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PRO AND ANTIOXIDANT SYSTEMS IN JUVENILE, RHEUMATOID AND OSTEO ARTHRITIS

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Abstract: Arthritis is a chronic inflammatory disease with an interaction of various genetic and environmental factors. It is a heterogenous group of disorder with different etiological and pathogenetic mechanisms. Lipid peroxidation is the prevailing mechanism in various inflammatory disorders. Hence the present study is aimed at evaluating the status of pro and antioxidants in the etiology of arthritis. Blood samples from 291 arthritis and 125 healthy controls matched for age and sex have been collected from Orthopedic Unit of Vaidya Vidhan Parishad, King koti , Hyderabad. Levels of malondialdehyde, ceruloplasmin and nitric oxide were analysed in serum and plasma samples by following appropriate methods. Mean levels of malondialdehyde was found to be significantly elevated in rheumatoid and osteoarthritis compared to controls where as a slight decrease was observed in juvenile arthritis compared to juvenile controls. Ceruloplasmin was also found to be elevated in juvenile arthritis compared to controls indicating the role of CP as an antioxidant in the etiology of Juvenile arthritis. Nitric oxide levels were significantly elevated in rheumatoid arthritis compared to control subjects. The findings can be correlated to the homeostatic role of pro/anti oxidant mechanisms resulting in delineating the subset of arthritis with juvenile arthritis being purely genetic and autoimmune rheumatoid arthritis being inflammatory change with oxidative stress and osteoarthritis resulting due to the disturbance in homeostasis of pro and antioxidants.

Key words: Arthritis, Ceruloplasmin, Malondialdehyde, Nitiric oxide, Lipid peroxidation

INTRODUCTION

Arthritis is a chronic systemic inflammatory disease predominantly affecting the diarthrodial joints and cartilage. Juvenile arthritis (JA) is a chronic arthritis beginning in childhood with musculocutaneous pain and other symptoms common in the childhood [1]. Rheumatoid arthritis (RA) is the most common connective tissue disorder with variable degrees of severity and disability with symmetrical inflammatory and destructive changes and tenosynovitis [2]. Osteo arthritis is also most common form of arthritis and a degenerative joint disease associated with cartilage degradation and loss of movement [3]. Thus arthritis is a heterogeneous disorder with genetic, biochemical and environmental factors involved in its etiology.

Oxygen derived free radicals and their products play an important role in the lipid peroxidative tissue damage. Malondialdehyde (MDA), a stable product of lipid peroxidation of membrane lipids is a known marker for tissue injury and plays a major role in various inflammatory diseases [4]. Ceruloplasmin (CP) a copper containing alpha 2 globulin of human plasma and is found to be elevated in chronic and acute arthritis [5]. Nitric oxide (NO) a short lived free radical has an important role in a number of biological processes and found to be elevated in various auto immune diseases[6]. Hence the present study is aimed at investigating the role of prooxidants like malondialdehyde, nitric oxide and antioxidant like ceruloplasmin in the etiology of arthritis which may throw light as prognostic indicators in the

inflammatory process associated with the condition.

MATERIAL AND METHODS

Blood samples from 291 arthritis and 125 healthy controls and 25 juvenile controls matched for age and sex have been collected from out patient unit of orthopedic department of Andhra Vaidya Vidhan parishad, Kingkoti, Hyderabad. Radiologically confirmed cases of arthritis were considered for the present study. Out of 291 patients 41 belonged to juvenile arthritis, 150 rheumatoid arthritis and 100 osteoarthritis. Information on epidemiological factors such as age, sex, age at onset, duration of the symptoms, familial status and addictions such as smoking, alcohol consumption were obtained from all the cases.

Serum and plasma were obtained from the blood samples for the analysis of MDA, CP and NO by following the method of Dahle et al [7], Ravin et al [8] and Green et al [9] respectively. In brief MDA was estimated by taking 0.5 ml of plasma diluted in 1ml distilled water. To this 3.5 ml of 10% trichloroacetic acid and 1.5 ml of 0.5% of thio barbituric acid were added and the mixture was heated to 100^0C for 15 minutes to develop a pink coloured precipitate. The supernatant was collected by centrifugation at 12000 rpm for 5 min and its optical density read at 532 nm against a water blank MDA was estimated using a standard curve prepared with 1, 1, 3, 3 tetraethoxy propane in nM/dL.

Ceruloplasmin was estimated by taking 0.5 ml of serum. To this add 1 ml of acetate buffer and 0.5 ml of freshly prepared 0.5% paraphenylene diamine solution, incubated for 1 hour at 37^0C and the reaction was terminated by adding 0.5 ml of sodium azide. The optical density was read at 530 nm and the levels were calculated in mg/dL.

Nitric oxide was analyzed by deproteinizing the serum with 100 µl of 35% sulfosalicyclic acid. The solution was centrifuged for 15 minutes, 200µl of supernatant was taken to which 300 µl of 5% aqueous ammonium chloride and 60 µl of 5% ammonium hydroxide and 530 µl of Griess reagent are added. Then the tubes were placed in water bath for 10 minutes maintained at 60^0C and cooled at 4^0C and the absorbance was read at 546 nm against blank.

Means and standard deviations were calculated and students 't' test was used for inter group comparisons at 5 % and 1 % level of significance [10].

RESULTS AND DISCUSSION

Rheumatoid arthritis is a heterogeneous group of disorder with an involvement of various biochemical and pathogenetic factors in the etiology of the disease. Hence the present study includes the study of pro and anti oxidants in the inflammatory process.

Mean levels of malondialdehyde was not found to be changed in juvenile arthritis group when compared to the juvenile control group while a significant increase of MDA was observed in rheumatoid and osteoarthritis compared to the control group (Table 1). The role of MDA as a sensitive marker of inflammation is thus suggested. These results further support the concept of oxygen free radicals playing an important role in the pathogenesis of inflammatory disorders which is in accordance with the earlier findings [4]. The high levels of MDA in juvenile control could be due to an imbalance between pro and anti oxidants. However, sample size is too small

Table 1: Serum levels of malondialdehyde (nm/dL), ceruloplasmin (mg/dL) and nitric oxide (µm/ml) in control and disease groups. *p<0.05,**p<0.01

Type	Control X± S.D. (n)	Disease X± S.D. (n)	t value
Juvenile arthritis			
MDA	634.44 ± 264.6(25)	519.13 ± 214.63(41)	1.937
CP	25.92 ± 8.88 (25)	38.66 ± 18.23 (41)	3.259**
NO	2.01 ± 0.59 (25)	3.00 ± 1.63 (41)	0.255
Rheumatoid arthritis			
MDA	385.15 ± 180.40 (125)	578.46 ± 232.30(150)	7.575**
CP	37.28 ± 18.30 (125)	33.24 ± 18.39 (150)	1.818
NO	3.48 ± 2.34 (125)	5.17 ± 3.37(150)	4.776**
Osteoarthritis			
MDA	385.15 ± 180.40 (125)	593.18 ± 279.72 (100)	3.275*
CP	37.28 ± 18.30 (125)	39.74 ± 22.06 (100)	0.916
NO	3.48 ± 2.34 (125)	3.902 ± 3.35 (100)	1.099

for a definite inference to be drawn.

Ceruloplasmin, a major copper containing plasma protein that is not only involved in iron metabolism but also functions as an antioxidant [5]. Ceruloplasmin levels were found to be significantly increased in juvenile arthritis compared to juvenile controls, while there was no significant difference with respect to rheumatoid and osteo arthritis. The increase in Cp levels could be explained on the basis of extensive free radical generation due to oxidative stress and inflammatory response thereby accounting Cp as an acute phase reactant. Increased oxidative stress could be due to compensating changes in the levels of antioxidants to maintain homeostasis of the pro/ anti oxidant system.

Nitric oxide, a free radical has important effects on bone cell function. The endothelial isoform of nitric oxide synthase is widely expressed in bone on a constitutive basis whereas inducible form is only expressed in response to inflammatory stimuli [11]. Mean levels of inducible nitric oxide was found to be significantly elevated in rheumatoid arthritis and an insignificant increase in osteoarthritis patients compared to control subjects. This suggests that the increased levels of NO may inhibit bone resorption and formation which in turn suppress bone turnover thus leading to the inflammatory condition [12]. The results also indicate that these levels can serve as a marker/indicator for disease progression in patients with arthritis. Further the rise of nitric oxide as an immunomodulator can also be highlighted, wherein the increase in nitric oxide levels may enhance the cytokines and TNFα secretions in arthritis which also suggests the disorder to be autoimmune in nature.

Thus the present study highlights the interaction of both pro/antioxidants and help in delineating the subset of arthritis with JA being purely genetic and autoimmune, RA being an inflammatory disorder associated with oxidative stress and OA resulting due to the disturbance in homeostasis of pro and antioxidant systems.

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