Urinary FDP, Heterophile Agglutinins IgG, IgM and C₃ in Renal Homotransplant Rejection and Glomerulonephritis

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It has been shown that urine fibrin and fibrinogen degradation products (FDP) may act as a useful index of active glomerular damage in proliferative glomerulonephritis (Clarkson et al, 1971; Davison et al, 1973) and in renal allograft rejection (Clarkson et al, 1970; Hume & Pitcher, 1973; Naish et al, 1973).

Serum heterophile agglutinin has been shown to be elevated during renal allograft rejection (Rapaport et al, 1968; McDonald, 1973) although this finding has been challenged by Tiong and Morris (1972) and Svehag et al (1973). The mechanism of this response is not known but it has been suggested that an immunological reaction to altered tissue antigens may be responsible. The haemagglutinins have been shown to be in the IgG, IgM fractions, (Salo, 1966).

There is ample evidence to suggest that immunological mechanisms are at play in both glomerulonephritis (Dixon, 1968; Berger et al, 1969; McCluskey, 1971; Macanovic et al, 1972) and in renal allograft rejection (Porter et al, 1968; McKenzie & Whittingham, 1968) and some measurements of urine immunoglobulins and complement have been made to support this view (Hermann et al, 1970).

It has been shown that the response of urine FDP to the anti-inflammatory agent indomethacin may serve as a prognostic index in patients with proliferative glomerulonephritis, a fall in FDP heralding a good prognosis. (Clarkson et al, 1971).

AIMS OF THE STUDY

1. To define the relationship between urine FDP and urine sheep heterophile agglutinin (SHA), IgG, IgM, C₃ and total protein.
2. To identify urine SHA in terms of its relationship with urine IgG, IgM, C₃ and total urine protein.
3. To assess the usefulness of urine SHA in the diagnosis of renal allograft rejection.
4. To assess the response in patients with glomerulonephritis of urine FDP, SHA, IgG, IgM and C₃ to indomethacin.

PATIENTS AND METHODS

Fifteen patients with proliferative glomerulonephritis and ten patients with a renal allograft were studied. Ninety urine samples were taken at random from these patients and from 100 normal control subjects. Seven patients with proliferative glomerulonephritis were subsequently monitored before and during indomethacin treatment, urine being analysed daily for from 16 to 50 days.

All urine samples were dialysed against tap water and concentrated approximately 25 times with polyethylene glycol. Urine FDP was measured using the tanned-red-cell haemagglutination inhibition immunoassay technique (TRCHII) (Hoq & Das, 1971), the urine heterophile agglutinin titre was established using the direct one-stage sheep red blood cell agglutination technique (Hoq et al, 1971) the urine IgG, IgM and C₃ was measured by the Mancini immunodiffusion technique (Mancini et al, 1965) and the total protein by the biuret method (Hiller et al, 1948).

RESULTS

There was no SHA, IgM or C₃ in the normal urines. FDP was present in amounts less than 0.5 µg/ml, IgG less than 0.5 mg/100 ml, and total protein less than 30 mg/100 ml. Table I shows the correlation coefficient (r) and P values for the relationship between the urinary components in the 90 samples taken from the patients. There was a significant correlation between urine FDP and urine SHA, IgG, IgM and C₃ but no correlation between

Table I. The correlations between urine FDP, SHA immunoglobulins, C₃ and total protein

<table>
<thead>
<tr>
<th>Relationship between</th>
<th>r</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>FDP/SHA</td>
<td>0.4891</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>FDP/IgG</td>
<td>0.6321</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>FDP/IgM</td>
<td>0.5238</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>FDP/C₃</td>
<td>0.6855</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>FDP/total protein</td>
<td>0.1256</td>
<td>N.S.</td>
</tr>
<tr>
<td>SHA/IgG</td>
<td>0.5551</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>SHA/IgM</td>
<td>0.6139</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>SHA/C₃</td>
<td>0.8319</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>SHA/total protein</td>
<td>0.1796</td>
<td>N.S.</td>
</tr>
</tbody>
</table>
Renal Homotransplantation

Figure 1. The changes in urine FDP, SHA, C₃, total protein, and immunoglobulins during a rejection episode

urine FDP and urine total protein. There was also a significant correlation between urine SHA and IgG, IgM and C₃ but no correlation between SHA and total protein.

In terms of the diagnosis of renal allograft rejection we were able to monitor 4 unequivocal episodes of clinical rejection in detail and found parallel elevation of urine FDP, SHA, IgG, IgM and C₃ on each occasion. Figure 1 shows an example of the changes in these urine measurements during a rejection episode and the return to normal when the rejection episode had responded to treatment.

In four of the seven patients with glomerulonephritis treated with indomethacin there was a fall in urine FDP, SHA, immunoglobulins and C₃. An example is shown in Figure 2. In all these patients the clinical progress was good with eventual disappearance of haematuria and proteinuria, and with no deterioration in renal function.

The fifth patient responded unfavourably to indomethacin with no fall in any of the urine components and progression of the renal disease to complete renal failure (Figure 3).
The remaining two patients responded to indomethacin in an atypical fashion. In the first patient (Figure 4) there was a fall in FDP, but no fall in heterophile agglutinin (immunoglobulins were not measured). In the second patient (Figure 5) there was a fall in FDP, and again no fall in heterophile agglutinin, and a rise in IgG, IgM and C₃. The ultimate clinical progress in these two patients was poor with progressive deterioration in renal function.

DISCUSSION

Previous studies have demonstrated a significant correlation between the activity of glomerular disease as assessed biochemically and histologically, and the excretion of urine FDP in proliferative glomerulonephritis, (Clarkson et al, 1971; Davison et al, 1973), and in renal transplantation (Clarkson et al, 1970; Hume & Pitcher, 1973; Naish et al, 1973). The demonstration in this study of a correlation between urine FDP and immunoglobulins and C₃ lends support to the concept that fibrin is laid down in glomeruli in proportion
Figure 3. An unfavourable response of urinary components to indomethacin

Figure 4. A diverse response of urine FDP and SHA to indomethacin
to the degree of immunological activity in these conditions. (Salmon et al, 1971; Luscher & Pfueller, 1971)

Heterophile agglutinin is found in the serum in response to a number of antigens. The mechanism responsible for its presence in the urine in glomerulonephritis and renal transplant rejection is not known, but it may represent an immune reaction to altered glomerular tissue antigens. This view is supported by the demonstration of a significant correlation between urine heterophile agglutinin and immunoglobulin and C3 which are assumed to be related to immunological events within the glomerulus (Gotoff et al, 1965; Michael et al, 1969; Berger et al, 1969; McCluskey, 1971; Macanovic et al, 1972).

The poor correlations between urine FDP, SHA and urine total protein tend to rule out the simple leakage of plasma through a damaged basement membrane as an explanation for the presence of these substances in the urine.

The demonstration of heterophile agglutinin in the urine in renal
transplant rejection and glomerulonephritis has practical implications in that
the assay is a simple, direct, one-stage agglutinin reaction, and as such
should be more applicable to the clinical situation than the laborious time
consuming FDP assay.

The examples of 'good' and 'bad' prognostic responses to indomethacin
have been demonstrated before by Clarkson et al (1971) in terms of urine
FDP. The demonstration in this study of a small sub-group of patients in
whom there was a diversity between urine FDP on the one hand, and urine
immunoglobulins and complement on the other suggests that in these patients
(1) the deposition of intra-renal fibrin may not be a key aetiological factor in
the progression of the renal failure, (2) urine FDP estimations are of limited
value in assessing progress, and (3) indomethacin may have no effect on the
immunological events leading to renal failure.

SUMMARY

It has been demonstrated that:
1. Urine FDP is related to the excretion of SHA, IgG, IgM and C3 and not
to total protein excretion in glomerulonephritis and renal allograft rejection.
2. Urine SHA may represent an immunological reaction to altered antigen
in these conditions.
3. Urine SHA is a practical method of monitoring renal allograft rejection
and the response of patients with glomerulonephritis to therapeutic agents.
4. There exists a sub-group of patients with glomerulonephritis in whom
intraglomerular fibrin deposition may not be a key aetiological factor in the
progression of renal disease.

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OPEN DISCUSSION

C L HALL (Birmingham): What is sheep heterophile agglutinin? What class of protein is it, what is its molecular size, and how does it get into the urine?

ANDERTON: We suggest on the basis of previous work that it is in the IgG/ IgM fraction, and we further think that its correlation with IgG and IgM in the urine suggests that it is the IgG and IgM that we are measuring. Heterophile agglutinin is an antibody which agglutinates the red cells of other species - in this case the sheep. It also agglutinates the red cells of rabbits and guinea pigs. It appears in the urine probably because of the immunological events that take place both in glomerulonephritis and renal transplant rejection, as a 'spill over' phenomenon, as do fibrin degradation products.

W ROWINSKI (Warsaw): Have you observed any variations in FDP excretion in patients treated with ALG?

ANDERTON: Yes. We have shown elevation of urine degradation products during ALG treatment.

ROWINSKI: The result therefore might be misleading when the patient is on ALG treatment.

ANDERTON: None of the patients were on ALG during the study.