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ORIGINAL ARTICLE

Upregulation of Proinflammatory Variables of Analytical Importance and their Potential Interplay in the Pathogenesis of Rheumatoid Arthritis

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ABSTRACT

Objective: Study role of the selected inflammatory markers and their importance in the development and progression of rheumatoid arthritis.

Study Design: Cross-sectional study

Place and Duration: Institute of Molecular Biology and Biotechnology, The University of Lahore, (11-months)

Methodology: A total of 50 patients diagnosed with rheumatoid arthritis in the Department of Orthopedics (Allama Iqbal Medical College, Jinnah Hospital, Lahore-Pakistan) and 50 healthy individuals of the age of 35-45 were included into the study. Glutathione, Catalase, Maliondialdehyde, Superoxide dismutase, Glutathione peroxidase, Nitric oxide (NO) and vitamin C, E and A was estimated spectrophotometrically. Serum C-Reactive Protein, IL-21, TNF-α, AOPPs, AGE's and MMP-3 was measured by ELISA method using Human-ELISA kits (Glory)

Results: A statistically noteworthy raise of all the biomarkers level in serum of rheumatoid arthritis (RA) was seen (p=0.001) comparable with healthy individuals. The correlation between joint damage progression diagnostic biomarker AGE and MMP-3 levels (r=0.485, 95% p=0.001) was highly significant. CRP and IL-21 also showed highly significant raise in joint inflammation (r=0.359, p=0.001). The results significantly depicted that model of MMP-3 levels at the onset of the disease and CRP and IL-21 were the robust interpreters of the erosion development. Similarly, antioxidants including Vitamin E, Vitamin C and Vitamin A were decreased in levels of (p<0.011), (p<0.011) and (p<0.022) respectively in RA subjects than in control subjects. Vitamin E, C and A are the most imperative free radical foragers within membrane. They also act as the major line of resistance against free radicals.

Conclusion: Development of pain in joints in rheumatoid arthritis condition is due to the increase in the oxidative stress which is the key contrivance in the pathogenesis of RA.

Keyword: Reactive oxygen species, Oxidative stress, oxidation of lipid peroxides, Rheumatoid arthritis, Osteoarthritis, osteocyte damage.

INTRODUCTION

Rheumatoid arthritis (RA) is an inflammation of multiple joints of unknown cause with yearly frequency of 0.5% to 1% of total world population stated every year. The frequency of this autoimmune disease is observed to be more in women than in men with its increased onset during the age of 40-50 years. The clinical manifestation of rheumatoid arthritis is the inflammation of peripheral joints due to increased ROS production in multiple small and large joints causing joint muscle swelling, early morning stiffness and deterioration of articular deformities and joint tissues breakage. Rheumatoid arthritis is not only the inflammation of multiple joints but it directly affects the whole heathy condition of the patient including inflammatory synovitis, fatigue, malaise and depression [1]. Biochemical markers of osteoclast degradation facilitates the early diagnosis of joint destruction. Several biochemical cascades are involved in the development of inflammation in RA involving several B cells, T cells, mitochondrial enzymes secretion and release of several proinflammatory cytokines including interleukins, MMPs, proteases and chemokines [2,3]. Several cellular cross talks including RANKL, NF-kB, MAPK and Akt signaling activate osteoclasts and bone resorption [4]. Fibroblasts are responsible for expressing RANKL in combination with macrophage colony stimulating factor (MCSF) promoting the differentiation of proosteoclasts into cartilage and bone [5]. Recent literature suggested that defective glycolytic enzymes releasing in T cells of the immune cells of the RA subjects oromotes the accumulation of NADPH which on oxidation produces a burst of ROS [6,7]. This condition pushes the cell in oxidative stress prevailing several regulating oxidative biomarkers [8]. Cellular citrulline levels associated with CD4+T cells are also observed to be high in RA patients [9]. Thus, targeting the immune cells B and T cells, chronic mediators, chemokines and cellular signaling molecules could be used for theranostic approach to the rheumatoid arthritis.

Rationaile: Primary aim of the study is to state the role and medicinal importance of pro-inflammatory markers that are involved in development and progression of rheumatoid arthritis among the patients.

MATERIALS AND METHODS

A total of 50 patients detected with rheumatoid arthritis in the Department of Orthopedics (Allama Iqbal Medical College, Jinnah Hospital, Lahore-Pakistan) following the criteria of age (45-70 years) was included into the study. Fifty healthy individuals were encompassed in the study as negative control. As per the inclusion and exclusion criteria patients with rheumatoid arthritis aging between 30-50 years were included in the current study while those on any other medication, alcohol or had any history of surgery or metabolic syndrome such as jaundice, diabetes or inflammation were excluded out from the study. The whole experimental protocol was carried out under the approval of Research Ethical Committee of Centre for research in Molecular medicine, The University of Lahore. 5ml venous blood sample of each RA patient as well as healthy control individual was taken from the antecubital vein. After collection, the blood was centrifuged and serum was alienated and stored in freezer at -70°C for further biochemical analysis. All the chemicals and reagents employed in the research project were purchased from the trademark company Sigma Chemical Company (USA). A needle was placed in an antecubital vein of the participant's armand 6 ml of blood was collected. 3 ml collected in an EDTA coated tube and performed hemoglobin and neutrophils count. The remaining 3 ml of blood was used in the evaluation of biochemical assays.

Estimation Diagnostic Variables of Medical Importance: Glutathione [10], Catalase [11], Thiobarbituric acid reactive substances [12], Superoxide dismutase [13], Glutathione peroxidase [14], Nitric oxide (NO) and vitamin C, E and A [15] was estimated spectrophotometrically. Serum C-Reactive Protein, IL-21, TNF-α, AOPPs, AGE's and MMP-3 was measured by ELISA method using Human-ELISA kits (Glory)

Statistical Investigation: The clinical data was expressed as mean ± SD (standard deviation) using SPSS version 5.0. The significance of the data was evaluated by the Student's t-test. A p-value <0.05 was considered statistically significant. One-way analysis of variance and spearman correlation (Two Tailed) was used to correlate the different variables. The difference was considered significant at p<0.05.

RESULTS

The present study comprises of fifty (n=50) clinically diagnosed subjects of Rheumatoid Arthritis and 20 normal healthy controls. The level of MDA (malondialdehyde) in controls was 0.96ng/dl±0.0392. The value in Rheumatoid Arthritis subjects was 4.57 ng/dl ±1.22. The value was significantly increased (p<0.015) in Rheumatoid Arthritis patients as compared to controls.

The value of SOD (superoxide dismutase) in normal healthy controls was1.056 ng/dl ±0.056. The level was slightly decreased (p<0.041) in Rheumatoid Arthritis patients than control group (0.118 ng/dl ±0.0018). SOD removes the toxic superoxide radical (O2) formed by the partial reduction of oxygen in tissues. Therefore, SOD is important for normal cell function. SOD is the antioxidant enzymes have also an antitoxic effect against superoxide anion. GSH was found to be significantly lower in patients compared to controls (p<0.005) in present study. In healthy controls the value of Catalase was 6.29 ng/dl ±1.99. The central non-enzymatic antioxidant tripeptide glutathione in its reduced form (GSH; c-L-glutamyl-L-cysteinyl-glycine). The value of Catalase was significantly decreased in patients 4.59ng/dl±1.088. The Catalase level was significantly decreased (p<0.006) in RA patients as compared to controls. Catalase enzyme converts the H_2O_2 to water and O_2. Catalase is the enzyme, which protects the cells from the collection of hydrogen peroxide. Statistically significant increase in levels of Nitric Oxide (p<0.036) in Rheumatoid patients compared to normal healthy controls. NO present at the sites of inflammation. Nitric oxide synthesis by endothelial cells, macrophages representing a role inflammatory process. NO was increased by 35.28ng/dl in RA patients as compared to normal subjects (19.98ng/dl). While non-enzymatic antioxidant Vitamin C and Vitamin E were slightly decreased in Rheumatoid Arthritis subjects. Statistically decrease in levels of Vitamin A, C and E were (p<0.011) than control subjects (Table: 01).

The level of Vitamin A in controls was652.35ng/dl±12.91. The value in RA patients was 324.76±10.88. There was a significant decrease in (p<0.022).Therefore non-enzymatic antioxidants: Vitamin E, Vitamin C and Vitamin A were decrease in levels of (p<0.011), (p<0.011) and (p<0.022) in RA subjects than in control subjects. Vitamin E is the most important free radical scavenger with in membrane. It is also the major line of defense against free radicals. The decrease in the levels on non-enzymatic antioxidant may be due to the increase of the reactive species. Vitamin A, E and C are well known antioxidants; they play an important role in protecting the lipids. Antioxidant vitamin E is the major chain breaking in human body. Antioxidants are developed in the body and can be taken from the food; the humans consume them. Vitamin E, A &C are required as micronutrients for human body. They were also highly decreased in RA patients as compared to the control group. (Table: 01)

MMP-3 and CRP levels at disease onset are the predictive of the joint damage progression as depicted by our results. The healthy control individuals showed the decreased levels of CRP and MMP-3 as compared to the RA patients. Advanced oxidation protein products (AOPPs) were significantly raised in serum of RA patients due to osteoclasts apoptosis. This effect is mediated through AGE's which is also observed to be higher in inflammatory bone cells which can be observed by the increased level (3.118ng/dl) in RA patients comparable with healthy individuals (1.78ng/dl) (Table: 01).

Tables 1: Profile of Different Variables in Patients Suffereing from Rheumatoid Arthritis

Variables	Control (n=50)	Subjects (n=50)	P-value
MDA	0.96±0.0392	4.57±1.22	0.015**
SOD	1.056±0.056	0.118±0.0018	0.035*
GSH	9.29±1.99	6.53±1.28	0.011***
CATALASE	6.29±1.45	4.59±1.088	0.006*
NO	19.98±3.58	35.28±6.58	0.000**
Vit-E	13.59±4.29	7.48±1.44	0.041**
Vit-C	4.58±0.956	2.48±0.956	0.019**
Vit-A	652.35±19.65	358.98±21.58	0.017*
CRP	1.05±0.043	1.85±0.046	0.036*
IL-21	4.358±1.09	8.59±2.55	0.001*
TNF-α	20.19±2.58	31.64±5.66	0.011**
AOPPs	0.659±0.018	1.88±0.012	0.017**
AGEs	1.78±0.114	3.118±0.956	0.004*
MMP-3	48.591±5.65	88.59±7.58	0.007*

Tables 2: Correlation Coefficients Matrix of Different Variables in Patients Suffering from Rheumatoid Arthritis

VAR.	MDA	SOD	GSH	CAT	NO	V-E	V-C	V-A	CRP	IL-21	TNF-α	AOPPs	AGEs	MMP-3
MDA	-	0.534	0.425	0.356	0.648	0.458	0.356	0.014	0.515	0.578	0.956	0.458	0.356	0.457
SOD		-	0.159	0.351	0.259	0.324	0.258	0.326	0.425	0.125	0.235	0.422	0.658	0.457
GSH			-	0.295	0.351	0.159	0.342	0.125	0.325	0.147	0.325	0.526	0.458	0.234
CAT				-	0.458	0.021	0.261	0.147	0.235	0.024	0.015	0.158	0.325	0.214
NO					-	0.259	0.458	0.265	0.425	0.325	0.254	0.021	0.254	0.235
Vit-E						-	0.245	0.156	0.025	0.235	0.024	0.254	0.015	0.348
Vit-C							-	0.269	0.325	0.148	0.259	0.322	0.145	.0294
Vit-A								-	0.158	0.235	.0451	0.358	.0147	0.269
CRP									-	0.359*	0.325	0.259	0.014	0.026
IL-21										-	0.441	0.562	0.268	0.165
TNF-α											-	0.425	0.256	0.258
AOPPs												-	0.326	0.452
AGEs													-	0.485*
MPP-3														-

DISCUSSION

Enormous research studies are available regarding MMPs and interleukins as predictive indicators of the synovial joint inflammation and joint damage development. Therefore, it is important to elucidate the key factors that regulate the bone invasion. It is reported earlier that proinflammatory biomarkers such as IL-21, TNF-α, MMP-3, AOPPS, AGEs and CRP result in the modulation of CD4+T lymphocytes in RA of both human and rat model. The present study found that IL-21 was highly expressed in RA patients leading to the activation of PI3K/AKT signaling. MMP-3 and adhesion molecules (STAT 3 and cadherin-11) also validate the progression of the disease. Hence, MMP-3

and IL-21 may therefore offer a novel target strategy to diagnose and control the aggression of rheumatoid arthritis. Patients with RA also showed increased expression of C-reactive protein in blood. CRP is released by the liver in response to the infiltration of distinct cytokines blocking CD32 to increase invasiveness and breaking of bone. P38 MAP kinase expression is upregulated which activate the cascade of NF-kB signaling in RA condition. Thus, blocking CRP signaling cascade may present a distinctive theranostic approach toward RA treatment. Pearson correlation matrix successfully depicted the relationship of various biomarkers analyzed in this study. AGE and MMP-3 showed high significant correlation (r=0.485, 95% p=0.001). This reveals joint narrowing and loss of articular cartilage. Similarly, a highly significant association was also observed between MMP3 and IL-21 (r=0.58, p=0.001). CRP and IL-21 were also highly raised showing a remarkable association of (r=0.359, p=0.001).These results

Depicted that MMP-3, IL-21 and all the above discussed parameters are responsible for the onset of the disease. CRP, MMP-3 and IL-21 are the strongest predictors of the bone resorption and erosion.

This study reveals that Malondialdehyde (MDA) valuation appears to be a subtle marker of inflammation in this chronic auto immune disorder and would help in understanding the nature of inflammatory impairment at a cellular level. A restraint reaction initiates the detrimental effect of the process which provides continued supply of free radicals which in turn causes endorsed peroxidation. It also leads to establishment of complex mixtures of lipid hydro peroxide aldehydes end products such as MDA. The existing study showed significant upsurge in lipid Peroxidation product MDA. MDA levels were found to be increased in both RA and OA patients than in healthy individuals, signifying an increase in the process of lipid peroxidation in these patients. The results of present study of MDA concentration was related to results obtained by previous studies [20] which advocated that serum MDA level in RA and OA patients increases significantly.

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Authors contribution: Every author played its part and worked professionally as a team, with their hard work present study was conducted.

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