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Increased incidence of glomerulonephritis following spleno-renal shunt surgery in non-cirrhotic portal fibrosis

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Increased incidence of glomerulonephritis following spleno-renal shunt surgery in non-cirrhotic portal fibrosis. In a prospective study of 200 non-cirrhotic portal fibrosis (NCPF) patients, 7% had mild proteinuria and their renal biopsies showed mild mesangial proliferative glomerulonephritis (mes-PGN). The remaining 93% biopsies were normal. However, following the insertion of a spleno-renal shunt (SRS) for portal hypertension 32% of these patients developed nephrotic syndrome in five years. Renal histology revealed mesangiocapillary glomerulonephritis (MCGN) (18.5%), mes-PGN (9%), minimal change nephropathy (3%), and chronic sclerosing GN (1.5%). Immunofluorescence showed granular deposition of IgA and C3. IgA2 was the predominant form of Ig in the glomerular deposits, indicating that IgA in the immune complexes was derived from the gastrointestinal tract. Electron microscopy revealed electron dense deposits in the mesangium. In contrast to the NCPF patients who underwent a SRS for portal hypertension, the 200 patients in our study who underwent spleno-renal shunting because of extra hepatic portal obstruction did not have renal disease, nor did they develop renal disease during the five-year post-operative follow-up. Fifty percent of the glomerulonephritis (GN) in the NCPF group progressed to renal failure in five years; 46.6% continued to have proteinuria. Low serum complement, C3 (40%) and circulating immune complexes (14.8%) were detected in the glomerulonephritis group. Our study shows that: (i) there is a high rate of the occurrence of GN following SRS in NCPF patients, but not in those with normal livers; (ii) the type of GN is primarily IgA nephropathy; and (iii) the GN could be the result of defective hepatic reticuloendothelial function in the NCPF group that is worsened by the shunting procedure.

Nearly 25 to 30% of all patients with portal hypertension in India who undergo surgery or sclerotherapy have non-cirrhotic portal fibrosis (NCPF) [1, 2]. The majority of these patients come from a lower or middle socioeconomic background, and variceal bleeding is the most common presenting feature. The spleno-renal shunt (SRS) has been found to be quite useful in controlling variceal hemorrhage. The disease seems to have a protracted benign course with good prognosis following the insertion of the SRS [3]. However, glomerulonephritis has been reported in patients with NCPF particularly following SRS surgery [4, 5]. In the present study, we prospectively determined the prevalence and characteristics of glomerular changes in patients with NCPF and the relationships of these renal lesions with portal hypertension and the SRS procedure.

Key words: glomerulonephritis, shunt surgery, fibrosis, nephrotic syndrome, hypertension, cirrhosis.

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METHODS

Two hundred consecutive, (earlier) biopsy-proven patients with NCPF and 200 with extra-hepatic portal obstruction (EHPO) admitted for the proximal SRS procedure were investigated. The cause of EHPO was portal vein thrombosis during childhood, a well-recognized condition in India. Alcoholism, systemic infection before or after surgery, and nephrotoxic drug therapy were criteria for exclusion. An episode of a major bleed from esophageal varices due to portal hypertension was the main indication for the SRS procedure. Each patient underwent splenectomy and anastomosis of splenic vein to the left renal vein, allowing part of portal circulation to directly enter the renal vein and then into the inferior vena cava. In this way the shunt decreased portal hypertension and increased portosystemic shunting. The mean age of NCPF patients was 28 years (range 14 to 44 years) and of EHPO patients was 19 years (range 9 to 23 years) with a male:female ratio of 3.1:1 and 6:1, respectively. The investigations routinely carried out in all the subjects included: liver function tests, hepatitis B virus (HBsAg) by radioimmunoassay, hemoglobin, total leukocyte and differential counts, reticulocyte and platelet counts, and erythrocyte sedimentation rate.

Serum complement C3 and circulating immune complexes were determined by the polyethylene glycol precipitation procedure [6]. Blood for cryoglobulinemia and anti–HCV (hepatitis C) were tested in those who developed GN during the post-operative period.

Renal function studies included routine urinalysis, 24-hour urinary protein estimation (by the Diacetyl monoxime method), blood urea, creatinine (using an Auto Analyzer; Medical System Polystat 2000) and creatinine clearance.

Following the necessary ethical approval and individual patient's consent, an intraoperative left renal biopsy was taken before splenectomy and spleno-renal shunting. There were no complications. Specimens were subjected to light microscopic (LM), immunofluorescence (IF) and electronmicroscopic (EM) examinations. In addition to the physical examination, the post-operative follow-up included urinalysis, and a biochemical assessment of renal, hepatic and hematologic functions every six months. Repeat percutaneous renal biopsies were performed in

Table 1. Renal function data in non-cirrhotic portal fibrosis before and after splenorenal shunting operation in 200 patients

	Pre-operative	Post-operative					
		1 Month	1 Year	2 Years	3 Years	4 Years	5 Years
Proteinuria *% of patients having > 300 mg/24 hrs*	7	8.5	11.5	26	27.5	29	32
Mean $U_{Pr} \times V$ *g/24 hr*							
M =	0.56 ± 0.09	0.6 ± 0.13	1.1 ± 0.28	2.5 ± 0.45	3.5 ± 0.46	3.8 ± 0.51	3.6 ± 0.56
N =	14	17	23	52	55	58	64
Hematuria *% 200 patients*	0	6	18	18	20	25	22
Average C_{Cr} *(ml/month)*[a]							
M =	79 ± 14	61 ± 5.0	60 ± 8.0	48 ± 9.0	41 ± 12	38 ± 16	32 ± 14
N =	14	17	23	52	55	58	64
Serum creatinine *mg/dl*	1.0 ± 0.1	1.0 ± 0.3	1.4 ± 0.4	1.5 ± 0.4	2.7 ± 1.4	2.8 ± 1.6	3.3 ± 1.7

Abbreviations are: C_{Cr}, creatinine clearance; M, mean value; *N*, number of patients.
[a] In patients who developed glomerulonephritis.

all those who developed significant proteinuria during the follow-up period.

Histological studies

Specimens were fixed in neutral buffered formalin and after processing, 3 μm paraffin sections were stained with hematoxylin-eosin (HE) and periodic acid-Schiff (PAS), silver methenamine (SM) and Maritus scarlet blue for light microscopical examination. A minimum of six glomeruli were taken as criteria for adequate biopsy.

Renal tissue for IF was available in 205 cases (122 NCPF, 83 EHPO). Tissue was snap-frozen and cut in Cryostat into 5 to 6 μm thick sections. The sections were stained with fluorescent monospecific antisera (Wellcome Reagents, UK) to demonstrate deposits of IgA, IgG, IgM, complement (C_3) and fibrinogen—fibrin (Fi). In addition, frozen sections of the biopsies of 10 patients with NCPF who developed GN during the post-operative follow-up were stained for IgGA1 and IgA2 and examined under an IF microscope. Similarly, 20 random biopsies from NCPF patients where light microscopy and IF showed mesangial hypercellularity or immune deposits were processed for the EM study. Five random samples from EHPO were taken for comparison. Small cubes of renal tissue were fixed in buffered glutaraldehyde for EM.

RESULTS

Renal function studies

There were no immediate post-operative complications and patients remained free of hematemesis during the follow-up period.

In the NCPF group, the incidence of proteinuria ($U_{Pr}V$) increased from 7% during the pre-operative period to 32% during the post-operative follow-up (Table 1). Twenty-eight percent of these clinically presented as nephrotic syndrome (mean $U_{Pr}V$ = 3.6 g) in five years. Microhematuria increased from 6% at one month post-operatively to 25% after four years. Pre-operative average serum creatinine of 1.0 ± 0.1 mg% in the 64 patients who developed glomerulonephritis following SRS operation increased progressively to 3.3 ± 1.7 mg% at five years, and creatinine clearance progressively declined to 32 ± 14 ml/minute during the corresponding period (Table 1). In EHPO patients no significant proteinuria (defined as > 300 mg in 24 hr) and/or hematuria were observed; the prevalence of significant proteinuria in the general Indian population is 0.01%. Renal biopsy showed mild mesangial proliferation in 3 (1.5%) EHPO patients that did not progress during the follow-up period.

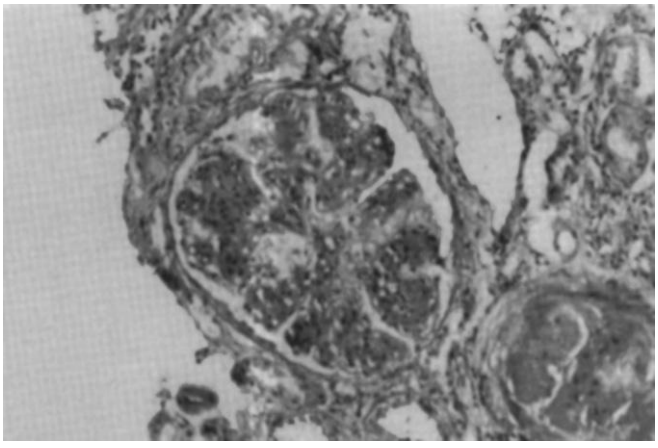

Fig. 1. Mesangiocapillary glomerulonephritis showing thickening of capillary walls, hypercellularity and lobular formation following the Leino renal shunting operation (hemaloxylin and eosin stain, ×200).

Renal biopsy (light microscopy)

Light microscopy revealed GN in 32% of NCPF patients, mesangiocapillary GN in 18.5% (Fig. 1), mesangial proliferative GN in 9% (Fig. 2), minimal change disease in 3%, and chronic sclerosing GN in 1.5%. One third of the patients with mesangiocapillary GN and 1/5 with mesangial PGN showed varying degrees of extra-capillary crescents.

Immunofluorescence

Glomerular mesangium was strongly positive for granular IgA and C_3 (Fig. 3) deposition in 52%. In 47% there was mixed deposition of IgA, C_3 and weakly positive IgM. All 10 tissue samples stained against IgA1 and IgA2 were positive for the latter and negative for the former. This finding suggested that the immunoglobulins were derived from gastrointestinal tract. Capillary walls did not reveal any deposits. Fibrin was detectable only where crescents were found. Mesangial IgA, C_3 and IgM deposits corresponded to diffuse mesangial proliferation seen on light microscopy.

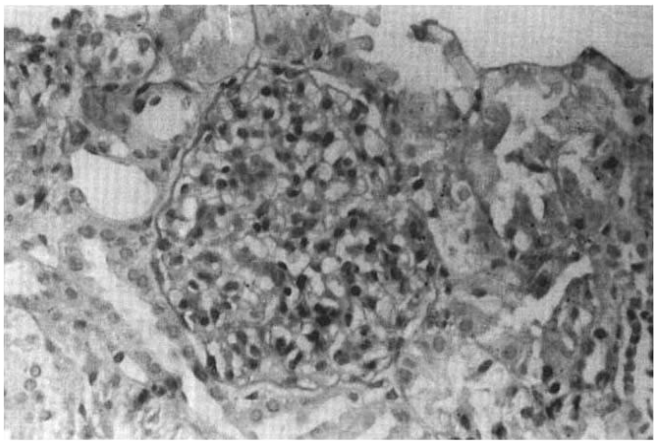

Fig. 2. Mesangioproliferative glomerulonephritis showing mesangial expansion with increased hypercellularity (hematoxylin and eosin stain, ×200).

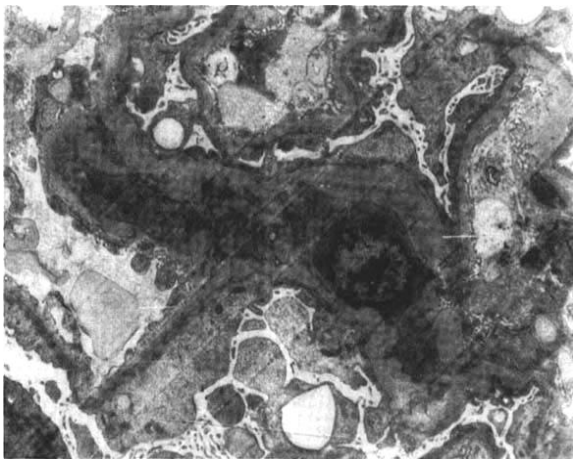

Fig. 4. Electron photomicrograph showing electron dense deposit in the mesangial cell cytoplasm (×4200).

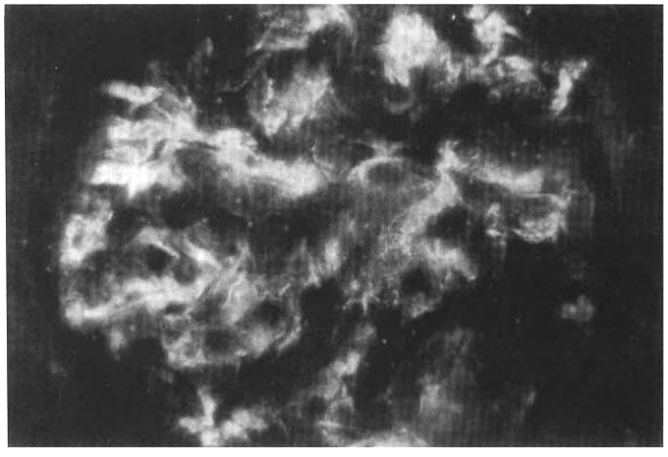

Fig. 3. Granular deposits of IgA in the mesangium (fluorescent antihuman IgA, ×350).

Electron microscopy

In 25 patients with NCPF there were electron dense deposits that looked discrete, granular and were confined to the mesangium, the capillary walls being free of any deposit (Fig. 4). Mesangial cells were increased in number whereas the endothelial cells were unremarkable. These features corresponded to the mesangial cell proliferation seen on LM and IgA and C_3 deposits. In one patient with EHPO, faint mesangial deposits were detected.

Hematological and immunological observations

Patients with NCPF tended to have lower leukocyte and platelet counts than patients with EHPO, which improved following splenectomy and the SRS operation (Table 2). Serum complement (C_3) and circulating immune complexes (CIC) were positive in 40% and 14.8%, respectively in NCPF; these were negative in EHPO patients. Hepatitis B and anti-HCV were positive in 18% and 12% of the NCPF group, respectively, but neither of these markers correlated with the occurrence of GN. HBsAg and anti-HCV were detected in 7% and 6% of the EHPO group, respectively. Cryoglobulins were negative in the serum of all those who developed GN. In the liver function tests hypoalbuminemia was the only abnormality observed in both groups, and the remaining parameters were normal (Table 2).

Outcome

At the end of five years the average GFR fell from preoperating value of 79 ± 14 ml/min to 32 ± 14 ml/min in 58% of the GN patients, 50% of whom showed elevated blood urea nitrogen. In 46.6%, proteinuria continued while 3% remained free of proteinuria.

DISCUSSION

We found that in NCPF patients, there was another 25% increase in the incidence of GN within five years following the SRS operation. More accurately it was IgA nephropathy in the most patients, with 27.5% showing MCGN and mesangial PGN apearance on light microscopy. IgA was the dominant immunoglobulin deposited along with C_3 in granular pattern indicating an immune complex type of GN. The demonstration of electron dense deposits in the mesangium suggested immune complexes and not mere trapping of IgA in the glomeruli. Further, in clinical terms NCPF patients presented a picture of nephrotic syndrome with significant proteinuria. In contrast, none of the EHPO patients had significant proteinuria prior to or following surgery.

Renal lesions have been described in cirrhotics for more than 25 years. These constituted a marked PAS positive thickening of glomerular mesangium [7]. Fisher and Perez also described mesangial PGN [8] with some correlation between the extent of renal lesions and duration of cirrhosis. Callard et al reported that granular deposits were mainly IgA and C_3 [9]. However, such lesions have not been described in NCPF following SRS surgery thus far.

The pathogenesis of an increased occurrence of glomerulonephritis in NCPF patients following spleno-renal shunting is not clear. In cirrhosis it has been related to presence of hepatitis B surface antigen [10]. Although 18% of the NCPF patients in the present study were positive for HBsAg and 12% for anti-HCV, the development of GN was not related to these markers.

Table 2. Hematological, immunological and liver function data

Investigation	NCPF		EHPO	
	Pre-op	Post-op (1 month)	Pre-op	Post-op (1 month)
Hematological				
Hemoglobin	9.6 ± 1.8	11.0 ± 1.4	9.7 ± 1.5	10.9 ± 1.8
TLC cells *mm*^{3}	3900 ± 1600	4800 ± 1700	4500 ± 1300	5100 ± 1600
Platelet count	88000 ± 22000	14000 ± 17000	130000 ± 2000	130000 ± 16000
ESR *mm/hr*	20	13	14	12
Immunological %				
Low serum C_3	ND	40	ND	0
CIC +ve	ND	14.8	ND	0
Cryoglobulinemia	ND	0	ND	0
Liver Function Test				
Serum bilirubin *mg/dl*	0.6	0.8	0.6	0.8
Albumin/globulin *g/dl*	2.6/3.7	2.8/3.1	2.6/3.2	3.6/3.2
SGOT/SGPT *IU*	32/38	24/18	32/28	24/20
SAP *KU*	10	12	14	13
HBsAg +ve %	18	18	0	7
HCsAg +ve %	ND	12	ND	6

Abbreviations are: ND, not done, C_3, Complement C_3; TLC, total leukocyte count; SAP, serum alkaline phosphatase.

Experimentally, saturation of the reticuloendothelial system (RES) in mice with colloidal carbon together with oral immunization produces mesangial IgA deposition, and inhibition of normal hepatic clearance mechanisms is probably the most important factor involved [11]. It is known that the function of hepatic RES in NCPF is poor where in EHPO it is intact. The spleno-renal shunt procedure is carried out to reduce portal hypertension and thus prevent gastrointestinal hemorrage from esophageal varices. However, the procedure enhances the direct portosystemic circulation and partly bypasses hepatic RES. Patients with marginal hepatic reticuloendothelial function, the first pass clearance of immune complexes emanating from the gastrointestinal tract is critical in preventing excessive levels of circulating immune complexes. When the first pass uptake of immune complexes is eliminated, there is excessive delivery of pathogenic immune complexes to the systemic circulation leading to immune complex GN. Demonstration of IgG2 in glomerular mesangium indicating that the IgA was derived from the gastrointestinal tract provides supportive evidence to the hypothesis outlined above. This hypothesis explains why there was increased incidence of GN following SRS procedure in the present series. However, SRS in EHPO patients with intact hepatic RES but not associated with GN suggests that the spleno-renal shunting procedure *per se* is not the predisposing factor, but it can be in the presence of poor hepatic clearance, as in NCPF patients.

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