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Research Article

RELATIONSHIP BETWEEN VITAMIN D3 AND DISEASE ACTIVITY IN IRAQI PATIENTS WITH ANKYLOSING SPONDYLITISAND THOSE TREATED BY INFLIXIMAB

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ABSTRACT

Background: In medicine, a 25-hydroxy vitamin D (calcidiol) blood test is used to determine how much vitamin D is in the body. The blood concentration of calcidiol is considered the best indicator of vitamin D status. It is the most sensitive measure, though experts have called for improved standardization and reproducibility across different laboratories , the normal range of calcidiol is 30.0 to 74.0 ng/mL. **Objective**: To evaluate vitamin D3 in iraqi patients with ankylosingspondylitis ,and represent its affect on disease activity. **Material and methods**: Eighty five AS patients are enrolled in this study with a mean age of 36 ± 41 years & age range from 16-56 years , mean duration equal to 12.08. **Results**: There was a slightly non significant increase of mean serum level of vitamin D3 in G2 & G3 (24.26 ± 16.51 ng/ml , p = 0.914 , 24.28 ± 16.16 ng/ml , p = 0.912) respectively compared with it in G1 (23.79 ± 14.00 ng/ml). This represented in table 3.2 . There is a highly significant decrease of mean ± SD of BASFI & BASDAI in G2 & G3 (5.87 ± 1.13 , 3.34 ± 0.585, P < 0.001 , 3.34 ± 0.78 , 1.35 ± 0.66 , P < 0.001) respectively compared in G1 (7.91 ± 0.78 , 5.51 ± 0.79) . **Conclusion:** Serum vitamin D levels can decrease in AS patients due to both nutritional deficiency and inadequate exposure to sunlight, or for etiopathogenetic reasons. They also led us to the conslusion that insufficiency of vitamin D may cause increased disease activity and fatigue, and decreased functional capacity and quality of life.

Keywords: AS , vit.D3 , Infliximab , BASFI , BASDAI.

INTRODUCTION

25 – hydroxyl vitamin D3 (25OHD3) Calcifediol , Calcifediol also known as calcidiol, 25-hydroxycholecalciferol, or 25-hydroxyvitamin D (abbreviated 25(OH)D),[1] is a prehormone that is produced in the liver by hydroxylation of vitamin D3 (cholecalciferol) by the enzyme cholecalciferol 25-hydroxylase . This metabolite is being measured by physicians worldwide to determine a patient's vitamin D status[2]. Calcifediol is then converted in the kidneys (by the enzyme 25(OH)D-1α-hydroxylase) into calcitriol (1,25-(OH)2D3), a secosteroid hormone that is the active form of vitamin D. It can also be converted into 24-hydroxycalcidiol in the kidneys via 24-hydroxylation[3,4].

In medicine, a 25-hydroxy vitamin D (calcidiol) blood test is used to determine how much vitamin D is in the body[5]. The blood concentration of calcidiol is considered the best indicator of vitamin D status[6]. It is the most sensitive measure[7], though experts have called for improved standardization and reproducibility across different laboratories[6], the normal range of calcidiol is 30.0 to 74.0 ng/mL[5]. The normal range varies widely depending on several factors, including age and geographic location. A broad reference range of 20–150 nmol/L (8-60 ng/ml) has also

been suggested[8] while other studies have defined levels below 80 nmol/L (32 ng/ml) as indicative of vitamin D deficiency[9].
Also calcidiol or 25 (OH) D blood test can be used to diagnose vitamin D deficiency, and it is indicated in patients with high risk for vitamin D deficiency and the results of the test would be used as supporting evidence for beginning aggressive therapies[10]. Patients with osteoporosis, chronic kidney disease, malabsorption, obesity, and some other infections may be high risk and thus have greater indication for this test[10]. Although vitamin D deficiency is common in some populations including those living at higher latitudes or with limited sun exposure, the 25(OH)D test is not indicated for entire populations[10]. Physicians may advise low risk patients to take over-the-counter vitamin D in place of having screening[10].
US labs generally report 25(OH)D levels as ng/mL. Other countries often use nmol/L. Multiplyng/mL by 2.5 to convert to nmol/L.
Increasing calcidiol levels are associated with increasing fractional absorption of calcium from the gut up to levels of 80 nmol/L (32 ng/mL). Urinary calcium excretion balances intestinal calcium absorption and does not increase with calcidiol levels up to ~400 nmol/L (160 ng/mL)[11].
A study by Cedric F. Garland and Frank C. Garland of the University of California, San Diego analyzed the blood from 25,000 volunteers from Washington County, Maryland, finding that those with the highest levels of calcifediol had a risk of colon cancer that was one-fifth of typical rates[12]. However, randomized controlled trials failed to find a significant correlation between vitamin D supplementation and the risk of colon cancer[13].

Subjects and Methods
Patient subjects
During the period from April 2013 to September 2013, sample subjects attending the out-patient clinic in Medical city – Baghdad Teaching Hospital – Rheumatology Consultation Unit , were subjected to the questionnaire .
Eighty five AS patients are enrolled in this study with a mean age of 36 ± 41 years & age range from 16-56 years , mean duration equal to 12.04 .

Blood Samples
Venous blood samples (5-10 mL) were taken in vacutainer tubes under sterile conditions from patients. Serum was obtained from freshly drawn, rapidly centrifugated. Serum was quickly frozen at - 70 ºC and stored until processed.

Biomarker test assessment
25 – hydroxyl vitamin D3 (25OHD3) Calcifediol levels were measured by enzyme- linked immunosorbent assay (ELISA) technique (enzyme-amplified sensitivity immunoassay (EASIA) kit.

METHOD
This assay employs the competitive inhibition enzyme immunoassay technique. The microtiter plate provided in this kit has been pre-coated with an antibody. Standards or samples are added to the appropriate microtiter plate wells with Horseradish Peroxidase (HRP) conjugated 25HVD3 Hapten. The competitive inhibition reaction is launched between with HRP-conjugated 25HVD3 Hapten and 25HVD3 in samples. A substrate solution is added to the wells and the color develops in opposite to the amount of 25HVD3 in the sample. The color development is stopped and the intensity of the color is measured.

Statistical Analysis
Statistical package for social science (SPSS) version 14.0 for Windows program on the computer was used to compare the significance in the mean values in the comparison groups. All data were given as mean ± standard deviation (SD). Student t-Test was applied, $p<0.05$ was considered statistically significant.
ANOVA test one way analysis of variance and Dunnett test was applied to compare differences between groups and within groups, $p <0.05$ was considered statistically significant.

RESULTS
Effect of Age and Duration
Eighty five subjects were involved in this study. Group 1 represents twinty two patients with ankylosing spondylitis without treatment of Tumor Necrosis Factor – α blocker Infliximab . Group 2 represents thirty four patients with (1 + 2) doses of the blocker . Group 3 represents twinty nine patients with (3 - 7) doses of the blocker . There was no significance related to age effect between these groups $P>0.05$. Table 1 represents that Mean duration equal to 12.08, there was also no significance related to duration P value > 0.05 . Table 2 represents that

Effect of Mean ± SD of Vitamin D3
In vitamin D3 there was a slightly non significant increase of mean serum level of

vitamin D3 in G2 & G3 (24.26 ± 16.51 ng/ml , p = 0.914 , 24.28 ± 16.16 ng/ml , p = 0.912) respectively compared with it in G1 (23.79 ± 14.00 ng/ml).Table 3 represents this effect.

Effect of Bath Ankylosing Spondylitis Function Index (BASFI) & Bath Ankylosing spondylitis Disease Activity Index (BASDAI)

Table 4 shows a highly significant decrease of mean ± SD of BASFI & BASDAI in G2 & G3 (5.87 ± 1.13 , 3.34 ± 0.585 , P < 0.001 , 3.34 ± 0.78 , 1.35 ± 0.66 , P < 0.001) respectively compared in G1 (7.91 ± 0.78 , 5.51 ± 0.79) .

DISCUSSION

In this cross – sectional study vitamin D3 , Bath Ankylosing Spondylitis Function Index (BASFI) and Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) were measured in three groups ofiraqi patients with ankylosing spondylitis.

Vitamin D has been found to have a role in the function of the immune system. There have been a lot of studies investigating a relation between vitamin D and disease activity in ankylosing spondylitis (AS). The function of vitamin D has been considered to be confined to the areas of calcium, phosphorus, and bone metabolism Deluca et al 2001[14] , but vitamin D has also been found to have a role in the function of the immune system . Hayes et al 2003[15], Griffin et al 2003[16]. For example, in vitro studies showed that vitamin D inhibits T-cell proliferation and decreases the production of Th1 cytokines interleukin-2 (IL-2), interferon-g (IFN-g), and tumor necrosis factor-α (TNF-α). Patel et al 2007[17]. Furthermore, in vivo studies suggested that vitamin D supplementation prevents the initiation and progression of inflammatory arthritis (collagen-induced arthritis) in rodents. Cantorna et al 1998[18].

Ankylosing spondylitis is one of the diseases that causes inflammatory arthritis . It is a chronic and systemic disease belonging to the spondylarthropathies group. Characteristically, AS involves the axial skeleton and the enthesis regions, although in some patients peripheral joints are also affected. Mermerci et al 2010[19].

The serum 25(OH)D3 level for normal is ≥30 ng/ml, deficiency is ≤20 ng/ml, and insufficiency is between 20.1–29.9 ng/ml. Brunvand et al 1998[20], Holick et al 2007[21].

The result of this study on 25 – hydroxyl vitamin D3 (25OHD3) Calcifediol was mean ± SD serum level in G1 (23.79 ± 14.00 ng/ml) . This result is considerd under insufficient level and this may negatively affect disease activity, functional status and quality of life. The serum vitamin D levels can decrease in AS patients due to both nutritional deficiency and inadequate exposure to sunlight, or for etiopathogenetic reasons. They also led us to the conslusion that insufficiency of vitamin D may cause increased disease activity and fatigue, and decreased functional capacity and quality of life.

There is a slightly non significant elevation of mean ± SD serum level of vitamin D3 in G2 & G3 (24.26 ± 16.51 ng/ml , p = 0.914 , 24.28 ± 16.16 ng/ml , p = 0.912) . These results revealed that mean serum level of vitamin D3 remain in low , and the only way to make the mean serum level of vitamin D3 reach the normal range in patients with ankylosing spondylitis is by increase of its supplementation in nutrition and adequate exposure to sunlight .

Bath Ankylosing Spondylitis Function Index (BASFI) and Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) have been evaluated through quesennaire on a scale of (0-10) .Their mean ± SD are high in G1 (7.91 ± 0.78 , 5.51 ± 0.79) respectively , and these results are in agreement with ones reported in other studies . Garrett et al 1994[22], Lukas et al 2009[23], van der Heijde et al 2009[24] , Calin et al 1994[25].

There are a significant decrease in mean ± SD of BASFI & BASDAI in G2 & G3 (5.87 ± 1.13 , 3.34 ± 0.585 . p < 0.001 , 3.34 ± 0.78 , 1.35 ± 0.66 , p < 0.001) respectively . In clinical practice, continuation of TNF-a blocking (infliximab) therapy is mainly based on subjective measures, such as a more objective measure of disease activity . Brouwer et al 2011[26], Kvien et al 2009[27] ,Landewe et al 2009[28] , Xu M et al 2011[29], Pedersen et al 2011[30]. However, a purely objective measure is still lacking in the evaluation process of TNF-a blocking therapy. The present study is consistent with the results that reported by studies above.

CONCLUSIONS

1. Serum vitamin D levels can decrease in AS patients due to both nutritional deficiency and inadequate exposure to sunlight, or for etiopathogenetic reasons. They also led us to the conslusion that insufficiency of vitamin D may cause increased disease activity and fatigue, and decreased functional capacity and quality of life.
2. The best way that make mean serum level of vitamin D3 reach to the normal range in patients with ankylosing

spondylitis is by increase its supplementation in nutrition and by adequate exposure to sunlight .

3. In this study, the BASFI, BASDAI scales, which are used to evaluate the functional status, quality of life, and fatigue in AS, showed a significant differences between the group 1 and group 2 & 3 , groups treated with infliximab. These findings showed that decreased vitamin D levels may lead to deterioration in functional capacity, quality of life, and fatigue in AS patients.

Table 1: Groups with Mean ± SD of Age

Age	Number	Mean ± SD	P value
Group 1	22	35.45 ± 8.75	
Group 2	34	37.47 ± 10.05	0.428
Group 3	29	35.89 ± 8.62	0.866
Total	85	36.41 ± 9.18	

Table 2: Groups with Mean ± SD of Duration

Duration	Number	Mean ± SD	P value
Group 1	22	10.44 ± 10.02	
Group 2	34	11.91 ± 7.80	0.518
Group 3	29	13.51 ± 7.04	0.189
Total	85	12.08 ± 8.19	0.707

Table 3: Mean ± SD of vitamin D3

Parameter	Group 1 (n= 22)	Group 2 (n=34)	P value	Group 3 (n=29)	P value
Vitamin D3	23.79 ± 14.00	24.26 ± 16.51	0.914	24.28 ± 16.16	0.912

Table 4: Groups with Mean ± SD of BASFI & BASDAI

Parameters	Group 1 (n= 22)	Group 2 (n= 34)	P value	Group 3 (n= 29)	P value
BASFI	7.91 ± 0.78	5.87 ± 1.13	0.000	3.34 ± 0.585	0.000
BASDAI	5.51 ± 0.79	3.34 ± 0.78	0.000	1.35 ± 0.66	0.000

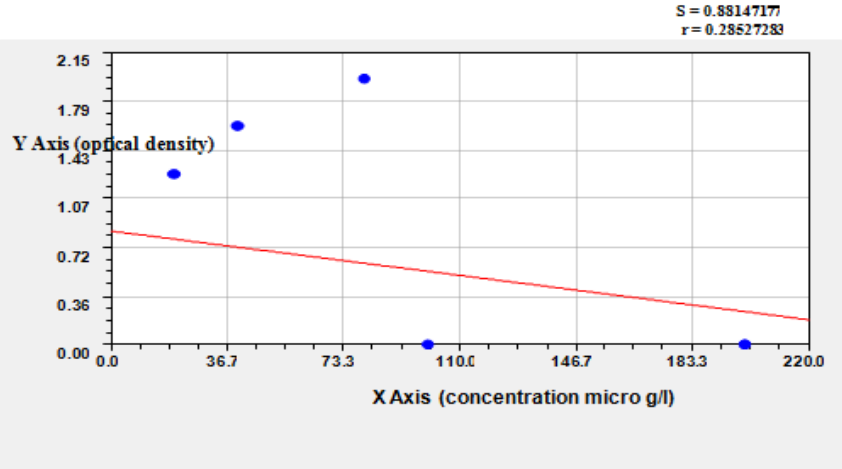

Fig. 1: Standard curve of 25-hydroxyl vitamin D3

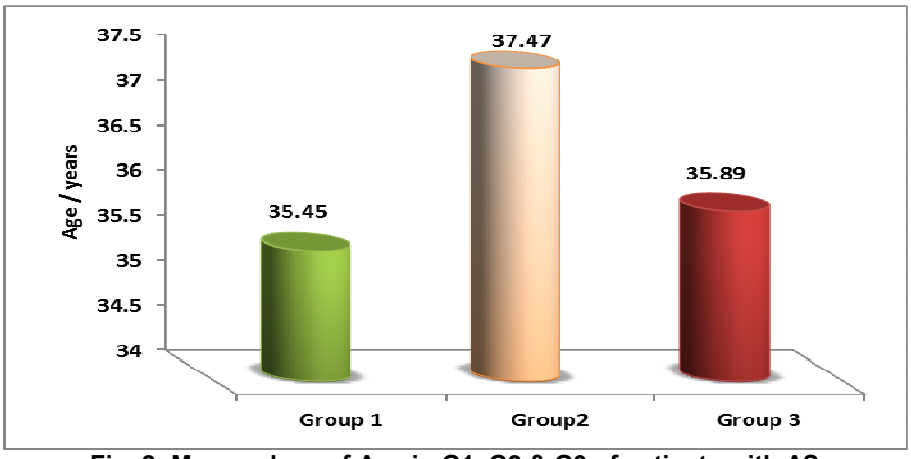

Fig. 2: Mean values of Age in G1, G2 & G3 of patients with AS

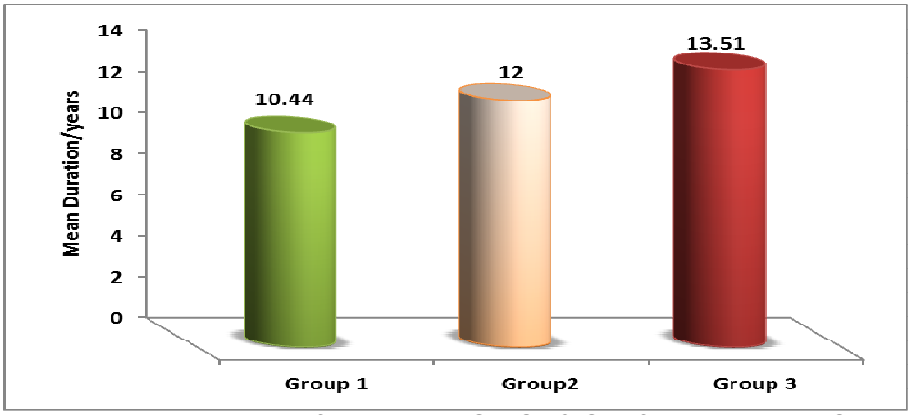

Fig. 3: Mean values of Duration in G1, G2 & G3 of patients with AS

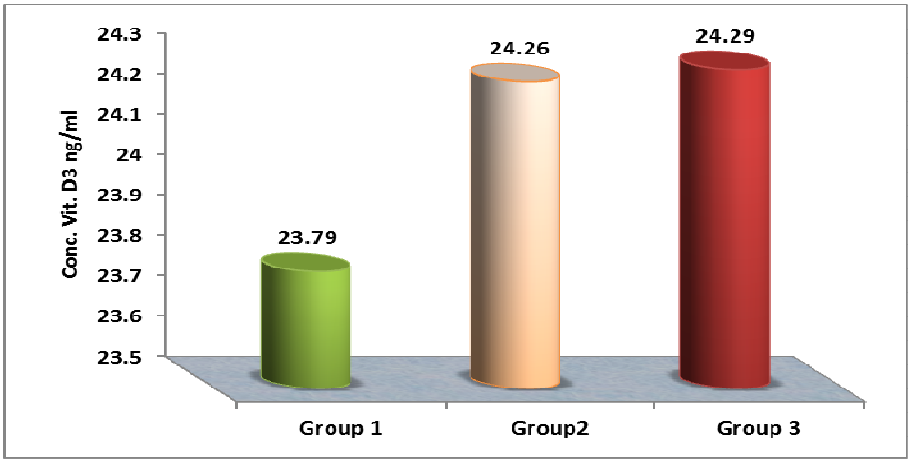

Fig. 4: Mean values of Vitamin D3 in G1, G2 & G3 of patients with AS

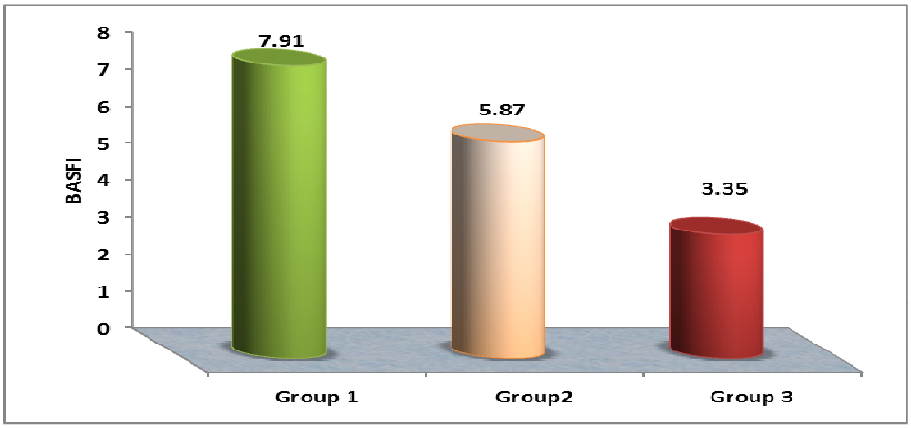

Fig. 5: Mean values of BASFI in G1, G2 & G3 of patients with AS

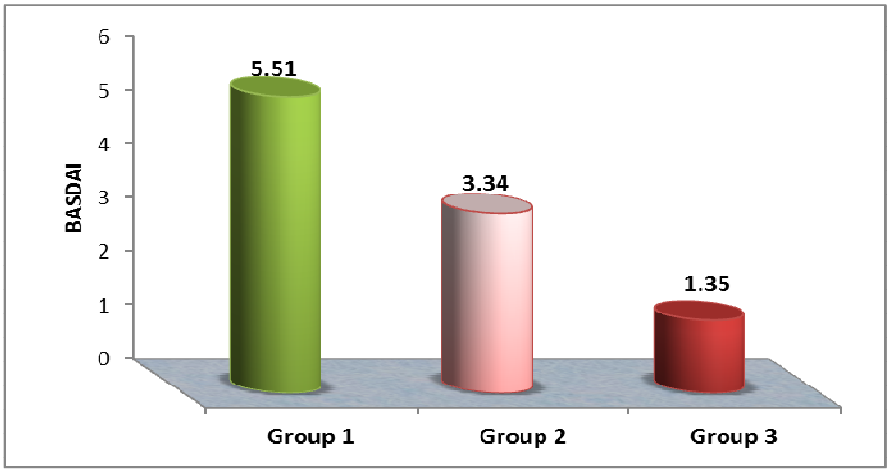

Fig. 6: Mean values of BASDAI in G1, G2 & G3 of patients with AS

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