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Research Article

SERUM BONE SPECIFIC ALKALINE PHOSPHATASE ININIRAQI PATIENTS WITH ANKYLOSING SPONDYLITIS AND THE EFFECT OF INFLIXIMAB THERAPY

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ABSTRACT

Background: Ankylosing spondylitis is a member of the group of the spondyloarthropathies with a strong genetic predisposition. Complete fusion results in a complete rigidity of the spine, a condition known as "bamboo spine". There is no cure for AS, although treatments and medications can reduce symptoms and pain. **Objective**: To evaluate bone specific alkaline phosphatase in Iraqi patients with Ankylosing Spondylitis, and evaluate the efficacy and safety of long term infliximab therapy. **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**: a significant decrease of mean serum level of ALP in group 3 patients who treated with infliximab (142.14 ± 4.68 ng/ml , $p < 0.05$ level) compared with mean serum level of ALP in G1(167.10 ± 10.37 ng/ml) , and a non significant decrease of mean serum level of ALP in G2 (154.08 ± 6.40 ng/ml , $p = 0.205$) compared with mean serum level of ALP in G1 (167.10 ± 10.37 ng/ml). **Conclusion:** An elevate serum level of bone specific alkaline phosphatase in Iraqi patients with ankylosing spondylitis , the attribution of this elevation is increasing bone production due to increase the activity of the disease lead to increase the ability of osteoblast to produce BALP . This cross - sectional study reveals the efficacy of infliximab and the good safety treatment in patients with activeankylosing spondylitis.

Keywords: AS, BALP, Infliximab.

INTRODUCTION

Ankylosing spondylitis (AS, from Greek ankylos, crooked; spondylos, vertebrae; -itis, inflammation), previously known as Bechterew's disease (or syndrome) and Marie-Strümpell disease, is a chronic inflammatory disease of the axial skeleton with variable involvement of peripheral joints and nonarticular structures. AS is a form of spondyloarthritis, a chronic, inflammatory arthritis[1] where immune mechanisms are thought to have a key role[2]. It mainly affects joints in the spine and the sacroiliac joint in the pelvis, and can cause eventual fusion of the spine.

Ankylosing spondylitis is a member of the group of the spondyloarthropathies with a strong genetic predisposition. Complete fusion results in a complete rigidity of the spine, a condition known as "bamboo spine[3]. There is no cure for AS, although treatments and medications can reduce symptoms and pain[4,5]. The overall prevalence of AS is 0.25 percent, and it is more common in men; three males are diagnosed with AS for every one female. However, many rheumatologists believe the number of women with AS is underdiagnosed, as most women tend to experience milder symptoms[6]. The majority of AS patients, including 95 percent of white patients, express

the HLA-B27 antigen[7] and high levels of immunoglobulin A (IgA) in the blood. The onset of the disease is typically between 15 and 25 years of age[7].

Alkaline phosphatase (ALP, ALKP) is a hydrolase enzyme responsible for removing phosphate groups from many types of molecules, including nucleotides, proteins, and alkaloids. The process of removing the phosphate group is called dephosphorylation. As the name suggests, alkaline phosphatases are most effective in an alkaline environment. It is sometimes used synonymously as basic phosphatase[8].

Normal ALP levels in adults are approximately 20 to 140 IU/L[9], though levels are significantly higher in children and pregnant women. Blood tests should always be interpreted using the reference range from the laboratory that performed the test. High ALP levels can occur if the bile ducts are obstructed[10]. Also, ALP increases if there is active bone formation occurring, as ALP is a byproduct of osteoblast activity (such as the case in Paget's disease of bone). Levels are also elevated in people with untreated Coeliac disease[11]. Lowered levels of ALP are less common than elevated levels.

Infliximab (INN; trade name Remicade) is a chimeric monoclonal antibody against tumour necrosis factor alpha (TNF-α) used to treat autoimmune diseases.

Infliximab was approved by the U.S. Food and Drug Administration (FDA) for the treatment of psoriasis, Crohn's disease, ankylosing spondylitis, psoriatic arthritis, rheumatoid arthritis, and ulcerative colitis. Infliximab won its initial approval by the FDA for the treatment of Crohn's disease in August 1998[12]. Infliximab works by binding to TNF-α. TNF-α is a chemical messenger (cytokine) and a key part of the autoimmune reaction. In rheumatoid arthritis, infliximab seems to work by preventing TNF-α from binding to its receptor in the cell.

Infliximab was developed by Junming Le and Jan Vilcek at New York University School of Medicine and developed by Centocor, (now Janssen Biotech, Inc.)[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

Serum bone alkaline phosphates were measured by enzyme- linked immunosorbent assay (ELISA) technique (enzyme-amplified sensitivity immunoassay (EASIA) kit.

Method

This assay employs the quantitative sandwich enzyme immunoassay technique. Antibody specific for BALP has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any BALP present is bound by the immobilized antibody. After removing any unbound substances, a biotin-conjugated antibody specific for BALP is added to the wells. After washing, avidin conjugated Horseradish Peroxidase (HRP) is added to the wells. Following a wash to remove any unbound avidin-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of BALP bound in the initial step. 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.
Mean duration equal to 12.08, there was also no significance related to duration P value > 0.05.

Effect of Mean ± SD of Bone Formation Marker Alkaline phosphatase

There is a significant decrease of mean serum level of ALP in G3 (142.14 ± 4.68 ng/ml , p < 0.05 level) compared with mean serum level of ALP in G1(167.10 ± 10.37 ng/ml) , and a non significant decrease of mean serum level of ALP in G2 (154.08 ± 6.40 ng/ml , p = 0.205) compared with mean serum level of ALP in G1 (167.10 ± 10.37 ng/ml) . Table 3 represents that.

DISCUSSION

In this cross – sectional study. Bone formation marker Alkaline Phosphatase was measured in three groups of patients with ankylosing spondylitis.
Group 1 consist of 22 patients with mean ± SD of age (35.45 ± 8.75) years, and mean ± SD of duration (10.44 ± 10.02) years. This group also showed an elevated of specific bone alkaline phosphatase mean ± SE (167.10 ± 10.37 ng/ml). This finding is in accordance with Kendall et al 1973[14] , who attributed this elevation to increase bone production due to increase the activity of the disease which cause increase the ability of osteoblast to produce BALP.

Group 2 consist of 34 patients with mean ± SD of age (37.47 ± 10.05) years , and mean ± SD of duration (11.91 ± 7.80) years . These patients were on (1-2) doses of tumor necrosis alpha inhibition (infliximab) through a period of (2 weeks to 1.5 months) . There was no significant decrease of mean ± SD serum level of ALP (154.08 ± 6.40 ng/ml , p = 0.205) , but a significant decrease of mean ± SD serum level of ALP (153.37 ± 4.68 ng/ml , p < 0.05) was shown in group 3 . Group 3 consist of 29 patients with mean ± SD of age (35.89 ± 8.62) years , and mean ± SD of duration (13.51 ± 7.04) years . They were on (3 – 7) doses of Infliximab through the period from (3 – 9) months . This can suggest that infliximab therapy in patients with active AS, was well tolerated and improved disease quickly and significantly. This finding is in consistent with the results done by these studies. Brandt et al 2000[15], Stone et al 2001[16], and others[17-20].

CONCLUSIONS

1. An elevate serum level of bone specific alkaline phosphatase in Iraqi patients with ankylosing spondylitis ,the attribution of this elevation is increasing bone production due to increase the activity of the disease lead to increase the ability of osteoblast to produce BALP .
2. This cross - sectional study reveals the efficacy of infliximab and the good safety treatment in patients with active ankylosing spondylitis.

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 ± SE of bone specific Alkaline Phosphatase

Parameter	Group 1 (n= 22)	Group 2 (n=34)	P value	Group 3 (n=29)	P value
Alkaline phosphatase	167.10 ± 10.37	154.08 ± 6.40	0.205	142.14 ± 4.68	0.02

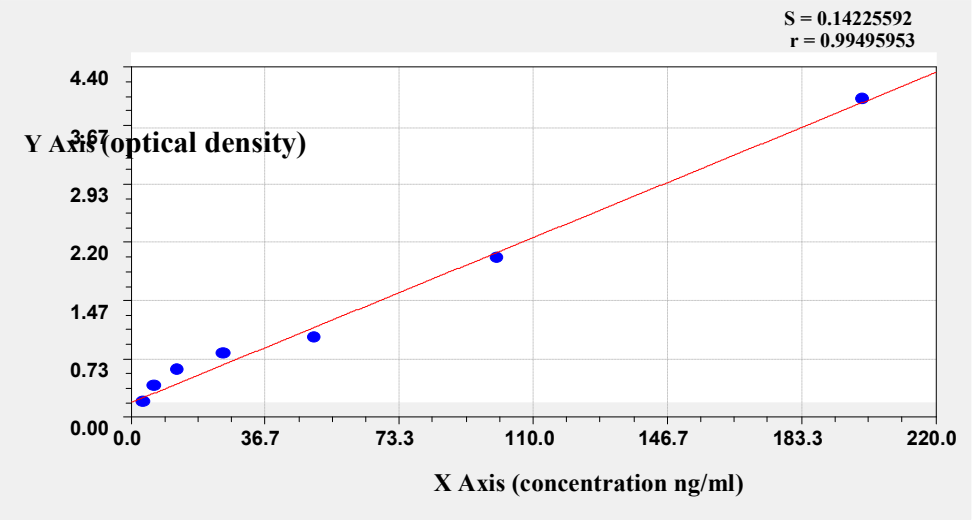

Fig. 1: Standard curve of Bone Alkaline Phosphatase

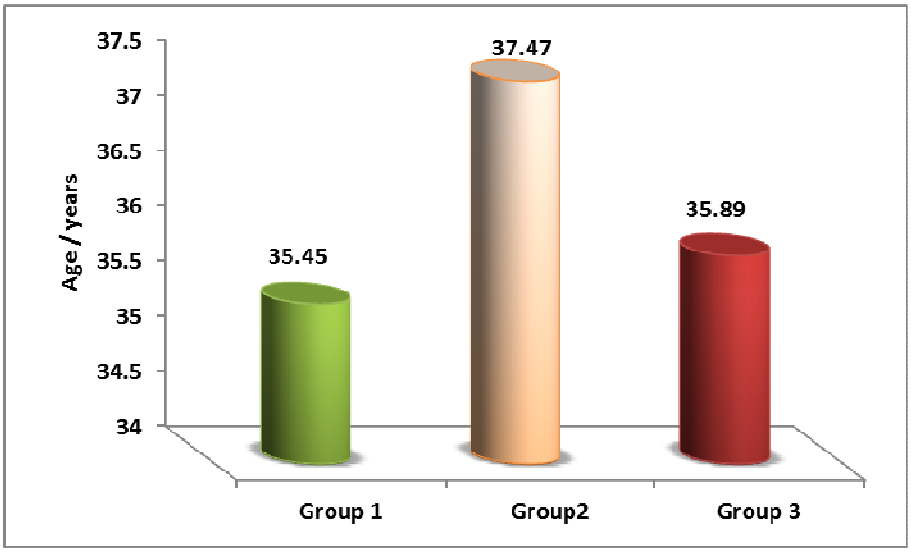

Fig. 2: Mean values of Age in G1, G2 & G3 of patients with AS

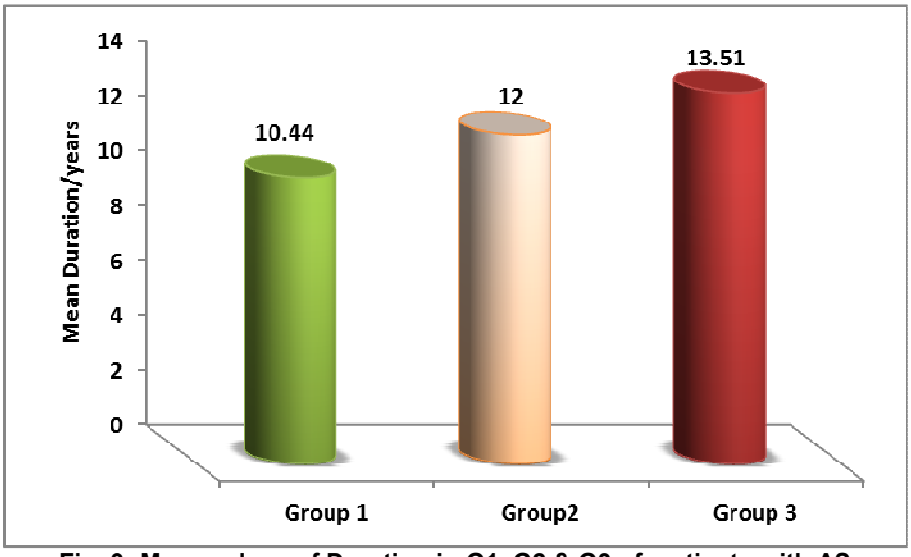

Fig. 3: Mean values of Duration in G1, G2 & G3 of patients with AS

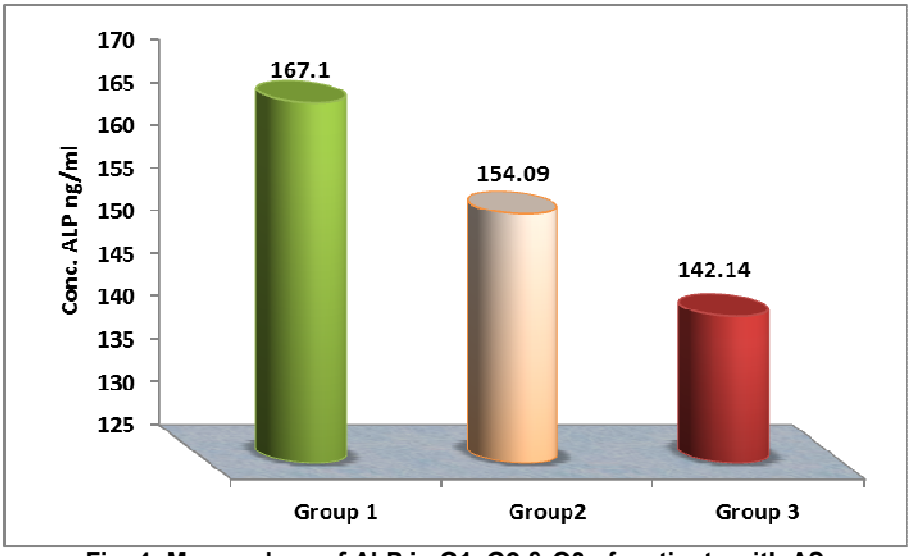

Fig. 4: Mean values of ALP in G1, G2 & G3 of patients with AS

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