Journal of Biology and Today's World
ISSN 2322-3308
http://www.journalbio.com

Received: 26 April 2014•Accepted: 20 June 2014

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doi:10.15412/J.JBTW.01030801

Protein oxidation biomarker (SH- group) in plasma of Rheumatoid Arthritis

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ABSTRACT

Rheumatoid Arthritis is the most common inflammatory disease of the joints. Approximately 1-2% of the general population worldwide suffers from Rheumatoid Arthritis. It is a chronic and progressive autoimmune disease in which joints are involved symmetrically; it is often accompanied by pain and fatigue. In this study, SH - group in patients with Rheumatoid Arthritis and healthy individuals has been observed. Around 30 Rheumatoid Arthritis patients and 30 healthy individuals as control subjects selected as control group. Five cc Heparin blood was taken from patients and the SH- group test was executed. This test depicts the level of oxidative stress. SH - group level was not significantly higher (P = 0.577) in healthy individuals compared to Rheumatoid Arthritis patients. Oxidation of Group SH - group is an indicator of stress, which is called thiol oxidation of proteins. Oxidative stress can influence the oxidation of proteins. Oxidative stress caused by free radicals is involved for resonance and can affect the thiol groups, which may cause damage to tissues.

Key words: Rheumatoid Arthritis, SH-group, Protein oxidation, Thiol

1. INTRODUCTION

The purpose of this study was to examine the amount of thiol in patients with Rheumatoid Arthritis. Rheumatoid Arthritis is a progressive systemic disease often present with polyarthritis cornea (1,2). Rheumatoid arthritis is the most common inflammatory disease of the joints. Approximately 1-2% of the general population of the world suffers from this disease. The risk of this disease increases with age, and women are affected three times more than men (3). This chronic destructive inflammatory disease targets the synovial membrane and it infiltrates synovial inflammatory cells. These inflammatory cells in the synovial membrane remain intact until the disease protests (4,5). The main cause of rheumatoid arthritis is still unidentified. Formation of reactive oxygen species (ROS) and lipid peroxidation as a pathogen plays an important role in rheumatoid arthritis. Bacteria or immune complexes consumption produces oxidative agents such as superoxide and hydrogen peroxide. It accumulates and produces reactive oxygen species (ROS).These reactive oxygen species, leads to membrane damage, degradation of hyaluronic acid, α-1 anti-proteinase inactivation and degradation of antioxidants in the synovial joint (6, 7). Hypoxia increases ROS pressure and can cause damage to the synovial cavity. ROS is involved as mediators of tissue damage in Rheumatoid Arthritis (8). In this disease, lack of antioxidants in the blood concentration was observed to be significantly increasing the possibility of Rheumatoid Arthritis. By strengthening the antioxidant defense system in these patients can partially prevent the induction and progression of these complications (9,10). In a healthy person, balance between free radical production and the antioxidant defense system maintained. An imbalance in the production of free radicals and antioxidant defense system is called the oxidative stress (11). Oxidative stress and free radicals in the pathogenesis plays a crucial role in causing this disease (12,13). Oxidation of SH - group as an

indicator of stress is called thiol oxidation of proteins. Thiol's are organic compounds containing sulfhydryl groups. Measurement of sulfhydryl groups of proteins, reflecting increased production of free radicals is considered appropriate. Antioxidants in the body like thiol's constitute a major part of the antioxidant effects and their role in defense against oxygen species play (14). Thiols are sensitive towards oxidative damage and reduction of oxidative stress is an important sign.

2. MATERIALS AND METHODS

This case-control study was conducted using random sampling. The study groups were matched for age and sex. Then 5CC Heparin blood was taken out from patients and centrifuged for 5 min at 3000 rpm. CRP, ESR and RF tests were done right after that. (Table 1, Table 2) show the result for experiments conducted on Thiol. To measure the Thiol, a well-known groups of the reagent Elman coefficient (inter-assay variation of measured output are 4% and 2/1%, respectively) were used. This represents a revival of Thiol groups, which can cause a yellow complex 5, 5′-dithiobis 2-nitrobenzoic acid (DTNB) is measurable at wavelength of 421 nm. Plasma 100 µm, 2800 µm pH8 PBS, and 300 µm 5, 5′-dithiobis 2-nitrobenzoic acid (DTNB) were then mixed and incubated for 15 min at room temperature; and OD is measured at 412 nm. The result were measured with a spectrophotometer Model EPOCH-Bio Tek and expressed as mean ± SD. Statistical significance was achieved if *p*-values were less than 0.05. All statistical analysis of independent – samples *t*-Testwas performed using the SPSS (Version 18). As for calibration, distilled water was used after each reading and the Inhibition is equal to OD-Blanc/136. Results are expressed as mean ± SD. Statistical significance was achieved if $p < 0.05$. Statistical analysis of independent – samples *t*-Test was performed using the SPSS (Version 18).

Table 1. CRP

Valid Negative	*Frequency*	*Valid Percent*	*Cumulative*	*Percent*
+	46	76.7	76.7	76.7
++	9	15.0	91.7	15.0
+++	2	3.3	95.0	3.3
++++	2	3.3	98.3	3.3
	1	1.7		1.7
Total	60	100	100	100

Table 2. RF

Valid Negative	*Frequency*	*Valid Percent*	*Cumulative*	*Percent*
+	46	76.7	76.7	76.7
++	4	6.7	83.3	6.7
+++	4	6.7	90	6.7
+++	5	8.3	98.3	8.3
	1	1.7		1.7
Total	60	100	100	100

3. RESULTS AND DISCUSSION

We observed non-significant (p=0.577) result when there was an increased in level of SH-group (Thiol)5, 5′-dithiobis 2-nitrobenzoic acid (DTNB) method in healthy individuals (control case) in comparison to Rheumatoid Arthritis patients. (Chart 1)(Table 3).

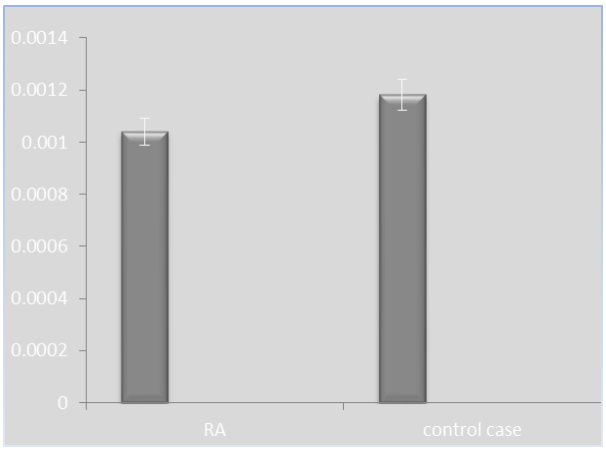

Chart 1. SH-group level in Rheumatoid Arthritis patients and control case
*P<0.05. The value is mean±SD

Table 3: SH-group (Thiol) levels in the study

p-value	*mean±SD control case*	*mean±SD Rheumatoid Arthritis*	
0.577	0.00096±0.00118	0.00076±0.00104	SH-group

From the results, we can see cysteine is highly susceptible to oxidative attack on different mechanisms - which can be used to create disulfide bonds. Measurement of SH-groups increased reflecting free radicals can be considered as appropriate. Anil Mahajan and colleagues study on patients with rheumatoid arthritis found that these patients had increased levels of oxidative stress (5). Ilaria Mazzetti and colleagues observed that in patients with rheumatoid arthritis, there would be an imbalance between oxidants and antioxidants observed (15). Arzu Seven and colleagues also studied on protein oxidation in patients with Rheumatoid Arthritis; and their result showed a significant decrease at the level of SH-group (8). In addition to that, antioxidant system in patients with rheumatoid arthritis will be jeopardized. The risk in these patients can cause permanent damage. In fact, a change in the balance oxidant or anti-oxidant is able to cause tissue damage among patients(3). The same result was seen in the amount of SH of a group increased in Rheumatoid Arthritis patients. This increase reflects towards an imbalance between oxidants and antioxidants, by which free radicals and oxidative

stress are increased. Results indicate that co administration of anti-oxidants is necessary with conventional drugs in order to be effective in patients with Rheumatoid Arthritis.

4. CONCLUSION

As one of the markers of protein oxidation, SH-group has an important role in oxidative stress. Oxidative stress can effect on patients with Rheumatoid Arthritis. In these patients, an increase in level of oxidative stress and antioxidant system will cause defect. This defect however, can be overcome to some extent and be prevented.

ACKNOWLEDGMENT

No acknowledgment mentioned by any authors.

AUTHORS CONTRIBUTION

This work was carried out in collaboration between all authors.

CONFLICT OF INTEREST

The authors declared no potential conflicts of interests with respect to the authorship and/or publication of this article.

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