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Original Article

Spectrum of Biopsy Proven Glomerular Disease: An Experience Based on Light Microscopy and Direct Immunofluorescence Microscopy

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Abstract

Glomerular diseases continue to be the leading cause of end-stage renal disease globally. In this study, 80 adequate renal biopsy specimens were subjected to histopathology and direct immunofluorescence (DIF) microscopy in the Department of Pathology, Rajshahi Medical College, from July 2008 to June 2009, to evaluate the spectrum of glomerular disease. Of them 42.5% were male and 57.5% were female with ratio 1: 1.35. The most frequent clinical presentation was nephrotic syndrome (62.5%). Primary glomerular disease was accounting for 91.25 % of all glomerular disease and of them; FSPGN was the most common histological lesion (27.4%). DMesPGN was the second most common lesion (17.8%) followed by MCD (15.07%), MGN (10.96%), FSGS (10.96%), IgA nephropathy (6.85%), CSG (2.74%) and Cres. GN (2.74%). Lupus nephritis was the most prevalent (71.43%) among secondary glomerulonephritis cases. Among 73 adequate cases for DIF, 63.01% were found to be DIF positive and IgG alone and in various combinations were found in 82.60 % cases (highest deposits).

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Introduction

Glomerular disease accounts for 10%–15% cases of ESRD in the USA[1] and comprises 25-45% cases of ESRD in developing nations including Bangladesh [2]. The patterns of the glomerular diseases are different in different countries and also, are changing with the passage of time in the same country, probably due to better infection control, changes in environmental pollution, increased awareness of the disease and changes in life expectancy. IgA nephropathy (IgAN) is common in the North West regions of Italy, [3] the Far East and Eastern Europe, [4, 5] while focal and segmental glomerulosclerosis (FSGS) seems most prevalent in the United States of America[6]. MesPGN is the most frequent pattern of glomerulonephritis in Bangladesh and it is 32.73% followed by FSPGN in 15.09%, MCD in 10% cases. Among these 97.27% are primary glomerulonephritis and remaining are due to systemic diseases [7]. Although a little is known about etiologic agents and triggering events, it is clear that immune mechanisms underlie most forms of primary glomerulonephritis and many of the secondary glomerular disorders [8, 9].

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The gold standard for diagnosis remains the renal biopsy. In order to evaluate renal biopsy the standard methods are light microscopy with routine Hematoxylin & Eosin (H&E) and special Periodic Acid Schiff (PAS) stain, Immunofluorescence microscopy (DIF) and Electron microscopy and correlating all these with clinical information to obtain the final diagnosis[10]. Most immune mediated glome-rulonephritis can be defined on the basis of DIF findings, and the pattern obtained is often diagnostic. DIF detects immunoglobulins within renal tissue and it has been used as an acceptable diagnostic tool for approximately four decades [9]. The present study is aimed at giving more recent insight into the prevalence of glomerular diseases as revealed by renal biopsy at Rajshahi Medical College Hospital. This is a tertiary care hospital with a heterogeneous population representing patients from almost all parts of the Northern districts of Bangladesh.

Material and Methods

Renal biopsy (needle biopsy) specimens were obtained from 80 clinically suspected and biopsy indicated patients of glomerulo-nephritis. The specimens were collected from the Department of Nephrology, Rajshahi Medical College Hospital, July 2008 to June 2009, for the study. Two renal biopsy samples were taken from each patient, which were processed for light microscopy (LM) and immunofluorence microscopy (EM). The specimen for light microcopy, preserved in 10% formalin was processed subsequently in the Pathology department of RMC, for paraffin impregnation. The paraffin blocks were sectioned serially at 2-3 μm thickness and one slide stained with Hematoxylin & Eosin (H & E) and another with Periodic acid Schiff (PAS). The other specimen for direct immunofluorescence (DIF) study, preserved in normal saline, was immediately processed for frozen section. Sections were cut in a -20^0 C cooled chamber of cryostat (*Leica* CM 1100, Heidelberger Strasse, Nussloch, Germany) at a thickness of 4-5 micrometer and the sections were air dried and stained with FITC conjugated rabbit antisera against human IgG, IgM and IgA (Astra, Italy) separately. The sections were examined under immunofluorescence microscope (Carl Zeiss Axioster plus HBO 50 fluorescent lamp, Gottingen, Germany). All biopsy specimens were interpreted according to World Health Organization (WHO) classification[11]. Statistical analysis was made using the statistical package for social sciences.

Results

Among the 80 cases 34 were male (42.5%) and 46 were female (57.5%). The male-female ratio was 1: 1.35. The age range of patients was 13 to 70 years with mean age of 24.15 years. The age and sex distribution of the study patients are shown in Table 1.

Table- 1: Age and sex distribution of study group (according to decades).

Age groups in year	Male	%	Female	%	Total	%
11-20	14	17.50 %	11	13.75%	25	31.25%
21-30	07	08.75 %	23	28.75 %	30	37.50 %
31-40	04	05.00 %	08	10.00 %	12	15.00 %
41-50	05	06.25 %	03	03.75 %	08	10.00 %
51-60	04	05.00 %	00	00.00 %	04	05.00 %
61-70	00	00.00 %	01	01.75 %	01	01.25 %
Total	34	42.50%	46	57.50 %	80	100.00 %

The most frequent clinical presentation was nephrotic syndrome 50 (62.5%) followed by nephritic syndrome 12 (15%), haematuria 10 (12.5%) proteinuria 4 (5%), RPGN 2 (2.5%) and renal failure 2 (2.5%) cases (Table 2).

Table-2: Relation of various patterns of glomerulonephritis with clinical presentation:

Clinical presentation	Histomorphological pattern of glmerulonephritis											
	MCD	FSPGN	DMesGN	MGN	MPGN	FSGS	CSG/CGN	IgAN	Lupus N	Cres. GN	TOTAL	Percentage %
Nephrotic syndrome	07	14	09	06	02	05	02	01	03	01	50	62.50
Nephritic syndrome	01	04	03	01	02	--	--	01	--	--	12	15.00
Haematuria	01	02	02	--	--	02	--	03	--	--	10	12.50
Proteinuria	02	--	--	01	--	01	--	--	--	--	04	05.00
RPGN	--	--	--	--	--	--	--	--	01	01	02	02.50
Renal failure	--	--	--	--	--	--	01	--	01	--	02	02.50
Total	11	20	14	08	04	08	03	05	05	02	80	

RPGN= Rapidly Progressively Glomerulonephritis

Among the 80 cases, 13 (16.25%) cases were in the age group of 15 years or below and 67 (83.75%) cases were in the age group of above 15

years. The most frequent pattern of GN in the age group of 15 years or below was MCD 4 (5.00 %) followed by FSPGN 3 (3.75 %) whereas in age group above 15 years, it was FSPGN 17 (21.25%) followed by DMesPGN 12 (15%). Out of 80 cases, 73 (91.25%) were primary GN, the remaining 7 (8.75%) were due to systemic diseases and infection (Table 3).

Table 3: Histopathological pattern of glomerulonephritis in different age groups.

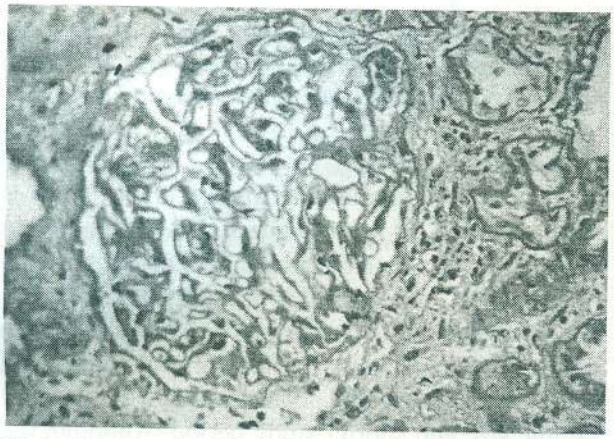
Types of GN	15 years or below				Above 15 years				Total	
	Male	Female	Total	%	Male	Female	Total	%	No	%
Primary GN										
MCD	02	02	04	5.00	04	03	07	8.75	11	13.75
FSPGN	01	02	03	3.75	05	12	17	21.25	20	25.00
DMesPGN	02	–	02	2.50	06	06	12	15.00	14	17.50
MGN	–	01	01	1.25	03	04	07	8.75	08	10.00
MPGN	–	01	01	1.25	02	01	03	3.75	03	3.75
FSGS	01	–	01	1.25	03	04	07	8.75	08	10.00
CSG/CGN	–	–	–	0.00	01	01	02	2.50	02	2.50
IgAN	–	–	–	0.00	02	03	05	6.25	05	6.25
Cres. GN	–	–	–	0.00	02	03	05	6.25	02	2.50
Secondary GN										
Lupus N	–	01	01	1.25	–	04	04	5.00	05	6.25
Amyloidosis (CSG)		–	–	–	01	–	01	1.25	01	1.25
HBV (MPGN)		–	–	–	01	–	01	–	01	1.25
Total	06	07	13	16.25	27	40	67	83.75	80	100

MCD= Minimal change disease; FSPGN= Focal segmental proliferative glomerulonephritis; DMesPGN= Diffuse mesangioproliferative glomerulo-nephritis; MGN= Membranous glomerulonephritis; MPGN= Membranoproliferative glomerulonephritis; FSGS= Focal segmental glomerulosclerosis; CSG= Chronic sclerosing glomerulonephritis; CGN= Chronic glomerulonephritis; IgAN= IgA nephropathy; Cres. GN= Crescentic glomerulonephritis.

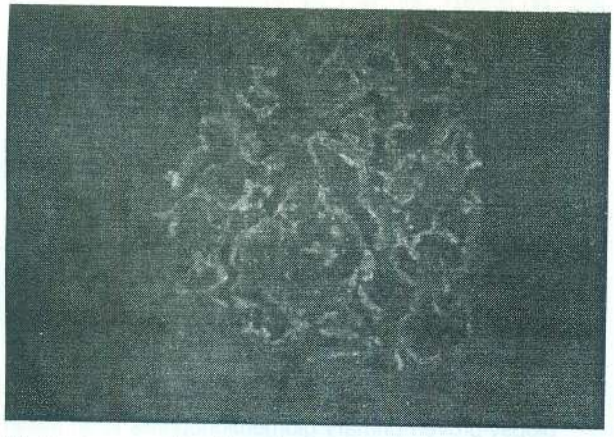
DIF study was done in 73 adequate cases, of which 46 (63.01%) cases DIF positive and 27 (36.98%) cases DIF negative. All of the MPGN, IgA nephropathy, Lupus nephritis and Cres. GN cases were DIF positive and none of the MCD and CGN showed positivity. About 91.66% of the DMesPGN, 55.55% of the FSPGN showed positivity. The most frequent combination of deposition of immunoglobulins were IgG + IgA , found in 10 (21.75) cases followed by IgG + IgA + IgM, in 06 (13.04%) cases, IgG + IgM, in 03 (6.25%) cases and IgA+ IgM, in 01 (02.17%) case respectively. The most frequent single deposition of immunoglobulin was IgG, found in 15 (41.30%) cases followed by IgA (Table-4).

Table 4: Results of histopathological findings with immunofluorescence microscopic findings.

Types of GN	Adequate DIF cases	IgG+ IgA+IgM		IgG+IgA		IgG+IgM		IgA+IgM		IgG		IgM		IgA		Positive	Negative
	No	No	%	No	%	No	%	No	%	No	%	No	%	No	%	%	%
Primary GN																	
MCD	11																100.00
FSPGN	18			01	(05.56)	01	(5.56)			06	(33.33)	02	(11.11)			55.56	44.44
DMesPGN	12	01	(08.33)	04	(33.33)					05	(41.66)	01	(8.33)			91.66	08.33
MGN	08	01	(12.50)	01	(12.50)					06	(75.00)					100.00	
MPGN	03	03	(100.00)													100.00	
FSGS	05											01	(12.50)			12.50	87.50
CSG/CGN	02																100.00
IgAN	05							01	(20.00)					04	(80.00)	100.00	
Cres. GN	02	01	(50.00)					01	(50.00)							100.00	
Secondary GN																	
Lupus N	05	01	(20.00)	01	(20.00)	01	(20.00)	01	(20.00)	01	(20.00)					100.00	
Amyloidosis (CSG)	01																100.00
HBV (MPGN)	01			01	(100.00)											100.00	
Total No of Cases	73	7		8		2		3		18		4		4		46	27



Photomicrograph of histological section showing thickening of basement membrane, a case of MGN.



Photomicrograph of a glomerulus of a case of IgA nephropathy showing fluorescence in the mesangium mild to moderate degree deposition of IgA.

Discussion

Primary glomerular disease (PGD) comprised 73 (91.25%) and secondary GN 7 (8.75%) of all the cases of glomerulonephritis in the present study and maximum number of cases 30 (37.5%) were found in age group 21-30 years. The most common clinical presentation was nephrotic syndrome (62.5%) followed by nephritic syndrome (15%). In a study on Bangladeshi population, Hossain (2000)[7] showed 59.1% cases as nephrotic syndrome. The most frequently diagnosed lesion in our study was MCD 4 (5.00 %) in the age group of 15 years or below, where as in the above 15 years age group it was FSPGN 17 (21.25%). In a study Nabiruddin et al (2001) [12] showed that FSPGN (23.49%) was the commonest pattern of GN in Bangladesh followed by MesPGN (16%). Present study showed that among 73 adequate cases for DIF, 46 (63.02%) cases were found to be DIF positive. But 50% DIF positive cases were observed by other two studies done in Bangladesh [13,7].

This study showed that the most frequent combination of deposition of different types of immunoglobulins were IgG + IgA, in 10 (21.75%), followed by IgG+ IgA+IgM, in 06 (13.04%), IgG + IgM, in 03 (06.52%) and IgA+IgM, in 01 (02.17%) respectively. It was also observed that IgG alone and in various combinations was found in 38 (82.60 %) cases (highest deposits). The highest frequency of IgG deposition in the mesangium of glomeruli strongly suggests evidence of immune complex deposition. In a study Tabassum (1988)[14] showed, deposition of IgG was found in 73.33% cases.

In the present study FSPGN was 20 (25.00%), most frequent pattern of glomerulonephritis in all age group. The highest incidence of FSPGN, 8 (10%) was in the 11-20 age group. Among them, 10 (55.55%) were DIF positive and showed focal and granular deposits of IgG in the mesangium. Tabassum (1988)[14] and Sharmin (1994)[13] showed similar DIF positivity (60% and 50%). In present study, DMesPGN, 14 (17.50%) was the second most common pattern of glomerulonephritis and highest incidence in 21-30 age group. Among them 11(91.66%) cases showed DIF positivity and deposit was IgG found in 10 (90.99%) cases in various combinations. In one case, there was deposition of IgM in the mesangium. Among the study group, 11 (10%) cases were diagnosed as minimal change disease and all were found to be DIF negative. In the present study, 8(10%) MGN cases were found. In Bangladesh, Rahman et al (1984) [15] showed that the incidence of MGN was 9%. In this study 100% of MGN showed DIF positivity in the BMZ and IgG was common in all cases along with IgA in two cases and IgM in one case. Similarly Hossain (2000)[7] and Rahman (2006)[16] showed 100% DIF positivity with predominance of IgG deposition in the BMZ. Among 4 cases of MPGN, three showed "Full house" deposition of IgG, IgM & IgA (75%) and one showed depositions of IgG & IgA (25%) in

the mesangium and BMZ. In the present study only 2 (2.73%) cases were histopathologically diagnosed as Crescentic GN. One of them was presented with nephrotic and other with RPGN. In DIF study, one case showed "full house" deposition and other showed IgG & IgM in mesangium and BMZ. In this study, among 8 cases of FSGS only one case showed mild deposition of IgM (20%) in DIF. Nabiruddin (1996)[17] showed 10% DIF positivity. Among 3 cases of CGN, no one showed any deposition of immunoglobulin. IgA nephropathy was found in 5 (6.25%) cases, which were very similar to other studies done by different Bangladeshi authors [Rashid et al (2003)[18] - 7% and Rahman (2006)[16], - 4.67%]. The most frequent pattern of glomerulonephritis in IgA nephropathy was MesPGN 3 (60%). Only IgA was found in 80% cases and IgA+IgM combination was found in rest 20% cases. It is the most common form of primary glomerular disease in Asia, accounting for up to 30% to 40% of all biopsies, for 20% in Europe, and for 10% in North America [19]. Incidence of IgAN in our country is very much lower (4.67%-7%) in comparison to other Asian countries (Singapore, China, Taiwan, up to 50%) may be explained by varying approaches to the use of renal biopsy in patient with mild urinary abnormalities such as asymptomatic microscopic haematuria [20]. Among the secondary causes of glomerulonephritis, lupus nephritis accounted for the majority of cases (5/7).

Histologically two cases were diagnosed as diffuse MPGN consistent with Lupus nephritis class IV, two as DMesPGN consistent with Lupus nephritis class IV and the remaining one diagnosed as chronic sclerosing GN consistent with Lupus nephritis class VI. One case (20%) showed "full house" granular deposition of immunoglobins (IgG+IgA+IgM) in mesangium from mild to moderate intensity. Remaining four showed granular deposition of IgG in all cases along with IgA in two cases and one with IgM. All cases the depositions were in the mesangium and in two cases also in the BMZ. In a study Rahman (2006)[16] showed 55.55% cases of "full-house" immune deposits and 22.22% cases of two types (IgG+ IgA or IgG + IgM) immune deposits and 11.11% cases of single type immune deposits. Amyloidosis was noted in one case, which represents 2.4% of all the biopsy specimens.

Conclusion

The pattern of glomerular disease in Rajshahi is similar to that of other studies done in home but differ from the western world. This difference may be possibly due to difference in predisposing factors, pathogenic mechanism and disease pattern in western world. This is not an epidemiological study, rather hospital based study with small sample size and shorter study period. Although the result is quite significant in comparison to other studies, but large scale study should be carried out for a longer period and a national registry for glomerulonephritis should be established for better planning and management of glomerular diseases.

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