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# GLOMERULONEPHRITIS IN SCHISTOSOMIASIS WITH MESANGIAL IGM DEPOSITS

AGEU DE GODOY MAGALHÃES FILHO*
ANTÔNIO VICTORIANO BARBOSA**
TERESA CRISTINA FERREIRA**

*Twenty one cases of hepatoesplenic schistosomiasis patients without clinical and laboratory evidence of renal disease, were studied by surgical biopsies using light microscopy and immunofluorescence. The cases were classified histologically as: normal pattern (6 cases); minimal changes (6 cases); and mesangial proliferative glomerulonephritis (9 cases). By the immunofluorescence microscopy using anti IgM, IgG, IgA and C3, the predominant finding in all biopsies, except the normal cases, was granular deposits of IgM in the mesangium along with C3.*

*On the other hand, IgG was present in all cases including normal biopsies along the capillary walls. However IgG was also present in the mesangium only in cases with glomerular lesions. This finding may well be similar to that recently described as IgM mesangial nephropathy.*

*According to our cases a mesangial proliferative glomerulonephritis, characterized by segmental cell proliferation and deposition of IgM in the mesangium, is probably the entity found in the early stages of mansonic schistosomiasis.*

Several papers have been published describing renal lesions in schistosomiasis mansoni; however some immunopathologic aspects still remain unknown. The first descriptions were Lopes (1964), Machado (1965) and Lima, Brito & Rocha (1969) who found proteinuria in patients with hepatosplenic schistosomiasis. The histologic aspects were described by Andrade & Queiroz (1968) as a basal membrane thickening and mesangial cellular proliferation.

More recently, ultrastructural evaluation was made of renal biopsies from patients with schistosomiasis and without clinical evidence of renal disease; Electron dense deposits, probably of gamma globulin immune complexes were described in the glomerular basal membrane, near mesangial cells (Brito et al 1969). Immunofluorescence studies

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*Centro de Pesquisas Aggeu Magalhães - FIOCRUZ - Caixa Postal 6034 - 50000 - Recife, PE Brazil.

**Deptº de Patologia - UFPE - Recife, PE - Brazil.

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of biopsies of patients with *S. mansoni* presenting clinical evidence of renal disease showed focal deposits of gamma globulin and complement (Brito et al 1970). Experimental studies were made in monkeys and mice and they all demonstrated gamma globulin deposition in the basal membrane and mesangial cells corresponding to slight hyperplasia of those cells (Brito et al, 1971).

Soluble antigen, together with IgG, IgM and C3 were detected in most of the severe lesions and in some of the mild lesions, these observations also indicating that renal lesions in *S. mansoni* infections may be attributable to the deposition of immune complexes preformed in the circulation and trapped in the glomerular capillaries. The same authors suggest that the glomerular injury in monkeys probably has a similar pathogenetic mechanism (Houba, Sturrock & Butterworth, 1977). Another alternative would be that antigens are deposited first in the capillary walls with subsequent binding of antibody and complement. The trapped antigen-antibody complexes would induce the mild hypertrophy and hyperplasia of mesangial cells, regarding the human glomerular injury seen in patients without evidence of clinical disease (Brito et al 1971). The purpose of this comunication is to report our findings in a group of patients with chronic schistosomiasis mansoni in the early stages of the renal lesions, without clinical or laboratorial evidence of renal disease.

## MATERIAL AND METHODS

Twenty one surgical biopsies from selected cases of schistosomiasis mansoni with clinical evidence of intensive and chronic disease were obtained. The average age was twelve years. All of them came from an endemic area and had viable eggs in the stool/or rectal biopsy examinations. No past history of repetitive sore throat or upper respiratory infections could be detected in them. None of the patients demonstrated clinical evidence of renal alteration. The renal specimens (with 3x5mm) were obtained during splenectomy and divided into two parts, for light microscopy and for immunofluorescence.

### a) *Light microscopy*

After the usual processing of fixation (alcoholic-Bouin's solution 8–24hs) the paraffin sections were stained by hematoxylin and eosin, periodic acid-Schiff, Massons's trichrome Azan and periodic acid-Schiff-methenamine silver (Jones).

### b) *Immunofluorescence microscopy*

A portion of each specimen was rapidly frozen (dry-ice-acetone) for frozen sections, after standard techniques for processing in a cryostat microtome at $3\mu$. The sections were stained with commercial fluorescein isothiocyanate-conjugated antisera using the technique (Hoechst Laboratories, S. Paulo, Brazil) with anti human IgG, IgM, IgA and C3, and were examined with a Leitz ultraviolet microscope. Fluorescence intensity was graded from (± minimal and ++ moderate fluorescence intensity), also kidney sections obtained a few hours *post-mortem* from a young accident-deceased individual were included for control. This control exhibited no evidence of *S. mansoni* infection at *post-mortem.*

### c) *Immunodiffusion Radial Tec. of Mancini*

Skin test using *S. mansoni* antigens; leucocyte migration inhibition test (modified from Federlin et al, 1971); and rosette formation by peripheral lymphocytes (Brain, Gordon & Willetts, 1970) were also performed from samples of peripheral blood from all cases.

## RESULTS

### a) *Light microscopy*

All biopsies were surgically removed during splenectomy and many glomeruli, about 30 to 35, were available for study. From the twenty one cases only *six cases* showed no demonstrable alterations and were considered as normal. Minimal changes characterized by a focal mesangial proliferation (4 to 6 cells per segment) were seen in *six cases. Nine cases* considered as mesangial proliferative glomerulonephritis showed a mesangial cell proliferation (above six cells per segment) usually associated with some focal increase of the mesangial matrix and basal membrane. This was particularly evident with the periodic acid-Shiff Stain (Fig. 1).

No evidence of sclerosis or other alteration was seen in any of the cases studied.

### b) *Immunofluorescence microscopy*

All normal cases showed a deposition of IgG (fine granular pattern) along the capillary walls only. No immunoglobulin or C3 fluorescence deposits were seen in the mesangium of the normal cases. Nor was complement (C3) fluorescence seen in the capillary walls (Table I). In the cases classified as minimal changes IgG (± to +) was found in the mesangium, as well as in the more advanced cases. IgM and C3 fluorescence deposits were seen predominantly in the mesangium in all cases of mesangial cell proliferation (Tables II, III and Fig. 2). IgA (+) occurred sporadically in three cases in the capillary and in one case it was present in the mesangium only. On the other hand, IgG was also present in the mesangium in all cases with mesangial cell proliferation and along the capillary walls (Tables II, III and Fig. 3). No antigen, however, was demonstrated in these cases.

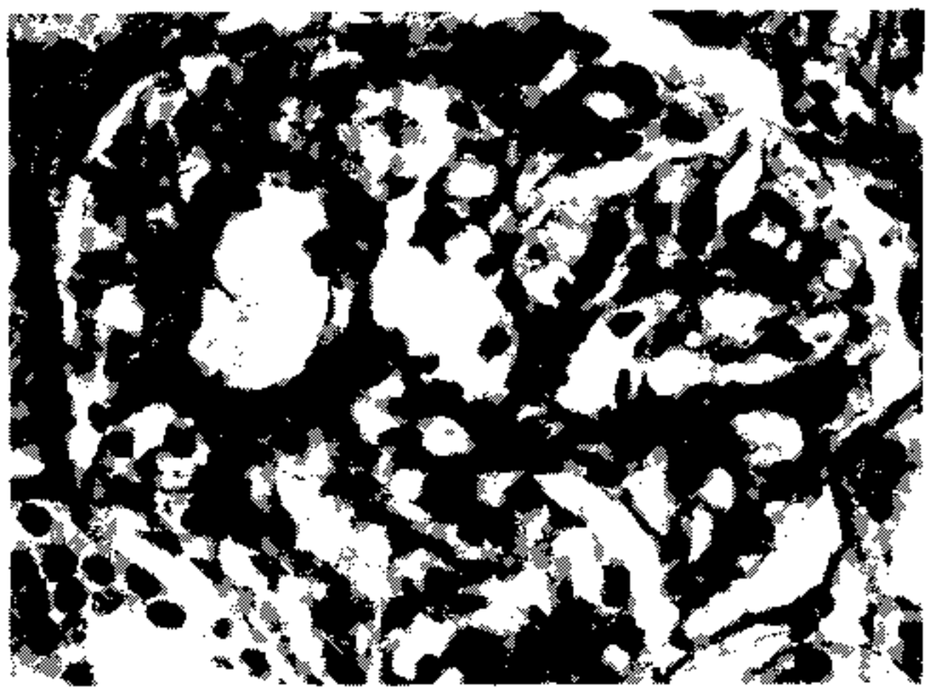
### c) *Immunodiffusion Radial Tec. of Mancini*

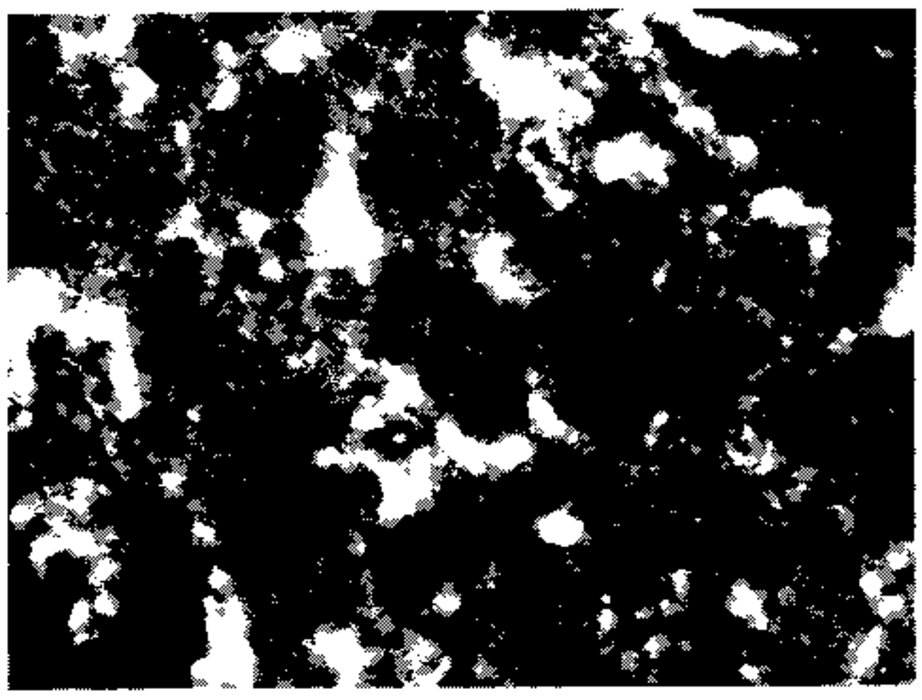
In all patients, immunodiffusion radial technique showed IgM level and IgG level relatively high: no significant diference in B and T peripheral lymphocytes; in cases of glomerular mesangial proliferation there was a low level of C3 and the skin test reaction was more severe.

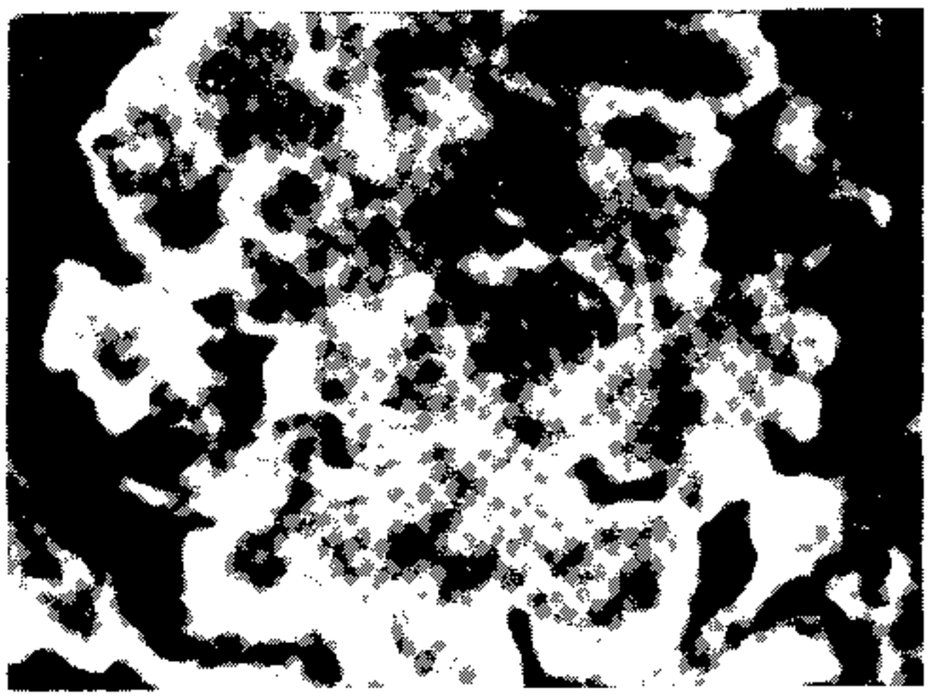
## DISCUSSION

A review of the clinical features of our 21 cases with chronic schistosomiasis shows that in spite of the advanced parasitic disease none of them had clinical or laboratory manifestations of renal alteration. It was our intention to study those cases of schistosomiasis in order to detect the early stages of renal morphologic changes by light microscopy and immunofluorescence. As indicated in Table I, the six cases with no histologic alteration showed diffuse deposition of IgG (fine granular pattern) along the capillary walls probably near the endothelial side of the glomerular basement membrane. On the other hand, coincidently in these cases there was no fluorescent material deposition (IgG, IgM or C3) in the mesangium.

Therefore, we suggest that the simple presence of IgG in the endothelial location, without complement deposition, might probably be the earliest manifestation, before glomerular alterations take place. This probably happens in all patients with the chronic stage of schistosomiasis. In fact, a high level of IgG is always found in the circulation. We believe that the fine granular pattern of IgG only present along the capillar walls in normal cases is a strong suggestion that the basement membrane still remains intact. According to our cases the patterns of immunoglobulin fluorescence did not correlate precisely with the degree of damaged renal function, although the fine granular pattern (IgG) was







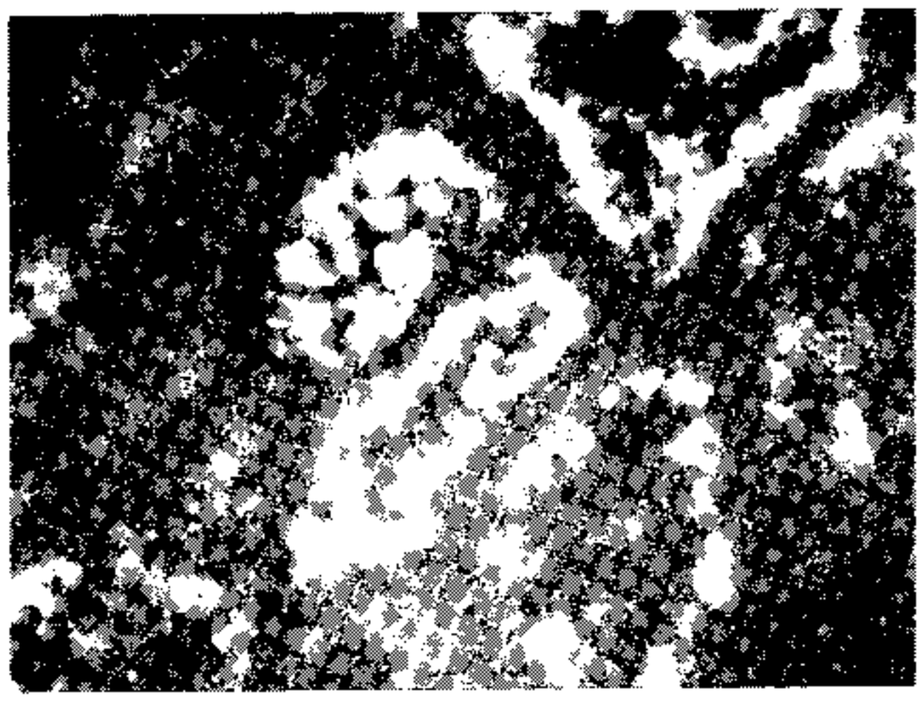


Fig. 1 Diffuse mesangial cellularity (moderate) small increase of glomerular basal membrane (periodic acid-shiff x 250).

Fig. 2. – Immunofluorescent preparation with anti-human IgM. There is deposition of the immunoglobulin in the mesangium at an intensity of ++ (x 250).

Fig. 3 – Immunofluorescent preparation with anti-human IgG (++) showing deposits in all glomeruli (x 250).

Fig. 4 – Immunofluorescent preparation with anti-human IgG (+) minimal changes (x 250).

associated with no histologic alteration by light microscopy. On the other hand, the transition from fine granular to course granular patterns was probably associated with the degree of renal changes; as observed in the initial stage of schistosomal nephropathy.

Some authors have suggested that coarse deposits were mostly positive for both immunoglobulins IgG and IgM and also for complement; and the majority of cases with fine granular pattern of fluorescence had IgG only and were complement negative. However, complement positivity was more closely associated with the presence of IgM than with individual subclasses of IgG. In conclusion, there has been found certain correlations between patterns of immunoglobulin fluorescence and the presence of complement in glomerular deposits. The finding of IgM and C3 with predominance in the mesangium in the cases of minimal changes and in all nine cases of mesangial proliferative glomerulonephritis, indicates that there is a strong possibility of its participation in this nephropathy and also a correlation with the degree of glomerular lesions (see Tables II and III). Cohen, Border & Glassock (1978) described a distintive entity which, because of the constant immunofluorescence findings, was described as IgM mesangial nephropathy.

TABLE I

Normal cases – immunofluorescence microscopy fine granular pattern: distribution

| *Patients* | *IgG* | | *IgM* | | *IgA* | | *C3* | |
|---|---|---|---|---|---|---|---|---|
| | *CW* | *MES* | *CW* | *MES* | *CW* | *MES* | *CW* | *MES* |
| 1 | + f | — | — | — | — | — | — | — |
| 2 | + d | — | — | — | — | — | — | — |
| 3 | + fs | — | — | — | — | — | — | — |
| 4 | + f | — | — | — | — | — | — | — |
| 5 | + f | — | — | — | — | — | — | — |
| 6 | + f | — | — | — | — | — | — | — |

f = focal; d = diffuse; s = segmental; cw = capillary wall; mes = mesangium; — = negative fluorescence

TABLE II

Minimal changes immunofluorescence microscopy granular deposits: distribution

| *Patients* | *IgG* | | *IgM* | | *IgA* | | *C3* | |
|---|---|---|---|---|---|---|---|---|
| | *CW* | *MES* | *CW* | *MES* | *CW* | *MES* | *CW* | *MES* |
| 7 | + d | + fs | + f | + d | — | — | + fs | + f |
| 8 | + d | + fs | ± f | + f | + f | — | ± f | + fs |
| 9 | + fs | ± fs | + fs | + f | — | — | + f | ++ f |
| 10 | + f | + f | ± fs | + f | — | — | ± f | + f |
| 11 | + f | + f | ± fs | + d | — | — | + f | ++ f |
| 12 | + f | + f | + f | ++ d | — | — | + f | + d |

f = focal; d = diffuse; s = segmental; cw = capillary wall; mes = mesangium; – = negative fluorescence

TABLE III

Mesangial proliferative glomerulonephritis immunofluorescence microscopy granular deposits: distribution

| *Patients* | *IgG* | | *IgM* | | *IgA* | | *C3* | |
|---|---|---|---|---|---|---|---|---|
| | *CW* | *MES* | *CW* | *MES* | *CW* | *MES* | *CW* | *MES* |
| 13 | + f | + f | + fs | + f | — | — | + f | ++ f |
| 14 | ± d | — | + d | ++ d | + f | — | + f | ++ f |
| 15 | + f | + f | + f | ++ f | — | — | + d | ++ d |
| 16 | + f | + f | + f | ++ f | — | — | + f | ++ f |
| 17 | ± f | ± f | — | + f | — | — | + f | ++ f |
| 18 | + d | + d | + d | ++ d | + f | + f | + f | ++ f |
| 19 | + f | ± fs | — | + f | — | — | + f | + f |
| 20 | + d | + f | + d | ++ d | — | — | + f | ++ f |
| 21 | + f | + f | — | + f | — | — | + f | + f |

f = focal; d = diffuse; s = segmental; cw = capillary wall; mes = mesangium; — = negative fluorescence

Our cases of schistosomiasis showed some similarity to the immunofluorescence findings described by Cohen, Border & Glassock (1978) but in the light microscopy our findings were characterized by proliferation of the mesangial cells focal or segmental or diffuse and a slight thickening of the glomerular basement membrane with a mild focal increase in the matrix.

Bhasin et al (1978), also described eleven cases of mesangial proliferative glomerulonephritis, with the presence of IgM by immunofluorescence.

It is possible that during a progressive transition the IgM is gradually lost from the mesangium and mesangial cellularity returns to normal. This would explain the variable presence of IgM and hypercellularity in the nonsclerotic glomerulli of patients with focal glomerulosclerosis. Roy, Westberg & Michael (1973) suggest that the meaning of IgM and C3 deposit in a mesangial distribution is unknown, and it seems be due to an increase of membrane permeability to proteins.

Previous studies in advanced schistosomiasis cases demonstrated a predominance of IgG and complement accompanied by mesangial cell proliferation (Brito et al 1970). As we studied early stages of renal lesions, the predominance of IgM with mesangial distribution, could be explained. According to our findings schistosomal nephropathy is probably due to initial IgM and C3 deposition in the mesangium. The IgG and C3 deposition in advanced stage of the renal lesions could probably be a result of constant sequence of IgM immune complex and C3 trapped in the mesangium which leaves an open path to the subsequent deposition of IgG. The occasional transition predominance of IgM to IgG

in the differents stages of the schistosomal nephropathy is unknown. The factors that participate in the progression from one form to another remain uncertain. Perhaps close examination of the clinical evolution and repeated biopsies in these cases will shed some light on this problem in the future.

RESUMO

Com o objetivo de observar as lesões renais iniciais na esquistossomose mansoni, foram selecionados 21 casos da forma hepato-esplênica, com idade média de 12 anos e que não apresentavam sinais clínicos ou laboratoriais de alterações renais.

As biópsias foram realizadas durante esplenectomia e em seguida examinadas à microscopia ótica e à microscopia de fluorescência, sendo empregados conjugados anti IgG, IgM, IgA e C3, isoladamente.

No exame microscópico, os 21 casos foram classificados em: 6 casos normais; 6 casos com lesões glomerulares consideradas mínimas e 9 casos com lesões mesangiais discretas ou moderadas. À imunofluorescência constatou-se presença de depósitos de IgM e de C3 predominantemente de localização mesangial, tanto nos casos de lesões mínimas, como nos casos de proliferação mesangial mais acentuada (Tabelas II e III). Constatou-se ainda depósitos de IgG em todos os casos, mesmo naqueles considerados normais, sendo entretanto localizados na parede capilar.

Os autores acreditam que na esquistossomose mansoni as lesões glomerulares são provavelmente provocadas pela deposição inicial de imunoglobulinas M, formando imunocomplexos ligados a C3. A presença de IgG no mesangio, já descrita nos estágios mais avançados da glomerulopatia esquistossomótica, seria resultante de uma seqüência evolutiva.

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