Immunological aspects of nephrotic syndrome in northern Nigeria

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SUMMARY  Immunological aspects of 40 northern Nigerian children with nephrotic syndrome of recent onset are reported. Eight out of 30 had hepatitis-associated antigen in their sera. Hypocomplementaemia was rare. Measurement of serum C3, C4, and ASOT was not of diagnostic value. Proteinuria selectivity index was poor in half of the patients, and appeared to depend on the severity of the kidney lesion. Abnormal immunofluorescence of kidney glomeruli to immunoglobulins, complement, Plasmodium malariae, and Plasmodium falciparum was found in 26 of the 29 children. The pattern of immunofluorescence was chiefly granular and was confined to the glomeruli. IgM was predominant. It was concluded that immunological reaction is involved in the pathogenesis of nephrotic syndrome in northern Nigerian children.

There is ample evidence that the pattern of nephrotic syndrome in tropical countries is different from that in temperate ones. Some of the characteristics of the disease peculiar to the tropics are—an older age of onset, the rarity of minimal change lesions, an association with quartan malaria, poor selectivity of proteinuria, and a poor response to corticosteroids.1–4

We have studied the immunological aspects of the syndrome in children attending this hospital. The study was approved by the Ethical Committee of the Faculty of Medicine, Ahmadu Bello University.

Patients and methods

Forty children who presented consecutively with nephrotic syndrome were studied. They had had no previous treatment. A diagnosis of the nephrotic syndrome was made in a patient if he (or she) presented with oedema and had had proteinuria of \( \geq 2g/24 \) hours, and had hypoalbuminaemia <25/g/l.

Routine investigations were made, but in addition repeated searches (3–6) were made for malarial parasites in peripheral blood smears before administration of chloroquine, and serum ASOT, C3, and C4 levels were determined. Sera were tested for HAA, urine protein selectivity was determined, and a kidney biopsy was performed.

Peripheral blood smears were examined for malarial parasites and sera were tested for HAA in another group of children; these children were matched for age and sex but they did not suffer from renal disease. Serum ASOT was determined with Wellcome Streptolysin-O commercial kit; C3 and C4 were determined by radial immunodiffusion using monospecific antisera, and the results were recorded as a percentage of a pooled adult Nigerian plasma standard. Sera were tested for HAA by counter-current immuno-electrophoresis. To find out the proteinuria selectivity index (SI), serum and urinary IgG and albumin concentrations were determined by radial immunodiffusion using monospecific antisera. The value of SI was calculated from the formula

\[
\text{SI} = \frac{\text{Urine IgG/serum IgG}}{\text{urine albumin/serum albumin}} \times 100
\]

and expressed as a percentage. SI was classified as high (1–15%), moderate (16–30%), or poor (\( \geq 31 \% \)).

<table>
<thead>
<tr>
<th>Abbreviations:</th>
<th>Meaning:</th>
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<tbody>
<tr>
<td>ASOT</td>
<td>antistreptolysin O titre</td>
</tr>
<tr>
<td>HAA</td>
<td>hepatitis-associated antigen</td>
</tr>
<tr>
<td>MPGN</td>
<td>membranoproliferative glomerulonephritis</td>
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<tr>
<td>PGN</td>
<td>proliferative glomerulonephritis</td>
</tr>
<tr>
<td>SI</td>
<td>proteinuria selectivity index</td>
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<tr>
<td>QMN</td>
<td>quartan malaria nephropathy</td>
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</table>
Table 1  Histological diagnoses related to other laboratory findings

<table>
<thead>
<tr>
<th>Histological diagnosis</th>
<th>Sex ratio (M : F)</th>
<th>Age (years) Mean Range</th>
<th>Malarial parasite</th>
<th>ASOT (U/ml) Mean Range</th>
<th>Complement (% of standard) C3 Mean Range</th>
<th>C4 Mean Range</th>
<th>HAA (n = 30)</th>
<th>SI (%) (n = 35)</th>
<th>Mean Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Membranoproliferative glomerulonephritis (n = 14)</td>
<td>1:3:1</td>
<td>6-8 3-11</td>
<td>4</td>
<td>4</td>
<td>202 20-1280</td>
<td>153 20-220</td>
<td>153 100-200</td>
<td>3</td>
<td>26</td>
</tr>
<tr>
<td>Quartan malaria nephropathy (n = 11)</td>
<td>1:4:5</td>
<td>5-3 3-9</td>
<td>4</td>
<td>6</td>
<td>62 10-80</td>
<td>123 18-184</td>
<td>140 130-150</td>
<td>2</td>
<td>12</td>
</tr>
<tr>
<td>Proliferative glomerulonephritis (n = 4)</td>
<td>3:1</td>
<td>1, 3, 4, 7</td>
<td>1</td>
<td>1</td>
<td>80, 180, 320, 640</td>
<td>131, 180, 201, 304</td>
<td>116, 120, 130, 132</td>
<td>0</td>
<td>5, 8, 34</td>
</tr>
<tr>
<td>Miscellaneous (n = 4)</td>
<td>3:1</td>
<td>3, 5, 7</td>
<td>2</td>
<td>0</td>
<td>20, 60, 72, 80</td>
<td>33, 43, 78, 224</td>
<td>75, 99, 115, 120</td>
<td>2</td>
<td>14, 39, 40</td>
</tr>
<tr>
<td>No biopsy (n = 4)</td>
<td>1:3</td>
<td>2, 6, 8, 10</td>
<td>1</td>
<td>2</td>
<td>40, 180, 250, 250</td>
<td>90, 112, 124, 170</td>
<td>66, 126, 130, 146</td>
<td>1</td>
<td>4, 15</td>
</tr>
<tr>
<td>Chronic glomerulonephritis (n = 3)</td>
<td>1:2:1</td>
<td>4, 8, 12</td>
<td>1</td>
<td>0</td>
<td>20, 40, 60</td>
<td>100, 100, 101</td>
<td>34, 97, 160</td>
<td>8</td>
<td>6, 15, 17</td>
</tr>
<tr>
<td>Total (n = 40)</td>
<td>1:2:1</td>
<td>6 1-12</td>
<td>1</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>

Patients without renal disease (n = 40) 1:5:1 6 1-12 1 10

<table>
<thead>
<tr>
<th>C3</th>
<th>C4</th>
</tr>
</thead>
<tbody>
<tr>
<td>153</td>
<td>20-220</td>
</tr>
<tr>
<td>153</td>
<td>100-200</td>
</tr>
</tbody>
</table>

ASOT, C3, and C4

Results

Children were divided into 6 groups according to the microscopic appearance of their kidney biopsies: the light microscope, OMN n = 11, PGN n = 4, miscellaneous, MPGN n = 4, and chronic glomerulonephritis (n = 3). The biopsy group comprised 4 children with membranous glomerulonephritis, one with rapidly progressive glomerulonephritis, one child with congenital tubular defect, one child with membranous glomerulonephritis, and one child with congenital tubular defect.

Malarial parasitaemia: The incidence of P. falciparum was not greatly different in children with nephrotic syndrome compared to those without disease. However, more children with nephrotic syndrome had P. malariae in their blood smears than healthy children without renal disease (P < 0.001), or patients with congenital nephrotic syndrome. The incidence of P. malariae in the blood smears of these children was 90% compared to 15% in the group of children without renal disease.

For the immunofluorescence studies, renal biopsy specimens were snap-frozen in liquid nitrogen and then cut into sections 6 μm thick. These sections were stained with fluorescein-labelled anti-IgG and anti-C3 conjugates to detect the presence of immunoglobulin and complement components. Immunofluorescence microscopy was performed to identify the presence of immunoglobulins and complement components in the biopsies.

The histological diagnoses were confirmed by light microscopy and immunofluorescence microscopy, and the results were compared to the clinical findings and other laboratory investigations.

For the electron microscopic examination, thin sections were cut from the biopsy specimens and stained with uranyl acetate and lead citrate. The sections were then examined using a transmission electron microscope.

Histological examination of the kidney biopsies showed varying degrees of glomerular and tubular abnormalities, including diffuse and segmental glomerulosclerosis, segmental and global sclerosis, and segmental capillary obliteration. Tubular atrophy and interstitial fibrosis were also observed.

The results of the histological examination were compared to the clinical findings and other laboratory investigations, and the correlation between the two was assessed.

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Table 2  Histological diagnoses related to immunofluorescence

<table>
<thead>
<tr>
<th>Membranoproliferative glomerulonephritis (n = 13)</th>
<th>Whole IgG IgA IgM C3 P. falciparum P. malariae</th>
<th>Total no of patients with abnormal immunofluorescence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glomerulonephritis (n = 9)</td>
<td>6 2 4 7 4 0 5</td>
<td>7</td>
</tr>
<tr>
<td>Quartan malaria nephropathy (n = 3)</td>
<td>3 2 0 2 1 0</td>
<td>2</td>
</tr>
<tr>
<td>Miscellaneous glomerulonephritis (n = 2)</td>
<td>2 2 0 2 1 0</td>
<td>2</td>
</tr>
<tr>
<td>Total (n = 29)</td>
<td>21 10 7 23 18 3 8</td>
<td>26</td>
</tr>
</tbody>
</table>

* Kidney tissue from 2 patients reacted only to anti-whole serum and to no other antiserum.

ASOT (mean 62 units/ml), and patients with PGN had the highest mean value of ASOT (305 units/ml). Two patients with MPGN had high ASOT levels—320 and 1280 units/ml. The fairly high C3 and C4 levels found were similar to the values in children in the local population.8

HAA. HAA was detected in 8 out of 30 sera of children with nephrotic syndrome, more often than the 2 out of 40 sera of children without renal disease (P < 0.01).

Proteinuria selectivity index. SI was determined in 35 patients: 19 patients had high, 9 had moderate, and 7 had poor selectivity. There was a wide range of values in each histological group. The degree of selectivity appeared to bear an inverse relationship to the severity of renal disease, but there was no relationship to age, sex, duration of symptoms, or initial laboratory findings.

Immunofluorescence. Abnormal fluorescence was found in 26 out of 29 biopsies. Immunofluorescence was predominantly granular (80%); it was linear in 5%, and mixed granular and linear in 15%. The fluorescence was confined to the glomeruli except for 2 patients in whom there was additional fluorescence in the tubules. Fluorescence was most intense with IgM. Seven patients showed positive IgA fluorescence except for IgA fluorescence. In children showing immunofluorescence the number with IgA fluorescence was 3 out of 10 in South Africa,11 3 out of 26 in east Africa,10 7 out of 26 in the present study, and none out of 41 children in southern Nigeria.9 We cannot account for these differences. In other continents the majority of cases of childhood nephrotic syndrome does not show abnormal immunofluorescence of kidney tissues.12

The evidence for immunological damage as demonstrated by immunofluorescence is supported by the finding of abnormal renal histology and poorly selective proteinuria. All the 36 renal biopsies showed pathological changes of varying severity; no biopsy showed minimal change lesions. 46% of the patients had poorly selective proteinuria. In other parts of Africa, pathological changes in kidney biopsies9 and poor SI6 have been reported. The findings in Africa are different from those in Europe and America, where a majority of cases of childhood nephrotic syndrome had minimal lesion on light microscopical examination of kidney biopsy13 associated with highly selective proteinuria in at least 90% of cases.5 14

Which antigens are involved in the immunological damage to the kidney? In the present study, P. malariae appeared to be one, as shown by (1) the high rate of P. malariae parasitaemia, (2) histology of the kidney biopsies, and (3) detection of P. malariae antigen in the glomeruli. The prevalence of P. malariae parasitaemia in children with nephrotic syndrome was higher than in patients without renal disease, or in children in the local population. The histology of kidney biopsy in 11 patients is compatible with the histology of QMN described by Edington and Gilles.15

P. malariae antigen was detected in the glomeruli of 5 of 9 patients with QMN, 2 of 13 patients with MPGN, and 1 of 2 patients with chronic glomerulonephritis. The 2 children in the MPGN group with P. malariae antigen in their kidney biopsies also
had *P. malariae* parasitaemia, and it is possible that they should have been diagnosed as having QMN.

About three-quarters of patients had evidence of immunological damage although *P. malariae* could not be demonstrated as the cause of the damage.

*P. falciparum* antigen was detected in the kidneys of 3 patients, all of whom had *P. malariae* antigen too. This could have been a cross-reaction with *P. malariae* antigen, although Berger et al.\(^1\) reported 3 cases of *P. falciparum* malaria associated with nephrotic syndrome who had renal histology similar to that seen in poststreptococcal acute glomerulonephritis. The histology of 4 biopsies in the present study was proliferative glomerulonephritis, associated with high ASOT levels in 3 of the children. The high levels of ASOT found in 2 patients with MPGN could have been an indication of recent infection with the streptococcus. Only 2 of the children with PGN and high ASOT values had histories that suggested recent poststreptococcal nephritis, but their clinical features were no different from the other children with the nephrotic syndrome. The role of anti-streptococcal antigens in the pathogenesis of nephrotic syndrome in our patients appears slight.

Abnormal kidney immunofluorescence has been described in patients with hepatosplenic Manson’s schistosomiasis with or without evidence of renal disease.\(^17\)–\(^18\) Schistosomiasis, both haematobium and Manson’s, occurs in Nigeria. We have not studied the role of schistosomiasis in the pathogenesis of nephrotic syndrome.

Eight (27%) out of the 30 sera tested had HAA. This figure is higher than for children without renal disease, and it is higher too than the figure of 6·3% for healthy children aged between 2 and 5 years in the local population.\(^19\) HAA has been detected by immunofluorescence in the glomeruli of patients with nephrotic syndrome associated with hepatitis-associated antigenaemia.\(^20\) Because of the high prevalence of HAA in the nephrotic syndrome found in this study, we plan to study the role of the antigen in the aetiology of nephrotic syndrome, using anti-HAA conjugates.

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References


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