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Original Article

Oxygen free radicals in children with acute rheumatic fever

Bülent Oran, Emre Atabek, Sevim Karaaslan, Ýsmail Reisli, Fatih Gültekin*, Ýbrahim Erkul

*Departments of Pediatrics and *Biochemistry, School of Medicine, Selçuk University, Konya, Turkey*

Abstract We have investigated the relationship between oxygen free radicals and acute rheumatic fever with regard to diagnosis of the disease process. At the time of diagnosis, we measured the levels of reactive oxygen molecules in the plasma, this being a parameter for oxygen free radicals, and discovered the levels to be significantly higher when compared with those measured in a control group ($P < 0.05$). The levels measured in the plasma, however, were not statistically different among patients with and without carditis.

We found a progressive decrease in the levels measured in the plasma when patients with acute rheumatic fever were tested on the 15th, 30th and 90th days subsequent to diagnosis. By the 90th day, levels measured in the plasma were still higher, but no longer significantly elevated, when compared with the control group. The present study is preliminary, but raises the possibility that measurement of oxygen free radicals in the plasma could be used as a laboratory test for active state of acute rheumatic fever. Further investigations will be needed, nonetheless, to determine the clinical application of this technique.

Keywords: Acute rheumatic fever, oxygen free radicals, reactive oxygen molecules; Group A β hemolytic Streptococcus

ACUTE RHEUMATIC FEVER IS WELL RECOGNIZED as a non-suppurative complication of streptococcal pharyngitis, but its pathogenesis still remains unclear.[1–3] Histological studies on biopsies and autopsy material obtained from patients with rheumatic heart disease have revealed infiltration of the myocardium and endocardium with monocytes, neutrophils and lymphocytes, along with other features of granulomatous myocarditis.[4–6] It has been suggested that these phagocytic cells which infiltrate the myocardium and endocardium may play a role in the pathogenesis of the disease by generating oxygen free radicals.[7,8] With this possibility in mind, we have measured the levels of reactive oxygen molecules in the plasma of patients with acute rheumatic fever before and during treatment. We have also compared these values, along with titres of antistreptolysin-O, erythrocytic sedimentation rate, and C reactive protein, between the patients and healthy subjects.

Correspondence to: Dr. Bülent Oran, Selçuk Üniversitesi, Tip Fakültesi Çocuk Klinigi, 42080 Konya, Turkey. Tel: 0 332 3232600; Fax: 0 332 323 26 41 or 323 26 43; E-mail: pedkar@ selcuk.edu.tr

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Materials and methods

The study was carried out in the Paediatric Cardiology Unit of Selçuk University, Turkey, from January, 1997, until December, 1998. We enrolled 23 patients with acute rheumatic fever, comparing the findings with those obtained from 25 healthy children. All those enrolled in the control group were in good health, having no evidence of any infectious, immunological, allergic or neoplastic disorders. The study was carried our after obtaining written fully informed consent from the parents of the patients and the controls. The protocol was approved by the ethics committee of our Hospital.

The diagnosis of acute rheumatic fever was based on the 1992 update of the Jones criterions.[9] All patients were examined by one of the two paediatric cardiologists in our study group. Chest radiographs and electrocardiograms were obtained from all patients. The cardiac diagnosis was confirmed by echocardiographic investigation using a Hewlett-Packard (Andover, MA, VSA) sonos 1000 system. Throat culture, along with antistreptolysin O, C reactive protein, and the erythrocytic sedimentation rate, were investigated in every patient, titres being determined by using

standard reagents (Rapitex ASL, N Latex CRP mono) and the Behring Nephelometer 100 analyzer. The titer of antistreptolysin O was considered to be elevated if it was greater than 320 Todd units per ml,[10] and C reactive protein if it was greater than 0,5 mg/dl. The sedimentation rate was determined by the Westergren method.[11] Levels of oxygen free radicals were measured in the plasma of healthy children, and also at the time of diagnosis, and on the 15th, 30th and 90th days of the treatment, for patients with acute rheumatic fever. The levels were measured colorimetrically using d-ROM's kit (d-ROMs test, Diacron s.r.j. Diagnostics Division, via zicrone n. 8–58100 Grosseto-Italy) and the Technico RA-XT otoanalyzer.

Statistical analysis was done by means of one-way analysis of variance, using the statistical package for social science computer program.

Results

We enrolled 23 consecutive patients with acute rheumatic fever who were admitted to our unit for Paediatric Cardiology, along with 25 healthy children as controls. The ages of the patients varied from 8 to 15 years, with a mean age of 11.2 ± 2.3 years, and those in the control group from 6 to 15 years, with a mean age of 10,2 ± 2,8 years. Of the patients, 10 were females and 13 males, while the control group was made up of 10 females and 15 males. The differences in age and gender between the patients and their controls were not significant.

The major manifestations encountered among our patients with acute rheumatic fever are listed in table 1. Carditis was present in 17 (74%) patients, all of whom also had arthritis. Only six (26%) patients had isolated arthritis. Isolated mitral regurgitation was present in 15 (88%) patients with carditis, and isolated aortic regurgitation in 2 (11%). One of the patients with mitral regurgitation had also prolapse of the mitral valve.

Cardiomegaly was noted on the chest radiograms of three patients, two of whom also had congestive heart failure (Table 1). Electrocardiograms were interpreted as showing left ventricular hypertrophy and left atrial enlargement in one of the patients with congestive heart failure. Patients with cardiomegaly were treated with prednisolone, and the others with salycilate. On day zero, prior to commencing treatment, titres of antisreptolysin O, along with the erythrocytic sedimentation rate and the levels of C reactive protein, and the measured levels of oxygen free radicals in the plasma, were significantly higher in the patients than in their

Table 1. Major manifestations and levels of oxygen free radicals in the plasma of patients and their controls.

	Levels of oxygen free radicals in the plasma (carr u/ml) Patients					
	Major Manifestations	0.	15 th	30 th	90 th days	Controls
1	C + A	778.9	685.9	623.5	598.0	433.3 +
2	C + A	417.3	398.5	266.4	245.3	389.2
3	A	707.2	580.2	455.8	338.4	425.4 +
4	A	622.8	601.2	564.8	469.7	324.1
5	C + A	735.8	605.6	551.3	505.9	324.3
6	C + A	681.7	608.4	477.6	229.3	275.4
7	A	781.7	610.3	551.4	507.8	385.4
8	C + A	497.4	465.2	446.3	428.0	424.0 +
*9	C + A	564.1	405.1	355.2	317.5	300.2
10	A	581.0	557.9	546.7	480.0	363.8
11	C + A	930.2	856.3	764.7	592.5	429.3 +
12	C + A	655.6	547.9	455.3	367.2	389.1
13	C + A	588.3	576.5	497.3	439.6	409.4
*14	C + A	616.5	597.0	428.1	225.3	420.2 +
15	C + A	645.3	576.1	503.2	372.6	353.9
16	C + A	467.6	331.5	287.1	277.2	389.5
17	C + A	597.2	546.0	489.3	420.6	315.1
18	C + A	708.0	564.9	477.6	432.0	391.2
19	A	621.9	579.4	496.0	336.6	401.3
*20	C + A	1068.1	731.3	674.2	—	329.0
21	A	752.5	694.1	586.3	498.6	375.9
22	C + A	449.4	418.3	367.5	328.0	308.2
23	C + A	654.0	624.3	586.0	498.6	359.4
24						320.3
25						351.1

C: carditis, A: arthritis, OFR: *Patients with cardiomegaly, + high levels in the controls.

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controls (P< 0,05) (Table 2). Every patient had higher anti-Streptolysin titers than the control group on day zero. There was then a significant decrease in these titers when measured on the 15th, 30th, and 90th days of treatment (P< 0,05). C reactive protein was positive in all patients but one. The concentrations of the protein then decreased on the 15th and 30th days of treatment, but a significant decrease was not observed until the period between the 30th and 90th days (p< 0.05). The sedimentation rate was elevated in all patients, but this showed a significant decrease at the 15th, 30th and 90th days(p< 0.05)

Following the anti-inflammatory therapy with either prednisolons or salycylate, the levels of reactive oxygen molecules decreased significantly at the 15th, 30th and 90th days (P< 0,05). This test no longer showed a significant difference between the control group and the patients on the 90th day of the treatment, and neither was there any significant difference between patients with carditis and isolated arthritis on day zero with regard to levels of oxygen free radicals (P< 0,05) (Table 3) (Figure 1)

Discussion

A free radical is any species of chemical capable of independent existence and containing one or more unpaired electrons. The superoxide anion radicals of oxygen, and hydrogen peroxide, the hydroxyl radical, and the oxides of nitrogen and hypochlorous acid are all known as free radicals.[12] Toxic metabolites of oxygen have now emerged as a major final common pathway of tissue injury in a wide variety of disease processes. A frequent cellular target is the lipid component of membranes, resulting in lipid peroxidation. Proteins are also subject to denaturation mediated by free radicals, which may lead to structural loss or enzymatic deactivation.[13] Experimental and clinical studies over the next few years will probably shed light on the role of such free radicals of oxygen in the mechanism of tissue injury. Rowe et al[14] demonstrated that activation of the carine neutrophil system results in the generation of such radicals, which are able to induce severe cardiovascular dysfunction. In acute and chronic active valvitis, the cellular infiltrates are primarily composed of T cells and macrophages, as demonstrated by Kemeny et al.[15] Kumar et al[7,8] suggested that these phagocytic cells, which infiltrate the myocardium, may play a role in the pathogenesis of cardiac disease seen in patients with rheumatic heart disease through the generation of oxygen free radicals.

We have now shown that patients with acute rheumatic fever and rheumatic heart disease have significantly higher levels of free radicals of oxygen in the plasma than do their controls. As yet, however, we have no evidence to show that the increased levels of radicals in the plasma are responsible for the cardiac damage observed in our patients, since we found no significant difference between patients with and without carditis. We did demonstrate, nonetheless, that there was a significant decrease in levels of free radicals in the plasma following anti-inflammatory therapy.

The most important problem is to determine the level measured in the plasma which should be considered as pathologic. Although it is well known that resting monocytes and neutrophils

Table 2. Patients and their controls.

Days	0	15 th	30 th	90 th	Control groups
ASO	922,91 ± 47,02	666,96± 322,0	384,78± 223,2	163,4 ± 43,13	158,5 ± 75,12
CRP	0,96 ± 0,21	0,91 ± 0,29	0,61 ± 0,50	0,00 ± 0,00	0,00 ± 0,00
ESR	57,09 ± 18,73	35,70 ± 14,39	16,7 ± 8,97	6,91 ± 5,09	6,74 ± 4,29
OFR	638,84 ± 121,0	572,26± 115,4	497,90± 115,3	404,73 ± 107,3	367,32 ± 107,3

ASO: Anti streptolysin-O titer (Todd U/ml), CRP: C-Reactive Protein (ng/ml), ESR: Erythrocytic sedimentation rate (mm/1 hour), OFR: Oxygen free radicals (reactive oxygen molecule, Carr u/ml)

Table 3. Levels of oxygen free radicals in the plasma of patients with carditis and isolated arthritis.

	Case number	%	Plasma OFR levels, mean ± SD (carr u/ml)
Isolated arthritis	6	26	533,5000 ± 48,3854
Arthritis + carditis	17	74	485,3294 ± 130,2974

P> 0.05
OFR: Oxygen free radical (reactive oxygen molecule)

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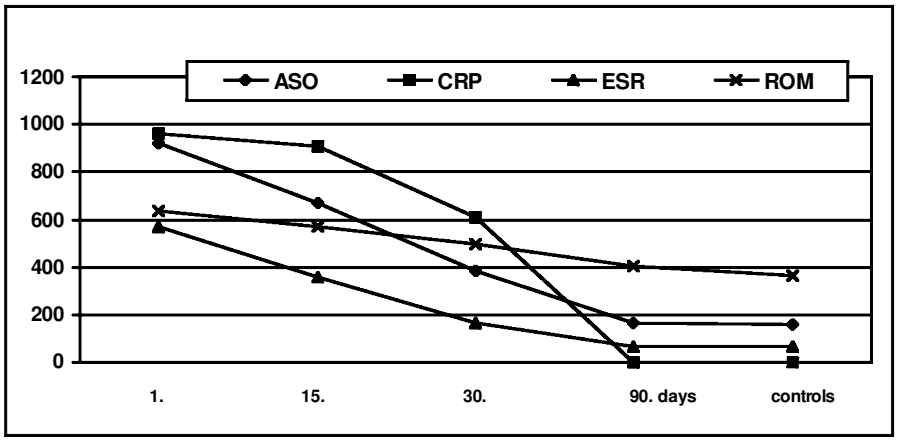

Figure 1.
Patient and control groups. ASO: Anti streptolysin – O titer (Todd u/ml), CRP: C-Reactive Protein (ng/ml); ESR: Erythrocyte sedimentation rate (mm/1hair): ROM: Reactive Oxygen Molecule (Oxygen free rad) Carr u/ml. ESR and CRP measures were multiplied by 10 and 1000 respectively to obtain clear depiction

produce little or no free oxygen radicals in normal subjects,[16] five children in the control group had similar concentrations in the plasma as found in the patients. Oxidative stress seems to be involved in the pathogenesis of several disorders in children. The oxidative phenomenon, in fact, interferes in a great number of pathological processes at several levels of the body, including the respiratory, cerebral, and cardiovascular systems. High levels of oxygen free radicals are expected in the presence of any inflammatory or degenerative condition which may have an immunological, infective or allergic basis, as well as being found in the presence of neoplasia.

We think that, one day, measurement of the levels of oxygen free radicals may be used as a nonspecific supplementary test in addition to the sedimentation rate and C-reactive protein so as to assess the activity of the rheumatic process, but we need more investigations, along with increased knowledge of the radicals themselves, before this can be achieved. It is also clear that, as yet, we have not provided sufficient data to establish the results as being of diagnostic value for acute rheumatic fever. In order to provide a more complete methodological approach, an increase in the number of controls could offer better support for our results, which we fully recognize as being no more than preliminary.

References

1. Rammelkamp CH, Denny FW, Wannamaker LW. Studies on the epidemiology of rheumatic fever in the armed service. In: Thomas (ed) Rheumatic Fever. Minneapolis Univ. Minnesota Press 1952: 72–89.
2. Stollerman GH. Rheumatic fever in the tropics. In: Shaper AG (ed) Cardiovascular disease in the tropics. London. Br Med Assoc 1974: 7–19
3. Ayoub EM, Kaplan E. Host-parasite interaction in the pathogenesis of rheumatic carditis. J Rheum 1992; 18: 6–13
4. Raizada V, Williams RC, Chopra P, Gopinath N, Prakash K, Sharma KB, Cherian KM, Arora R, Nigam M, Zabriskie JB, Husby G. Tissue distribution of lymphocytes in rheumatic heart valves as defined by monoclonal anti-T cell antibodies. Am J Med 1983; 74: 90–8
5. Narula J, Chopra P, Talwar KK, Reddy KS, Vasan RS, Tandon R, Bhatia ML, Southern JF. Does endomyocardial biopsy aid in the diagnosis of active rheumatic carditis? Circulation 1993; 88: 2198–2205
6. Chopra P, Narula J, Kumar AS, Sachdeva S, Bhatia ML. Immunohistochemical characterization of Aschoff nodules and endomyocardial inflammatory infiltrates in left atrial appendages from patients with chronic rheumatic heart disease. Int J Cardiol 1988; 20: 99–105
7. Kumar V, Ganguly NK, Anand IS, Wahi PL. Release of oxygen free radicals by macrophages and neutrophils in patients with rheumatic fever. Eur Heart J 1991; 12: 163–165
8. Kumar V, Ganguly NK, Sethi AK, Anand IS, Verma J, Wahi P. Role of oxygen free radicals generated by blood monocytes and neutrophils in the pathogenesis of rheumatic fever and rheumatic heart disease. J Mol Cell Cardiol 1990; 22: 645–651
9. Diagnosis of rheumatic fever-special writing group. Guidelines for the diagnosis of rheumatic fever Jones criteria, 1992 update JAMA 1992; 268: 2069–2073.
10. El-Said GM, El-Refaee MM, Sorour KA, El-Said HG. Rheumatic Fever and Rheumatic Heart Disease In: Garson A, Biricker JT, Fisher DJ, Neish SR. Eds. The Science and Practice of Pediatric Cardiology. The Williams and Wilkins CO, Baltimore 1998: 1691–1721.
11. Dacie JV, Lewis SM. Practical Hematology, 7th edition, Churchill Livingstone, Singapore, 1991
12. Gutleridge JMC. Lipid peroxydation and antioxidants as biomarkers of tissue damage (European Beckman Conference) Clin Chem 1995; 41/12: 1819–1828
13. Rangan U, Bulkley GB, Prospects for treatment of free radical-mediated tissue injury. Br Med Bull 1993; 49: 700–718.
14. Rowe GT, Eaton LR. Hess ML Neutrophil-derived, oxygen free radical-mediated cardiovascular dysfunction. J Mol Cell Cardiol 1984;16: 1075–1079
15. Kemeny E, Grieve T, Marcus R, Sareli P, Zabriskie JB. Identification of mononuclear cells and T cell subsets in rheumatic valvulitis. Clin Immunol Immunopathol 1989; 52: 225–237
16. Badwey JA, Karnovsky ML. Active oxygen species and function of phagocytic leukocytes. Ann Rev Biochem 1980; 49: 695–726

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