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ORIGINAL ARTICLE

Assessment of Prognostic Variables of Medical Importance and their Impending Role to Develop Rheumatoid Arthritis

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ABSTRACT

Aim: To assess the role of different prognostic variables of medical importance having potential role in the progression of rheumatoid arthritis (RA).

Study Setting: This cross sectional case control study was carried out in the Institute of Molecular Biology and Biotehnology, The University of Lahore-PakistanDuring the period of from Jan 2019 to Jan 2021

METHODOLOGY: Fifty patients of rheumatoid arthritis and fifty healthy age matched males were selected to analyse the Levels of MDA (nmol/ml), SOD (U/ml), GSH (µmol/L), CAT (U/L), IL-6 (pg/ml), TNF-α (pg/ml), MMP-9 (ng/ml) and NO (µmol/L) using ABCAM elisa kits.

RESULTS: Current study observed significant rise in concentrations of MDA ($p = 0.025$) and significantly lower concentrations of SOD ($p = 0.021$), GSH ($p = 0.054$) and CAT ($p = 0.001$) in the RA patient. The mean values of IL-6, TNF-α,MMP-9 and NO were 11.59±3.29 pg/ml, 26.59±5.29 pg/ml, 91.99±14.529 ng/ml and 22.259±5.29 µmol/Lin RAsubjects' verses 3.59±0.254 pg/ml, 13.29±3.59 pg/ml, 42.29±5.88 ng/ml and 6.58±1.59 µmol/Lin healthy controle was observed respectively.

CONCLUSION: Upregulation of MDA, MMP-9 and TNF-α and downregulation of SOD, GSH and CAT portrays that antioxidants have impending role in disease (RA) development and proression as depicted from strong corelations coefficients matrix. The results of different variables in the present study shows that these markers may be used as predictive markers of prognostic importance in future to predict the disease severity and theranostic importance.

Keywords: MDA, Interleukin-6, MMP-9, SOD, CAT, TNF-α

INTRODUCTION

Rheumatoid arthritis is autoimmune disease characterized by systemic as well as chronic inflammation, it may affect different organs and tissue but primarily affect the cartilage and joints. Although RA development and its progression is still remained to be defined, different therapeutic routes are presented, and they have completely changed disease prognosis[1,2].In RA pathogenesis, several cell types including macrophages, osteoclasts, T and B lymphocytes, as well as synovial fibroblasts, are known to be involved. As these cells are coordinated, many inflammatory mediators are released, maintaining the disease's inflammatory response. [3]. There are four stages of RA progression, at early stage there is only inflammation can be seen in synovial membrane instead of bone/cartilage damage and this is called stage [1]. Stage 2 of RA is characterised by synovial membrane inflammation, which damages cartilage. Stage 3 of RA is marked by bone and joint degeneration can lead to arthritis. It's the end stage of RA, when swelling in the joints has lessened but function in the joints has gotten worse along with muscle weakness, joint structural deterioration and bone fusion.

In RA patients, the synovial membrane is characterised by hyperplasia, angiogenesis, proliferation and CD4+ T helper cell infiltration. Inflammation and destructive process are caused by the two important pro-inflammatory cytokines comprising interleukin-6 and tumour necrosis factor alpha However, several supplementary cytokines too play main role in RA pathogenesis[5,6]. In recent years, the role of interleukin-6 in health and disease has been under a lot of scrutiny predominantly in the course of recent pandemic COVID-19. IL-6 mediated various inflammatory pathways are also relatively accountable in the development and progression of rheumatoid arthritis[7]. It has been found that cytokines, both pro- and anti-inflammatory, play an important role in the course of RA, resulting in inflammation and cartilage damage, 8. Drugs that target cytokines in the body have shown promise in RA patients as disease-modifying agents[9].

In polypeptide chains of different target protein, the peptide bond hydrolysis is catalysed by the enzymes named as proteases that can regulate several essential physiological processes such as protein degradation, apoptosis, immune response, signal transduction and autophagy[10]. Any dysregulation in the activity of protease enzymes can cause the development of different diseases such as RA. As the disease progresses, bone and cartilage are lost from the joints.[11]. ECM components such as collagen and proteoglycans in the articular cartilage of afflicted joints have been found to breakdown by MMPs. The higher amounts of MMPs found in RA patients are thought to be to blame. Because of the degradation of fibrils in the basal area and the deterioration of proteoglycans on the surface, it is possible that MMPs play a special role in joint degeneration in RA patients.[12],[13]

MATERIALS AND METHODS

Research and Ethics Committee at the University of Lahore approved this study. Fifty adult males (RA Positive) in this study ranged in age from 40 to 70 years on average, and they were tested for a variety of physiological and biochemical characteristics. In this investigation, 50 healthy adult males of the same age and sex were used as positive controls. For the analytical assessment, venous blood samples were obtained from the anticubital vein of each participant and the blood samples were then centrifuged within one hour after extraction. The separated serum was then stored at -70°C for the future assays.

Biochemical Analysis Of Different Variables In Ra Patients: Levels of MDA (nmol/ml), SOD (U/ml), GSH (µmol/L), CAT (U/L), IL-6 (pg/ml), TNF-α (pg/ml), MMP-9 (ng/ml) and NO (µmol/L) in patients of RA and healthy controls patients were estimated using ABCAM Elisa kits.

Excel spreadsheets were used to gather all of the readings and do statistical analysis (SPSS v.16 or later). (MeanS.D) where (p0.05) indicated significant results were taken. T-tests and Spearman correlations were applied in the study.

RESULTS

Current study comprised fifty RA patients as cases and fifty healthy males as controls. The serum MDA level was found 4.025±1.059 nmol/ml and 0.915±0.059 nmol/ml) in RA patients and controls respectively, which was significant statistically ($p=0.025$). The activity of antioxidant enzymes including SOD, GSH and CAT was found to be lower in patients with RA than controls (0.047±0.001 Vs. 0.17±0.005 U/ml), (4.59±1.084 Vs. 10.25±3.29 µmol/L) and (2.195±0.956 Vs. 6.49±2.18 U/L) respectively.

Table 1: Levels Of Circulating Variables In Rheumatoid Arthritis

Variables	Subject (n=50)	Control (n=50)	P- value
Mda (nmol/ml)	4.025±1.059	0.915±0.059	0.025
Sod (u/ml)	0.047±0.001	0.17±0.005	0.021
Gsh (µmol/l)	4.59±1.084	10.25±3.29	0.054
Cat (u/l)	2.195±0.956	6.49±2.18	0.0014
Il-6 (pg/ml)	11.59±3.29	3.59±0.254	0.026
Tnf-α (pg/ml)	26.59±5.29	13.29±3.59	0.014
Mmp-9 (ng/ml)	91.99±14.529	42.29±5.88	0.005
No (µmol/l)	22.259±5.29	6.58±1.59	0.026

DISCUSSION

The current study aims to investigate the oxidative stress markers (MDA), activities of antioxidant enzyme (SOD, GSH and CAT) and (IL-6, TNF-α and NO) in RA patients as well as determining the interaction behaviors among all these variables that may play a key role in RA pathogenesis. In present study, an increased level of MDA was recorded in RA patients than in controls, which indicates the elevated oxidative stress in RA patients. Several studies have described that in RA patients the MDA levels are considerably elevated when compared to healthy controls[14]. Shakir et al. reported a significant rise in MDA levels of their RA patients[15]. Antioxidant enzymes provide defense against free radical induced damage by the inhibition of lipid peroxidation. Current study found that the levels of SOD, CATand GSH was decreased significantly in RA patients as compared to controls. In several studies, reduced SOD activity has been shown in RA patients[16].

RA is a chronic and systemic inflammation, increased proliferation of synovial cell, and bone loss are all symptoms of rheumatoid arthritis. Inflammatory cytokines have been linked to the progression of RA, and IL-6 has been found to play a significant role in this process.[8]. One of the many biological effects of IL-6 is the induction of B cell and plasma cell proliferation, as well as an increase in the production of IgG, M, and A antibodies, as well as the mediating of acute-phase protein production by hepatocytes and the beginning of T helper cell differentiation. A number of proinflammatory cytokines, are present in RA, making it an inflammatory condition. Peripheral blood IL-6 levels in RA patients above the age of 50 were considerably elevated[17]. In RA patients, IL-6 is also implicated in the degeneration of joints. In the current investigation, IL-6 levels were shown to be elevated in both RA patients and healthy individuals. RA pathogenesis has been linked to elevated levels of pro-inflammatory cytokines in earlier investigations, according to the findings of this study. RA patients had higher levels of TNF- than controls in the current study, indicating that it may play a role in disease aetiology.

Table 2: Prognostic Variables In Rheumatoid Arthritis

Variables	(r)	P-value
Sod vs mmp-9	0.652***	0.015
Sod vs mda	0.516**	0.001
Sod vs tnf- α	0.859**	0.004
Il-6 vs mmp-9	0.678**	0.025
Tnf- α vs no	0.659***	0.014
Mmp-9 vs tnf- α	0.658***	0.023
No vs mmp-9	0.589***	0.011
Tnf- α vs no	0.658**	0.000
Tnf- α vs il-6	0.648***	0.015
No vs sod	0.659***	0.0323

Study after study has found evidence that endogenous NO synthase is elevated in people with rheumatoid arthritis (RA). There is a high concentration of NO18 in the inflamed joint of RA patients. According to the results of a recent study, people with RA have higher serum NO levels than healthy controls. Even while NO is critical to a number of physiological activities, excessive production of it can be harmful. Apoptosis, signal transduction, mitochondrial activities, and apoptosis are all affected by NO at the site of synovial inflammation. The effects of NO are dependent on the concentration of the gas. Overproduction of NO is a significant factor in the development of RA. Research into how different inflammatory mediators influence immune cell activity needs to be done further[19].

CONCLUSION

Based on the correlation between different variables current study determined that increase in MDA levels, reduction in antioxidant enzyme activity, inflammatory mediators and MMP-9 can induce chronic inflammation, which play an important role in the pathogenesis of RA. Further studies are required to know about exact etiological mechanism of RA pathogenesis. Inhibition of provocative mediators such as IL-6, TNF-α may represent a noval therapeutic target in RA management.

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