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Original Research Article

Assessment and Comparison of Serum Biomarkers in Patients with Ankylosing Spondylitis

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Abstract: ***Background:*** To evaluate bone metabolism in patients with ankylosing spondylitis (AS) and test the hypothesis that nitrite, fetuin A with vitamin D and TNF-α serum concentrations are correlated with the severity of bone loss as assessed by bone mineral density (BMD) and biochemical markers of bone turnover. Osteoporosis occurs frequently in patients with AS and OPG represents a soluble decoy receptor that neutralizes receptor activator of nuclear factor-κB ligand (RANKL), an essential cytokine for osteoclast function. ***Materials and Methods:*** In our study, patients with ankylosing spondylitis (AS) who were visiting or admitted in the OPD and Emergency departments of Mahavir Institute of Medical Sciences, Vikarabad were formally enrolled for this study. Clinical data, radiographs of the spine, BMD of lumbar spine and the femur, biochemical markers of bone turnover, and serum levels of feutin A, nitrite were evaluated in 60 patients with AS (72% men) and 60 age-matched healthy controls (76% men). The estimation and comparison in serum biomarkers' levels have been analysed in concern with their ability to predict impaired healing at an early stage. All this consolidated data was analysed using SPSS software. ***Results:*** The result showed significant variations in measuring haemoglobin (Hb), Serum nitrite but significant increase of white blood cells (WBCs), platelets count and erythrocyte sedimentation rate (ESR) with increasing of TNF-α (P value<0.01) among different group. ***Conclusions:*** From the result can be accomplish that fetuin A with vitamin D and TNF-α play essential role in prognosis and aetiology of AS. Thus, measurements of these biomarkershold promise in differentiating between inflammatory and mechanical low back pain.

Keywords: Serum nitrite, fetuin A, vitamin D, ankylosing spondylitis.

INTRODUCTION

Ankylosing means stiff or rigid, spondyl means spine, and itis refers to inflammation. The disease causes inflammation of both cartilaginous joints of the spine and the sacroiliac joints, resulting in stiffness and pain [1]. The occurrence of a major dominant gene in AS was supported by the finding of a strong association with the (HLA-B27) human leukocyte antigen [2]. A genetic predisposition exists for the development of the disease, as evidenced by its strong association with HLA-B27, especially in the case of AS, where 90% of the patients are positive for this allele [3].

Biomarkers plays an important role in providing the information that promotes understanding of the prognosis, activity of the disease, and pathogenesis of the AS. The biomarkers such as the tumor necrosis factor-alpha and interleukin-17, ESR and C-reactive protein are the currently used biomarkers for the evaluation of inflammatory activity of the disease [4]. However, these biomarkers do not have the most ideal specificity, sensitivity, and reproducibility characteristics. These inflammation markers offer a poor correlation to the degree of activity in patients with AS [5].

Several studies have explained the role of macronutrients and micronutrients foods in the progression and development of chronic diseases. Active vitamin D 1 excites absorption of intestinal calcium. Fetuin-A is divergently controlled by different mediators like TNF, IFN-γ, and HMGB1, and functions as a positive or negative acute phase protein in injury and infection [6].

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Recently, the role of IL-23, IL-1α and IL-7 has showed associations with polymorphisms in AS [4]. The main challenges for the management of AS are related to the lack of biomarkers associated with disease activity as well as the inability to predict joint damage and response to treatment. So the present study was aimed to assess and compare of serum biomarkers in patients with AS.

MATERIALS AND METHODS

In our study, the normal individuals accompanying orthopaedic patients who were visiting/admitted in the OPD and Emergency departments of Mahavir Institute of Medical Sciences, Vikarabad were formally enrolled for this study. A total number of 60 ankylosing spondylitis (AS) patients and 60 healthy individuals considered as control subjects, from both ender between the age of 22 and 65 years, were included in this study. The aim of the present study was to assess and compare serum Biomarkers among healthy subjects and in Patients with Ankylosing Spondylitis**.** All participants were briefed and adequately advised in the local language, and their written informed, voluntary consent was obtained. All the enrolled subjects were subjected to a careful history, general and systemic physical examination. The questionnaire recorded information on gender, age, height, weight and comorbidity status (such as hypertension, diabetes mellitus and any other metabolic disorder) and medication use, including and nutritional supplementation. The height and weight of the individuals were used to calculate the body mass index (BMI).

Our study was a prospective longitudinal study; this was designed to study persons who were suffering from Ankylosing Spondylitis (AS), and these patients were routinely followed up to 3 months post AS. The diagnosis of AS patients was done by rheumatologist according to Bath Ankylosing Spondylitis Functional Index (BASFI) and Bath Ankylosing Spondylitis Disease Activity Index (BASDAI). Many cases were excluded: chronic diseases, rheumatic diseases, endocrine and metabolic disorders.

Biochemical Estimation: 10 ml of blood obtained from every individual in this study. These samples of blood samples were processed and separated using a serum separator tube, which allowed clotting the blood samples within 30 minutes and then centrifuged for 15 minutes (1000 g). Serum samples were stored in a laboratory deep-freezer at -80°C, by using commercially available kits; different ELISA assays performed for estimation of haematological profile, serum nitrite, fetuin A with vitamin D and TNF-α.

Statistical Analysis: All the consolidated data were analysed using SPSS software. The Student t-test and the Mann-Whitney test used for comparative analyses of serum biomarkers. The p-value of <0.05 was taken to be statistically significant.

RESULTS

Table 1: Demographic data of the Ankylosing Spondylitis patients of the present study

Demographic data	n=60
Age	38.5 ± 7.4
Age at onset of symptoms	32.6 ± 8.5
Evolution time	4.51 ± 3.08
Gender	43
HLA-B27 (+)	32 (53.3%)

The study group comprised 60 individuals, with a mean ± SD age of 38.5 ± 7.4 years. All patients had adult-onset AS (mean ± SD age at onset 32.6 ± 8.5 years). Out of 60 cases, only 32 patients (53.3%) were positive for HLA-B27. Seven of the female patients were positive for HLA-B27, whereas 25 male out of 60 patients were positive for HLA-B27. Correlation of joint involvement with HLA-B27 antigen positivity revealed that in HLA-B27-positive (Table 1).

Table 2: Baseline characteristics of healthy Control Group and Ankylosing Spondylitis patients' group

Parameters	Control Group (n=60)	Patient Group (n=60)
BMI	24.3±3.5	25.3±2.9**
Months of Severe Back Pain	1±0.24	20±1.57***
BASDAI score (0–10)	0	37 (61.67%)
BASFI score (0–10)	0	23 (38.33%)

**P Value < 0.01, ***P Value <0.001

Table 2 shows the statistically difference in BMI of control group compared with AS group, the severity of the back pain was also assessed in between the groups, there was a statistically difference in the patients group when compared with healthy control group. 61.6% of the patients group showed BASDAI score whereas, 38.3% individuals from the patients group also showed BASFI score (Table 2).

Table 3: Assessment and Comparison of Haematological profile between Control Group and Ankylosing Spondylitispatients' group

Parameters	Control Group (n=60)	Patient Group (n=60)
Hb (g/dl)	14.02±6.31	16.81±4.28**
ESR (mm/ H)	5.71±1.34	11.53±0.96*
RBCs count ($x10^3$/µl)	431.562±87.21	523.66±87.6**
Platelets count ($x10^3$/µl)	271.43±87.6	212±34.2***
WBCs count ($x10^3$/ µl)	35.7±11.2	47.23±8.7**
25-Vitamin D_3 (µg/l)	21.3±9.8	22.3±9.8*
1,25-Vitamin D_3(ng/l)	58.51±12.57	47.2±11.53**

Data presented as Mean ± SD, *P Value <0.05, **P Value <0.01, ***P Value <0.001

The results depicted in Table 3 shows the statistically significant correlation between haematological parameters and vitamin D levels in Control, Ankylosing Spondylitis patients' group.

Table 4: Assessment and Comparison of Serum Biomarkers between Control Group and Ankylosing Spondylitispatients' group

Parameters	Control Group (n=60)	Patient Group (n=60)
TNF-α (pg/ml)	2.5±0.9	9.8±1.4***
Phosphate (mmol/L)	1.3±0.7	0.94±0.24*
Serum nitrite (µmol/ L)	7.56±6.57	39.52±18.55***
Fetuin A (ng/ml)	140.1±32.8	92.37±17.2**

Data presented as Mean ± SD, *P Value <0.05, **P Value <0.01, ***P Value <0.001

The results depicted in Table 4 shows the statistically significant corelation between TNF-α, Serum nitrite, and Fetuin Alevels in Control, Ankylosing Spondylitis patients' group.

DISCUSSION

Spondyloarthritis is a group of chronic, inflammatory, rheumatic diseases characterized by overlapping clinical signs and symptoms and a common genetic background. Ankylosing spondylitis shows a strong correlation with human leukocyte antigen (HLA-B27), and number of evidence suggests that the B27 gene may have a pathogenic role in the development of AS and HLA-B27 has a tendency for familial association [7].The aim of the present study was to assess and compare serum Biomarkers among healthy subjects and in Patients with Ankylosing Spondylitis.

The present study shows that the mean age group of the patients group was found to be 32.6 years. The present findings are in accordance with earlier studies [8]. The human leukocyte antigen (HLA-B27) is a class I antigen of the major histocompatibility complex, and it is strongly associated with ankylosing spondylitis and other related spondyloarthropathies [9]. In this study, we found that the strong association of HLA-B27 was seen in 53.3%, similar to our observation, earlier study by Khan has also reported in AS patients [10].

For better diagnosis, clinical and prognostic assessment and evaluation of the response to treatment of AS, biomarkers like tumor necrosis factor (TNF)-α inhibitors (TNFi) are the key proteins and proved to play a significant role in the inflammatory chain reaction and joint inflammation, pain, and damage in ankylosing spondylitis [11]. IL-6, similarly to TNF-α, is one of the most extensively studied cytokine in rheumatic diseases, especially in rheumatoid arthritis (RA), but it has also been evaluated in axSpA. IL-6 is produced by a variety of immune cells that further induce the production of several acute-phase proteins [12].

Our study documented elevated serum concentration levels of TNF-α in patients with As compared to healthy individuals. An important data set has evaluated serum TNF-α as a biomarker that reflects disease activity. BMI, BASDAI and BASFI scores showed statistically difference in the patients group when compared with healthy control group.

Present study also evaluates tat ESR, haematological parameters and vitamin D levels were significantly increased in early AS compared to controls. This finding is consistent with a series of previous studies suggesting that the above investigations may slightly outperform as an supportive marker of tissue inflammation in AS [13].

Fetuin A is glycoprotein of alpha-2-HS type detected in the plasma of fetus and due to injury or infection response it can also synthesized principally by liver in adult life. In current study: fetuin A records high significant decreasing of serum level in AS, when compared to healthy group. This finding is in the line of recent study reported lower serum fetuin-A levels in AS compared to controls [14]. This study is in parallel with other study: Fetuin A serum concentration decreased in

AS significantly with increased C-RP and not significant with ESR in both genders [15].

The importance of the Serum nitrite in inflammatory joint disease is studied extensively. Several studies suggest that tissue injury in inflammation involves NO production. Present observations shows that serum nitrate levels were found to be higher in patients with AS when compared with control group, these observations are in accordance with earlier studies [16].

To conclude, the result can be accomplish that gender distribution, HLA-B27 positivity, fetuin A with vitamin D and TNF-α and serum nitrate play essential role in the pathogenesis and progression of AS which should be more comprehensively investigated.

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