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Electron Microscopic Findings in Patients with Glomerulonephritis in Sudan

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Abstract: Glomerulonephritis (GN) is a common cause of end stage renal disease (ESRD). Electron microscopic studies bestowed significantly to the understanding of the pathogenesis of the disease process in GN. Its value has been strongly confirmed in diagnosis of glomerular diseases. To establish the role of electron microscopy in the diagnosis of glomerular diseases, in order to introduce services and to improve the histopathological standards of diagnosis of renal disease. Different patterns of EM in the different types of glomerular diseases in 132 renal biopsies from patients with glomerular diseases were evaluated. These specimens were stained and examined under the light microscopy (LM) then compared by transmission electron microscopy (TEM) findings. The most frequent types of glomerular disease was minimal change disease (22.7%), followed by lupus nephritis (20.5%). LM failed to diagnose the minimal change disease and Alport's syndrome. In this study EM was found necessary in diagnosis of 37 cases (28%), supportive in diagnosis of 17 cases (12.9%) and not required in 79 (59.8%) of cases. The current study supported what had been concluded in the previous studies; EM still has an integral role and necessary for diagnosis of certain glomerularpathies *e.g.* minimal change disease.

Keywords: Electron microscopy, Kidney, Biopsy, Glomerulonephritis.

INTRODUCTION

Glomerulonephritis (GN) is a heterogeneous group of illnesses that are generally, characterized by inflammatory process affecting primarily the glomerulus, with infiltration and proliferation of acute inflammatory cells. GN is a common cause of end stage renal disease (ESRD) worldwide especially in developing countries [1]. The incidence and prevalence of end-stage kidney disease vary globally. Glomerular diseases usually diagnosed as primary glomerulopathy such as minimal change disease, focal segmental glomerulo-sclerosis (FSGS), membranous glomerulonephritis (MGN), or as secondary glomerulopathy as a manifestation of a systemic disease (e.g, diabetes, hypertension, and amyloidosis). However, infections, genetic diseases (e.g, Alport syndrome), drugs, malignancy, vasculitis, and other conditions should be considered in the differential diagnosis of secondary causes [2]. The past decade has witnessed major advances in understanding the etiology and pathogenesis of glomerulonephritis.

The clinical and laboratory findings are important for diagnosis of GN. Renal Glomerular diseases are routinely diagnosed by histological evaluation of kidney biopsies doing light microscopy (LM), immunofluorescence microscopy, and transmission electron microscopy (TEM), which provide a two-dimensional visualization of glomeruli is the gold standard and which allowed pathologists to characterize renal diseases and developing treatment strategies [3-5]. Electron microscopy allows the most comprehensive assessment of the manifold basement membrane changes. It has been most widely used for the interpretation of renal biopsies and the examination of tumors that present a diagnostic problem with light microscopy [6]. Electron microscopy (EM) has been used for the morphological diagnosis of glomerular diseases, and its value has been strongly confirmed [6]. In major medical centers where kidney biopsies are performed, EM is routinely done together with LM and immunofluorescence study for the evaluation of the specimens. Some investigators had observed that about

85% of kidney biopsies had an indication of EM for diagnostic confirmation [7]. However, the use of EM in Sudan has markedly ignored in spite of the role of EM in pathologic examination of specimens from kidney biopsy is well established. Several studies have evaluated routine use of EM in kidney biopsy evaluation [8-10].

In Sudan the number of existing cases of end-stage renal disease (ESRD) caused by glomerulonephritis is increased and is the leading cause in Sudanese population [11]. So, the goal of this study was to establish the role of EM in the diagnosis of glomerular diseases in patients with GN.

MATERIALS AND METHODS

Patients

This a descriptive study performed in Alzaiem Alazhari University, Khartoum, Sudan, from July 2012 to July 2013. A total of 132 renal biopsies from patients with glomerular diseases were collected. For each patient, the demographics data (name, age and gender) were documented. These specimens were examined and diagnosedusing light microscopy (LM) and transmission electron microscopy (TEM) by nephropathologists. Ethical approval was obtained from the ethical and scientific committees of the ethical review board of Alzaiem Alazhari University.

Light microscope examination

Renal biopsies were fixed in 10% phosphate-buffered formalin, dehydrated in gradual ascending ethyl alcohol begin with 50%,60%, 70%, 80%, 90% finally two changes of absolute ethanol (100%) at room temperature. Clearing step was done by using xylene. 58^{o}c melted paraffin wax was used for tissue impregnation and embedding. 4µm transparent tissue sections were obtained by using Leica rotary microtome.The tissue sections stained with regressive Harri's hematoxylin and 0.5% eosin, periodic acid-Schiff (PAS), Silver methanamine, Masson's trichrome and Congo red according to standard protocols.Finally renal biopsies were examined under an ordinary LM by two well qualified nephropathologistsfor diagnosis.

Transmitted electron microscope (TEM) examination:

For EMbiopsies were selected by LM, which containing at least one glomerulus. Biopsies were fixed with 2.5% glutaraldehyde in 0.1M cacodylate buffer (pH = 7.2) at 4°C, post-fixed in 4% osmium tetroxide (OsO4) in 0.l M cacodylate buffer incubated in fume hood at room temperature followed by washing in 1 M cacodylate buffer pH=7.2.Tissues were dehydrated through ascending grades of ethanol starting from 70%, 85%, 96%, and finally two changes of absolute ethanol (100%) at room temperature. Tissues were cleared with two changes of propylene oxide (1,2-epoxy propane) on a rotating mixer at room temperature.Infiltration was achieved by mixing equally volume of resin (Epon-812) and propylene oxide (1,2-epoxy propane). The embedding blocks containing resin were polymerized at 60°C.Semi thin sections were cutted by using an ultra-microtome with glass knives, stained at 100°c with toluidine blue, then LM examination was performed to select an area which contained glomeruli, and further ultra-thin sectioning with thickness of 500 micron was done by using Leica ultra-cut microtome with diamond knifes (Leica EM UC7-Germany). Uranyl acetate and lead citrate were used for staining of ultra-thin section and finally examined under transmitted EMby same nephropathologists in order to reach the final diagnosis.

Statistical analysis

Data obtained from this study were analyzed by using statistical package for social science software (SPSS) version 20.

RESULTS

In this study 132 renal biopsy samples were randomly collected from patients with glomerulonephritis. The mean age of the patients was 23.8 years, their age ranged from 1-67 years (Table-1), 41.7% were male and 58.3%were female out of them 32.4% children and 60.6% were adults (table-2). The most frequent types of glomerular disease were found was minimal change disease (22.7%), followed by lupus nephritis (20.5%). Whereas the focal segmental glomerulosclerosis, Alport's syndrome, amyloidosis, diabetic glomerulopathy, diffuse mesengial sclerosis, hemolytic uremic syndrome, hypertensive nephropathy, IgA nephropathy, mild mesangial nephritis, membranoproliferative glomerulonephritis, Membranous glomerulonephritis, Pauci-immune crescentic glomerulonephritis, crescentic glomerulonephritis, post infectious glomerulonephritis and transplant glomerulopathy were found with the percentage of 18%, 1.5%, 5.3%, 1.5%, 3%, 0.8%, 3.8%, 1.5%, 0.8%, 3%, 9.8%, 0.8%, 1.5%, 3%, and 2.3% were found respectively, out of these cases EM was found necessary in diagnosis of 37 cases (28%), supportive in diagnosis of 17 cases (12.9%) and not required in 79 (59.8%) of cases (table-3).The importance to use EM in diagnosis of glomerular diseases was appeared when the LM failed to diagnose the minimal change disease and Alport's syndrome (Figures 1-6). As showed in (table-3), the cases of focal segmental glomerulosclerosis, hemolytic uremic syndrome, hypertensive nephropathy, IgA nephropathy, mild mesangial nephritis, membranoproliferative glomerulonephritis and crescentic glomerulonephritis the EM was found to be neither necessary nor supportive for reach the final diagnosis.

Table-1: Age distribution of the patients included in this study

Minimum	Maximum	Range	Mean	Total
1	68	67	23.8	132

Table-2: Age groups and gender in studied subjects

		Gender		Total
		Male	Female	
Age groups	Children (%)	26 (19.7%)	26 (19.7%)	52 (39.4%)
	Adult (%)	29 (22%)	51 (38.6%)	80 (60.6%)
Total (%)		55 (41.7%)	77 (58.3%)	132 (100%)

Table-3: Contribution of light and electron microscopy methods in diagnosis of glomerulopathies

Glomerular disease	**Light microscope (%)**	**Electron microscope finding**			**Total (%)**
		Essential (%)	**Supportive (%)**	**Not required (%)**	
Minimal change disease	-	30(22.7%)	-	-	30(22.7%)
Focal segmental glomerulosclerosis	24 (18%)	-	-	24(18%)	24(18%)
Alport's syndrome	-	2(1.5%)	-	-	2(1.5%)
Amyloidosis	6 (4.5%)	1(0.8%)	3(2.3%)	3(2.3%)	7(5.3%)
Diabetic glomerulopathy	1(0.8%)	1(0.8%)	-	1(0.8%)	2(1.5%)
Diffuse mesengial sclerosis	4(3%)	-	1(0.8%)	3(2.33%)	4(3%)
Hemolytic uremic syndrome	1(0.8%)	-	-	1(0.8%)	1(0.8%)
Hypertensive nephropathy	5(3.8%)	-	-	5(3.8%)	5(3.8%)
IgA nephropathy	2(1.5%)	-	-	2(1.5%)	2(1.5%)
Lupus nephritis	26(19.7%)	1(0.8%)	8(6%)	18(12.9%)	27(20.5%)
Mild mesangial nephritis	1(0.8%)	-	-	1(0.8%)	1(0.8%)
Membranoproliferative glomerulonephritis	4(3%)	-	-	4(3%)	4(3%)
Membranous glomerulonephritis	12(9%)	1(0.8%)	4(3%)	8(6%)	13(9.8%)
Pauci-immune crescentic glomerulonephritis	1(0.8%)	-	-	1(0.8%)	1(0.8%)
Crescentic glomerulonephritis	2(1.5%)	-	-	2(1.5%)	2(1.5%)
Post infectious glomerulonephritis	3 (2.3%)	1(0.8%)	-	3(2.3%)	4(3%)
Transplant glomerulopathy	3(2.3%)	-	1(0.8%)	3(2.3%)	3(2.3%)
Total	95 (72 %)	37(28%)	17(12.9%)	79 (59.8 %)	132(100%)

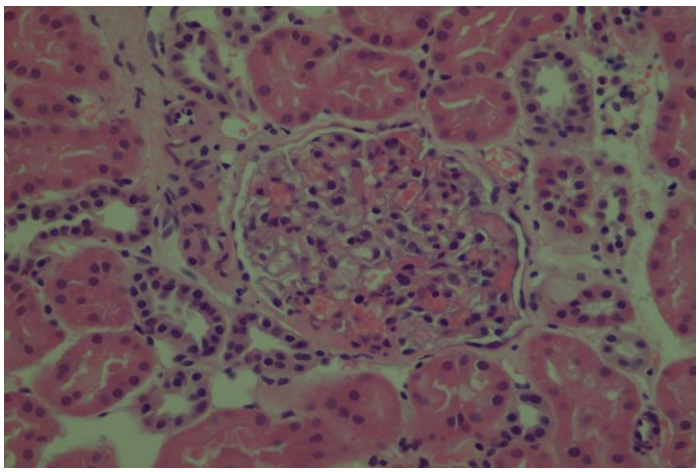

Fig-1: Minimal change disease, the glomerulus in this H&E section looks normal without an increase in cellularity or an increase in basement membrane thickness. X40

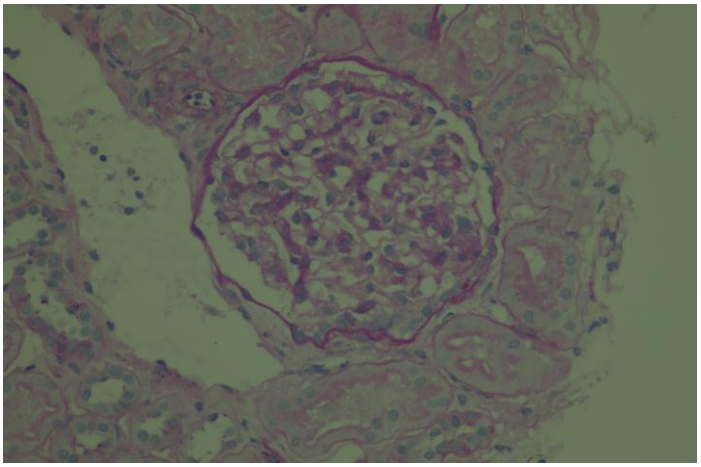

Fig-2: Minimal change disease, the glomerulus in this PAS to highlight basement membranes of glomerular capillary loops and tubular epithelium.x40

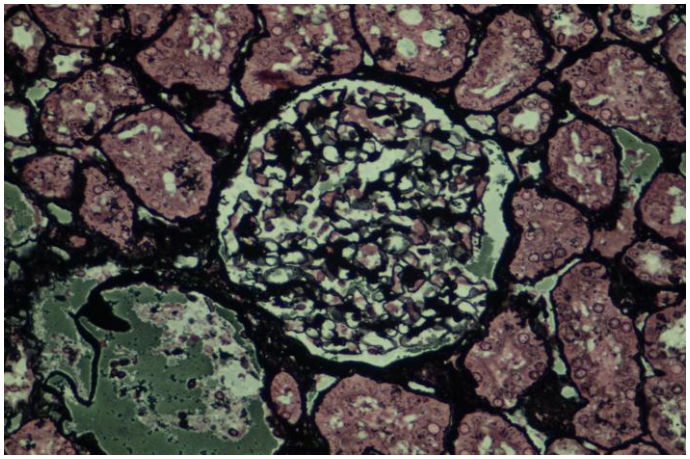

Fig-3: Minimal change disease, the glomerulus in John's technique (silver stain) looks normal. x40

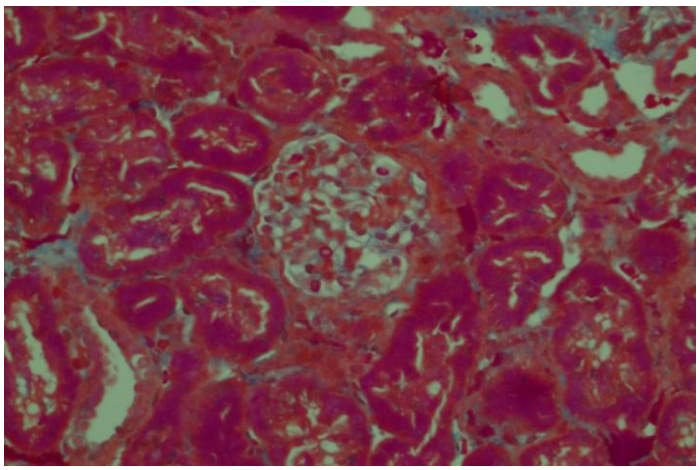

Fig-4: Trichrome stain of a glomerulus in a patient with minimal change disease demonstrates few blue collagen deposition.x40

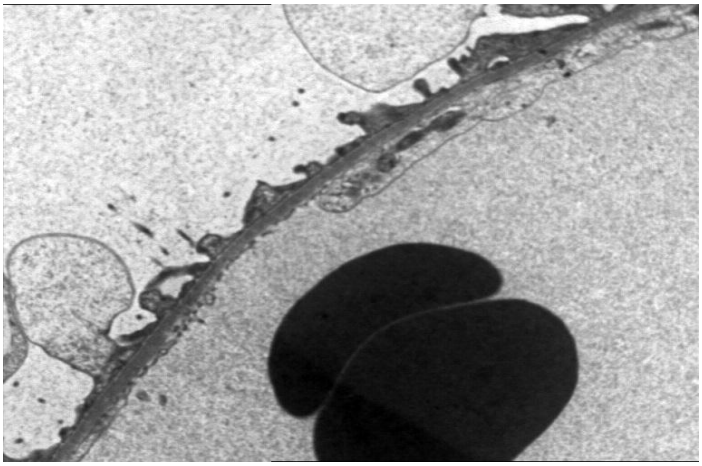

Fig-5: Minimal change nephropathy: diffuse loss of epithelial cell foot processes = enfacement E/M micrograph. x1000

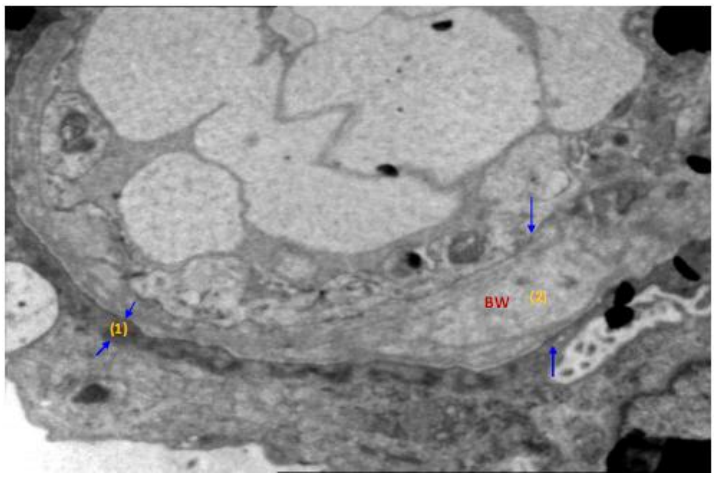

Fig-6: Alport's syndrome, alternately thick and thin basement membranes; BW, thick areas with irregular lamination of lamina densa - "basket weave". (1) Normal basement membrane. (2) Thick basement membrane. E/M micrograph. x12.000

DISCUSSION

The role of electron microscopy in renal biopsy diagnosis is well established and has been used in the pathological identification of renal glomerular disease over the past few decades and its diagnostic value has been strongly accented. Nevertheless, it soon became obvious that ultra-structural examinations remain essential in both research and diagnosis. Renal biopsies are still widely examined with the electron microscopy together with light microscopy and immune histology required for optimum pathologic evaluation of glomerulonephritis. EM findings confirm the diagnosis rendered that was recognized by LM and immunohistology, or provide new information that is valuable for patient's management. The strength of the ultra-structural study hinges on the presence of glomeruli in the biopsy material and unveils the morphological features of this disorder and may also improve the understanding of their pathogenesis.

Deciding to choose between electron or light microscopic studies has been a field for debates [12]. Also EM was considered most useful in the current study in the diagnosis of minimal change disease, mainly in its differential diagnosis from other disease that can have normal morphology by LM, such as early membranous glomerulonephritis, also considered essential in establishing the diagnosis of focal segmental glomerulosclerosis. In these diseases, LM can be normal at first but EM can detect the alteration at glomerular basement membrane. On other hand LM failed to diagnose all cases of Alport's syndrome because in Alport's syndrome LM can be normal or only shows mesangial hyper cellularity and immunofluorescence study is negative for any deposition, so EM is necessary for diagnosis [13]. Differentiation of Alport's syndrome from thin basement membrane disease can be difficult because these two diseases are closely related, however the major abnormalities of the glomerular basement membrane are visualized only by EM [14].

In current study the role of EM in diagnosing the glomerular disease was graded into three groups: (1)

Necessary: If final diagnosis could not have been achieved without EM, (2) Supportive: If the ultrastructural study agreed with primary diagnosis, and (3) Not required: If the EM was not needed to confirm the diagnosis. Accordingly EM was necessary in 37 cases out of 132 (28%), supportive in 17 cases (12.9%) and not required in 79 (59.8 %).These findings was in line with other recent studies; Isa Jahanzad *et al.,* [15], they were found that EM study was necessary in 51 cases out of 134 (38%), supportive in 40 cases (≅ 30%) and not required in 43 (32%). Another study in 2010 by Darouich *et al* revealed that EM was essential for diagnosis in 8 cases (40%) and was helpful in 12 cases (60%), they concluded that: the ultra-structural study provides essential or helpful information in many cases of glomerular diseases, and therefore EM should be considered an important tool of diagnostic renal pathology [16]. In Rivera et al. study about value of EM in the diagnosis of childhood nephrotic syndrome, EM was essential in diagnosis of 73% cases and supportive in a further 27% [17]. Siegel *et al.* showed that EM contributed to diagnosis in 48% of cases [8], and Sementilli *et al.* concluded that ultrastructural study is necessary in diagnosis of hereditary nephropathies [18].

EM is considered helpful in accurate determination of the stage of the disease [19], and excluding the secondary causes, especially in association of lupus nephritis by observing mesangial deposits and tubuloreticular inclusion. In case of lupus nephritis, EM was important to establish the diagnosis of combined classes. Although the LM was useful in the diagnosis of hypertensive GN, hemolytic uremic syndrome, membranous GN, post infectious diseases, diffuse mesangial sclerosis, and transplant nephropathy. In case of amyloidosis most of cases diagnosed by LM except one case , the LM failed to diagnosed this case of a 5 years girl, this may due to early stage of amyloidosis in this H&E and Congo red failed to detect the abnormality.

In the current study, the routine use of EM in conjunction with the light microscopic findings was considered to be essential in reaching a definitive diagnosis in 12.9% of cases, these results are in line with other studies; Collan and associates [20], who found EM to be helpful for diagnosis in 18.3% of cases. In 2011, Ghadeer & Sawsan they were found the EM was important for diagnosis in 17% of renal biopsy cases [21].

The evaluation of renal biopsy specimens without access to EM results can lead to missed diagnosis [12]. In contrast there was study published in 2014 by Simin Torabinejad *et al.* they analyzed pathologic reports from electron and LM of 985 cases of renal biopsies with variable glomerular diseases during years 2000-2010, their results showed that only in MCD and alport nephrology there is no significant difference between the group in which EM provided valuable additional information and the group that consisted of cases in which EM did not add any useful information to LM, they concluded that EM is an expensive process to be performed routinely for evaluation and diagnosis of kidney diseases, it should be wisely selected for diagnosis of those disorders that need to be evaluated by electron microscopy [22].

GN requires prompt diagnosis and early diagnosis of glomerulonephritis (GN) is important in initiating appropriate treatment and controlling chronic glomerular injury that may eventually lead to end-stage renal disease (ESRD). Electron microscopy findings confirm the diagnosis that was rendered by light microscopy and immunohistology, or provide new information that is valuable for patient's management. In conclusion, electron microscopy having been used for a glomerulonephritis cases appeared to benefit directly from this investigation in Sudanese population.

In conclusion: The current study supported what had been concluded in the previous studies; EM still has an integral role and necessary for diagnosis of certain glomerularpathies *e.g.* minimal change disease.

CONFLICTS OF INTEREST

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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