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Research Article

Evaluation of Serum Eta Protein, Sclerostin, and Calcitonin Level in Arthritis Patients on Vitamin D Therapy

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ABSTRACT

Background: Arthritis is an inflammatory condition affecting the diarthrodial joints. A joint's bone, cartilage, and structural design all preserve its integrity, and arthritis alters that integrity, resulting in joint activity loss and discomfort. The primary symptom of arthritis is joint pain, although other symptoms include stiffness, inflammation, and impaired joint motor function. **Methods:** The cross-sectional study comprised 90 subjects: 70 arthritis sufferers and 20 controls, ages 25-60, both sexes. From September 2023 to March 2024, patients were referred to Kirkuk city in Azadi hospital and Kirkuk general hospital. The research participants were placed into three groups: Group 1 for arthritic patients without Vit D (35). Patients with arthritis who received vitamin D for at least two months (G2), n (35), were the second group. The third group comprised healthy individuals without arthritis n (20). **Result:** Significant (P<0.05) increase in ETA protein levels in G1 and G2 compared to G3. G1 had ETA protein levels of 28.05±5.34 ng/L, G2 had 24.10±3.67 ng/L, and G3 had 8.92±2.80 ng/L. Sclerostin levels peaked in G1 (0.4273±0.3023 pg/mL) and declined in G2 (P<0.05) compared to G3. Calcitonin levels were higher in G1 (34.72±4.72 pg/mL) and G2 (27.06±5.85 pg/mL) than G3 (14.71±3.71 pg/mL) at (P<0.05). **Conclusion:** The study found a rise in ETA protein and calcitonin levels in arthritic patients before and after therapy with vitamin D, which was not influenced by vitamin supplementation. Sclerostin levels increase in arthritic patients and decrease following therapy with vitamin D.

Key words: Arthritis, ETA protein, Calcitonin, Sclerostin, Vitamin D.

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INTRODUCTION

Arthritic disorders involve inflammation and reduced function in the joints and connective tissue, leading to serious health problems and death.[1] There are about 100 types of arthritic disorders, which combined pose a significant health and economic burden on communities. Common arthritic disorders comprise rheumatoid arthritis (RA), osteoarthritis (OA), ankylosing spondylitis (AS), gout, lupus, and psoriatic arthritis.[2]

Arthritic conditions are prevalent globally. Epidemiological research from 2010 showed that the prevalence of arthritic illnesses globally varied between 11.6% and 46.4% during the preceding two decades. This variation depended on geographical regions, study methods, and the ages of the individuals examined.[3,4] The family of 14-3-3 regulatory proteins has seven isoforms: Beta (β), epsilon (ε), gamma (γ), eta (η), tau (τ), zeta (ζ), and sigma (σ), sharing around 50% amino acid similarity among them.[5] Serum 14-3-3 η is a new pro-inflammatory substance found in joints that is believed to play a role in the development of rheumatoid arthritis.[6] An connection has been found between the blood levels of 14-3-3 η and the extent of joint destruction in individuals with rheumatoid arthritis and gout.[7,8] Sclerostin is a glycoprotein mostly secreted by mature osteocytes. It acts as a suppressor of osteoblast growth and specialization from mesenchymal stem cells by inhibiting the Wnt/β-catenin pathway. Sclerostin has a role in regulating bone tissue metabolism at a physiological level. Sclerostin levels were markedly reduced in osteoarthritis.[9]

Calcitonin (CT) is a 32-amino-acid monomeric peptide primarily generated by the thyroid C-cells through the cleavage and posttranslational processing of procalcitonin (PCT).[10] Combining PCT and CT with other RA-related biomarkers may enhance the diagnosis accuracy of early RA.[11]

MATERIAL AND METHODS

The cross-sectional study involved 90 participants, comprising 70 arthritis sufferers and 20 controls aged between 25 and 60, of both sexes. Patients were referred to two primary hospitals, Azadi Hospital in Kirkuk city, and Kirkuk General Hospital (Iraq), between September 2023 and March 2024. The participants in this study were categorized into 3 groups: In the first group (G1) of 35 individuals with arthritis without taking vitamin D, the diagnosis was confirmed by medical staff consultation. The second group consisted of 35 Arthritis patients who had been taking vitamin D for a minimum of 2 months (G2). The third group consisted of 20 healthy individual volunteers with no previous arthritis history. A brief questionnaire was used to gather clinical history data, including age, sex, weight, height, family history of arthritis, chronic conditions, and treatment details. The exclusion criteria included people with kidney illness, diabetes mellitus, liver and pancreatic disease, documented diagnostic grounds for malabsorption (gastrointestinal abnormalities), pregnancy, and alcohol intake. Using a sterile disposable syringe, 5 ml of each person's venous blood was extracted. The blood was then placed into gel tubes and allowed to coagulate for 20 minutes at room temperature. For fifteen minutes, the samples were centrifuged at 3000

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rpm. After that, the sera were divided into three 500 µl Eppendorf tubes and stored at -20°C until they were needed for the test, which had the following parameters: 14-3-3 protein eta, calcitonin (CT), and sclerostin (SOST).

Statistical analysis: Both SPSS 22 and Microsoft Excel 2019 were utilized to enter and analyze the data. In the form of frequencies, descriptive statistics were utilized to elucidate the attributes of the participants. The study groups were compared utilizing the t-test and Chi-Square test. A P-value below 0.05 is deemed to indicate statistical significance.

RESULT

The current study included 90 participants. The findings represented statistical values of clinical parameters measured in serum of two groups: G1=arthritis patients before treatment with vitamin D(35), G2= arthritis patients before treatment with vitamin D(35), G3=normal individual without history of arthritis (20), with divided into Age (A1 = 25-36, A2 = 37-48, A3=49-60years).

The total number of a subject that participate are total 90 subjects (35 arthritis patients before treatment, 35 arthritis patients after treatment and 20 controls healthy subjects). This study show increase incidence of arthritis in age between 49-60 years and its percentage was 45.7%, followed age groups 37-48 years and its percentage which was found to be 38.6%, the least age group was 25-36 and its percentage was 15.7%% (Table 1).

This study show increase incidence of arthritis in women that were 46(65.71%) in compared with male 24(34.29%) (Table 2).

The current study included 90 participants. The findings represented statistical values of clinical parameters measured in serum of two groups: G1=arthritis patients before treatment with vitamin D(35), G2= arthritis patients before treatment with vitamin D(35), G3=normal individual without history of arthritis(20), with divided into Age (A1 = 25-36, A2 = 37-48, A3=49-60years).

Table 3 explains the mean and stander deviation of ETA protein in serum of G1, G2,and G3 (Table 1). There were significant increase (P<0.05) in levels of ETA Protein in G1 and G2 compared with G3. The level of ETA protein in G1 (28.05±5.34 ng/L),while in G2 (24.10 ±3.67 ng/L), in G3 was (8.92±2.8 ng/L). As for sclerostin the highest (mean±SD) levels was (0.43 ±0.3 Pg/ml) in G1, then decreased in G2 in compared with G3 at (P<0.05). Furthermore, increase Calcitonin level in G1(34.72 ±4.72 Pg/ml) and G2 (27.06±5.85 Pg/ml) in compared with G3 (14.71 ±3.71 Pg/ml) at (P<0.05).

Table 4 explains the mean and stander deviation of ETA protein, sclerostin, and Calcitonin in serum according to age among arthritis patients before and after treatment. The age groups divided into 3 groups for each patients groups, as A1 (25-36) years, A2 (37-48), and A3 (49-60) years.

Table 1: Relation the number of arthritis patients with Age.

Age group	Patients	
	No.	%
25-36	11	15.7
37-48	27	38.6
49-60	32	45.7
Total	70	100

Table 2: Relation the number of arthritis patients with Age.

Gender	Patients	
	No.	%
Male	24	34.29
Females	46	65.71
Total	70	100

Table 3: Comparison between arthritis patients and control regarding the mean of ETA protein, sclerostin, and Calcitonin.

Studied groups	G1(n=35)	G2(n=35)	G3 (n=35)	P value
ETA (ng/L)	28.05 ± 5.34a	24.1±3.67a	8.92±2.8b	0.01
Sclerostin (Pg/ml)	0.43±0.3a	0.38±0.2ab	0.26±0.25b	0.05
Calcitonin (Pg/ml)	34.72±4.72a	27.06±5.85a	14.71±3.71b	0.01

Table 4: Serum level of in ETA protein, sclerostin, and calcitonin in two groups of arthritis patients according to age groups.

Group	Age	n	ETA (ng/L)	Sclerostin (pg/ml)	calcitonin (pg/ml)
G1	A1	10	21.01±4.14ab	0.3124±0.151a	39.85±7a
	A2	14	30.58±3.56a	0.4673±0.331a	35.19±8.31a
	A3	13	29.68±6.16ab	0.456±0.345a	30.51±5.81b
G2	A1	9	26.66±2.09ab	0.37±0.162a	29.97±5.27b
	A2	15	17.43±6.54b	0.4196±0.221a	30.57±6.71b
	A3	11	26.36±5.49ab	0.37±0.25a	29.75±4.11b

Data expressed as mean±SD.

There was significant increase (P<0.05) in levels of ETA Protein in A2 (30.58±3.56 ng/L) in compared with A1 (21.01±4.14 ng/L) and A3 (29.68±6.16 ng/L), in the G1 group, while significant decrease in A2 (17.43±6.54b) as compared with A1 (26.66±2.09) and A3(26.36±5.49) in the G2 group.

There was no significant differences (P>0.05) in levels of sclerostin among age groups (A1, A2, A3) in G1 that were (0.3124±0.151, 0.4673±0.331a, 0.4560±0.345) Pg/ml. In addition no significant differences (P>0.05) in levels of sclerostin among age groups(A1, A2, A3) in G2 that were (0.37±0.162, 0.4196±0.221, 0.3674±0.250) Pg/ml. Furthermore, There was significant increase (P<0.05) in levels of calcitonin in A1 (39.85±7.00) Pg/ml and A2 (35.19±8.31) Pg/ml in compared with A3 (30.51± 5.81b) Pg/ml, in the G1group.

This study found that there was no correlation between sclerostin level and calcitonin Level in patients with arthritis before treatment (r=0.1) (Figure 1A). This study found that there was no correlation between sclerostin Level and ETA protein Level in patients with arthritis before treatment (r = 0.2) (Figure 1B). This study found that there was no correlation between sclerostin Level and PTH Level in patients with arthritis before treatment (r = 0.1) (Figure 1C).

DISCUSSION

When compared to controls, the study indicates that individuals with arthritis had higher means of ETA protein both before and after receiving vitamin D therapy. The study's P value of 0.01 indicated significance. The study done by,[12] show increase level of ETA protein in patient with RA in compared with healthy individual while no differences between osteoarthritis and control. Other study done by[13] show a relationship between 14-3-3 ETA protein and both ankylosing spondylitris(AS) and rheumatoid arthritis(RA), but with (RA) more than (AS). Levels of 14–3-3 η protein in the blood were notably elevated in gout patients in comparison to the control group. The presence of erosive alterations in patients indicates the significance of the 14–3-3 η protein in inflammatory and structural damage pathways, suggesting its usefulness as a marker for disease severity.[14] A study conducted by Maksymowych et al. found that the median levels of 14-3-3 η are notably greater in patients with developed rheumatoid arthritis compared to healthy individuals.[15] Levels of 14-3-3η were elevated in individuals who develop specific types of arthritis. The 14-3-

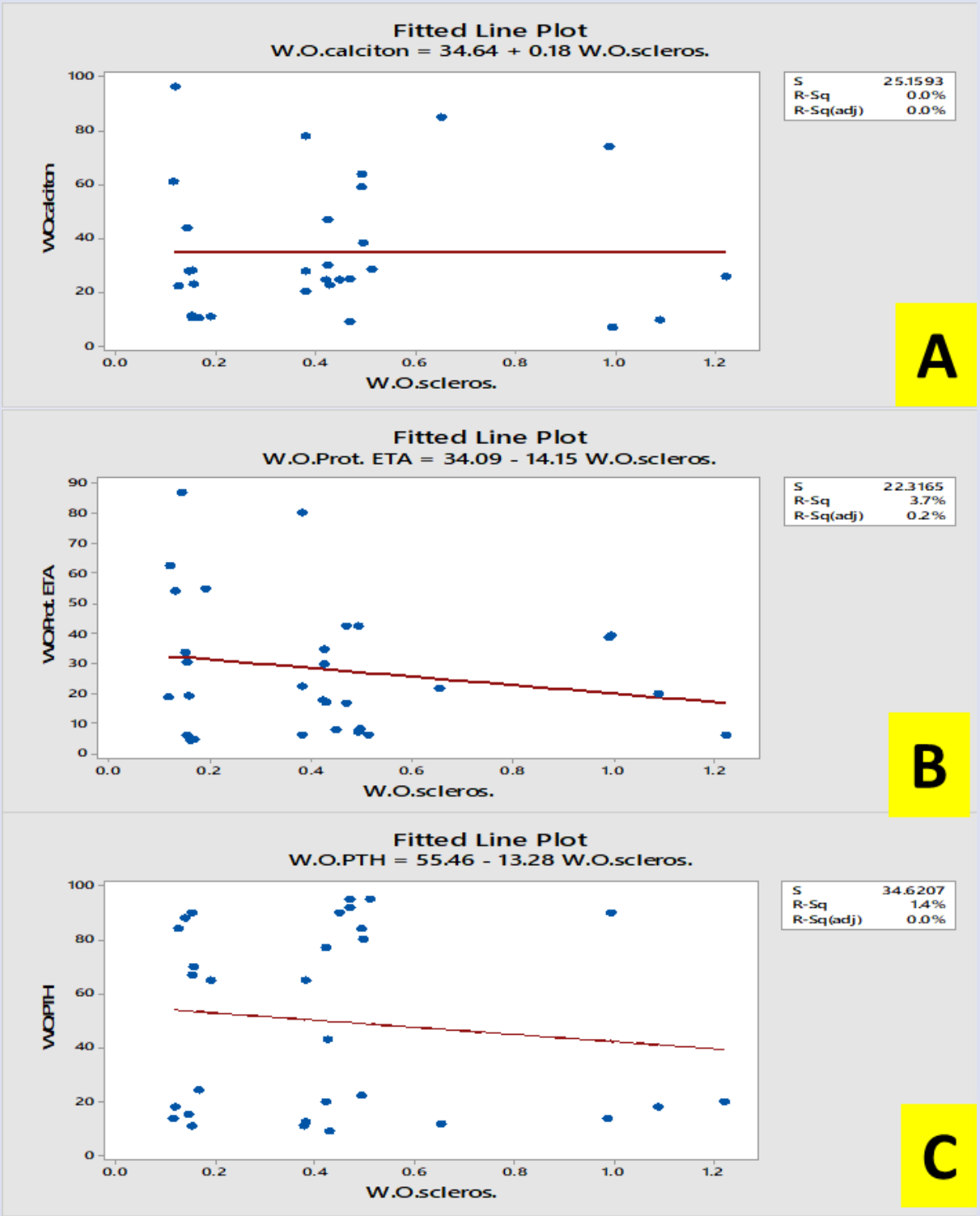

Figure 1: (A) Correlation between between sclerostin level and calcitonin level in patients with arthritis before treatment. (B) Correlation between between sclerostin level and ETA level in patients with arthritis before treatment. (C) Correlation between sclerostin level and PTH level in patients with arthritis before treatment.

3 ETA protein is found outside the cell and leads to many detrimental consequences, directly contributing to the disease process. Soluble 14-3-3 η protein in the extracellular environment, at amounts seen in the serum of RA patients, has ligand activity and selectively activates cells of the innate immune system.[16]

The study show the highest mean of sclerostin was in patients with arthritis before treatment with vitamin D, followed by patients with arthritis after treatment with vitamin D, then the lowest mean of sclerostin was in control. The study were significant at P. value=0.05

This study supports the findings of reference[17] which demonstrate elevated levels of sclerostin in arthritis patients, indicating a potential link between arthritis and bone damage. Serum sclerostin levels were higher in individuals with rheumatoid arthritis, but it did not serve as a reliable indicator for disease activity, bone erosions, or atherosclerosis.[18]

Sclerostin plays a crucial role in regulating the Wnt pathway by preventing Wnt from attaching to its receptor, which leads to the inhibition of bone growth. Serum levels of sclerostin are influenced by genetic factors, age, gender, obesity, renal function, and the presence of diabetes mellitus[19]. The study demonstrated an increase in the mean level of calcitonin in arthritis patients before and after therapy with vitamin D compared to the control group. The study was statistically significant with a P-value of 0.01. This study,[20] supports the findings that indicate elevated levels of calcitonin in arthritis patients with vitamin D deficiency. It also aligns with,[21] showing increased calcitonin levels in arthritis patients with chronic kidney disease, suggesting that low vitamin D levels may be a contributing factor in the development of rheumatoid arthritis. Vitamin D deficiency or insufficiency was more prevalent in patients with RA than in healthy individuals. The average blood vitamin D levels in RA patients were considerably lower compared to healthy subjects. Vitamin D has regulatory functions and its receptors are found in several immune system cells. This study show highest level of ETA protein in arthritis patients Before treatment were in age group A2.[22] While in arthritis patients after treatment the highest level of ETA protein were in A1 and 3, as well as noting a decrease of ETA protein in group A2 in arthritis patients after treatment from group A2 of arthritis patients Before treatment. A study by[23] discovered that high levels of 14-3-3-ETA protein can trigger variables linked to the development of RA at amounts typically seen in RA patients. The result show no differences in the mean of sclerostin among ages group for both before and after treatment with vitamin D. This study agree with,[24] that show in differences in sclerostin according to age and no correlation between them. Other study show Serum sclerostin had significantly positive correlations with the age of onset and weight of rheumatoid arthritis patients and negative correlation with erythrocyte sedimentation rate.[25]

This study show highest level of calcitonin in arthritis patients Before treatment were in age group A1 and A2. While no differences in calcitonin level among age group of arthritis patients after treatment. Variations in vitamin D metabolism may lead to mineral and bone diseases, elevated PTH levels, hypertension, systemic inflammation, and ultimately kidney impairment.[26-28] The study done by.[29] show increase calcitonin in SLE in compared with RA. This study disagree with[30] that showed no differences in calcitonin level according to age groups which might be related other electrolyte disturbances.[31]

CONCLUSION

This study concluded increase ETA protein and calcitonin in arthritis patients after and before treatment with vitamin D, that not affected by vitamin supplemented. While increase sclerostin in patients with arthritis and decrease after treatment with vitamin D.

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