythrites which is a type of autoimmune disease (Alamanos et al., 2005). If left untreated, persistent synovial inflammation develops, which may result in joint injury, systemic complications, progressive disability, or death (Heidari et al., 2011). People with RA are more prone to cardiovascular complications than healthy people, (Zegkos et al., 2016). anti-citrullinated and rheumatoid factor (RF) are common Autoantibodies often found in patients with rheumatoid arthritis (Rantapää-Dahlqvist et al., 2003). The etiology of rheumatoid arthritis is unknown. calcium-phosphate balance and the maintaining of healthy bones and body requires the presence of Vit.D (Audran et al., 2010). Additionally, vitamin D’s extraskeletal effects include immune system modulation (pleiotropic effects). rheumatoid arthritis. Calabresi and associates (2018) Cardiovascular disease (CVD), inflammatory bowel disease (Crohn’s disease), osteoarthritis, infections, obesity, insulin resistance, type 2 diabetes, and connective tissue disorders are all examples of connective tissue illnesses which are all connected to the Vit.D deficiency, including. a high number of pro-inflammatory cytokines are generated by Activated immune cells and synovial fibroblasts, these cytokines play a critical role in the development and the progression of RA (McInnes et al., 2007).
other inflammatory mediators in synovial tissue are controlled by a primary proinflammatory mediator the TNF-cytokine (Brzustewicz et al., 2015). Additionally, it promotes bone and cartilage degradation by activating chondrocytes and osteoclasts (Vasanthi et al., 2007). IL-1 and IL-6 are two more cytokines that have a role in the development of rheumatoid arthritis. IL-17F and IL-17A are Th17-specific cytokines. Additionally, these cytokines are involved in the production of matrix metalloproteinases (MMPs), osteoclast differentiation, chemokines, inducible nitric oxide synthase, and cell adhesion molecule expression (Mateen al et, 2016). compensatory reaction develops Due to the presence of anti-inflammatory cytokines such as IL-4 and IL-10 in the RA synovium, (Cicuttini et al., 1995).

In vitro, vitamin D promotes monocyte differentiation into macrophages while suppressing pro-inflammatory cytokine production and restricting antigen delivery to lymphocytes via decreasing MHC-II expression on the cell surface (15). Vitamin D also inhibits T cell and monocyte proliferation and activation while increasing the production of anti-inflammatory cytokines such as IL-10.

Examine the quantifiability of 25-hydroxyvitamin D and inflammatory cytokine levels in RA patients to see whether there is a link between the two. Additionally, these measures were evaluated across a range of severity levels in individuals. There is just one study that we are aware of that establishes a link between inflammatory cytokines and 25-hydroxy vitamin D levels in northern Iraq.

**Patients and Methods:**

We conducted case-control research. All (100 RA patients) were recently diagnosed between November 2019 and April 2021, using the 2010 ACR/EULAR diagnostic criteria for RA (Neogi et al., 2010), and were recruited from the Rizgary teaching hospital’s rheumatology department. rheumatoid arthritis Patients who have a concurrent autoimmune illness or viral hepatitis are excluded. None of the rheumatoid arthritis patients or controls had previously taken vitamin D. The Research Advisory Committee and Institutional Ethics of the Faculty of Medicine, Hawler medical university/college of medicine, Erbil, Iraq, authorized the research.

Venipuncture was used to get aseptic peripheral venous blood samples into sterile vacutainers. Each participant in the present research had a liver, blood count (CBC), renal function tests, and (ESR) erythrocyte sedimentation rate Following that, total serum 25 OH vitamin D analysis was performed on VIDAS using enzyme-linked fluorescence assay (ELFA)

the vitamin D normal range defined by The (IOF) International Osteoporosis Foundation's Nutrition as follows: more than 100 nmol/L is possibly dangerous, ≥75 nmol/L (≥30 ng/ml) is normal, 50 to 75 nmol/L, (20–30 ng/ml) is insufficient and below 50 nmol/L (below 20 ng/ml) is inadequate,

The plasma was utilized to detect TNF- and IL-1 levels (KOMA BIOTECH INC.), according to the manufacturer’s procedure. TNF-, IL-10, IL-4, IL-1 IL-6, and IL-17 A levels were estimated using human ELISA kits following the procedure of the manufacturer. The sensitivity of detecting TNF-α, IL-1β, IL-4, IL-6, IL-10, IL-17A by the ELISA kits for was 5.5pg/ml, 1pg/ml, < 2.2pg/m, < 2pg/ml, < 5pg/ml, < 2.3pg/ml respectively.
Disease severity measurement:

28-Joint Count disease activity score (DAS28) was utilized to determine the severity of the disease. It is based on the (ESR) erythrocyte sedimentation rate number of the painful and swollen joints, and a visual analog scale (VAS) of a patient’s health evaluation (Prevoo et al, 1995). DAS 28 ≤ 3.2 DAS 283.2 indicates a mild illness severity, DAS 28 > 3.2 and ≤ 5.1 indicates a moderate disease severity and DAS 28 > 5.1 indicates severe disease.

Results:

This research included 100 rheumatoid arthritis patients aged (47.4 ± 2 years). All of the patients were freshly diagnosed. The control group comprised 40 healthy people (46.6 ±10.4) of same-sex and age. mean of body mass index (BMI) was calculated for patients which was 23.4±1.21, compared to 23.3±1.35 in controls. As demonstrated in the variations in sociodemographic factors such as BMI, gender and age, were statistically insignificant between controls and patients (Table 1).

Table 1: socio-demographic comparisons were made between the control group and RA patients.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Control</th>
<th>RA-pt.</th>
<th>P value</th>
<th>Variables</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean (+, - SD) Years</td>
<td>46.6(± 10.4)</td>
<td>47.4(± 10.2)</td>
<td>0.102</td>
<td>Age, mean (+, - SD) Years</td>
</tr>
<tr>
<td>Height</td>
<td>162.8( ±5.4)</td>
<td>160.9 (± 5.1)</td>
<td>0.052</td>
<td>Height</td>
</tr>
<tr>
<td>Weight</td>
<td>57.15( ± 4.12)</td>
<td>58.5 (± 4-5)</td>
<td>0.103</td>
<td>Weight</td>
</tr>
<tr>
<td>BMI</td>
<td>23.1(± 1-35)</td>
<td>23.4(± 1-21)</td>
<td>0.202</td>
<td>BMI</td>
</tr>
<tr>
<td>ESR</td>
<td>13.1(±4.42)</td>
<td>37.8(± 8.4)</td>
<td>0.001</td>
<td>ESR</td>
</tr>
<tr>
<td>Vit. D3 level</td>
<td>39.95(± 9.8)</td>
<td>16.85(± 8.7)</td>
<td>0.001</td>
<td>Vit. D3 level</td>
</tr>
</tbody>
</table>

Regarding ESR in the RA, the group was (37.8±8.4), while in the control group was (13.1±4.42), a statistically significant difference was realized as shown in table no.1.

Lower levels of Vitamin D are correlated with RA patients (16.85 ± 8.7) than in controls (39.95 ± 9.8) (Table 1). Only ((20 %) of controls had vitamin D insufficiency and (61 %) of RA patients; this variation was statistically significant (p = 0.001). In this research, rheumatoid arthritis was negatively correlated with 25-hydroxyvitamin D. as shown in fig. 1,
Regarding the level of cytokines among the participants: there is a significant difference between the level of proinflammatory cytokines like IL-17, IL-6, IL-1, and TNF in RA. In patients compared with the normal control group, anti-inflammatory cytokines (IL-4, IL-10) levels were higher in RA. Patients compared with the normal control group. As shown in table (2).

Table 2: Comparison of anti-inflammatory cytokines and pro-inflammatory between RA patients and a control group.

<table>
<thead>
<tr>
<th>Level of cytokines</th>
<th>Control</th>
<th>RA-patient</th>
<th>P-value</th>
<th>Level of cytokines</th>
<th>Control</th>
<th>RA-patient</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1 (≤6-7)</td>
<td>4.3 ± 2.6</td>
<td>23 ± 9.43</td>
<td>0.001</td>
<td>IL-1 (≤6-7)</td>
<td>4.3 ± 2.6</td>
<td>23 ± 9.43</td>
</tr>
<tr>
<td>IL-6 (≤20 p 9 /ml)</td>
<td>1.14 ± 0.7</td>
<td>4.56 ± 2.1</td>
<td>0.001</td>
<td>IL-6 (≤20 p 9 /ml)</td>
<td>1.14 ± 0.7</td>
<td>4.56 ± 2.1</td>
</tr>
<tr>
<td>IL-17 (≤1-4 p g /ml)</td>
<td>0.9 ± 0.81</td>
<td>5.3 ± 3.52</td>
<td>0.001</td>
<td>IL-17 (≤1-4 p g /ml)</td>
<td>0.9 ± 0.81</td>
<td>5.3 ± 3.52</td>
</tr>
</tbody>
</table>

Patients were then subdivided, based on the level of vitamin D, into the low vit.D and normal Vit.D groups. The severity of the disease and the level of anti-inflammatory and proinflammatory cytokines were compared in both groups. The mean DAS-28 was higher in the low vit.D group compared to the normal Vit.D group as shown in figure number 2.

Figure 2: Vitamin D levels in RA patients and the disease activity

low vitamin D group had a higher level of proinflammatory cytokines than the group with the normal vitamin D, normal vitamin D group had a higher level of cytokine IL-4 than in the group with the low vitamin D, but IL-10 was not affected by the normal vitamin D group, as shown in table 3.

Table 3: Comparison of anti-inflammatory cytokines in RA patients and pro-inflammatory according to the level of Vitamin D.
catalystines | IL – 1 | 10.4 ± 5.8 | 21.4 ± 7.33 | 0.001 | IL – 6 | 2.09 ± 1.95 | 5.36 ± 2.91 | 0.001 | IL – 17 | 1.87 ± 1.3 | 4.97 ± 1.87 | 0.001 | TNF | 9.25 ± 2.16 | 20.15 ± 5.45 | 0.001 | IL – 4 | 5.28 ± 1.35 | 1.94 ± 1.2 | 0.001 | IL – 10 | 4.2 ± 1.76 | 3.7 ± 0.86 | 0.093

Discussion

As is the case in many other Middle Eastern nations, Vitamin D deficiency is common in northern Iraq, particularly among women. The current research discovered that 77% of females had rheumatoid arthritis and 70% of females have control. Although they live in sunny regions, these conditions may be caused by inadequate skin exposure to sunshine (traditional clothing, insufficient dietary intake of vitamin D) and ethnicity, additionally, Theres one possible explanation for vitamin D deficiency among nations is latitude. While the prevalence of rheumatoid arthritis rises with latitude (Ponsonby et al, 2005). Nevertheless, vitamin D levels are low in low latitudes (Lee et al, 2016). It was previously believed that vitamin D could not be synthesized via exposure to sunshine during certain winter months. We compared rheumatoid arthritis patients to healthy controls in the present research and discovered that patients with rheumatoid arthritis had substantially lower blood vitamin D levels. This conclusion is consistent with other research results, such as a VD deficiency in a sample of people with rheumatoid arthritis was correlated with the cross-sectional study that examined blood VD levels (Grazio et al., 2015).

Another research on Caucasian women showed that RA patients had lower levels of VD than controls (Brance et al., 2015). A recent meta-analysis showed an inverse association between serum VD levels and disease severity ratings in RA patients and healthy controls (Davies et al, 2016).

Another Iranian study discovered an inverse connection between blood VD levels and rheumatoid arthritis severity of 93 RA patients and 31 healthy controls, suggesting that VD supplementation should be used in conjunction with other established rheumatoid arthritis treatments (. Rajaee et al, 2017). Additionally, an inverse connection between blood VD levels and susceptibility to RA was discovered in the Turkish population (Senel et al, 2012).

Numerous more investigations, particularly those conducted in Egypt, discovered no statistically significant difference between rheumatoid arthritis patients and controls (Sabbagh et al, 2013). Additionally, this was verified by (Raczkiewicz et al., 2015).

Our study also showed an inverse relationship between pro-inflammatory cytokines and vitamin D3 and disease severity, which has been confirmed in many other studies. A recent meta-analysis of 963 controls and 1,143 RA patients showed the same negative association between disease severity and blood VD levels (Bae et al.,2016). A research of 894 RA patients and 861 healthy controls found an inverse relationship between blood VD levels and disease activity (Cecchetti et al., 2016). Researchers found a substantial VD deficit in 4,793 Japanese rheumatoid arthritis patients, as well as an inverse association between clinical symptoms and VD levels (Furuya et al., 2013).

A similar relationship was discovered between the serum VD levels and disease activity score Numerous other investigations found an inverse relationship between illness severity and VD levels in a similar fashion (Kostoglou-Athanassiou et al., 2012).
immune-mediated responses require Vitamin D for the proper control. Another study found that Vitamin D is necessary for the regulation of the adaptive and innate immune systems, according to Schwalfenberg and colleagues (Cantorna et al., 2010). VD affects both the innate and adaptive immune systems, primarily via toll-like receptors (TLRs) and T-cell development, particularly T17 cells, which have a role in the pathogenesis of RA (RA) (Higgins et al., 2013). VD affects how immune cells are regulated and differentiated. It is responsible for regulating the synthesis and secretion of autoantibodies by B cells (Heidari et al., 2012). By triggering apoptosis in activated B cells, it inhibits B-cell growth and differentiation (Ritterhouse et al., 2011). VD decreases T-cell proliferation and the generation of pro-inflammatory cytokines such as IL2, INF-γ, and TNF (Ranganathan et al., 2009).

By contrast, VD is focused on achieving an optimal balance of T1 and T2 cells to inhibit the autoimmune response mediated by T cells. This is accomplished by regulating the generation and activation of CD4+ T cells. Additionally, it prevents antigen representation (Szekely et al., 2012). VD enhances regulatory T cell activity to counteract the effects of autoreactive T cells (Luo et al., 2009).

VD has been shown to inhibit estrogen synthetase function, thus diminishing estrogen’s ability to stimulate the immunological response in synovial tissue. As a result, it is now possible to regulate the autoimmune reaction (Villaggio et al., 2012).

The majority of cells that produce TNF express VDR (activated macrophages, fibroblasts, monocytes, natural killer cells, and mast cells). VD levels may influence VDR binding to target sequences in the TNFA gene’s upstream regulatory regions, thus regulating TNFA mRNA production. However, VD levels are unrelated to the stability of TNFA mRNA. As a result, VD exerts transcriptional regulation over TNF (Hakim et al., 2003).

A variety of cytokines production can be inhibited by VD has, it has the reverse impact on IL-10 and IL-4, increasing their synthesis (Correale et al., 2009). Numerous studies have shown that 1,25 (OH) 2D3 increased T2 synthesis by CD4+Mel14+ T cells, resulting in increased production of IL-4 and IL-10.

**Questionnaire and data collection:**

The researchers conducted an interview and used a questionnaire they developed to gather the data. The questionnaire included many questions covering sociodemographic information, educational attainment, risk factors such as smoking, infection, and alcohol use, any history of vitamin D intake, and any chronic condition such as kidney, liver, or diabetes.

The ethical committee at Hawler medical university granted permission, and the Erbil directorate of health provided a letter of facilitation (DOH). This research was performed with the informed verbal permission of all patients before their participation. Each participant was thoroughly informed of the study’s aim.

IBM SPSS 20.0 was used to analyze the statistical data. Statistical Percentages and numbers are considered categorical variables, while standard deviation and mean are considered continuous variables. 100 rheumatoid arthritis patients and 40 healthy controls were evaluated using a 5% significance level. The study has a 99.98 percent power and an effect size of 1.125. Comparing groups using quantitative data that is given regularly. The Student t-test was used for normally distributed data, while the Mann-Whitney test was used for non-normally distributed data. To assess qualitative variables, the Chi-square, Monte Carlo, and Fisher’s exact tests were employed. The receiver operating characteristic (ROC) curve was used to evaluate VDR gene expression as a predictor of rheumatoid arthritis. All results were interpreted at a significance level of 5%.
References


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