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The Relation between Biochemical Parameters and Rheumatoid Arthritis Disease

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Authors' contributions

This work was carried out in collaboration between all authors. Authors HGA and FS designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors SMRP and DHA managed the analyses of the study and also the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Rheumatoid arthritis is a self-immunity, progressive, and chronic disease the cause of which is still not clear. This disorder is mainly characterized with non-exclusive inflammation of local joints or joints inflammation, morning stiffness, destruction of articular tissues, and transformation of joints. Studies have shown that development of rheumatoid arthritis is related to increased production of oxygen reactant species and decreased the ability to suppress oxidative stress. In rheumatoid arthritis patients, increased free radicals of oxygen (ROS) act as mediators of tissue damage. This point emphasizes on the necessity of applying appropriate methods for examining tissue oxidative condition and antioxidant compounds capabilities in patients with rheumatoid arthritis.
Methodology: We considered its relation with biochemical parameters. In surveying 130 patients

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with rheumatoid arthritis, the index rate of oxidant was compared in test group and control group using independent T Test by prooxidant-antioxidant balance (PAB).
Conclusion: Due to the fact that *P* Value <0. 001, we observed a meaningful difference with rheumatoid arthritis. In examining biochemical parameters in patients with rheumatoid arthritis, urea and has decreased while uric acid content has increased.

Keywords: Reumatoid arthritis; biochemical factors; prooxidant-antioxidant balance.

1. INTRODUCTION

Rheumatoid arthritis (RA) is a self-immunity, progressive, and chronic disease the cause of which is still not clear. This disorder is mainly characterized with non-exclusive inflammation of local joints or joints inflammation, morning stiffness, destruction of articular tissues, and transformation of joints [1]. Studies have shown that development of rheumatoid arthritis is related to increased production of oxygen reactant species and decreased ability to suppress oxidative stress [2]. In rheumatoid arthritis patients, increased free radicals of oxygen (ROS) act as mediators of tissue damage.

In recent years, the oxidative role of stress has been confirmed in many chronic diseases including rheumatoid arthritis [3]. Therefore, laboratorial review of oxidative stress in a patient's follow-up as well as interventions for adjusting this condition can help greatly to treatment of the patient in future. We arranged clinical examining, biochemical and blood parameters in patients with rheumatoid arthritis. This study was aimed at possibility of applying PAB method in diseases occurring on the several fields. We expect this study can be used routinely to reduce the intensity of osteo-arthritis and Rheumatoid arthritis diseases.

2. MATERIALS AND METHODS

2.1 Sampling and Bleeding

In this study, 130 patients with RA (115 female and 15 male patients bedridden in Gha'em Hospital (Mashhad, Iran) considering use of drugs and smoking) were bled. 130 healthy people also were used as control group. The study protocol was approved by the Ethical Committee of the Research Council of Ferdowsi University of Mashhad and was performed in conformance with the Declaration of Helsinki ethical guidelines, as reflected in a prioriapproval by the committee.

After gathering blood samples, we studied its relation with clinical, biochemical parameters especially hepatic enzymes, creatinine, urea, uric acid, and bilirubin serum.

2.2 Methods of Measuring

To assess biochemical factors, blood serum was separated and was measured by using spectrophotometer (OPTIZEN 2120+) according Pars Azmoon Company directions and by using laboratorial kits. Measurement of urea was conducted by calorimetric method, creatinine by enzyme method, bilirubin by photometric method using 2,4-dichloroaniline (DCA), uric acid by enzyme method, and Alkaline Phosphatase (ALP) by DGKC method (the standard of German Biochemistry Society). Commercially available kits (Pars Azmoon, Iran) were used for measuring plasma biochemical parameters. An assay for the determination of prooxidant…antioxidant balance (PAB) was used in this study, in which the prooxidant burden and the antioxidant capacity were measured simultaneously in a single assay.

2.3 PAB Assay Chemicals

TMB powder (3,3',5,5'-tetramethylbenzidine, Fluka), peroxidase enzyme (Applichem: 230 U/mg, A3791, 0005, Darmstadt, Germany), N-chloro 4-methylbenzenesulfonamide, sodium salt (chloramines T trihydrate) (Applichem: A4331, Darmstadt, Germany), hydrogen peroxide (30%) (Merck), as well as all the other reagents used were reagent grade and were prepared in double distilled water.

2.4 PAB Assay

This method is based on two different oxidation–reduction reactions which take place simultaneously. In the enzymatic reaction the chromogen TMB is oxidized to a colored cation by peroxides and in the chemical reaction the colored TMB cation is reduced to a colorless compound by antioxidants. The photo- metric

absorbance is then compared with the absorbance given by a series of standard solutions that are made by mixing varying proportions (0–100%) of 250mmol/L hydrogen peroxide – as a representative of hydroperoxides which is an indicator of total oxidantstatus, with 3 mmol/L uric acid – as a representative of the antioxidant capacity (in 10 mmol/L NaOH). It should be underlined that hydrogen peroxide and uric acid do not interact with each other, and do not neutralize the activity of each other. This photometric comparison is carried out using an ELISA reader.TMB powder (60 mg) was dissolved in 10 mL dimethyl sulfoxide (DMSO). For preparation of the TMB cation, 400 mL of the TMB/DMSO solution was added to 20 mL of acetate buffer (0.05 mol/L buffer, pH 4.5), and then 70 mL of fresh (chloramine-T) (100 mmol/L) solution was added to this 20 mL. The solution was mixed well and incubated for 2 hours at room temperature in a dark place. Then 25 units of peroxidase enzyme solution were added to 20 mL of TMB cation solution, dispensed in 1 mL and stored at –20°C. In order to prepare the TMB solu-tion, 200 mL of TMB/DMSO was added to 10 mL of acetate buffer (0.05 mol/L buffer, pH 5.8) and the working solution was prepared by mixing 1 mL TMB cation with 10 mL of TMB solution. This working solution was incubated for 2 min at room temperature in a dark place and immediately used. Ten mL of each sample, standard, or blank (distilled water) were mixed with 200 mL of working solution in each well of a 96 well plate, which was then incubated in a dark place at 37°C for 12 minutes. At the end of incubation, 100 mL of 2 normal hydrochloric acid (2 N HCl) was added to each well, and the optical density (OD) was measured in an ELISA reader at 450 nm with a reference wavelength of 620 or 570 nm. A standard curve was provided from the values relative to the standard samples. The values of the unknown samples were then calculated based on the values obtained from the above standard curve.

2.5 Statistical Analysis

Statistical analysis, including calculation of averages and standard deviations, was conducted using SPSS software. The independent T-test was used for comparing the results. Pearsonian correlation test was applied to determine the correlation between measured parameters and values ($p < .05$) were considered as meaningful.

3. RESULTS AND DISCUSSION

Results from examining biochemical parameters in both case and control group including people with rheumatoid arthritis and healthy people were shown in Table 1.

The results from Table 1 in both groups were compared using T-test and according to the results, it was found that the urea and uric acid contents have a meaningful difference in both groups.

The results from comparing biochemical indices in both patients with rheumatoid arthritis group with normal balance and patients with rheumatoid arthritis group with increased oxidant are shown in Table 2.

In this study, by examining the relationship between biochemical parameters and RA, we found that urea and uric acid contents have a meaningful difference in both case and control groups and that urea content has decreased and uric acid content has increased in case group. These results suggest the increase in reactive oxygen species and reflect the tissue damages such as arthritis in RA patients. In a study conducted in India, it was proved that there is an oxidative stress in rheumatoid arthritis disease course [4]. It was also reported an increase in oxidants contents in rheumatoid arthritis disease [5]. In a comparison between healthy people and people with rheumatoid arthritis, was proved

Table 1. Measured plasma biochemical parameters (Mean ± SEM) in experimental groups (n= 130 in each group)

Plasma biochemical parameters	Patients with rheumatoid arthritis	Control group (healthy people)	P value
Urea (mg/dl)	21.39±6.483	25.01±9.035	0.015
Uric acid (mg/dl)	9.78±2.977	8.81±3.712	0.034
Bilirubin (mg/dl)	0.78±0.47	0.67±0.33	0.260
Creatinine	0.79±0.17	0.78±0.14	0.887

Table 2. Measured plasma biochemical parameters in experimental groups (n= 130 in each group) regarding prooxidant-antioxidant balance

Plasma biochemical parameters	Patients with rheumatoid arthritis with normal balance	Patients with rheumatoid arthritis with increased oxidant	P value
Urea (mg/dl)	40.12±15.67	32.20±13.97	0.004
Uric acid (mg/dl)	6.6±2.27	6.5±2.34	0.861
Bilirubin (mg/dl)	0.7±0.46	0.7±0.44	0.758
Alkaline Phosphatase (U/L)	171.1±90.91	149.4±45.94	0.256

decreased antioxidant content in patients group [6-8]. This correlation is negative (reverse) about urea and the oxidant content would increased by decreasing urea. In studies and reviews conducted, biochemical indices have been less often compared with oxidant content. Biochemical indices changes were compared with oxidant index with Pearsonisn Test and there was only a reverse linear correlation between urea and oxidant content.

In another study in Jordan, the glutathione defense system in patients with rheumatoid arthritis were examined and it was found that antioxidant defense system has been damaged in patients with rheumatoid arthritis [7]. In another study, the oxidants and antioxidants contents were reviewed. They observed that in patients with rheumatoid arthritis, the antioxidant contents has decreased and Malondialdehyde content had increased and thus the antioxidant system is defected in these patients [8-11].

4. CONCLUSION

Finally, it is suggested that, by using this method, we can quickly discover the extent of tissue damage and perform therapeutic interventions for recovery of domesticated animals from different diseases prevalent in veterinary science such as osteoarthritis and rheumatoid arthritis which are most prevalent.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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