

VOLUME 21 – AUGUST 1984

## HUMORAL IMMUNITY IN ACUTE POST- STREPTOCOCCAL GLOMERULONEPHRITIS

---

S. Rajajee  
P.R. Narayanan  
S.G.P. Moses  
N. Sundaravalli

### ABSTRACT

*Humoral immune response was assessed in 60 children with acute post-streptococcal glomerulonephritis in parallel with 15 children with only skin streptococcal infection and 20 normal children.*

*B-lymphocytes as assessed by EAC rosettes estimation was significantly elevated in patients. Markedly high anti-DNase B antibody titres were demonstrated in patients and to a less extent in skin infection controls, as compared to normal controls.*

*C<sub>3</sub> levels were decreased in all the patients and C<sub>4</sub> levels were decreased in 76%. The levels returned to normal 2 months later. C<sub>3</sub> and C<sub>4</sub> levels were normal in skin infection and normal controls.*

*Serum IgG, IgM, IgA were normal in patients and Rheumatoid factor was positive in only 24% of patients, all of whom had a low positive titre.*

*These studies indicate marked humoral immune response to streptococcal infection in patients with APSGN. IgG anti-IgG immune complex did not play a significant role in our patients.*

**Key words:** *Acute post-streptococcal glomerulonephritis, Humoral immunity.*

---

*From the Department of Immunology, TB Research Centre and Institute of Child Health & Hospital for Children, Madras.*

*Reprint requests: Dr. Sarala Rajajee, 19 II Main Road, C. I. T. Colony, Madras- 600 004.*

Acute post streptococcal glomerulonephritis (APSGN) is a well recognised sequelae to nephritogenic streptococcal(1-5) infection. It is generally regarded as an immune complex disease (6-8) but the nature of the antigen is not known. The antigen has been variously reported as being in the streptococcal cell wall, protoplasmic membrane or extra cellular product (7,8). Some reports mention that alteration of the immunoglobulin (Ig) (9,10) or glomerular basement membrane (7) may occur as a result of streptococcal infection so that the altered IgG or glomerular basement membrane acts as an antigen.

Apart from the nature of the antigen, the nature of the antibody response in the host is of crucial importance in the formation of pathogenic immune complexes (11).

There have been very few reports in India regarding the immune response in children with APSGN. The present study aims at assessing the humoral immune response in APSGN in comparison to skin infection and normal controls.

### Material and Methods

#### Patients

60 patients admitted at the Institute for Child Health, Madras, with the clinical picture of APSGN and 15 children (siblings of patients) with pyoderma but no nephritis were studied. 20 other children with no skin or pharyngeal infection and normal urinary findings served as normal controls. The ages of the patient and controls ranged from 2 to 12 years, and they came from the same socio-economic strata.

The clinical criteria for APSGN was:  
1. Acute onset of oliguria, edema

with proteinuria and gross or microscopic hematuria.

2. Hypertension with or without other features, as cardiac failure, hypertensive encephalopathy or acute renal failure.
3. No history of previous renal disease.
4. History of recent skin or throat infection.

Patients with features of Nephrotic syndrome such as massive albuminuria, hypoalbuminemia and hypercholesterolemia were excluded.

*Blood samples*

On admission, blood was collected for anti-DNase-B, ASO titres, C<sub>3</sub>, C<sub>4</sub>, immunoglobulins and rheumatoid factor estimations. Subsequently samples were collected from patients 2 months later. Storage of sera was at -20°C in small aliquots.

*Methods*

1. EAC Rosettes: C<sub>3</sub> receptor lymphocytes were determined using the technique as outlined by Bianco *et al.* (12).
2. Anti streptococcal antibodies anti-DNase-B and ASO titres were estimated by the micro technique determination( 13).
3. Complement factors C<sub>3</sub> and C<sub>4</sub>, immunoglobulins IgG, IgM, IgA levels were determined by the single radial immunodiffusion method of Mancini *et al.* (14) using monospecific antisera (immuno diagnostics).
4. Rheumatoid factor was assessed by the Rosewaler test.

**Results**

*Surface markers for E-lymphocyte*

The results are summarised in *Table 1*. Patients with APSGN had significantly elevated EAC rosettes as compared to the skin infection and normal controls. The proportion of EAC rosettes was nearly 32% in patients as compared to 18.9% and 19.6% in the other 2 groups.

**TABLE I** – *Proportion of B-lymphocytes (EAC rosettes) in APSGN and controls*

Group	No.	B-lymphocytes mean, SD and range
AGN	30	31.9 ± 7.4* (20–48)
Normal control	20	18.9 ± 4.19 (10–28)
Skin infection controls	5	19.6 ± 5.5 (14–28)

Significant (+) p< .001.

*Anti-DNase-B and ASO titres*

The mean titre for normal controls was 401.66 with a range of 75 to 800.

The levels in patients and skin infection controls were significantly higher than that in normal controls. 100% of patients had elevated anti-DNase-B levels compared to 87 % in skin infection controls and 0% in normal controls.

The titres were as high as 76,800 in 10% of patients (*Table II*).

The mean titre of ASO was not significantly increased in patients or skin infection controls. Only 3 patients and 2 skin infection controls had elevated ASO titres.

*Serum immunoglobulins and complement factors C<sub>3</sub> and C<sub>4</sub>*

The levels of IgG, IgM and IgA were within normal limits in patients, skin infection and normal controls. C<sub>3</sub> levels were decreased in all the patients during the acute phase (p<.001). C<sub>4</sub> levels were low in 46 patients (76.6%) and normal levels in 14 patients. The levels of C<sub>3</sub> and C<sub>4</sub> in skin infection and normal controls were within the normal range.

C<sub>3</sub> and C<sub>4</sub> returned to normal levels when the sera of 25 patients were tested 2 months after onset of disease (Table III).

*Rheumatoid factor*

Differential agglutination titres (DAT) of less than 1/8 was taken as negative. 76% of the patients were negative for rheumatoid factor. The remaining 24% were positive in the acute phase. 10 had titres of 1/16 and 4 had titres of 1/32.

**TABLE II** - Anti-DNase-B and SAO titre in APSGN and controls

Group	No.	ASO	Anti-DNase-B
		Mean, SD and range	
AGN	51	210 ± 206 (85-1360)	9080.39 ± 14860.3* (1200-76,800)
Contact (skin infection) controls,	15	302 ± 365 (85-1360)	2886.6 ± 1626.94** (300-4800)
Normal controls	15	134 ± 121 (85-340)	401.66 ± 316.01 (75-800)

\*Highly significant P < .003 when compared to normal controls.  
 \*\*Highly significant P < .001 when compared to normal controls.

**TABLE III** - C<sub>3</sub>, C<sub>4</sub> levels in APSGN and controls

Group	No.	Acute phase		2 months later (25 patients)	
		C <sub>3</sub>	C <sub>4</sub>	C <sub>3</sub>	C <sub>4</sub>
AGN	60	35.8* ± 15.3	15.9** ± 7.9	61.8 ± 20.6	34.7 ± 14.1
Skin infection controls	14	108.3 ± 21.4	26.8 ± 6.7	-	-
Normal controls	14	103.2±40.9	28.4±8	-	-

\*P < .001 significant

\*\*P< .001 significant.

## Discussion

Proportions of B-lymphocytes, as estimated by EAC rosettes were significantly higher in patients with APSGN as compared to skin infection and normal controls. B-lymphocytes estimated by fluorescein-labelled aggregates for cells bearing FC receptors were found to be elevated in children with APSGN in a study by Williams *et al.* (15). They also reported that EAC rosettes were reduced in the same patients which they attributed to the saturation of EAC receptors by complexes or other factors present in the sera.

Our data show markedly elevated anti-DNase-B titres in patients with APSGN. There was also good response in skin infection controls but not to the extent as in patients. The strikingly high antibody titres in patients suggests that attack rates are perhaps related to the magnitude of immune response (16,17). Hyperimmune anti-DNase-B responses in patients with APSGN have been reported after pyoderma (3,16,17,19). Dillon reported that streptococcal immune response in patients with AGN is significantly greater than in comparable groups of infected controls (16). The magnitude of the immune response in streptococcal infections may be related to genetic factors in the host, variables such as chronicity of infections and serotypes of infecting strains (17).

In our study only 3 patients and 2 skin infection controls had elevated ASO titres. Therefore, anti-DNase-B estimation is superior to the ASO titres as an indicator of streptococcal skin infection. This has been attributed to the fact that skin lipids may inhibit streptolysin-O (20).

C<sub>3</sub> levels were decreased during the acute phase in all our patients, C<sub>4</sub> was

decreased in 46 patients and normal in 14. Both returned to normal 2 months later. Other investigators have demonstrated decreased levels of C<sub>3</sub> in patients with APSGN (6,21). This suggests that the low C<sub>3</sub> in APSGN occurred as a result of activation of the classical pathway. Recent reports have shown that C<sub>19</sub> and C<sub>4</sub> levels were normal and C<sub>3</sub> was lowered due to activation of alternate complement pathway (5,22,23). Onyewottu *et al.* (5) reported increased levels of immune complexes and low C<sub>3</sub> levels in APSGN but C<sub>4</sub> was normal. It was postulated that C<sub>4</sub> levels could be normal, because of increased synthesis of C<sub>4</sub> so that low levels are detected only during early stages of nephritis. In our patients the mean duration of illness before hospitalisation was 4 days, hence C<sub>4</sub> could be estimated early in the course of the disease. Our data showed that C<sub>3</sub> and C<sub>4</sub> levels were normal in skin infection and normal controls.

IgA, IgG, and IgM levels were normal in patients, skin infection controls and normal controls. Rheumatoid factor was positive in only 24% of our patients. 10 of them had DAT of 1/16 and 4 had 1/32, Mohammed *et al.* (4) have made similar observations in their study.

Bernardo-Rodriguez *et al.* (9) and McIntosh (10) reported increased levels of serum IgG in majority of their patients with elevated rheumatoid factor in a large percentage of patients.

The low percentage of positive rheumatoid factor and low levels of positive titres indicate that IgG anti-IgG immune complex may not play a significant role in the pathogenesis of the disease in our patients.

## Acknowledgement

The authors are indebted to the Staff

at the Streptococcal Reference Laboratory, CMCH, Vellore for kindly doing the estimation of anti-DNase-B antibody and ASO titres.

## REFERENCES

1. Svartman M, Potter EV, Finklea JF, Poon King T, Earle DP. Epidemic scabies and acute glomerulonephritis. *Lancet* 1972, i: 249.
2. Dillon HC. Pyoderma and Nephritis. *Ann Rev Med* 1967, 18: 207.
3. David Earle. Acute Glomerulonephritis. The Biologic and Clinical Basis of Infectious Diseases 2nd Ed. Ed Guy P, Youmans, Philip Paterson Herbert Summers 1980, pp 223-235.
4. Mohammed I, Barbara MA, Holborow EJ, Bryceson ADM. Circulating immune complexes in subacute infective endocarditis and post-streptococcal glomerulonephritis. *J Clin Pathol* 1977, 30: 308-311.
5. Onyewotu II, Jenny Mee. Circulating immune complexes and complement level in relation to the clinical presentation of Nigerian children with acute post-streptococcal glomerulonephritis. *J Clin Pathol* 1978, 31: 817-822.
6. I Van De Rijn Fillet H, Brandeis WE, Reed H, Poon-King T, McCarthy M, Dey NK, Zabriskie JB. Serial studies on circulating immune complexes in post-streptococcal sequelae. *Clin Exp Immunol* 1978, 34: 318.
7. Zabriskie JB. The role of streptococci in human glomerulonephritis. *J Exp Med* 1971, 134: 180-192.
8. Lyampert I, Vvedenskaya OL, Danilova TA. Study on streptococcal group A antigens common with heart tissue elements. *Immunology* 1966, 11: 313.
9. Bernardo Rodriguez Iturbe, Garcia R, Rubio L. Etiologic, pathogenic, clinical and immunopathologic considerations in acute post-streptococcal glomerulonephritis. In: Streptococcal diseases and the immune response. Eds Stanley Reed, John Zabriskie. Academic Press 1980. pp 537-551.
10. McIntosh RM. The role of interactions between streptococcal products and immunoglobulins in the pathogenesis of glomerular and vascular injury. In: Streptococcal disease and the immune response. Eds Stanley Reed, John Zabriskie. Academic Press 1980, pp 585-596.
11. Wardle EN. Glomerulonephritis In: Guidelines in medicine. Vol 2, International Medical Publishers 1979, pp 89-96.
12. Bianco C, Patrick R, Nussenzweig V. A population of lymphocytes bearing a membrane receptor for antigen-antibody complement complexes. I. Separation and characterisation. *J Exp Med* 1970, 132: 702.
13. Nelson J, Ayoub EM, Wannamaker LW. Streptococcal antideoxyribonuclease-B microtechnique determination. *J Lab Clin Med* 1965, 71: 867.
14. Mancini G, Carbonara AO, Heremans JF. Immunochemical quantitation of antigens by single radial immune diffusion. *Int J Immunochem* 1965, 2: 235-254.
15. Williams DG, Peters DK, Fallows J, Petric A *et al.* Studies of serum complement in the hypocomplementaemic nephritis. *J Exp Med* 1974, 34: 180-192.
16. Dillon HC, Reeves MC. Streptococcal immune response in nephritis after skin infection. *Am J Med* 1974, 56: 3.
17. Wannamaker LW. Difference between streptococcal infection of throat and skin. *N Engl J Med* 1970, 282: 23-31, 78-85.
18. Alan Bisco. Factors influencing serum antibody response in streptococcal pyoderma. *J Lab Clin Med* 1973, 81: 410.
19. Jerath VP, Ratan Singh, Sudershan Kumari.  $\beta$ -hemolytic streptococci group A in the skin and throat of patients of scabies with nephritis. *Indian J Med Res* 1979, 69: 933-936.
20. Peter G, Smith AL. Group A streptococcal infections of the skin and pharynx. *N Engl J Med* 1977, 297: 311.
21. Cameron JS, Vick RM, Ogg CS, Seymour WM, Chantler C, Turner DR. Plasma C<sub>3</sub> and C<sub>4</sub> concentrations in the management of glomerulonephritis. *Br Med J* 1973, 3: 688, 672.
22. Kohler PF, Ten Binsel R. Serial complement component alterations in acute glomerulonephritis and systemic lupus erythematosus. *Clin Exp Immunol* 1969, 4: 191-202.
23. Lewis EJ, Carpenter CB, Schur PH. Serum complement component levels in human glomerulonephritis. *Ann Intern Med* 1971, 75: 555-560.