ISSN : 2348- 8069 Int.J.Adv.Res.Biol.Sci.2014; 1(1):162-165

International Journal of Advanced Research in Biological Sciences

www.ijarbs.com

Short Communications

Immunological and biochemical studies on Rheumatoid Arthritis before and after treatment

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Abstract

The present study of serum markers carried out in 96 patients of rheumatoid arthritis revealed significant result to lipid peroxidation, NO and TNF-α.. Patients on treatment showed decreased levels of the above parameters than the patients before treatment. The study finds its importance in identifying the abnormalities of the serum markers in patients with RA. Identifying the possibility of these serum markers may help in the early diogonsis and medical management of the patients.

Keywords: Rheumatoid Arthritis; lipid peroxidation, NO and TNF-α.

Introduction

Rheumatoid arthritis is a chronic autoimmune disease. Its onset is frequently heralded by fatigue, diffuse myalgia, fever, loss of appetite, and vague malaise. Women are affected more frequently than men, in a ratio of 2 or 3 to 1.Predominance in women parallels the effect of hormones on the immune system. The ability of females in many mammalian species, including the human, to surpass males in both humoral and cellular immune response is well documented (Maiden and Mazonson, 1993). Thus if RA is somehow linked to an abnormal or harmful immune response, the result may be accentuated in females. Compared with controls, a considerably higher than expected frequency of women with onset between 50 and 55 years was recorded. At present there is body of evidence indicating that aging may have an interesting diversity of effects on the immune response (Paimela, 1995). The incidence of autoantibodies such as rheumatoid factors or anti-nuclear antibodies increases with age. In most patients the disease starts gradually and insidiously, but in a few the onset can be extremely acute, occurring within 24 to 48 hours. Prodromal symptoms of fatigue, myalgias, and malaise may be be present for weeks or months before onset of joint symptoms, but in most cases joint pain, stiffness, and swelling arise early in the disease. At time, initial joint involvement may be spotty, but evolution over weeks or months to a relatively symmetric pattern of polyarthritis is almost always observed. A study of the onset of RA in 102 patients showed that in 2 or 3 patients, the disease began in the winter. About 10% of patients showed

an acute polyarticular onset that could be pinpointed to the day; 18%could identify the week of onset, while the remainder could isolate the onset only to the nearest month. Older patients fared worst during the mean four to five year follow up(Scott and Symmons,1987) More severe eventually developed when the large joints were also involved initially, or when metatarsophalangeal (MTP) joints 1 and 3 were involved early. MTP joint involvement at onset also correlated with the early occurrence of erosions.

Materials and Methods

The subjects of the present study were patients presenting Rheumatoid arthritis along with age and sex matched controls. Blood samples were collected from the patients attending the Rheumatology center hospital. 10ml of venous blood was collected. Aliquoted in to two tubes.Plasma was collected from hepararinised blood. Centrifuged at 1500rpm for 10 minutes. Serum was separated from the whole blood; centrifuged at 1500rpm for 10minutes.Plasma and serum samples were stored.

Estimation of plasma MDA levels was carried out and lipid peroxidation product were quantified by the thiobarbituric acid (TBA) method of Gavino et al, in 1981. 0.5ml of plasma was made up to1 ml with 0.9% Saline and an equal volume of TCA . was added and incubated at 37°c for 20 min and centrifuged at 3000rpm for 10 min. To 1 ml of protein free supernatant 0.25ml of TBA was added and heated in water bath at 95°c for 1hr till a faint pink colour appears. After cooling the intensity was read at 532nm against water with a SHIMADZER UV 240 spectrophotometer. The lipid peroxidation activity is expressed in n moles of MDA equivalents /ml 1,1,3,3, Tetra ethoxypropane (1-100n moles/used as a standard)

Estimation of Nitric Oxide levels was done using Griess reagent by Green *et al*.(1982).1 ml of serum was added to 0.1ml of Sulphosalicylic acid and mixed every 5-min for every 30 min. Centrifuged at 3000rpm for 20min. 200 microliters of supernatants were added to30 ml of 10% NaOH and 300microliters of Tris HCl buffer [PH at 9.0]. 530microliters of Griess reagent was added. Kept in dark for 10min and read at 540nm against water blank

Coat micro wells with 100µ1 per well of capture antibody diluted in coating buffer. Seal the plate and incubate over night at 4°c. Aspirate wells and wash 3 times with wash buffer. Add assay diluent 200lit per well. Incubate at room temperature for 1 hour. Prepare standard and sample dilutions in assay diluent. Pipette 100µ1 of each standard, sample and control in to appropriate wells. The plate incubate for 2 hours at a room temperature. Aspirates / wash the wells 5 times with wash buffer. Add 100µl pf working detector to each well. Seal the plate and incubate for l hour at room temperature. Aspirate/wash the wells 7 times with wash buffer. Add 100µl of substrate solution to each well. Incubate the plate for 30 minutes at room temperature in the dark. Add 50µl of stop solution to each well. Read absorbance at 450nm. Within 30 minutes of stopping reaction.

Results and Discussion

The results of this investigation carried out in 96 RA patients and 15 controls are presented in tables 1-3numbers. All the patients were examined clinically and information pertaining age, sex, habits and health status were recorded in special case.Preforma clinical examinations were fouled by a series of laboratory investigation to carry out biochemical and immunological studies.

In this present study patients of RA belonged to the age group of 20-90 years. Among 96 RA patients 84 were females and 12 were males. All the patients in the study had a habit of drinking tea and coffee (1-4cups).out of 12 man 6 were smokers and 5 were alcoholic. Medical history of all the patients showed that 20 were diabetic. Nitric oxide levels estimated in the patients of RA before and after treatment and controls are presented in the table no.2.The mean ± SD level in serum were found to be 4.26±0.18 and 2.9± 0.17 respectively. The mean ±SD level in serum of control is 1.44±0.34. NO levels are high

Table.1 Plasma malondialdehyde levels (n moles/ml) of a RA patients and controls.

Category	No. of cases	MDA Level Mean ± SD
Before treatmemt RA patients	78	6.51 ± 1.76
After treatment RA patients	18	5.55 ± 1.81
Controls	15	2.07 ± 0.17

Table.2 Nitric oxide levels (µm of nitrate) of RA patients and controls.

Category	No. of cases	No levels Mean ± SD
Before treatment RA patients	78	4.93 ± 0.53
After treatment RA patients	18	4.26 ± 0.18
Controls	15	1.44 ± 0.34

Table.3 serum TNFα (pg/ml) of RA patients and controls.

Category	No. of cases	TNF~Levels Meanï SD
before treatment RA patients	78	25.75 ± 8.3
after treatment RA patients	18	16.58 ± 4.32
controls	15	8.2 ± 1.86

when compared to controls and the values were significant. Malondialdehyde levels estimated in RA patients before and after treatment and controls are presented in table no: 1. The mean ± SD of MDA level in the plasma were found to be 6.51±1.76 and 5.55±1.81 respectively.The mean±SD level in serum of control is 2.07±0.17. The MDA levels in the patients are significant high when compared to control.

Serum TNFα levels estimated in the patients of RA before and after treatment are presented in the table no.3.The mean ± in serum were found to be 25.75± 8.3 and 16.58±4.32 respectively. The mean and SD level in serum of control is 8.2±1.86.Serum TNFα levels are high when campared to controls and the values were significant.

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