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CIRCULATING IMMUNE COMPLEXES IN CHILDREN WITH RHEUMATIC FEVER*

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An autoimmune mechanism is claimed in the pathogenesis of acute rheumatic fever and rheumatic carditis on the basis of studies performed by several investigators[1,2]. Depression of cell-mediated immunity plays an important role in the development of autoimmune diseases[3,4]. In some ways rheumatic fever resembles an immune complex-mediated disorder[5]. It is known that one of the most important factors causing immunological tissue injury is immune complexes[6]. A fifty percent or more inhibition of the blastic transformation response of the normal lymphocytes to phytohemagglutinin by the plasma from the patients with active rheumatic fever was observed[7]. This suggests the presence of an inhibitory factor in the plasma of patients with active rheumatic fever causing some degree of depression in the lymphocytic functions or cell-mediated immune response in these patients[8]. It is believed that these inhibiting factors may be the antigen-antibody complexes[8].

In the literature, it has been shown that circulating immune complexes were frequently present in adults and children with acute rheumatic fever[9,10] but, the immune complex values for various clinical presentations of rheumatic fever have not been reported.

The aim of this study is to investigate the role of the circulating immune complexes in rheumatic fever patients with various clinical presentations in regard to the evaluation of the disease's activity.

Material and Methods

Our study group consisted of 38 patients with rheumatic fever whose ages ranged between 6-17 years who were diagnosed in the Department of Pediatrics at Hacettepe University Children's Hospital.

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The Jones criteria was used for guidance in the diagnosis of acute rheumatic fever[11]. The acute cases were observed for one week after the onset of an acute episode of rheumatic fever. The patients had not received any steroids or salicylates. Hemoglobin values were above 10 g/dl in all the patients and control group. The patients and controls were divided into five groups:

Group I consisted of sixteen patients who had acute rheumatic fever and carditis. There were eleven males and five females whose ages ranged between 6-17 years, with a mean age of 10.8 years. All the patients had positive acute phase reactants, radiologic evidence of cardiomegaly and valvular lesions. Three patients had pericardial friction rub in addition to mitral regurgitation. Ten patients revealed congestive heart failure. The diagnosis of acute rheumatic carditis was made on the basis of the presence of a significant murmur plus two or more of the following: pericardial friction rub, radiologically evident cardiomegaly, congestive heart failure, increased antistreptolysin 0 titer and positive acute phase reactants such as an increased erythrocyte sedimentation rate, C-reactive protein and leukocytosis. The patients were treated with prednisolone for a 4-6 week duration. Circulating immune complexes were determined on three occasions such as before treatment, 15 days after prednisolone therapy, and 10 or 15 days following withdrawal of prednisolone therapy.

Group II consisted of eight patients who had acute rheumatic fever without carditis. There were four males and four females whose ages ranged between 11-12 years, with a mean age of 13.5 years. All the patients had migratory polyarthritis, an elevated erythrocyte sedimentation rate and positive C-reactive protein. Most of them had an elevated antistreptolysin 0 titer.

Group III consisted of nine patients who had inactive rheumatic fever with valvular lesions. There were eight females and one male whose ages ranged between 9-17 years, with a mean age of 13.2 years. Positive acute phase reactants were not evidenced in this group; acute phases of their illness were previously observed in our hospital. This group was studied between six months to two years after the onset of the acute phase of the disease.

Group IV consisted of five patients with rheumatic chorea. There were four males and one female whose ages ranged between 8-14 years, with a mean age of 10.6 years. None of the patients with rheumatic chorea had positive acute phase reactants. Two patients had mitral regurgitation.

Group V consisted of 13 controls in good health with no history of rheumatic fever. There were eight females and five males whose ages ranged between 5 and 15 years.

The circulating immune complexes were detected by the Raji-cell radioimmunoassay method[6].

IMMUNE COMPLEX STUDY BY RAJI-CELL RADIOIMMUNOASSAY METHOD

Abbreviations:

AHG : Aggregated Human Gammaglobulin
NHS : Normal Human Serum
PBS : Phosphate Buffered Saline
HSA : Human Serum Albumin
MEM : Minimal Essential Medium

I. *lymphoblastoid cell cultures (Raji-cells)*

Raji-cells extracted from Burkitt's lymphoma were maintained in Eagle's MEM: These cells were supplied by the research laboratories of Sweden's Karolinska Institute and multiplied in the Virology Department by continous passages. AHG was obtained from the Cohn Fraction II of Sigma and each AHG used in the study was centrifuged at 1500 g for 20 minutes. NHS was collected from normal individuals and stored at −70 °C. MEM was supplied by Flow Laboratories. HSA was supplied by the Blood Bank. Radioactive I^{125} was bound to IgG in accordance with Theofilopoulos et al's method[6].

II. *preparation of the standards*

1. AHG was diluted in varying proportions between 10 ng-10 µg with PBS (phosphate buffered saline, pH 7.3): (1/1, 1/50, 1/100, 1/500, 1/1000).
2. NHS was diluted in 1/2 proportion with PBS (pH: 7.3).
3. 1/2 diluted 50 AHG was mixed with 50 1/2 diluted NHS. The final concentration of NHS became 1/4.
4. 25 µl of the AHG-NHS mixture was placed into the test tubes.
5. These test tubes were kept in a 37 °C water bath for 30 minutes.
6. After incubation 50 µl (2×10^6) Raji cells were added to the tubes and left in a rocking water bath at 37 °C for another hour.

III. *preparation of patients' sera*

1. 1-2 cc sera which were taken from patients were stored at −40 °C and removed from the deep freezer 30 minutes before the experiment.
2. The sera were diluted with PBS in 1/4 proportion. 25 µl of each specimen was transferred to counting tubes.
3. 50 µl (2×10^6) Raji-cells were added to the above mixture.
4. Test tubes were incubated in rocking water bath for one hour at 37 °C. All of the specimens were studied in a duplicated manner.

IV. *preparation of the background*

25 µl of the NHS which was diluted in 1/4 proportion was placed into the counting test tube. The standards and the specimens obtained from the patients were exposed to the same procedures in further steps of the study.

1. The cells were washed three times with MEM after incubation.
2. 1 percent HSA was prepared in MEM solution (MEM-HSA).
3. Optimum amounts of I^{125} diluted with MEM-HSA in a proportion of 1/10 were added to the test tubes.
4. The test tubes were kept in a freezer at −4 °C for a period of 30 minutes.
5. The cells were washed with a 1 percent HSA-MEM solution three times after incubation.
6. I^{125} cell precipitates remaining in the test tubes were counted by the gamma counter.

V. *calculation of the results*

1. The values obtained from the gamma counter cpm (count per minute) for the standard tubes were placed on the vertical axis and the AHG amounts in µg were placed on the horizontal axis of a graph.
2. The location of the values (cpm) obtained from the patients' plasma was found on the curve and the projection of this location on the horizontal axis yielded the amount of immune complexes in these test tubes. These amounts were expressed as µg AHG/ml of the sera.
3. Immune complex values outside the limits of 6 µg-1800 µg were omitted.
4. The Mann Whitney U test was used for a statistical comparison between the groups.

For statistical analysis the significance test between two matched-pairs was used in the cases of active rheumatic carditis.

Results

Immune complexes were found to be strongly positive in 27 (71%) of the 38 rheumatic fever patients (Table I and Fig. 1).

Immune complexes were positive in 13 (81.2%) of the 16 patients with active rheumatic fever carditis before prednisolone therapy (mean 282.08 ± 285.7 mg/ml). Immune complexes were detected in 53 percent of the patients after 15 days of prednisolone treatment (mean 361.53 ± 538.6 mg/ml). After a 4-6 week period of prednisolone treatment, the drug started to be gradually withdrawn and the immune complex average 10-15 days after the conclusion of the therapy was

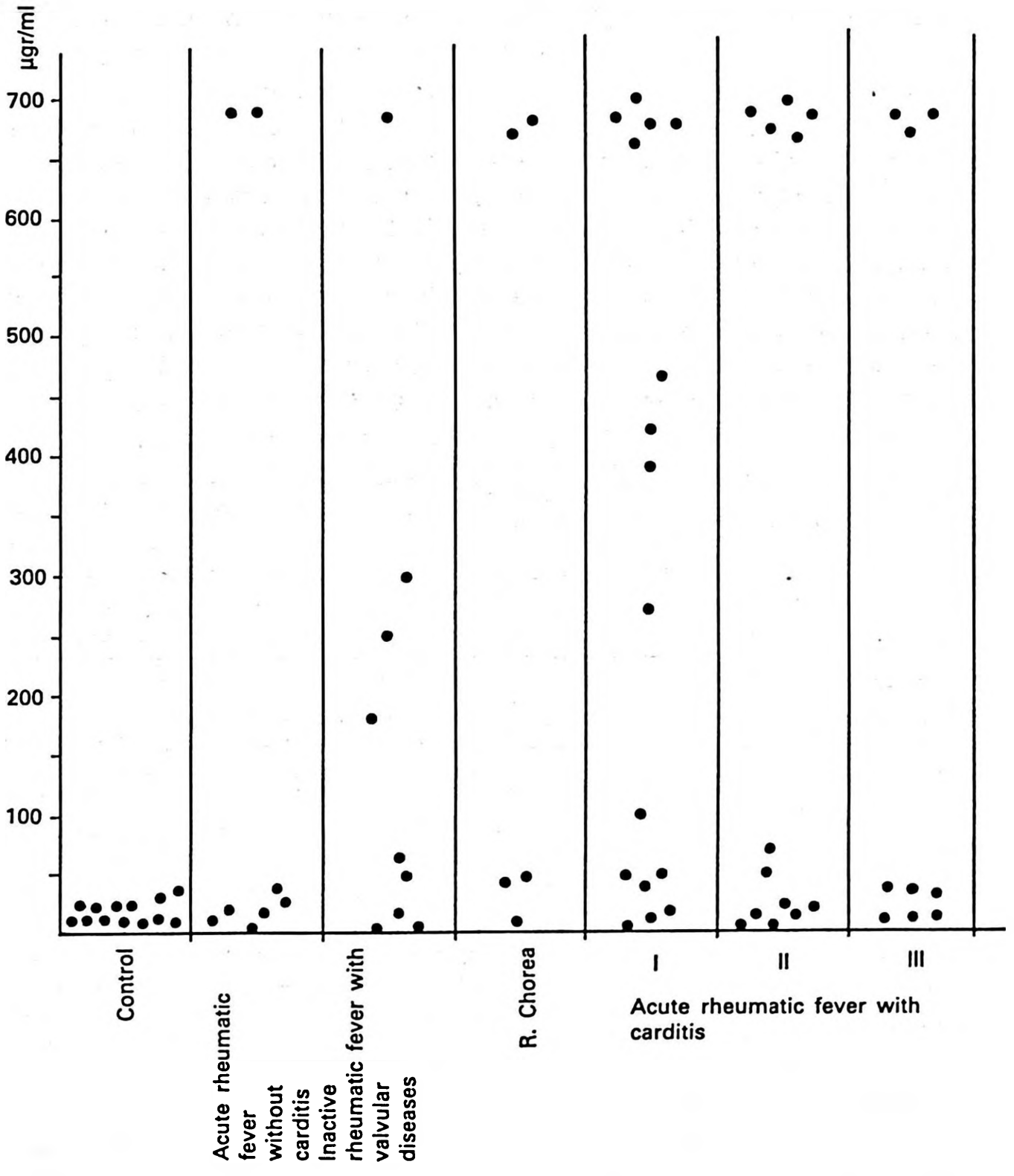

Fig. 1: Circulating Immune complexes in patients with rheumatic fever and the control group

found to be positive in 66.6 percent of the patients (mean 245.6 ± 337.7 mg/ml). The values of the circulating immune complexes in the first, second and third sera

of this group demonstrated statistical importance when compared with the control group ($p<0.05$). The significance test between two matched-pairs was found not to be significant in patients with acute rheumatic fever carditis ($p>0.05$).

In four of the eight patients (50%) with acute rheumatic fever without carditis, the immune complexes were positive (mean 185.25 ± 315.3 mg/ml). The comparison with the control group was statistically significant ($p<0.05$). Immune complexes were positive in six (66.6%) of nine patients with inactive rheumatic fever with valvular diseases (mean 170.13 ± 226.9 mg/ml). A comparison with the control group was significant ($p<0.05$). Immune complexes were positive in four (80%) of the five rheumatic chorea patients (mean 297.73 ± 363.4 mg/ml). The statistical significance was important ($p<0.05$). Only in one of the controls (7.6%) immune complexes were positive (mean 5.99 ± 3.38 mg/ml).

TABLE I: Circulating Immune Complexes in Children with Rheumatic Fever and the Control Group

	Number of cases	Number of cases positive	Mean µg/ml	Range
Acute rheumatic fever with carditis				
1. Before treatment	16	13 (81.2%)	282.08 ± 285.7	4.34 – 695.65
2. After 15 days	13	7 (53.0%)	361.53 ± 538.6	3.47 – 1800
3. Ten days following withdrawal of therapy	9	6 (66.6%)	245.6 ± 337.7	3.47 – 695.65
Acute rheumatic fever without carditis	8	4 (50.0%)	185.28 ± 315.3	4.34 – 695.65
Inactive rheumatic fever with valvular diseases	9	6 (66.6%)	170.13 ± 226.9	4.34 – 695.65
Rheumatic chorea	5	4 (80.0%)	297.73 ± 363.4	10.43 – 695.65
Control group	13	1 (7.6%)	5.99 ± 3.38	3.6 – 14.6

Discussion

An immunologic response to group A streptococcus has been strongly implicated in the etiology of acute rheumatic fever, although the precise pathogenic mechanisms have not been clearly defined[12,13]. The concept of immunologic cross-reactivity is suggested by the presence of organ specific antigens in the mammalian heart; sharing of common antigens by group A streptococci and the mammalian heart; antibodies reactive with the myocardial tissue in some patients with rheumatic carditis, and diffuse deposits of gammaglobulin in the hearts of

patients having active carditis[12-15]. Depression of cell-mediated immunity plays an important role in the development of autoimmune diseases[3,4].

It is known that one of the most important factors causing immunological tissue injury is immune complexes[5]. The development of a number of assays for the detection of immune complexes in man has led to the observation that circulating immune complexes may be detected in various diseases such as Henoch-Schönlein purpura,[16] infective endocarditis,[17] retinal vasculitis,[18] acute glomerulonephritis[19] and rheumatoid arthritis[20].

Levels of circulating immune complexes have been determined in children with acute rheumatic fever and acute poststreptococcal nephritis by Van de Rijn et al[10]. Clq solid phase assay employing radiolabelled protein A and the Raji-cell radioimmunoassay have been used in the detection of immune complexes. Both groups of patients had significantly elevated levels of immune complexes during the acute phase and showed equivalent decline to slightly elevated levels during the six month observation period. Employing the five radioimmunoassays for immune complexes, sera from patients with adult acute rheumatic fever were examined by Yoshinoya and Poge[9] in acute and postacute phases. Immune complexes were detected in 89 percent of acute phase sera by one assay. Immune complex values decreased significantly during the follow-ups, although some abnormalities persisted[9]. In these studies, the patients had clinical evidence of either carditis and/or arthritis.

In our study, circulating immune complexes were detected in 71 percent of the total 38 patients with rheumatic fever. Our findings are in agreement with these reports. In the present study, circulating immune complexes were detected in 50 percent of the patients with acute rheumatic fever without carditis, whereas, the incidence of circulating immune complexes was 81.2 percent in the sera of patients with carditis. These immune complex values decreased during the follow-up (average 2.5 months) and were found to be positive in 66.6 percent of the patients.

Immune complexes were found in 66.6 percent of patients with inactive rheumatic fever with valvular diseases. In this group, no patient had positive acute phase reactants. Intervals between acute disease and testing in patients of this group ranged from six months to two years (average 15 months). In comparison, the follow-up results of acute rheumatic fever patients with carditis and inactive rheumatic fever patients with valvular diseases are interesting. In experimental animals, IgG containing immune complexes whose sedimentation rates ranged between 14 and 22 S were cleared rapidly by the reticuloendothelial system; whereas smaller complexes persisted much longer[21].

Van de Rijn et al[10] have detected both large and small molecular weight complexes in two patients with acute rheumatic fever and observed a similar

distribution of complexes in patients with poststreptococcal glomerulonephritis[10]. Whereas, the circulating immune complexes detected by Yoshinoya and Pope[9] were primarily of low molecular weight (<19 S) although larger complexes have also been found[4]. They have suggested that variability of immune response to the streptococcal antigens or the rate of immune complex clearance between the two studies may have accounted for the differences in the relative quantities of large-size complexes[9]. It has been suggested that multiple antibody systems can persist for from six months to two years or longer[11,22,23], especially in patients with valvular heart disease[22].

In our study, we were not able to characterize the molecular weight of immune complexes. These small complexes may be responsible for the high immune complex values of inactive rheumatic fever patients with valvular diseases.

Husby[24] has reported that antibodies gave cross reactions with the human caudate and subthalamic nucleus cytoplasm in the sera of rheumatic chorea patients. These antineural antibodies were responsible for the clinical episodes and the duration of the disease. The presence of circulating immune complexes in the sera of 80 percent of the patients with rheumatic chorea as high as values of patients with acute rheumatic fever with carditis is another finding of interest in our study.

The development of rheumatic heart disease after group A β-hemolytic streptococcal infection is considered to involve an immune response that is altered in some way[25]. However, the precise pathogenic mechanisms responsible for the various presentations of acute rheumatic fever may be different. Humoral or cell-mediated immunity directed against the streptococcus that is cross-reactive with the heart and other tissue antigens, immune complexes or a combination of these, may be responsible for the various presentations of the disease.

However, in spite of the accumulation of a large source of data on these antigens, the pathogenesis of rheumatic fever remains an enigma. No specific antigen has been identified as of yet[2].

Summary

The circulating immune complexes in rheumatic fever patients with various clinical presentations were investigated by the Raji-cell radioimmunoassay technique. The Jones Criteria was used for guidance in the diagnosis of 38 rheumatic fever patients and 13 age-matched healthy controls. Immune complexes were found to be strongly positive in 27 (71%) of the 38 rheumatic fever patients. Immune complexes were positive in 13 (81.2%) of 16 patients with acute rheumatic fever carditis, in four (50%) of the eight patients with acute rheumatic fever without carditis, in six (66.6%) of the nine patients with inactive

rheumatic fever with valvular diseases, in four (80%) of the five patients with rheumatic chorea and in one (7.6%) of the 13 controls. The presence of circulating immune complexes in patients with rheumatic fever may be responsible for the severity of the clinical presentation and the duration of the disease.

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