FALCIPARUM MALARIA IN NATURALLY INFECTED HUMAN PATIENTS : X. ULTRASTRUCTURAL PATHOLOGICAL ALTERATIONS OF RENAL GLOMERULI

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Summary :

The ultrastructural pathological alteration of the renal glomeruli is described in nine patients with acute *Plasmodium falciparum* infection. Glomeruli were large and hypercellular with various types of blood cells in their capillary lumina. These were occasionally occluded with enlarged endothelial cells. Epithelial cells, or podocytes, were hypertrophied with extensively diffused foot processes fusion and numerous villous transformation, or appeared aedematous filling the urinary space. The glomeruli basement membrane was irregular in thickness or focally missing. Mesangial cells were undergoing proliferation and contained electron-dense deposits. Subendothelial "hump"-like deposits, similar to those previously described in immune complex nephritis, were detected in most cases; but the largest amount was found in the three youngest patients in whom parasitaemia levels were the highest. This study showed that the kidney may become the target damage mediated by immunopathologic mechanism, and the developed lesions may correspond, for certain extent, to membranoproliferative glomerulonephritis with abnormal deposits, during the acute infection with falciparum malaria. The amount and density of the immune complex deposit could be correlated with the degree of parasitaemia and, in return, with the degree of glomerular immunopathological alterations.

KEY WORDS : Falciparum malaria, acute infection, renal glomeruli, humans, pathological alterations, ultrastructure.

MOTS CLES : paludisme à falciparum, infection aiguë, glomérules rénaux, altérations pathologiques, ultrastructure.

Résumé : PALUDISME À FALCIPARUM CHEZ DES PATIENTS SPONTANÉMENT INFECTÉS : X. ALTÉRATIONS PATHOLOGIQUES ULTRASTRUCTURALES DES GLOMÉRULES RÉNAUX

Les altérations pathologiques ultrastructurales des glomérules rénaux sont décrites chez neuf patients souffrant d'un accès de paludisme aigu à *Plasmodium falciparum*. Les glomérules sont volumineux et le siège de nombreuses cellules, avec différents types de cellules sanguines dans la lumière capillaire. Celle-ci est parfois obstruée par des cellules endothéliales augmentées de volume. Les cellules épithéliales, ou podocytes, sont hypertrophiées, présentant une fusion des pseudopodes diffuse et de nombreuses transformations villoses, ou présentant un aspect œdémateux, comblant l'espace urinaire. La membrane basale glomérulaire est d'épaisseur irrégulière ou manque par endroits. Les cellules mésangiales prolifèrent et contiennent des dépôts denses aux électrons. Des dépôts sous-endothéliaux "en dos d'âne", semblables à ceux précédemment décrits au cours des néphrites à complexes immuns, sont détectés dans la majorité des cas; mais la plupart ont été trouvés chez les trois patients les plus jeunes, dont les taux de parasitémie étaient les plus élevés. Cette étude montre que le rein peut devenir la cible des lésions par le biais d'un mécanisme immunopathologique, et que les lésions développées peuvent correspondre dans une certaine mesure à une glomérulo-néphrite membranoproliférative à dépôts anormaux, au cours d'un paludisme aigu à falciparum. L'importance et la densité du dépôt de complexes immuns pourrait être corrélée au degré de la parasitémie et au degré des altérations immunopathologiques glomérulaires.

INTRODUCTION

Glomerular disease in human malaria has long been recognized (Goldie, 1930; Laha, 1945). However, it was only in the last two decades that this subject has attracted considerable interest again. Two distinct groups of glomerulonephritis responsible for acute renal failure were reported to be specific for *Plasmodium falciparum* and *P. malariae*, respectively (Hartenbower *et al.*, 1972) and their causation has to be regarded as species-specific interaction between host and parasite (Houba, 1979). The first group is characterized by a glomerular inflammation that develops during the clinical course of acute falciparum malaria, and a nephrotic syndrome may occur (Berger *et al.*, 1967). Granular deposits of IgM and BIC- globulin with or without IgG accompanied by various types of inflammatory

cells were detected in morphologically altered glomeruli, indicative of an immunopathological process (Bhamarapravati *et al.*, 1973). Granular deposits of *P. falciparum* antigens were demonstrated in autopsy tissue. The second group is represented by the chronic progressive glomerulonephritis associated with *P. malariae* infection, and known as malarial nephropathy (Lancet, 1972; Hendrickse *et al.*, 1972; White, 1973). Despite the several immunopathological investigations on the renal glomeruli in humans and experimental animals infected with the malaria parasite (Houba *et al.*, 1971, 1976; Boonpucknavig *et al.*, 1972, 1973, 1979; Bhamarapravati *et al.*, 1973; Suzuki, 1974), studies on the ultrastructural pathological alterations of human glomeruli during acute infection with *P. falciparum* is still limited.

This is the tenth paper in this series on human falciparum malaria (El-Shoura, 1993 a,b,c; El-Shoura, 1994 a,b; El-Shoura & Al-Amari, 1993 a,b; El-Shoura *et al.*, 1993 a,b), and presents the result of our detailed

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study based solely on ultrastructural findings of glomerulonephritis developing during acute infection with *P. falciparum*. Details of immunofluorescent study of the present cases will be presented elsewhere.

MATERIALS AND METHODS

Nine patients aged 5 (2M + 1F), 10(1F), 12 (1M + 1F), 25(1M + 1F) and 56 (1M) years were admitted with a diagnosis of acute falciparum malarial infection. All patients had a history of fever with rigors of less than one week's duration before admission. Falciparum malaria parasites were seen in the peripheral blood films of all patients. Parasitaemia, i.e. the parasite count (PC) was expressed as the absolute number of parasitized erythrocytes per microliter (μ L) blood. The mean PC was 63.720 PC/ μ L in all patients. Laboratory examination showed variable degrees of proteinuria with or without microscopic haematuria in all patients, in addition to oedema, the main diagnostic criteria of the nephrotic syndrome. All patients were treated with oral chloroquine alone and none showed the presence of malarial parasites at the time of kidney biopsy. Patient 4 had a glucose-6-phosphate dehydrogenase deficiency of the erythrocytes and developed acute renal failure that required peritoneal dialysis before she recovered.

For examination by transmission electron microscopy (TEM), percutaneous (needle) kidney biopsies were performed 10 days after the antimalarial treatment in all patients. Each biopsy was directly fixed in 2.5% glutaraldehyde in 0.1M sodium cacodylate buffer, pH 7.2, for 2 h at room temperature, postfixed in 1% osmium tetroxide, dehydrated in an ascending series of ethanol, and embedded in Spurr's resin through propylene oxide. Thin sections were stained with uranyl acetate and lead citrate, and examined in a Jeol 1200 EX TEM at 80 kV.

RESULTS

Except for a few differences, the detected glomerular ultrastructural alterations were similar in all cases. Glomeruli were generally large and hypercellular (Figs. 1,2) due to an increase in endocapillary cells and an infiltration types of blood cells. The capillary lumen in the first four cases were characterized by the presence of erythrocytes and polymorphonuclear cells (Figs. 1-4), in addition to monocytes, lymphocytes and blood platelets in case 4

(Fig. 5). Leukocytic cytoplasmic processes extended to abut the endothelial cell surrounding the capillary lumen (Fig. 4). Many erythrocytes were also observed in the urinary space of case 5 glomeruli (Fig. 6). The capillary lumina were occasionally occluded either by swollen, hypertrophied, or by enlarged, projected endothelial cells (Figs. 1-3). The overlying epithelial cells, or podocytes, appeared normal in some glomeruli; their cytoplasm was rich in cell organelles including Golgi bodies, rough endoplasmic reticulum, mitochondria, submembranous pits and vesicles, and numerous free and polyribosomes (Fig. 7). Their foot processes were conspicuous and abutting the capillary basement membrane (Fig. 7). In altered glomeruli, the epithelial cells were hypertrophied, with a moderate to electron-dense cytoplasmic matrix (Fig. 4), or appeared oedematous filling the urinary space (Figs. 8, 9). These cells showed extensive diffused foot process fusion and numerous villous transformation (Figs. 1-4, 6, 8, 9). The glomerular capillary basement membranes were, in places, extremely thin, thick or completely missing (Fig. 8), or contained membranous structures. Three to five large mesangial cells with increase cytoplasmic matrices were apparently undergoing cell proliferation as indicated by the presence of centrioles in vicinity to the cell nuclei (Fig. 10). Increase of the marginal mesangial matrices obviously leads to the distortion of the surrounding capillaries (Fig. 1). Bowman's capsule was slightly thickened and showed poorly differentiated electron-dense deposits (Fig. 6). Characteristic subepithelial coarse granular deposits in the form of "hump-like" were seen in most cases (Figs. 4, 5). In addition, varying amounts of coarse and fine granular deposits were also detected in some mesangial matrices (Figs. 3, 10), and occasionally within the basement membrane. The largest amount of deposit was detected in cases 1-3 in which the patients were 5 years old (2M+1F) and their parasitaemia levels were the highest (Mean 165.230 PC/ μ L).

DISCUSSION

The present study showed that various glomerular histopathological alterations were developed during the acute infection with *P. falciparum*. Although these alterations were similar in all studied cases, the deposited material varied in amount and density from one case to another. With regard to glomerular histopathology, previous reports showed considerable variation. Proliferate glomerular lesions including minimal focal and lobular involvement in 29 of 31 cases with parasitaemia were found during studies of the malaria nephropathy in Uganda

Figs. 1 et 2. – Hypercellular glomeruli (Cases 1 and 2, respectively). Note the enlarged endothelial cells (E) projected into (Fig. 1) or occluding (Fig. 2) the capillary lumen (C). Note also the numerous villous transformation (*) of the podocyte (P) foot processes. **Fig. 3.** – A part of a glomerulus (Case 3) showing capillary lumina occluded by endothelial cells (E). Note the mesangial deposit (D), R, luminal erythrocytes. **Fig. 4.** – A blood capillary of a glomerulus (case 4) showing a polymorphonuclear cell (N) projecting cytoplasmic processes (thin arrow) abutting the endothelial cell (E). Note the subepithelial deposit (D) in the form of "hump-like". Bend arrow points to fused foot processes. Asterisk represents villous transformation. **Fig. 5.** – A capillary lumen (C) enclosing blood platelets (case 4). D, subepithelial "hump"-like deposits. **Fig. 6.** – A part of a glomerulus (case 5) showing erythrocytes (R) in the urinary space (U). Short solid arrow points to fused foot processes. Long empty arrow represents poorly differentiated deposits in the Bowman's capsule (B). Other letterings as in Figs. 1 and 2.

Fig. 7. – A normal looking podocyte (P) (case 6) with conspicuous foot processes beneath the regular capillary basement membrane (B). C, the capillary lumen. **Fig. 8.** – A part of altered glomerulus (case 7) showing aedematous epithelial cells (P). Note the extremely thin (straight solid arrow), missing (bend empty arrow) and thick (winged arrow) places of the capillary basement membrane (B). Other letterings as in Fig. 2. **Fig. 9.** – As in Fig. 8 but showing a rather regular capillary basement membrane (B) (case 8), but the endothelial cell (E) appears damaged. P, aedematous podocytes. **Fig. 10.** – Mesangial cells (m) (case 9) showing a centriole (thick arrow) indicating proliferation. Note also the mesangial deposits (thin arrows).

(Kibukamusoke & Hutt, 1967). Membranous lesions were less common and noted only in patients who had apparently no parasites in the peripheral circulation. In contrast, contributive clinical and renal biopsy study in Nigerian children (Hendrickse *et al.*, 1972) revealed a marked thickening of glomerular capillary walls. Such thickening involved the subendothelial aspects of the basement membrane giving rise to a double contour of plexiform arrangement of fibrils. These lesions were progressive from a local to a diffuse thickening of the capillary wall until finally the whole glomerulus became hyalinized and sclerosed. However, the morphology of the present glomerular pathological alterations correspond, for certain extent, to those previously described for the membrane proliferative glomerulonephritis (Anderson, 1985).

The subepithelial and mesangial deposits revealed in all cases, are ultrastructurally similar to those seen in immune complex nephritis in both *P. berghei* infected mice (Boonpucknavig *et al.*, 1972) and in humans naturally infected with *P. falciparum* (Bhamarapravati *et al.*, 1973). The immune complex was demonstrated to be antigen, antibody, and complement, and known to cause various degrees of glomerular immunopathological alterations (Bhamarapravati *et al.*, Loc. Cit). Both size and pattern of immune complex deposits varied considerably in various cases of monkey and man (Ward & Conran, 1969). Subepithelial coarse granular deposits along the capillary walls were the most common form, while varying amounts of coarse and fine granular deposits occasionally occurred in mesangial matrices. However, the largest amount of deposit was detected in the three youngest patients in whom parasitaemia levels were the highest. In addition, it has been reported that parasitaemia levels increase in young patients, possibly due to less immature immune system (El-Shoura, 1993a). Accordingly, one may suggest that the amount and density of the immune complex deposits could be correlated with the degree of parasitaemia during the acute infection with falciparum malaria and, in return, with the degree of glomerular immunopathological alterations.

Red and white blood cells were observed in the capillary lumen of the present cases. The presence of the immune complex deposit was suggested to trigger the formation of reaction products which are chemotactic to leukocytes in general and to neutrophils in particular in the kidneys of mice (Boonpucknavig *et al.*, 1972). This leads to damage of the glomerular basement membrane and to leakage of plasma proteins.

While the present study has focused primarily on glomerular damage, one cannot exclude the possibility

that tubular changes (hemoglobinuric nephrosis and hemosiderosis) and sequelae may be an additional feature of malarial nephropathy, as has been noted in many early studies of malaria.

CONCLUSION

This study showed that the glomerular pathological alterations during acute infection with falciparum malaria are as follow :

1. Hypercellular glomeruli with capillary blood cells.
2. Increase of endothelial cells in number and size.
3. Hypertrophied epithelial cells with fused podocytes and villous transformation.
4. Irregularly thickened BM.
5. Mesangial proliferation and increase cell matrices.
6. Presence of subepithelial "hump-like", mesangial, and membranous fine and granular deposits.
7. The largest amount of deposits were detected in the youngest patients, in whom parasitaemia levels were the highest, indicating that the amount of immune deposits may correlate with both age of patients and the degree of parasitaemia. However, these pathological alterations correspond, for certain extents, to the membranoproliferative glomerulonephritis with abnormal deposits and seem to be specific morphological features during the course of acute infection with falciparum malaria.

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