PREVALENCE OF 25-HYDROXY VITAMIN D DEFICIENCY AND SOME BIOCHEMICAL PARAMETERS IN IRAQI PATIENTS WITH RHEUMATOID ARTHRITIS AND THEIR ASSOCIATIONS WITH DISEASE ACTIVITY

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ABSTRACT

Objectives: The objectives of the study were to evaluate changes in 25(OH) Vitamin D levels and some biochemical parameters in rheumatoid arthritis (RA) patients compared with healthy controls and assess the correlation of 25-hydroxy Vitamin D, calcium, magnesium, and disease activity. Study the effects of anti-RA drugs on these biochemical parameters and also the role of supplements calcium and 25-OH Vitamin D in RA patients.

Methods: This study conducted between 60 patients for RA and 20 healthy controls according to the American College of Rheumatology standards in 2010. In this study, 25-hydroxy Vitamin D was measured using an enzyme-linked immunosorbent assay, and also some biochemical parameters were measured with a spectrophotometer (Humalyzer 2000).

Results: Serum 25(OH) Vitamin D, calcium, magnesium, and albumin levels were significantly lower in RA patients compared with healthy controls. Serum alanine aminotransferase aspartate aminotransferase levels were significantly increased in RA patients compared with healthy controls. The correlation was non-significantly among 25-hydroxy Vitamin D and clinical disease activity index (CDAI), while the results showed significantly inverse correlation calcium and magnesium concentrations with CDAI.

Conclusion: 25-OH Vitamin D, calcium, albumin, and magnesium deficiency appear to be widespread in patients with RA. Thus, biochemical changes in RA are reflected in the pathogenesis of RA. Furthermore, in these results, there is no relationship between Vitamin D and the disease activity, while there is a relationship between calcium and magnesium with disease activity.

Keywords: Rheumatoid arthritis, 25-hydroxy vitamin D, Calcium, Magnesium, Albumin, Aspartate aminotransferase, Alanine aminotransferase.

INTRODUCTION

Rheumatoid arthritis (RA) is serious and threatens to health worldwide, where RA is considered a pathological condition that attacks the immune system and affects the articular membrane leads to the secretion of cytostatic glycoproteins that cause cartilage erosion and osteoporosis adjacent to the affected joint [1]. Vitamin D is one of the main vitamins that soluble in fat [2,3]. Given the important roles of Vitamin D at the human corpus including maintaining healthy bone, activates the body’s immune system, prevents osteoporosis, and increases bone density, and promotes the absorption of minerals and calcium from the intestine [4]. Recently, Vitamin D deficiency has become difficult to ignore, where the previous studies have shown that long-term Vitamin D deficiency causes RA, cancers, obesity, and affects the work efficiency of the insulin [5-7]. Calcium has benefits in bone building and its role in the health of the muscles, heart [8], but the role of calcium is not yet clear in RA, perhaps this is due to the changes occurring in the bone as a result of affected by RA [9]. Magnesium (Mg) is an essential mineral needed by the body to perform its functions [10]. Magnesium has multiple functions in the body, including contributes to bone formation, supports the immune system, and increases its ability to resist diseases [11]. Albumin (Alb): Is one of the essential proteins found in the blood. Albumins are produced in the liver and stimulation by hormones such as cortisol, insulin, and growth hormone [12,13]. The aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are enzymes found at the cells of the body, especially in the liver and also found in the heart, kidney, brain, pancreas, red blood cells, and muscle tissue. High ALT and AST levels are mainly associated with liver damage or body tissue, so when the liver cells or body tissues to are exposed to damage, the higher rate of ALT and AST is released into the bloodstream [14].

METHODOLOGY

Study design
The present study was conducted at Baghdad Teaching Hospital/City of Medicine from September 2018 to January 2019. This study included 80 blood samples, 60 of which were Iraqi patients with RA (49 females and 11 males), and 20 samples for healthy controls (16 females and four males). Patients with RA were diagnosed by consultant rheumatologists; according to American College of Rheumatology (ACR) standards in 2010, information related to RA disease was collected such as gender, age, duration of disease, type of treatment, and measurement disease activity by clinical disease activity index (CDAI).

This study was approved by the ethics committee before work begins.

Sample collection
Five milliliters of blood were withdrawn from RA patients and healthy controls. The blood sample was placed at a gel tube and left at 25°C until blood clotted and then placed in a centrifuge at 3000 rpm for 10 min. After that, the serum was placed at small tubes (Eppendorf) and stored at −40°C. Enzyme-linked immunosorbant assay according to CALBIOTECH was utilized to detect antibody 25-hydroxy-Vitamin D and using a spectrophotometer (Humalyzer 2000) for the measurement of calcium, magnesium, albumin, AST, and ALT.
Table 1: Comparison between serum 25-OH Vitamin D and biochemical parameters in RA patients and healthy controls

| Parameters                  | RA patients (n=60) mean±SD | Healthy control (n=20) mean±SD | p-value |
|-----------------------------|-----------------------------|--------------------------------|---------|
| 25-OH Vitamin D (ng/mL)     | 8.39±2.37                   | 10.14±4.01                     | 0.045** |
| Calcium (mg/dL)             | 6.75±0.79                   | 7.93±0.72                      | 0.001***|
| Magnesium (mg/dL)           | 2.04±0.34                   | 2.26±0.20                      | 0.007***|
| Albumin (g/L)               | 39.57±8.21                  | 43.99±4.28                     | 0.003***|
| ALT (U/L)                   | 28.04±18.45                 | 21.95±5.10                     | 0.002***|
| AST (U/L)                   | 25.72±13.10                 | 20.1±0.50                      |         |

RA: Rheumatoid arthritis, SD: Standard deviation, n: Number, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, *p<0.05, **p<0.01

Exclusion criteria
In this study, patients with epilepsy, heart disease, migraine, and thyroid disorders were excluded from the study.

Statistical analysis
Data analysis was performed using the SPSS statistical program, (Version/11.5; SPSS Inc., Chicago, IL). Analysis of variance (ANOVA) was used to determine whether there are any statistically significant differences among the means. Data were presented as mean±standard error. Pearson’s correlation (r-correlation) was used between 25-hydroxy-Vitamin D, calcium, magnesium, and CDAI. p≤0.05 was considered statistically significant.

RESULTS
In this study, the results showed a significantly decreased in serum 25-OH Vitamin D, calcium, magnesium, and albumin levels in RA patients compared with healthy controls, while the results showed a significantly increased in serum ALT and AST levels in RA patients compared with healthy controls in Table 1.

Fig. 1 shows the serum level of 25-OH Vitamin D in RA patients with different drugs. The mean and standard deviation (SD) values for patients treated with methotrexate (MTX) 10.03±1.84 ng/mL; patients treated with Etanercept-MTX 9.22±2.96 ng/mL; patients treated with Etanercept 7.05±1.38 ng/mL; and patients treated with prednisolone-Etanercept-MTX 7.14±1.07 ng/mL. These results noted that a significantly low (p=0.021) serum level of 25-OH Vitamin D is more in both patients treated with Etanercept and patients treated with prednisolone-Etanercept-MTX compared with patients treated with MTX and patients treated with Etanercept-MTX.

Fig. 2 shows the serum level of calcium in patients with different drugs. The mean and standard deviation values for patients treated with (MTX) 7.15±0.60 mg/dL; patients treated with Etanercept-MTX 7.07±0.55 mg/dL; patients treated with Etanercept 5.85±1.52 mg/dL; and patients treated with prednisolone-Etanercept-MTX 6.62±0.94 mg/dL. These results noted that a significantly low (p=0.045) level of calcium is more in both patients treated with Etanercept and patients treated with prednisolone-Etanercept-MTX compared with patients treated with MTX and patients treated with Etanercept-MTX.

Fig. 3 shows the serum level of albumin in RA patients with different drugs. The mean and standard deviation values for patients treated with MTX 44.06±6.38 g/L; patients treated with Etanercept-MTX 43.69±7.74 g/L; patients treated with Etanercept 33.50±9.32 g/L; and patients treated with prednisolone-Etanercept-MTX 35.65±7.60 g/L. In these results, noted that a significantly low (p=0.001) level of albumin is more in both patients treated with Etanercept and patients treated with prednisolone-Etanercept-MTX compared with patients treated with MTX and patients treated with Etanercept-MTX.

Fig. 4 shows the serum level of ALT in RA patients with different drugs. The mean and standard deviation values for patients treated with MTX 26.86±9.47 U/L; patients treated with Etanercept-MTX 35.45±25.51 U/L; patients treated with Etanercept 22.65±13.08 U/L. In these results, noted that the increased level of ALT is more in both patients treated with MTX and patients treated with Etanercept-MTX compared with patients treated with Etanercept and patients treated with prednisolone-Etanercept-MTX.

Fig. 5 shows the serum level of AST in RA patients with different drugs. The mean and standard deviation values for patients treated with MTX 25.80±7.44 U/L;
patients treated with Etanercept-MTX 27.73±13.08 U/L; patients treated with Etanercept 21.98±5.63 U/L; and patients treated with prednisolone-Etanercept-MTX 24.47±10.29 U/L. In these results, noted that the increased level of AST is more in both patients treated with MTX and patients treated with Etanercept-MTX compared with patients treated with Etanercept and patients treated with prednisolone-Etanercept-MTX.

Table 2 shows the comparison of serum level of 25-OH Vitamin D between RA patients with Vitamin D supplementation and RA patients without Vitamin D supplementation. The mean and SD values for RA patients with Vitamin D supplementation 20.03±5.21 ng/mL and RA patients without Vitamin D supplementation 8.39±2.37 ng/mL. The results showed a significantly increased (p=0.001) in RA patients with Vitamin D supplementation compared to RA patients without Vitamin D supplementation.

Table 3 shows the comparison of serum level of calcium between RA patients with calcium supplementation and RA patients without calcium supplementation. The mean and SD values for RA patients with calcium supplementation 8.28±0.72 mg/dL and RA patients without calcium supplementation 6.75±0.7 mg/dL. The results showed a significantly increased (p=0.001) in RA patients with calcium supplementation compared to RA patients without calcium supplementation.

Data, as shown in Table 4, the correlation between the concentration of 25-OH Vitamin D, calcium, magnesium, and disease activity (CDAI) in RA patients were assessed. The results showed a non-significantly correlation between 25-OH Vitamin D (r=0.18, p=0.385) and CDAI in RA patients, while in the results showed a significant inverse correlation between calcium (r=−0.67, p=0.0001), magnesium (r=−0.52, p=0.0001), and CDAI in RA patients.

DISCUSSION

The results of this study agree with the previous, where these studies showed a low level of 25-OH Vitamin D in patients with RA compared with healthy controls [1,5,16]. Recently, low levels of 25-OH Vitamin D have been observed in RA patients and as known that Vitamin D is a stimulant for immune tolerance [17] and therefore Vitamin D deficiency affects immune tolerance, causing the development of autoimmune diseases (e.g., RA). Vitamin D is also known to have immune properties [18], so Vitamin D regulates the immune response through different mechanisms (e.g., stimulate regulatory T cells, decrease antigen presentation, and inhibit the pro-inflammatory T helper type 1 profile) [19,20] so, the cause of 25-OH Vitamin D deficiency in RA patients was due to several reasons; autoimmune diseases, which played a role in the low level of Vitamin D, and Vitamin D deficiency was associated with musculoskeletal pain [21,22], as well as found that RA patients treated with corticosteroids had a low level of Vitamin D, although the treatment of corticosteroids has a small effect on the level of Vitamin D, corticosteroids have lowered the level of Vitamin D in RA patients [23,24]. Besides, 25-OH Vitamin D deficiency was not limited to RA patients, but the healthy controls also suffered from 25-OH Vitamin D deficiency, but the decline was only in healthy females, as shown in Table 1. The results of this study agree with a previous study [25] and they found decreased 25(OH) Vitamin D level in veiled women; therefore, 25(OH) Vitamin D deficiency in women was caused by the veil, little exposure to the sun, contraceptive pills and neglecting Vitamin D-containing foods [26]. As shown in Table 2, the results showed significantly increased in RA patients with Vitamin D supplement compared to RA patients without Vitamin D supplement, and the results of this study agree with the previous study [27]. Vitamin D supplement has contributed to the high level of Vitamin D in patients with RA as well as has a role in reducing the risk of osteoporosis [28].

As shown in Table 4, the results showed a non-significantly correlation between 25-OH Vitamin D and CDAI. The results of this study agree with previous studies, where the previous studies showed no association between Vitamin D and disease activity. Lack of Vitamin D may be a negative effect of bone health in RA patients [29,30]. Unlike, the previous study, the previous study showed a significant inverse relationship among serum Vitamin D levels and disease activity, and it
considered that Vitamin D deficiency may be one of the reasons that lead to the development of the disease RA [31].

As shown in Table 1, the decreased calcium level in RA patients a result agree with the literature [32] and the present study agrees with a previous study [33]. The reason of low level of calcium in RA patients was due to poor absorption of calcium from the intestines or the effect of glucocorticoid therapy, which given to RA patients caused osteoporosis and bone loss. In addition to the glucocorticoid treatment prevents the absorption of calcium in the intestines and renal tubules, and this is done through mechanisms that depend on Vitamin D [34]. Besides, some authors point out that the lack of calcium level may not be due to the disease itself, sometimes it is because of the medications used in the treatment of RA [35], the data in Fig. 2 show increased bone loss in both RA patients treated with Etanercept and RA patients treated with MTX-Etanercept-Prednisolone, these drugs cause weakness of the intestinal calcium absorption and RA patients more at risk of osteoporosis complications [36]. As shown in Table 3, calcium supplementation contributed to high calcium levels in RA patients and patients with RA need calcium supplements to maintain healthy bones and teeth and prevent osteoporosis [37].

As shown in Table 4, the results showed a significant inverse correlation between calcium and CDAL. The result of this study agrees with the previous study [36], where the previous study showed the correlation of low level calcium with disease activity in patients treated with corticosteroids. The reason for this was due to osteoporosis correlation with RA, besides that, the risk of rheumatic drugs (MTX-Prednisolone), which caused a decline in calcium level in the blood. Osteoporosis is prevented by the use of active calcium therapy [38,39].

As shown in Table 1, the results showed a significantly decreased serum level of Mg as well as found a significant inverse correlation of Mg and CDAL. Inflammation, regardless of its etiology, can cause noticeable changes in the distribution of minerals, and therefore, RA is one of the chronic infections associated with mineral disturbances in serum and oxidative stress [33]. Low magnesium levels in RA patients may be linked to several reasons included chronic inflammatory conditions that change the level of Mg and potential mechanism for reducing Mg in RA due to severe stress on cells in immune processes and the effect of RA disease on the bones directly causing osteoporosis and therefore decrease magnesium and calcium in the blood. The result of this study agrees with the previous studies [33,40].

As shown in Table 1, the results showed a significantly decreased albumin in RA patients compared with healthy controls and the results of this study agree with the previous studies [41,42], where the previous study shows that the decrease of albumin in RA patients was not limited to liver disease and malnutrition because in both cases the albumin circulation rate decreases when there was a decrease of albumin in the blood. Thus, the lower albumin in RA patients was due to several reasons; impaired kidney function and reduced albumin synthesis, loss or increased catabolism, as well as corticosteroid therapy, leads to a lower level of albumin and maybe the loss of albumin in the fluid of inflammatory joints leading to the depletion of fluid in the intravascular, therefore decreased concentration of serum albumin [42].

As shown in Table 1, the results showed a significantly increased ALT and AST in RA-patients compared to healthy controls. The results of this study agree with the previous study [43]. Rise of liver enzymes ALT and AST in RA patients was due to several reasons; damage to liver cells, which increases the effectiveness of AST/ALT enzymes in the blood [42]. It may be due to the building of glycogen in liver cells as a result of fat accumulation and thus lead to cirrhosis of the liver and also abnormal changes in hepatocytes, where these changes lead to the release of liver enzymes in the blood. In addition to the effect of rheumatism treatment, which contributes to rising levels of ALT and AST, where the previous study showed that MTX treatment raised ALT and AST levels in RA patients [44].

Limitations of the study

The limitations of this study are small sample size as well as not available time to measure potassium, phosphorus, parathyroid hormone, and other signs that have a link to the inflammation of RA. In addition, no specific information was obtained from RA patients on dietary intake of magnesium.

CONCLUSION

In this study, the RA patients had lower levels of 25(OH) Vitamin D, calcium, magnesium, and albumin comparison with healthy controls. Increased levels ALT and AST in RA patients compared to healthy controls. Supplements helped raise 25(OH) Vitamin D and calcium levels in RA patients. The supplements can be used as adjunctive therapy with rheumatic treatment. Furthermore, it can be considered a substance that helps strengthen and maintain joints and bones. A negative correlation among 25-hydroxy Vitamin D and disease activity by CDAL was found in patients with RA, while a positive correlation was found among calcium, magnesium levels, and CDAL. Therefore, we conclude from this study that the reason for the decrease is due to changes in the joints as a result of the development of the disease RA.

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AUTHORS’ CONTRIBUTIONS

Authors Abdulnasser M., Al-Gebori and Mohammed Hadi Munshed Alosami contributed to the original idea design of the study and facilitate the acquisition of samples of healthy controls and RA patients according to ACR standards in 2010. Nawal Haider Al-Hashimi was responsible for carrying out the work and performing all the tests of the above-mentioned patients. All authors contributed to the interpretation of data and manuscript writing.

CONFLICTS OF INTEREST

Nil.

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