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Research Article

The spectrum of renal changes in patients with liver diseases: an immunofluorescent and light microscopic study

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ABSTRACT

Background: There are divergent observations on renal function tests and renal morphology in patients with liver diseases. The present study was designed (1) To study the morphological changes in kidney in patients with various types of Liver diseases; (2) To study the correlation between the renal histology, clinical and other laboratory parameters in these cases; (3) To suggest the possible mechanisms of renal injury secondary to liver diseases.
Methods: This prospective study was carried out at the department of pathology of a tertiary care centre in Delhi. A total of 30 patients admitted with liver diseases were included in the study. All kidney samples were evaluated by light microscopy and immunofluorescence microscopy. The sections were stained by fluorescent labeled antisera for human IgG, IgA and IgM. Postmortem kidney biopsy from 10 patients dying of unrelated diseases served as controls.
Results: Glomerular changes on light microscopy were present in twenty four patients (80%) as compared to two controls (20%). The difference was statistically significant (x^2= 11.75; p<0.001). Nine out of 13 patients with severe impairment of liver function tests (LFTs) showed specific glomerulopathies whereas only one out of 17 patients with mild to moderate impairment of LFTs, showed specific glomerular lesion. It was found to be statistically significant (x^2= 13.4; p<0.001). Immunofluorescent study showed the presence of immune deposits in 21 out of 30 patients (70%). IgA positivity was seen in 18 cases, IgG in 9 cases and IgM in 10 cases.
Conclusions: There is a wide spectrum of morphological lesions in the kidney in patients with liver diseases. These were mainly glomerular lesions and were directly related to the severity and chronicity of liver diseases. Immune deposits were commonly present in patients with chronic liver disease.

Keywords: Chronic liver disease, Cirrhosis, Glomerular lesions, Immunofluorescence

INTRODUCTION

Though the importance of kidney in hepatic diseases has been mentioned as early as in 1685 by John Brown yet the interest of the various workers to study the renal function as well as renal morphology in liver diseases has started increasing only in the recent few decades.[1] Bloodworth and Sommers were first to suggest that cirrhosis produced specific glomerular changes which they named "cirrhotic glomerulosclerosis".[2] These changes consisted basically of numerical increase in epithelial and endothelial cells and pronounced periodic acid schiff (PAS) positive fibrillar thickening of the glomerular stroma or the matrix. As glomerular changes were not necessarily limited to cirrhosis, but also to other liver disorders, Sakaguchi et al called them "hepatic glomerulosclerosis" and with the use of electron microscopy, it was revealed that mesangial stalk thickening was due to both an increase in mesangial matrix and amorphous deposits with clear halos.[3] Immunofluorescence studies have shown glomerular deposits to be composed mainly of IgA and C3 and also

of IgM and IgG. IgA was demonstrated as the main immunoglobulin deposited in the glomerular mesangium.[4] Callard P et al also demonstrated mesangial and subendothelial deposits of IgA along with mesangial thickening in the large majority of renal biopsies taken during portocaval anastomosis which were characteristic of "cirrhotic glomerulonephritis".[5] Newell GC found increased serum IgA levels in over 90% of cirrhotic patients with glomerular IgA deposition.[6] It was found that glomerular deposits almost always contained IgA.[7,8] The pathogenetic role of HBV in renal disease has attracted much attention, since Combes et al reported glomerulonephritis with immune complexes of HBsAg and its antibody (anti- HBs) in a patient infected with HBV.[9] Various morphological patterns including membranous nephropathy (MN), mesangiocapillary glomeruonephritus (MCGN), minimal change nephropathy (MCN), mesangioproliferative glomerulonephritis (MPGN) have been described.[10,11] In 1863, Austin Flint made a special reference to oliguria in cirrhotic patients.[1] Silent glomerulopathies were observed by earlier workers while other reports showed increasing incidence of urinary abnormalities or clinical manifestations indicative of renal dysfunction.[7,8,12,13] Liver cirrhosis glomerulonephritis is a silent disease and routine urine examination for proteinuria and hematuria should be done in search of possible glomerulopathy.[14] The absence of significant proteinuria and hematuria does not rule out the presence of renal lesions in cirrhotic patients.[15]

Keeping in view the scarcity of published data on spectrum of renal morphological changes in patients with liver diseases in India, a prospective study was carried out in the Department of Pathology, MAMC, New Delhi, India.

METHODS

This prospective study included 30 patients of liver diseases admitted to the medical wards of LNJP and GB Pant Hospitals, New Delhi. These patients belonged to the following groups of liver diseases.

(1) Acute liver disease

This group included patients of acute hepatitis and patients with past history of acute hepatitis and comprised 6 patients who were diagnosed on the basis of clinical features e.g., prodromal symptoms, jaundice, tender hepatomegaly and laboratory investigations such as liver function tests (LFTs), urine examination etc.

(2) Chronic liver disease

Patients in this category belonged to either chronic hepatitis or cirrhosis of liver. (i) Chronic hepatitis: There were two patients of chronic hepatitis and both belonged to chronic active hepatitis. One of these patients was HBsAg positive. The diagnosis of chronic hepatitis was made once it was established that hepatitis had been present on clinical or other grounds for a period of at least six months; (ii) Cirrhosis of liver: The diagnosis of cirrhosis of liver was made clinically, biochemically and/ or histologically. A total of 22 patients were included in this group. They were further categorized into compensated (7) and decompensated (15).

(a) Compensated cirrhosis

Patients belonging to this group either had no ascites and edema or had just detectable ascites. These patients never had malena or hemetemesis. They did not have palpable spleen and there was no other evidence of portal hypertension. Out of seven patients in compensated stage, one patient was HBsAg positive.

(b) Decompensated cirrhosis

Patients of this group presented with well-established clinical picture of cirrhosis. All the 15 patients had marked ascites as well as edema. All of them had evidence of portal hypertension. Out of these 15 patients, ten were in hepatic coma. Kidney tissue was obtained by biopsy/ autopsy. In 7 patients, kidney tissue was obtained by antemortem kidney biopsy while in 23 patients; it was obtained by post-mortem needle biopsy/ autopsy. Antemortem kidney biopsy was done only in patients with normal prothrombin time. Meticulous effort was made to exclude cases who had any history of pre-existing renal disease and cases with systemic diseases or diseases which simultaneously affect liver and kidney e.g., DIC, Drugs, Toxins, Collagen vascular diseases, Diabetes mellitus etc as judged by history, physical examination, radiological and laboratory findings. Antemortem biopsy was done in all cases of chronic hepatitis, patients with past history of acute hepatitis, and in two cases of compensated cirrhosis and one case of decompensated cirrhosis. Ten patients who died of unrelated diseases, e.g., Myocardial Infarction and Cerebrovascular events served as controls. From these patients, renal tissue was obtained through post- mortem biopsy or at autopsy. After admission in medicine wards, each case was studied in detail as per proforma. Data was entered in excel spread sheet and statistical analysis was done using epidemiological information package. KRUSKAL- WALLI'S chi square test was used. P value <0.05 was taken to represent significant difference. In most of the cases and controls, two biopsies were attempted each for light microscopic and immunofluorescent studies, but in 4 cases immunofluorescence was done on paraffin section as enough tissue was not available. For light microscopic studies, the tissue was fixed in 10% formalin and routinely processed for paraffin embedding. The sections were stained with Hematoxylin & Eosin (H& E), PAS, and methenamine silver stain. For immunofluorescence studies, the fresh kidney tissue was snap frozen promptly by immersing in a container of liquid nitrogen, at temperature of -196 ^{0}C for less than a minute. The frozen

tissue was then inserted into a small air tight plastic tube including a suitable label for identification of the tissue. The tubes were then placed in deep freezer at a temperature of -70^0 C or lower. Direct method of immunofluorescence staining was used in which the tissue was reacted directly with a monospecific antisera labelled with FITC (Fluorescein isothiocyanate). Four sections of each kidney specimen were taken, three of which were stained by fluorescent labelled antisera for human IgG, IgA and IgM (Wellcome Research Laboratories, England or Sigma Chemicals Company, USA). Fourth section was stained with phosphate buffer saline (PBS).

RESULTS

Out of 30 patients with various liver diseases included in the study, 21 were males and 9 were females. Maximum patients (15 out of 30) were of Cirrhosis (decompensated) category (Table 1). On the basis of LFTs, the patients were divided into two groups (i) patients with mild-moderate impairment of LFTs, included 17 patients. (ii) patients with severe impairment of LFTs, included 13 patients. The degree of impairment of renal function was estimated from blood urea, uric acid and serum creatinine levels. Maximum patients with deranged renal functions were seen in cirrhosis (decompensated group) (Table 2). Similarly lower serum sodium levels were found in patients with marked as well as rapidly accumulating ascites and edema (decompensated cirrhosis). Histomorphological features were divided into glomerular, tubular, vascular and interstitial changes. Glomerular changes were present in 80% cases (24/30) as compared to 20% in controls (2/10) (Table 3). The difference was statistically significant (x^2=11.75; p<0.001). Tubular changes were seen in 11 cases (36.7%) as compared to two controls (20%). The difference was statistically insignificant (x^2=0.97; p>0.1). There were 17 patients with mild to moderate impairment of liver function, out of which one showed specific glomerular lesion, while out of 13 patients with severe impairment of LFTs, 9 showed specific glomerulopathies. It was found to be statistically significant (x^2=13.4; p<0.001). Basement membrane thickening was found to be present in 15 cases (50%). In three cases, it was diffuse (Figure 1a, 1b) and in remaining 12 cases, it was patchy and uneven (Figure 1c, 1d, 1e). Classical 'spikes' could be demonstrated on silver methenamine staining in one of the cases of membranous glomerulonephritis (Figure 1f). Double contouring was seen in one case of MPGN and was well demonstrated by special stains. Hypercellularity was present in ten cases (33.3%) (Figure 2a). Diffuse mesangial and endothelial proliferation was seen in two cases of DPGN (Figure 2b). Interstitial changes mainly included focal lymphonuclear infiltrate in the interstitium. Focal hyalinosis was seen in one case. Two cases showed focal interstitial fibrosis. Large clusters of foam cells were seen in two cases. Vascular changes were mainly in the form of intimal proliferation with reduplication of elastic lamina. As glomerular changes were found in 80% of the cases, these were studied in more detail. They were further subdivided into specific and non- specific groups. Specific groups included 3 cases of membranous glomerulonephritis (MGN), one case of membranoproliferative glomerulonephritis (MPGN), 2 cases of diffuse proliferative glomerulonephritis (DPGN) and 4 cases of focal segmental glomerulosclerosis (FSGS). Non- specific group was further divided into changes like patchy thickening of capillary walls, global glomerulosclerosis, mesangial matrix expansion etc. Five cases showed focal proliferation of mesangial cells with segmental accentuation in one. Mild capsular epithelial proliferation was seen in five cases. One case of DPGN also showed formation of cellular crescents in 4 out of 19 glomeruli (Figure 2c). Adhesion of glomerular tuft to the capsular wall was seen in one case each of DPGN and MPGN (Figure 2d). Glomerulosclerosis was seen in 11 cases, out of which four showed focal and segmental involvement. Seven cases showed focal global sclerosis. Age range of these patients was from 19-55 years (mean age; 30 years). Mesangial matrix expansion was seen in 12 cases. Diffuse mesangial matrix expansion was seen only in one case. Age range of these patients was from 18- 60 years (mean age; 36.5 years). Out of four cases of FSGS, one showed hyalinosis in the glomeruli, i.e., bright extra eosinophilic rounded foci within the areas of sclerosis (Figure 2e). Other minor non- specific glomerular changes were- the congestion of glomeruli (4 cases) (Figure 3a), dilatation of glomerular capillary loops (1 case) (Figure 3b), periglomerular fibrosis (Figure 3c) (1 case) and increase in urinary space with presence of polymorphs and fibrinous material (1 case). One case of MPGN showed hyaline thrombi in few capillary loops, and focal fibrinoid swelling of the capillary walls (Table 4). Three of the six patients of acute liver disease showed glomerular changes whereas 21 out of 24 patients with chronic liver disease had glomerular changes (Table 5). The difference was found to be statistically significant (x^2=4.212; p<0.05). Renal failure was present in 14 cases. Of these, 9 were of oliguric type. In 6 out of 9 cases of oliguric renal failure, no tubular changes were seen. One case showed characteristic appearance of ATN. Of the remaining two cases, one showed tubular atrophy and dilatation and the other showed hydropic change in the proximal tubular epithelial cells and RBC casts in the collecting tubule. Out of the 5 cases with non- oliguric renal failure, four were devoid of any tubular changes. One case showed tubular atrophy, dilatation, regeneration and hyaline casts (Figure 3d). Incidence of tubular changes in patients with renal failure (5/14) and without renal failure (6/16) was found to be statistically insignificant (p>0.5). The incidence of glomerular changes was 11/14 in patients with renal failure and 13/16 in patients without renal failure. The difference was statistically insignificant (x^2=1.92; p<0.1). Urinary examination was done in 25 patients. It could not be done in 5 patients as they were in critical condition and died within few hours of admission in the hospital. Urinary abnormalities were found in eight patients, seven patients

had proteinuria and five patients showed microscopic hematuria; two patients had moderate proteinuria and five showed mild proteinuria. Twenty four hours urinary protein estimation was done in only two of these patients and had 2.4g and 2g of proteins in urine respectively. Both of these patients had past history of acute hepatitis and on renal histology showed appearance of DPGN and MGN respectively. All the 8 patients with urinary abnormality showed glomerular changes while 16 out of remaining 22 patients showed these changes. No statistically significant correlation was seen between the urinary abnormality and glomerular changes. (x^2=2.76; p>0.05).

Table 1: Age and sex distribution of patients with various types of liver diseases.

Type of Liver Disease	Age		Sex		Total
	Range	Mean±S.D.	Male	Female	n = 30
Acute Hepatitis	24-50	41.25±10.43	3 (75%)	1 (25%)	4 (13.33%)
Patient with past h/o acute hepatitis	22-45	33.50±16.25	-	2 (100%)	2 (6.67%)
Chronic Hepatitis	10-30	20.00±14.15	2 (100%)	-	2 (6.67%)
Cirrhosis (compensated)	20-48	37.50±9.79	6 (85.7%)	1 (14.3%)	7 (23.3%)
Cirrhosis (decompensated)	18-16	39.30±11.50	10 (66.6%)	5 (33.3%)	15 (50%)
Controls	18-55	38±2	8 (80%)	2 (20%)	10

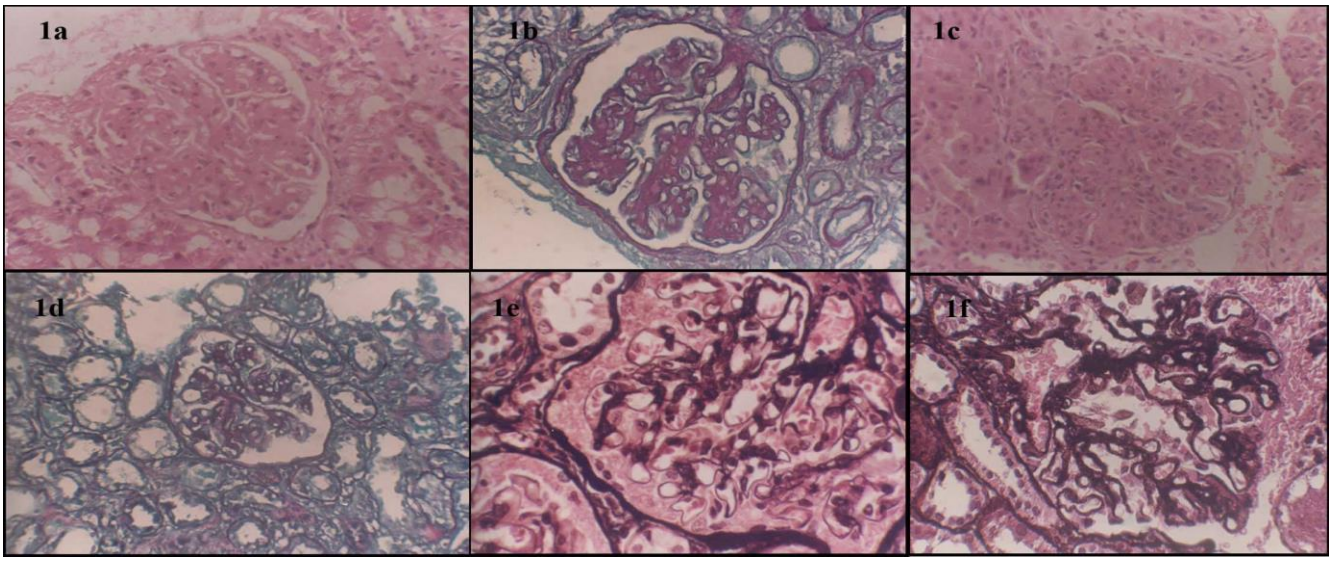

Figure 1: (1a): Microphotograph from a case of membranous glomerulonephritis showing diffuse uniform thickening of glomerular basement membrane (H& E; x400); (1b) : Microphotograph from a case of membranous glomerulonephritis showing diffuse uniform thickening of glomerular basement membrane (PAS;x400); (1c): Microphotograph from a case of membranoproliferative glomerulonephritis showing mesangial hypercellularity and patchy basement membrane thickening. A lobular appearance is also discernible (H&E; x400); (1d): Microphotograph showing patchy thickening of glomerular basement membrane (PAS; x200); (1e): Microphotograph showing patchy thickening of glomerular basement membrane (Silver methenamine stain; x400); (1f): Microphotograph showing 'spikes' seen in a case of membranous glomerulonephritis (Silver methenamine stain; x400).

Table 2: Manifestation of renal functions in various groups of liver diseases.

	Acute Hepatitis (n = 4)	Patients with past h/o acute hepatitis (n = 2)	Chronic hepatitis (n = 2)	Cirrhosis (Compensated) (n = 7)	Cirrhosis (Decompensated) (n = 15)
Blood urea above 40 mg%	2	-	1	2	14
Blood uric acid above 6.0 mg%	2	-	-	-	10
Serum creatinine above 1.5 mg%	2	-	-	-	11

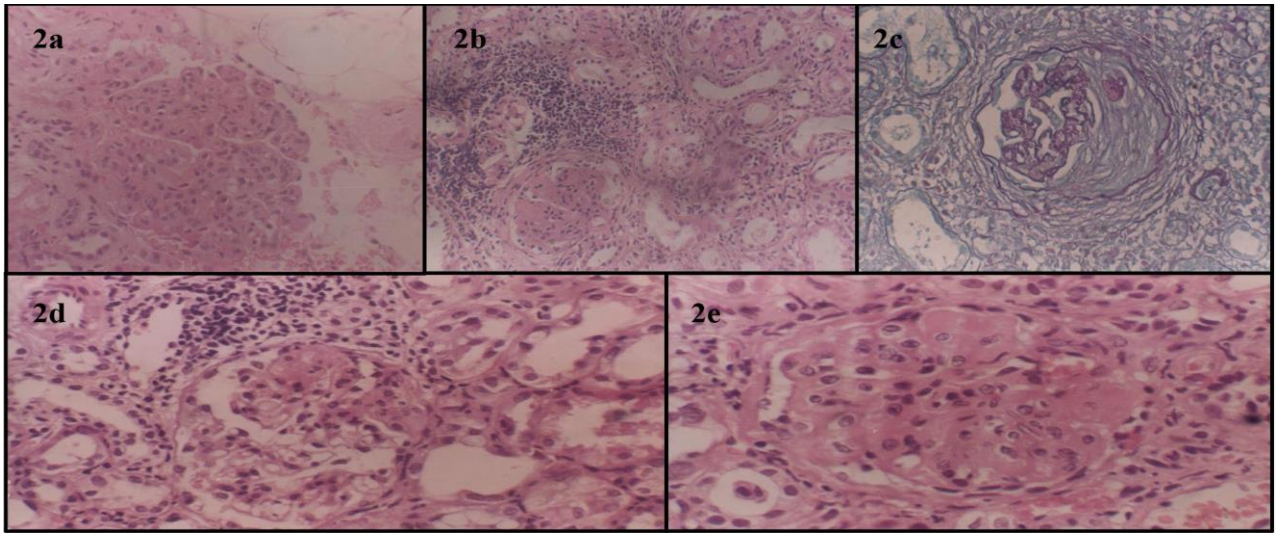

Figure 2: (2a): Microphotograph from a case of membranoproliferative glomerulonephritis showing focal fibrinoid thickening of capillary walls and mesangial hypercellularity (H& E; x400); (2b): Microphotograph showing glomerular sclerosis, tubular atrophy, interstitial inflammation and arteriolar sclerosis in a case of diffuse proliferative Glomerulonephritis (H& E; x200); (2c): Microphotograph showing a crescent. Mild periglomerular fibrosis is seen (PAS; x400); (2d) Microphotograph showing segmental glomerulosclerosis. Sclerotic segment is adherent to the capsule. Mild mesangial matrix expansion is seen in other segment of the tuft (H& E; x400); (2e): Microphotograph showing advanced glomerulosclerosis with hyalinosis (H& E; x400).

Table 3: Histomorphological features in kidney in liver diseases.

Case / Controls	Glomerular changes	Tubular changes	Interstitial changes	Vascular changes
Controls (n=10)	2 (20%)	2 (20%)	1 (10%)	2 (20%)
Acute hepatitis (n = 4)	2 (50%)	1 (25%)	1 (25%)	1 (25%)
Past history of acute hepatitis (n = 2)	2 (100%)	1 (50%)	2 (100%)	---
Chronic hepatitis (n = 2)	2 (100%)	1 (50%)	---	1 (50%)
Cirrhosis (compensated) (n = 7)	5 (71.4%)	3 (42.9%)	4 (57.2%)	1 (14.3%)
Cirrhosis (decompensated) (n = 15)	13 (86.7%)	5 (33.3%)	3 (20%)	4 (26.7%)
Total	24 (80%)	11 (36.7%)	10 (33.3%)	7 (23.3%)

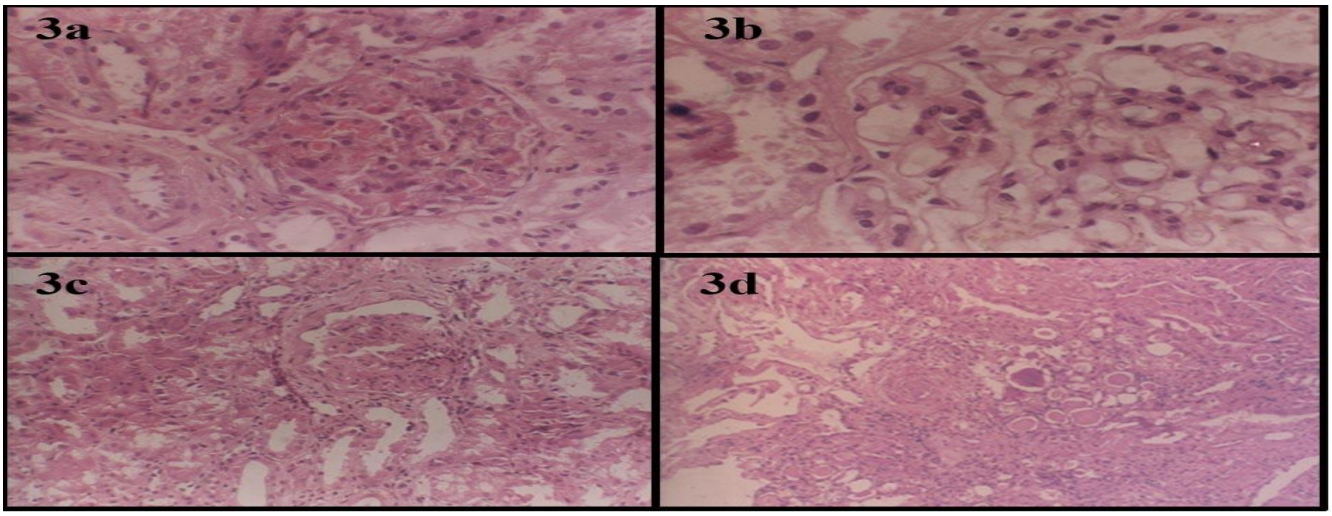

Figure 3: (3a): Microphotograph showing congestion of glomerular tuft (H& E; x400); (3b): Microphotograph showing dilatation of glomerular capillary loops (H& E; x400); (3c): Microphotograph showing periglomerular fibrosis (H& E: x200); (3d): Microphotograph showing tubular epithelial denudation as well as regeneration and hyaline casts (H& E; x200).

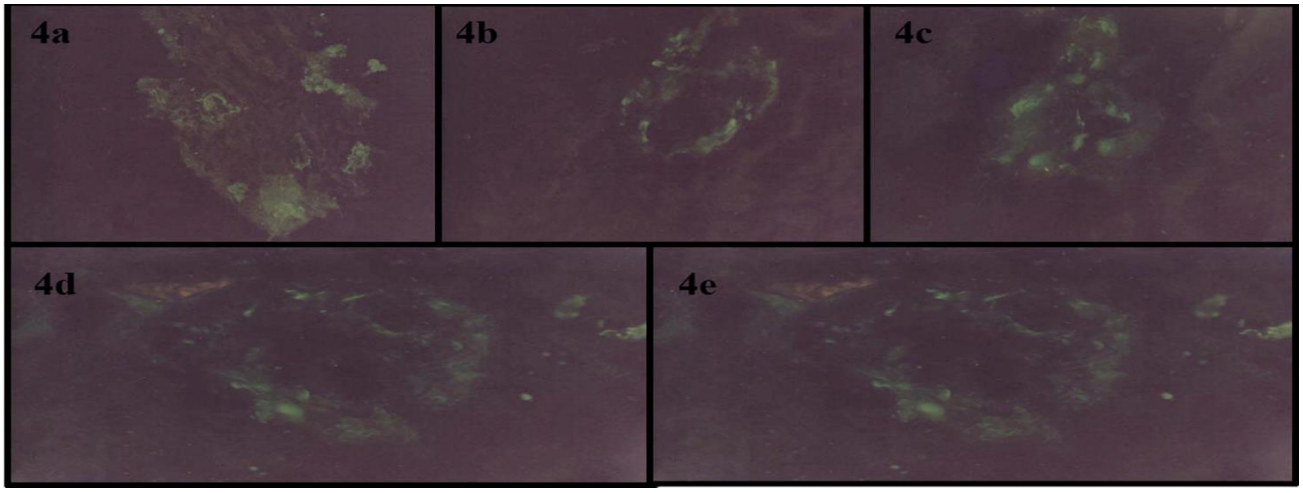

Figure 4 (a): Microphotograph showing moderately dense granular deposits of IgA in the glomeruli (Direct Immunofluorescence- FITC x50); (4b): Microphotograph showing IgA deposits, more predominantly in the mesangium (Direct Immunofluorescence- FITC x200); (4c): Microphotograph showing IgA deposits in the glomerular capillaries, mesangium and in tubular walls (Direct Immunofluorescence- FITC x200); (4d): Microphotograph showing IgA deposits mainly along the glomerular capillaries. Faint positivity is also seen in tubulointerstitial area (Direct Immunofluorescence- FITC x280); (4e): Microphotograph showing irregular IgG deposits in the glomerulus (Direct immunofluorescence- FITC x360).

Table 4: Frequency of various morphological lesions in kidney in patients with liver diseases.

Morphological lesions	Focal	Diffuse	Total no. of cases
Glomular basement membrane thickening	12	3	15
Hypercellularity	8	2	10
Glomerulosclerosis	11	-	11
Mesangial cell proliferation	5	1	6
Capsular epithelial cell proliferation	5	-	5
Cellular Crescents	1	-	1
Mesangial matrix expansion	11	1	12
Polymorphs	2	-	2
Periglomerular fibrosis	1	-	1
Capsular synechia	3	-	3
Tubular atrophy	10	-	10
Tubular dilation	4	-	4
Tubular regeneration	5	-	5
Hydropic change	2	-	2
Casts	4	-	4
Interstitial inflammation	9	-	9
Foam cells	2	-	2
Intimal Vascular thickening	6	-	6

Immunofluorescence

Eighteen cases (60%) were found to be positive for IgA in the glomeruli, IgG deposits were seen in 9 cases (30%) and IgM in 10 cases (33.3%) (Table 6). Immune deposits were not seen in control group. These deposits were granular and were present along the capillary wall and in the mesangial areas in the glomeruli. They were subjectively graded as mild (+) to moderate (++). None of the cases showed heavy immune deposits. Deposits of IgA were more common in chronic liver disease (15/ 24) than acute liver disease (3/ 6). The difference was found to be statistically insignificant (x^2= 0.2628; p>0.5). Out of two HBsAg positive cases, one showed immune deposits of all the three types namely IgA, IgG and IgM while other was negative for all the three. The correlation between the degree of sclerotic changes and the degree of deposition of immune complexes in each case was also examined. Immune deposits were seen in 15 of the 18 cases with sclerotic changes. All cases of MPGN and MGN showed immune deposits along the GBM and in the mesangium (Table 7). There were two cases in which immune deposits were present in the glomeruli in the absence of light microscopic glomerular lesions. One of these was a case of compensated cirrhosis while other was that of decompensated cirrhosis, both of which showed IgA and IgG deposits. Overall age wise immune deposits showed that immune deposits were present in 72.2% of patients below the age of 40 years and in 66.7% of patients above 40 yearrs of age. The difference was statistically insignificant (x^2= 0.16; p>0.05).

Table 5: Distribution of various renal morphological lesions in various categories of liver diseases.

Morphological group		Acute hepatitis (n = 4)	Patient with past h/o acute hepatitis (n = 2)	Chronic hepatitis (n = 2)	Cirrhosis (compensated) (n = 7)	Cirrhosis (decompensated) (n = 15)	Total n=30
Specific	MGN	-	1	1	1	-	3
	MPGN	-	-	1	-	-	1
	DPGN	-	1	-	1	-	2
	FSGS	1	-	-	2	1	4
Non specific		-	-	-	2	12	14
Within normal limits		3	-	-	1	2	6

Table 6: Immunofluorescence findings in patients with liver diseases.

Result	IgA	IgG	IgM
Negative	12	21	20
Positive (+)	11	5	6
Positive (++)	7	4	4
Total positive cases	18	9	10

Table 7: Immune deposits in various categories of lesions.

Renal Lesion	IgA	IgG	IgM
MGN	3/3 (++)	2/3 (+ to ++)	1/3 (+)
MPGN	1/1 (++)	1/1 (++)	1/1 (++)
DPGN	1/2 (+)	1/2 (+)	--
FSGS	2/4 (+ to ++)	--	1/4 (++)
Patchy GBM thickening	5/8 (+ to ++)	3/8 (+ to ++)	4/8 (+ to ++)
Global glomerulosclerosis	4/8 (+ to ++)	1/8 (+ to ++)	3/8 (+ to ++)
Mesangial matrix expansion	6/11 (+ to ++)	3/11 (+ to ++)	5/ 11 (+ to ++)
Others	2/6 (+ to ++)	1/6 (+)	--

DISCUSSION

Renal function and morphology in liver disease is being studied with more and more interest, particularly in many European countries and Japan.[1-9] However, many intriguing issues remain to be resolved. The first is the frequency and the morphological spectrum of the glomerular lesions associated with various liver diseases. The incidence reported varies from 25% to 90%.[13,15-17] There is very wide morphological spectrum of glomerular changes described by different workers and it overlaps with other well-known morphological entities like diabetic glomerulosclerosis and IgA nephropathy.[17] The second issue is the incidence of urinary abnormalities or clinical manifestations indicative of renal dysfunction. Earlier workers reported silent glomerulopathies.[12,13] Later on, many workers found glomerular changes with urinary and other clinical or laboratory abnormalities.[7,8] Variable correlations with degree of hepatic dysfunction and type of liver disease have been described. Morphological and immunomorphological kidney glomerular changes were found more often in chronic hepatitis patients by Morzycka et al.[18] The third issue is establishment of the mechanism of development of glomerular lesions. There are many different schools of thoughts regarding the pathogenesis of glomerular lesions The deposition of immune complexes along GBM and in the mesangium has been suggested.[19,20] The association of HBsAg with immune complex mediated glomerulonephritis in liver diseases is highly variable by different authors. Some have rarely detected HBsAg in the glomeruli of cirrhosis patients, while others showed very strong relation of HBsAg with these renal changes.[3,8,19] The role of IgA has also been shown to play a part in the pathogenesis of renal changes. IgA levels are high in alcoholic cirrhosis, so higher incidence of IgA nephropathy type has been found.[4]

In our study, various aspects of renal morphology were studied in thirty patients of Liver diseases. The pathological findings showed that twenty four (80%) had glomerular changes. Similarly, high incidence of glomerular changes has been reported by many workers. Kawaguchi et al found glomerular abnormalities in 90% of cases, which were of liver cirrhosis only. Nakamoto et al reported glomerular abnormalities in 69.2% of cases with variety of liver diseases in a mixed autopsy and biopsy study.[17,20] Another autopsy study showed these changes in 64.6% of the cases of liver cirrhosis.[8] Callard P et al found glomerular lesions in nine of ten patients with liver cirrhosis.[5] Trawale et al found glomerular lesion in 77% of patients with cirrhosis.[15] Later, Jones et al found low incidence of glomerular lesions in 28 of the 100 cases (28%) with cirrhosis in autopsy study.[13] Similarly Patek et al noted low incidence (11.7%) of glomerulonephritis in cases of cirrhosis in an autopsy

study.[21] Morzyka et al in a study reported glomerular lesion in 16 out of 99 necropsy cases of various forms of hepatitis and liver cirrhosis and more than 50% of these cases were HBsAg positive.[18] Bloodworth and Sommers found glomerular changes in 78 out of 100 patients of cirrhosis.[2]

Membranous glomerulonephritis (MGN)

MGN is uncommon but well documented complication in liver diseases. In the present study, we found three cases (10%) of MGN. Notchy et al described two cases of MGN in a study of 34 patients with overt glomerulonephritis and chronic liver disease. This lesion was found in 7.6% of cirrhosis cases in another study.[7,8] Clinically, proteinuria and microscopic hematuria were found in two patients, and one showed only proteinuria, whose 24 hr urinary protein was done and found to be 2.0g/ 24 hrs. But we did not find the complete picture of nephrotic syndrome in any case. However, many workers have noted the complete clinicomorphological picture of membranous glomerulonephritis.[9,22,23] Generally, HBsAg has been implicated in chronic membranous glomerulonephritis in liver diseases. Kneisser et al, Kohler et al and Takekoshi et al found MGN in HBsAg positive cases whereas in some other studies, HBsAg was not found in sera of patients of MGN seen in association with liver diseases.[7,8,22-24] Also, in our study, none of the cases of MGN showed HBsAg positivity in the serum. We found MGN in patients with chronic active hepatitis, cirrhosis of liver and one with past history of acute hepatitis. Kohler et al, Combes et al found MGN in patients with past history of acute hepatitis, while Kneisser et al, Mistilis et al and Bridi et al reported MGN in patients of chronic active hepatitis.[9,22,23,25,26] Another study found MGN only in patients of cirrhosis.[8] Therefore, it appears that MGN may be associated with a variety of liver diseases. Histologically, one of our cases of MGN showed early uniform basement membrane thickening. On immunofluorescence (IF), the deposits of IgA and IgG along the GBM and in the mesangium were present in this case. Second case of MGN showed 'spikes' when stained by silver methenamine stain. Similar 'spiky' projections have been described in single cases.[8,18] IF study in this patient showed the presence of moderate degree of IgA deposits and IgM deposits, which were granular in nature and were present along the GBM and in the mesangium. Kidney biopsy in the third case revealed diffuse uniform thickening of basement membrane and mild focal interstitial inflammatory infiltrate. On IF, moderate degree of IgA and mild degree of IgG deposits were found.

Membranoproliferative glomerulonephritis (MPGN)

In our study, only one case (3.33%) of MPGN was found. It was a 10 year old boy with chronic active hepatitis and was HBsAg positive. The incidence of MPGN varied from 4.96% to 46.8% in other studies.[7,8,17,20] MPGN was also observed by other workers in HBsAg positive cases.[18,22,27-30] The renal histology showed diffuse mesangial proliferation and glomerular basement membrane thickening. Other changes seen in this case were presence of polymorphs, fibrinoid swelling of capillary walls giving a wireloop appearance, double contouring of basement membrane and thrombi in few capillary loops. All these findings are compatible with the diagnosis of MPGN but are seen more often in multisystem collagen vascular disorders. In this case of MPGN, no urinary abnormality was seen. Nakamoto et al and Kawaguchi et al also found poor correlation of urinary abnormality with MPGN lesions.[17,20] On the contrary, a few other studies found marked urinary abnormality e.g. proteinuria, hematuria, pyuria, red cell casts etc.[7,8,27,29,30] On IF, this patient showed moderate degree of IgA, IgG and IgM deposits along GBM and in the mesangium. These observations are consistent with those of Brzosko et al, Hirschel et al while only IgG deposits were found by Knetcht et al.[28-30] On the other hand, out of five cases of MPGN (mesangiocapillary) of Kawaguchi et al, none showed IgG deposits, IgA deposits were seen in four cases with or without IgM and one case showed only IgM.[17]

Focal segmental glomerulosclerosis (FSGS)

The term 'glomerulosclerosis' is being used in relation to kidney lesions secondary to liver diseases since 1959 when Bloodworth and Sommers first of all named the glomerular lesion in cases of cirrhosis as "Cirrhotic glomerulosclerosis".[2] Later, Sakaguchi et al also described changes in liver disease under the term 'Hepatic glomerulosclerosis'.[3] Ideally, the term focal segmental glomerulosclerosis should be restricted to only those cases which show capillary wall collapse and/ or mesangial matrix expansion and when it is focal and segmental in distribution. In our study, there were four cases of FSGS. Three cases belonged to cirrhosis of liver and one was of acute fulminant hepatitis. The later patient died of renal failure and the histology of the kidney tissue showed focal segmental proliferation and hilar type of segmental sclerosis. There was sclerosis of mesangial stalk in one of the glomerulus. Focal tubular atrophy and regeneration was also seen at places. Oliguria and hypertension were present in our cases but urinary protein examination could not be done. Another patient with FSGS showed segmental glomerulosclerosis in 9 glomeruli out of which six showed early segmental and three showed advanced segmental glomerulosclerosis. Focal mesangial proliferation in one of the glomeruli was seen. Hyalinosis was also seen in the glomeruli. There were capsular synechia in four glomeruli. Tubular atrophy and regeneration with mild interstitial inflammation were also seen. The patient had cirrhosis of liver with portal hypertension and hepatic coma. Patient died of renal failure. Other two patients had cirrhosis of liver. Both of them showed segmental sclerosis and one of them showed hilar sclerosis, a feature of early FSGS. None of these patients had urinary abnormalities.

Diffuse proliferative glomerulonephritis (DPGN): DPGN has also been reported in cases of cirrhosis and other liver diseases. Notchy et al reported DPGN in 8.8% of cases while Kawaguchi et al found this lesion in 33.3% of cases.[7,17] In our study, DPGN was found in 2 patients out of 30 (6.7%). One patient was HBsAg positive and had cirrhosis of liver while the other had past history of acute hepatitis. Brzosko et al also reported the presence of DPGN in HBsAg positive cases.[28] Both the cases in our study showed diffuse endocapillary proliferation. One of these cases showed cellular crescents and polymorphs while other showed adhesions of glomerular tufts to Bowman's capsule. Tubular atrophy was seen in both the cases and focal mononuclear inflammatory infiltrate in interstitium was also present. Blood vessels showed intimal proliferation in one of the cases while other showed arteriolar sclerosis. Clinically, one of these patients showed no urinary abnormalities while other patient who past history of acute hepatitis had showed proteinuria (2.4g/ 24hr). Kawaguchi et al found low incidence of urinary abnormality in their cases of DPGN.[17] Patchy Basement membrane thickening: The GBM thickening was observed in cases of cirrhosis by several other workers.[1,12,13,31] We found focal GBM thickening in 12 cases. Associated mesangial matrix expansion was also seen in most of the cases. Clinically, we found proteinuria (3 cases) and microscopic hematuria (2 cases) in our patients with patchy GBM thickening. On the contrary, Jones et al found no urinary abnormality in such cases.[13] Global glomerulosclerosis: In the present series, global glomerulosclerosis was found in seven patients. In these cases, total or global sclerosis of some of the glomeruli was seen. This was associated with other types of lesions like DPGN, MME, glomerular patchy basement membrane thickening. Six patients were under 40 years of age while one was above 40 years of age. Presence of global sclerosis in young individuals (mean age: 30 years) undermines the possibility of it being just an age related change. Mesangial matrix expansion: We observed mesangial matrix expansion in patients with an age range of 18- 60 years (mean age 36.5 years). This again does not appear to be an age related change. Isolated mesangial matrix expansion is a non specific lesion noted by various workers.[1,12,18,19] Bloodworth and Sommers found mesangial matrix expansion in 68 out of 100 cases of cirrhosis in an autopsy study.[2] Solomon et al found increase of mesangial matrix in all of his 24 cases of various liver diseases in a biopsy study.[1] Here it can be noted that there is some morphological overlap of hepatic glomerulopathy lesions with certain categories of the lesions of DM and IgA nephropathy. However, the former usually occurs in an entirely different set up. Moreover, we had excluded cases of DM from our study and therefore cannot comment on any possible cases in which Diabetes and liver disease coexist. Focal proliferation of mesangial cells and capsular epithelial cells was also noted in 5 cases each. This lesion was also kept under non- specific group of lesion. Other non specific glomerular lesions observed were congestion of glomeruli (4 cases), dilatation of capillary loops (1 case), periglomerular fibrosis (1 case). Overall, we found a wide spectrum of glomerular lesions with various specific and non specific lesions discussed above and the incidence of these lesions was found to be statistically significant when compared to control cases ($p<0.001$). There was no correlation of these changes with presence or absence of renal failure ($p>0.1$). Jones et al found glomerular changes in 28% of cases as compared to 10% in controls.[13] The difference was statistically significant. But these changes were found to be correlated with the severity and type of liver disease. These changes were more common in chronic liver diseases and the difference was statistically significant ($p<0.05$). Similarly, specific lesions were more commonly associated with severe liver disease and the difference was found to be statistically significant ($p<0.001$). We found no significant statistical correlation of glomerular lesions and urinary abnormality. Jones et al found no correlation of glomerular changes to urinary abnormality.[13] Similarly, Callard et al found urinary abnormality in only one out of nine patients.[5] Trawale et al found that proteinuria was significantly lower in the entire group of patients with cirrhosis than in patients without liver disease.[15] They further suggested that the threshold for clinically significant proteinuria may be lower in patients with cirrhosis than in patients without liver diseases. Tubular changes observed were mainly tubular atrophy (10 cases), tubular regeneration (5 cases) and tubular dilation (4 cases). Hydropic change and casts were seen in 2 and 4 cases respectively. Tubular changes were found in 36.7% cases as compared to 20% in control group. The difference was found to be statistically insignificant ($p>0.1$). This change was also compared in two groups of patients with and without renal failure but the difference was found to be statistically insignificant ($p>0.05$). Wilkinson et al also suggested that renal failure in liver diseases may occur with or without tubular changes.[32] Interstitial changes mainly showed focal lymphomononuclear infiltrate. Focal hyalinosis (1 case) and focal interstitial fibrosis (2 cases) were seen. Foam cells were seen in clusters in the interstitium in two cases. In one case, it was accompanied by FSGS and with mild non- specific glomerular changes in the other. Renal function: High uric acid and serum creatinine levels were observed in 12 and 13 patients respectively. Low serum sodium was seen in 14 patients. Oliguria was observed in nine patients and 14 patients ultimately developed azotemia and renal failure. Hyponatremia is a well-known feature in cirrhotic patients especially in those whom ascites becomes refractory to treatment. In the present study, it was a striking feature in patients with decompensated cirrhosis. Also, higher incidence of renal failure was seen in patients with decompensated cirrhosis. Similar observations were made by Baldus et al, Shear et al.[33-34] No statistically significant correlation could be found between renal failure and glomerular or tubular changes. Immunofluorescence: Direct IF with FITC labelled antisera was done in all thirty cases. Highest frequency of IgA immune deposits (60%) was found which is consistent with that of Berger et al.[4] The

presence of high incidence of immune deposits suggests a high possibility of immune complex mediated glomerular injury in patients of liver diseases. Similar views had been put forward by Callard et al, Morzyka et al and Fukuda Y.[5,8,18] The deposits were mainly granular and were present in the capillary walls and mesangium. Eight cases showed moderate amount of deposits while rest showed mild amount. Four cases, in which immunofluorescence was done on paraffin sections showed mild amounts of deposits. This could be attributed to the damage of the immune deposits during the dewaxing of the sections. The incidence of immune deposits was apparently more in chronic liver diseases (62.5%) than in acute liver disease (50%) but the difference was found to be statistically insignificant. Hence, there is no correlation between the immune deposits and type of liver disease. Kneisser et al and Moryzcka et al observed the presence of immune deposits more frequently in chronic liver diseases.[18,22] No correlation of age with immune deposits has been found. In patients below 40 yrs of age, the incidence of immune deposits was found to be 72.2% against 66.7% in those above 40 yrs of age. It was found to be statistically insignificant. Thus this could not be merely age related phenomenon. Most of these deposits were found in patients with specific glomerular lesions. All cases of MGN and MPGN showed glomerular immune deposits along GBM and in the mesangium. While only half of the cases of FSGS and DPGN showed these deposits. The deposits were also seen in non- specific lesions like GBM thickening and mesangial expansion. HBsAg was positive in sera of two cases only. One showed immune deposits of IgA, IgG and IgM types, while other was negative. Combes et al, Myers et al and Nagy et al have strongly stressed on the role of HBsAg in the pathogenesis of glomerular lesions in liver diseases.[9,27,35] A geographical analysis of the problem shows that most of the studies related to renal changes in liver diseases have been done in France and Japan. In India, there has been no such dominant series so far, to the best of our knowledge. French workers have suggested that the mechanism of development of these renal lesions was related to deposition of immune complexes in the glomeruli and that too, more frequently of IgA type. They found most of these deposits in the paramesangial regions and tried to correlate them with IgA nephropathy. Moreover, IgA levels are high in alcoholic cirrhosis. So, they stated that alcohol is one of the etiologic factors for renal lesions seen in the cases of alcoholic cirrhosis.[4] They also stressed on the role of HBsAg in the pathogenesis of immune complex mediated glomerulonephritis. Pouria et al suggested that abnormalities of liver clearance of IgA production described in liver disease may influence the distribution and behaviour of IgA, leading to a common pattern of disease by differing mechanisms.[36] There is also evidence that in alcoholic liver disease the expression of Fcα receptors is reduced on circulating monocytes and that endocytosis of circulating IgA immune complexes is defective.[37] On the other hand, Japanese workers have not commonly found IgA nephropathy type picture. Although they also suggested the role of immune complex deposit in the glomeruli leading to morphological and clinical renal changes. Also, HBsAg was rarely detected in the glomeruli by these workers and they suggested that although HBsAg could be a cause but other antigens from alimentary tract including bacterial, viral and dietary could be responsible for these renal glomerular changes.[8]

We found that IgA deposits were highest (60%) in our cases and they included all the ten patients with history of alcohol intake, but again there were substantial number of patients in which there was no history of alcohol intake. So, it is difficult to say that there is definite relationship of alcohol with the increased levels of IgA in sera leading to renal changes. Moreover, the immune deposits, we found were not paramesangial, they were mainly mesangial and along the GBM. However, the total number of cases studied by us is small and we have not measured serum IgA levels. We could also not implicate HBsAg in causation of renal glomerular lesions as we could find HBsAg in the sera of only two of our patients. Thus, on the whole, there is a fair degree of agreement on renal glomerular lesion in liver diseases being an immune complex mediated disease; the precise antigen(s) responsible for this phenomenon continue to be a matter of further research.

CONCLUSION

There is a wide spectrum of morphological lesions in kidney in patients of liver diseases. The predominant lesions are glomerular and are directly related to the severity as well as chronicity of liver diseases. Renal failure and urinary abnormalities are not well correlated with these glomerular changes. There is no relation of age with immune deposits and these are more often present in patients with chronic diseases. The renal glomerular injury in liver disease appears to be an immune complex disease. However, no particular antigen could be implicated.

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REFERENCES

1. Solomon P. The role of the kidney in Laennec's cirrhosis of the Liver. Medicine. 1958;37(4):299-316.
2. Bloodworth JM, Sommers SC. Cirrhotic glomerulosclerosis: a renal lesion associated with hepatic cirrhosis. Lab invest. 1959;8:962-5.

3. Sakaguchi H, Dachs S, Grisman E, Paronetto F, Solomon M, Churg J. Hepatic glomerulosclerosis: An electron microscopic study of renal biopsies in liver diseases. Lab Invest. 1965;14:533-41.
4. Berger J, Yaneva H, Nabarra B. Glomerular changes in patients with cirrhosis of Liver. Adv Nephrol Necker Hosp. 1977;7:3-14.
5. Callard P, Feldmann G, Prandi D, Belair MF, Mandet C, Weiss Y. Immune complex type glomerulonephritis in cirrhosis of the liver. Am J Pathol. 1975;80(2):329-40.
6. Newell GC. Cirrhotic Glomerulonephritis: Incidence, Morphology, Clinical Features and Pathogenesis. Am J Kidney Diseases. 1987;9(3):183-90.
7. Nochy D, Callard P, Bellon B, Bariety J, Druet P. Association of overt glomerulonephritis and liver disease: a study of 34 patients. Clin Nephrol. 1976;6(4):422-7.
8. Fukuda Y. Renal glomerular changes associated with liver diseases. Acta Pathol Jpn. 1982;32(4):561-74.
9. Combes B, Shorey J, Stastny P, Eigenbrodt EH, Hull AR, Carter NW. Glomerulonephritis with deposition of Australia antigen- antibody complexes in glomerular basement membrane. Lancet. 1971;2(7718):234-7.
10. Lai KN, Lai FM, Chan KW. The clinic-pathological features of hepatitis B virus associated glomerulonephritis. Q.J. Med. 1987;63:323-3.
11. Johnson RJ, Couser WG. Hepatitis B infection and renal disease. Clinical immunopathogenetic and therapeutic considerations. Kidney Int. 1990;37:663-76.
12. Fisher ER, Hellstrom HR. The membranous and proliferative glomerulonephritis of hepatic cirrhosis. Am J Clin Path. 1959;32(1):48-55.
13. Jones WA, Rao GDR, Brunstein H. The renal glomerulus in cirrhosis of Liver. Am J Pathol. 1961;39:393-404.
14. Dash SC, Bhowmik D. Glomerulopathy with Liver Disease: Patterns and management. Saudi J Kidney Dis Transplant. 2000;11(3):414-20.
15. Trawale JM, Paradis V, Routou PE, Francoz C, Escolano S, Sallee M. The spectrum of renal lesions in patients with cirrhosis: a clinicopathological study. Liver Int. 2010;30(5):725-32.
16. Noel LH, Droz D, Gascon M, Berger J. Primary IgA nephropathy from the first described cases to the present. Semin Nephrol. 1987;7:351-4.
17. Kawaguchi K, Koike M. Glomerular lesions associated with liver cirrhosis: an immunohistochemical and clinicopathologic analysis. Hum Pathol. 1986;17(11):1137-43.
18. Morzycka M, Slusarczyk J. Kidney glomerular pathology in various forms of acute and chronic hepatitis. Arch Pathol Lab Med. 1979;103:38-41.
19. Sakaguchi H. Hepatic glomerulosclerosis-Light microscopic study of autopsy cases. Acta Pathol Jpn. 1968;18(4):407-15.
20. Nakamoto Y, Lida H, Kobayashi K, Dohi K, Kida H, Hattori N, et al. Hepatic glomerulonephritis characteristic of hepatic IgA glomerulonephritis as the major part. Virchows Arch A Pathol Anat Histol. 1981;392(1):45-54.
21. Patek AJ, Seegal D, Bevans M. The coexistence of cirrhosis of the liver and glomerulonephritis. Am J Med Sci. 1951;221(1):77-85.
22. Knieser MR, Jenis EH, Lowenthal DT, Bancroft WH, Burns W, Salhoub R. Pathogenesis of renal disease associated with viral hepatitis. Arch Pathol. 1974;97(4):193-200.
23. Kohler PF, Cronin RE, Hammond WS, Olin D, Carr RI. Chronic membranous glomerulonephritis caused by hepatitis B antigen- antibody immune complexes. Ann Intern Med. 1974;81(4):448-51.
24. Takekoshi Y, Shida N, Saheki Y, Tanaka M, Satake Y, Matsumoto S. Strong association between membranous nephropathy and hepatitis B surface antigenemia in Japanese children. Lancet. 1978;2:1065-8.
25. Mistilis SP, Blackburn CR. Active chronic hepatitis. Am J Med. 1970;48(4):484-95.
26. Bridi GS, Falcon PW, Brackett NC, Still WJ, Sporn IN. Glomerulonephritis and renal tubular acidosis in a case of chronic active hepatitis with hyperimmunoglobulinemia. Am J Med. 1972;52(2):267-78.
27. Myers BD, Griffel B, Navch D, Jankielowiiz T, Klajman A. Membranoproliferative glomerulonephritis associated with persistent viral hepatitis. Am J Clin Pathol. 1973;60(20):222-8.
28. Brzosko WJ, Nazarewicz T, Krawczynski K, Morzycka M, Nowoslawski A. Glomerulonephritis associated with Hepatitis – B surface antigen immune complexes in children. The Lancet. 304(7879):477-81.
29. Hirschel BJ, Benusiglio LN, Favre H, Chalelanat F, Humair L, Zubler RH, et al. Glomerulonephritis associated with hepatitis B, report of a case and review of literature. Clin Nephrol. 1977;8(3):404-9.
30. Knetchel GL, Chisari FV. Reversibility of hepatitis B virus – induced glomerulonephritis and chronic active hepatitis after spontaneous clearance of serum hepatitis B surface antigen. Gastroenterology. 1978;75(6):1152-6.
31. Baxter JH, Ashworth CT. Renal lesions in portal cirrhosis. Arch Pathol(Chic.). 1946;41:476-88.
32. Wilkinson SP, Hirst D, Day DW, Williams R. Spectrum of renal tubular damage in renal failure secondary to cirrhosis and fulminant hepatic failure. J Clin Pathol. 1978;31(2):101-7.
33. Baldus WP, Feichter RN, Summerskill WH. The kidney in cirrhosis. I. Clinical and Biochemical features of azotemia in hepatic failure. Ann Intern Med. 1964;60:353-65.
34. Shear L, Kleinerman J, Gabuzda GJ. Renal failure in patients with cirrhosis of the liver. I. Clinical and pathologic characteristics. Am J Med. 1965;39:184-98.

35. Nagy J, Bajtai G, Brasch H, Sule T, Ambrus M, Deak G, et al. The role of hepatitis B surface antigen in the pathogenesis of glomerulopathies. Clin. Nephrol. 1979;12(3):109-16.
36. Pouria G, Feehally J. Glomerular IgA deposition in liver disease. Nephrol Dial Transplant. 1999;14:2279-82.
37. Silvain C, Patry C, Launay P, Lehuen A, Monteiro RC. Altered expression of monocyte immunoglobulin A Fc Receptor is associated with defective endocytosis in patients with alcoholic cirrhosis: Potential role for IFN- γ. J Immunol. 1995;155:1606-18.

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