Original Article

Immunohistochemical Analysis of Glomerular Phospholipase A2 Receptor in Primary and Secondary Membranous Glomerulonephritis

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Abstract

Background: A common cause of adult nephrotic syndrome (NS) is membranous glomerulonephritis (MGN). The larger portion of MGN in adults is the idiopathic/primary MGN (iMGN). So many conditions like autoimmunity, malignant tumor, drugs and infections can cause a secondary form of MGN. We can diagnose primary/ idiopathic MGN after the omission of all known secondary causes. As we know M-type phospholipase A2 receptor (PLA2R) on podocytes is the target antigen in the pathogenesis of primary MGN. Phospholipase A2 receptor (PLA2R) can be detected by immunohistochemically. Differences between primary MGN (iMGN) and secondary MGN are essential because of treatment implications.

Objective: To evaluate the histopathological pattern of MGN and to correlate with PLA2R deposition in primary and secondary MGN in a group of Bangladeshi people.

Methods: The research method is descriptive cross-sectional and observational. The study population was 60 patients in the department of Histopathology, AFIP, Dhaka Cantonment, Dhaka for duration of six months period from January 2019 to June 2019. At first, adequate clinical information was taken and categorized as primary and secondary MGN on clinical basis, thereafter immunohistochemistry of anti-PLA2R was done from formalin-fixed paraffin block of those cases.

Results: A total of 60 diagnosed cases of MGN, 56 patients were found primary, and 04 were secondary based on clinical data. In this study, all primary MGN was found as PLA2R positive.

Conclusion: The present study exhibits that immunohistochemistry with anti-PLA2R antibodies can be the best diagnostic method for diagnosis of idiopathic MGN which offers an opportunity for personalized medicine.

Keywords: Membranous glomerulonephritis, Phospholipase A2 receptor, Immunohistochemistry, Nephrotic syndrome, Direct immunofluorescence.

Introduction

Subepithelial immune deposition in membranous glomerulonephritis (MGN), can induce an extent of changes in the glomerular basement membrane (GBM).[1,2] In adult causes of nephrotic syndrome due to idiopathic MGN, the worldwide incidence is 20-30% and in children, it is 1-9%.[3] Differentiation of primary and secondary MGN is very important for the clinical management of the patient. Idiopathic MGN is more common than secondary MGN. The autoimmune basis of primary human MGN appears to be largely and it is approximately 70%-80% with in situ glomerular immune complex formation. Primary MGN patients have auto-antibodies and it is directed against PLA2R expressed in podocytes and proximal tubules.[3] The model of Heymann nephritis has been thoroughly studied as a model of MGN. Heymann's nephritis model was first described in 1959 and played a vital role in the identification of suspected antigens.[4] Thrombospondin type-1 domain–containing 7A (THSD7A) is another recently described second podocyte antigen which is also responsible for primary MGN and comprises approximately 5%–10%. In patients with antenatal MGN, neutral endopeptidase (NEP) has been identified as the target antigen of antibodies deposited in the subepithelial space. Approximately sixty to eighty percent of MGN patients presented with features of nephrotic syndrome. Macroscopic hematuria is rare in MGN.[3] Hypertension is usually found when renal insufficiency has developed.[5] GBM changes in MGN are homogeneous, typically showing little variation among glomeruli in renal biopsy specimens of MGN. The light microscopic examination can demonstrate the pathologic spectrum of glomerular capillary wall changes but it is best understood and staged at the ultrastructural level. Immunofluorescence and electron microscopy (EM) easily establish the diagnosis. All stages of MGN reveal a generalized, peripheral granular pattern of deposition of IgG, C3, Kappa and Lambda, sometimes with IgM in immunofluorescence microscopic study. Up to now, idiopathic MGN has been diagnosed by the omission of secondary causes along with detailed history, physical examination and laboratory studies. Tissue evaluation of idiopathic MGN, by using antiphospholipase A2 receptor (PLA2R) antibody can be done by immunohistochemistry (IHC).[6]

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Little work has been done on renal pathology in our country. This type of study, especially on MGN and focusing on IHC detection of target antigen in iMGN, has not been done previously in Bangladesh. Diagnosis is usually confirmed by histopathological examination (morphology) of the specimen, along with pattern of immune deposition (DIF & IHC). The result of this study can provide an insight in pathological diagnosis of iMGN in a group of people in our country which will help physician about treatment and patient outcome.

Materials and Methods

This was a descriptive cross-sectional observational study undertaken in the department of histopathology, Armed Forces Institute of Pathology, Dhaka Cantonment, Dhaka from January 2019 to June 2019. Study population was sixty (60) renal biopsy proven specimen of membranous glomerulonephritis patients with five cases of minimal change disease taken as control.

Inclusion criteria: Patient diagnosed as a case of membranous glomerulonephritis with adequate renal tissue available for IHC.

Exclusion criteria:

1. Patients who would refuse to be included in this study.
2. Known to be hypertensive and diabetic patient.
3. Patients known to have other glomerulonephritis.

Initially light microscopy (LM) of renal biopsies was assessed with the help of Hematoxylin and Eosin, Periodic Acid-Schiff, Massion Trichrome and Silver methenamine stain. The findings studied on LM were thickening of glomerular basement membrane (GBM), presence/absence of spikes and crater, segmental sclerosis, mesangial hyper cellularity, and chronic tubulointerstitial changes including tubular atrophy and interstitial fibrosis presented as chronicity in cortical core. Then direct immunofluorescence (DIF) study was done. Finally immunohistochemistry was done using PLA2R antibody (Thermofisher, MA5-24608, 1:200-1:500 dilution) and interpreted as follows[7]:

- PLA2R positive = Granular staining along basement membrane alone; absent in podocyte.
- PLA2R negative = Staining present in podocytes; absent along basement membrane.
- PLA2R equivocal = Staining present both along basement membrane as well as in podocytes.

All the cases were correlated clinically and diagnosed on clinical basis as either iMGN or sMGN and then findings were correlated with IHC findings.

Results

In this study PLA2R positive MGN was 56 and PLA2R negative MGN was 04 shown in Table-I. PLA2R negative cases were all stage-v Lupus nephritis. Among the PLA2R positive cases 36 were male and 20 were female. Male to female ratio was 1.8:1. PLA2R negative cases shown 1 male and 3 female patients with male to female ratio of 0.33:1 (Table-II).

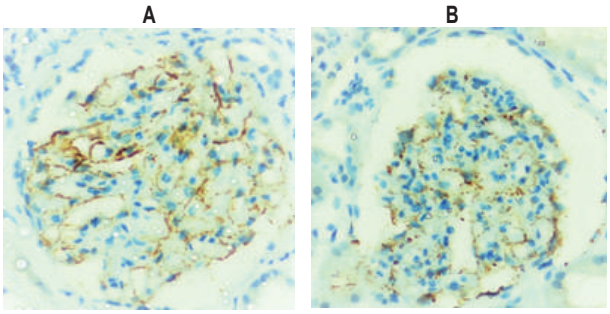

Figure-1: Immunohistochemical glomerular expression of PLA2R positive cases (A & B)

Table-I: Frequency distribution of PLA2R positive and PLA2R negative MGN cases in primary and secondary MGN

Membranous glomerulonephritis	PLA2R positive	PLA2R negative	P value
Primary MGN	56	0	<0.001
Secondary MGN	0	4	

Table-II: Gender wise frequency distribution of MGN

Variables	Male	Female	Ratio
PLA2R positive MGN	36	20	1.8:1
PLA2R negative MGN	01	03	0.33:1

It was observed out of 60 patients, 18 patients (30%) belonged to age group 31-40 years followed by 15 patients (25%) belonged to age group 41-50 years; mean age of patient was 36.45±8.5 years with a range of 13 to 72 years. So, more than half of the patients in this study were between 31-50 years (Table-III). Out of 60 patients 37 were male (61.66%) and 23 were female (38.33%). Male to female ratio was 1.6:1 (Figure-2).

Table-III: Distribution of the study population by age (n=60)

Age (in years)	number	Percentage
≤ 20	06	10
21-30	12	20
31-40	18	30
41-50	15	25
≥ 50	09	15
Mean ± SD	36.45±8.5	
Range (min-max)	(13-72)	

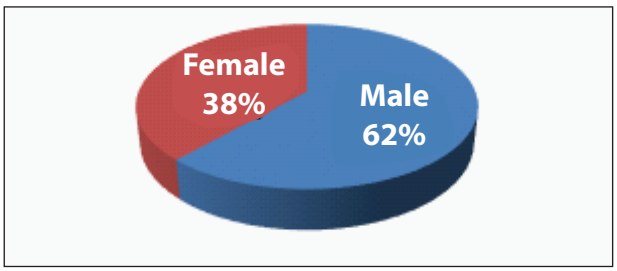

Figure-2: Sex distribution of the patients, study population (n=60)

It was observed that almost half of the (45.00%) patients had oedema followed by 14(23.33%) had raised BP, 12 (20%) had microhaematuria and 07(11.66%) had oliguria. In this study, 50 patients (83.33%) were presented with nephrotic syndrome, 6(10%) with nephritonephrotic syndrome and rest 4 patients (6.66%) with nephritic syndrome. Table-IV shows the clinical characteristics of the study population. In light microscopic study shows GBM thickening was present in all 4 cases of PLA2R negative and 50 cases of PLA2R positive MGN. DIF study revealed IgG deposit in all 60 cases whereas none showed IgA deposit. We have observed higher sensitivity and specificity of PLA2R by IHC on renal biopsies for the diagnosis of primary MGN (Table-V).

Table-IV: Clinical characteristics of the patients with PLA2R positive and PLA2R negative MGN

Variables	PLA2R Positive MGN	PLA2R Negative MGN	p values
Number of patients	56	04	<0.001
Mean age, years	36.45	41.5	0.11
Male: Female	1.8:1	0.33:1	0.002
Mean serum creatinine, mg/dl	1.8	1.2	0.044
Mean UTP (Gm/24 hrs)	6.26	3.28	0.038
Mean serum cholesterol, mg/dl	382	220	<0.001
Mean serum albumin, mg/dl	2.7	3.2	0.028
Raised blood pressure (yes/no)	13	01	<0.001

Table-V: Comparison review of previous studies on PLA2R on renal biopsy

First author (Ref.), year	Subjects n	Assay Method	Sensitivity (%)	Specificity (%)
Larsen (11), 2013	165	IIF	75	83.0
Segarra-Medrano (15), 2014	64	IHC	76	94.0
Hoxa (16), 2012	88	IHC	84	100
Svobodova (18), 2014	84	IIF	80	84.0
Barrett (19), 2014	07	IIF	67	100
Gasim (20), 2014	41	IIF	77	94.0

Discussion

Membranous glomerulonephritis in adult is the most common etiologies of idiopathic nephrotic syndrome. One-third of patients can develop end-stage renal disease in MGN.[8,9] Treatment modalities of MGN depend upon whether the disease is primary or secondary. Immunosuppression regimens are practiced for the treatment of primary membranous glomerulonephritis. The immunosuppressive regimen can cause significant treatment complications. Therapeutic management of underlying diseases can cure secondary membranous glomerulonephritis.[10] Clinically we can differentiate primary and secondary membranous glomerulonephritis after a thorough evaluation. This study was carried out to give an overview of the demography, incidence and immunomorphological pattern of membranous glomerulonephritis. We here studied 60 cases of renal biopsy-proven MGN over 06 (six) months.

Here we get 56 patients who were primary MGN (PLA2R positive) and 4 patients who were secondary MGN (PLA2R negative). The findings are clinically significant (P-value is <0.001). Out of sixty studied subjects 37 were male (61.66%) and 23(38.33%) were female with a male-female ratio of 1.6:1. Present study reveals males are affected more than females. Similar male prevalence is reported in various other studies conducted in different parts of the world.[7,11] Age range of the study was from 13 years to 72 years. More than fifty percent of patients in this study were between 31-50 years. The mean age of all patients was found 36.45±8.5 years. This present study indicates that MGN was most frequent in the 4th and 5th decades. Gudipati et al also reported almost the same mean age and most prevalent decade.[7] Clinically most of the patients presented with features of nephrotic syndrome and it is adult onset. The present study showed a statistically significant difference between clinical features of primary and secondary MGN. Massive proteinuria, impairment of renal function, hypoalbuminemia, hyper-cholesterolemia and raised BP were more marked in primary MGN (p<0.05) (Table-IV). A Light microscopic study was done and analysis of glomerular, tubulointerstitial, and vascular compartments was carried out. Cases of secondary MGN show more segmental sclerosis and mesangial hypercellularity in comparison to idiopathic MGN. No noteworthy difference is observed in tubular atrophy/ interstitial fibrosis between primary and secondary MGN. A Comparison of DIF findings reveals no striking differences between the two types of MGN. A good number of studies were done on serological testing for PLA2R antibodies for the detection of primary MGN with varying sensitivity and specificity. Serum titre of the immune complex can fluctuate, that's why serologic diagnosis may not always be workable and suitable.[12,13] In comparison to tissue and serum, the sensitivity of PLA2R is higher in tissue sections. Some studies also reported sensitivities of the serum and biopsy tests to be 57% and 74% respectively.[14] As there is no workability of serologic diagnosis in our country we studied only the IHC expression of PLA2R on renal biopsy sample. If we can do both together it could allow us to do a comparative study.

A comparative study from Spain[15] showed that the sensitivity of PLA2R IHC was 76.6%, comparable to 72.3% using IIF and 74.5% using ELISA. PLA2R IHC has been found to have a sensitivity ranging from 84%[16] to 77%[15] with a specificity of 100%[16] to 94%[15] for the differentiation of primary from secondary MGN. To the best of the knowledge no data exist related to its efficiency and validation as a diagnostic tool in Bangladesh. The majority of the tissue based studies about PLA2R are DIF assays performed on paraffin blocks. However these studies have shown to be technically cumbersome with requirement of special equipment like a fluorescence microscope. Another disadvantage of DIF based

test is that the stain fades away with the passage of time. Assandri et al in their review on PLA2R have mentioned the limitations of DIF studies for PLA2R including difficulties in standardization and interpretative errors.[17] In comparison IHC is more pathologist friendly and cost effective technique which is widely available across the globe in majority of the laboratories as well as most of the laboratories deal with renal biopsy samples in Bangladesh. Hence studies of PLA2R can be carried out by IHC on tissues in our country.

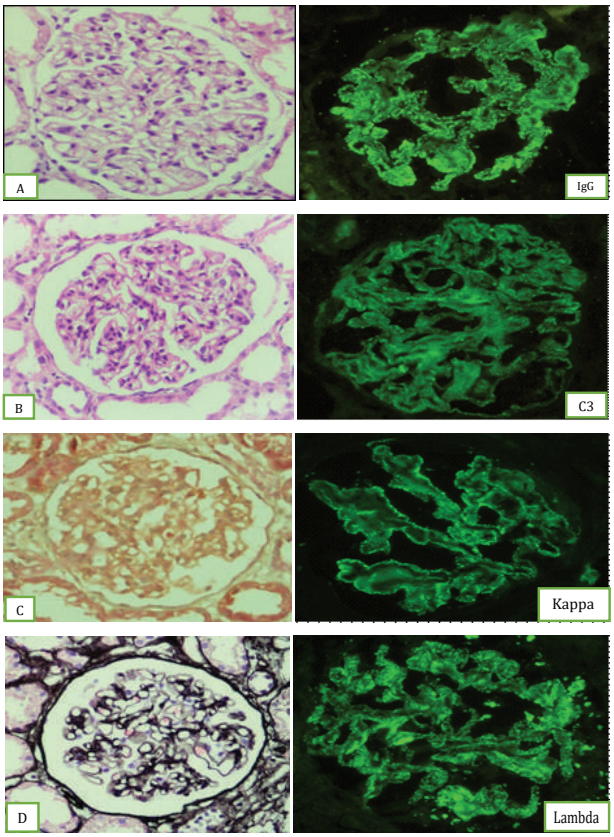

Figure-3: Light microscopic and direct immunofluorescence study of a case of Membranous Glomerulonephritis.

Clinical note: Female 37 years presented with nephrotic range of proteinuria.

Lab Inv: Urine alb-3+, RBC-nil /HPF, UTP-7.5 Gm/L, Serum albumin-2.3 Gm/L, Serum cholesterol 588 mg/dl, Serum creatinine-1.8 mg/dl, ANA-negative, HbsAg- negative.

Histopathologic findings: Basement membrane of glomerulus appears thickened. Mesangial cellularity and matrix appears normal. Crater formation is present. (A) H&E, (B) PAS, (C) Masson's trichrome and (D) Silver stain.

DIF: IgG (3+), C3 (1+), Kappa (2+) and Lambda (3+) in the mesangium. No IgA, IgM or C1q deposit is seen.

Histologic diagnosis: Membranous Glomerulonephritis.

Conclusion

Anti-PLA2R antigen is a specific marker to distinguish primary MGN from secondary MGN and its detection can be done serologically or in the biopsied tissue by IHC or IF study. One of the biggest advantages of IHC over DIF is the fact that IHC staining is permanent and does not fade like DIF staining does. Negative IHC expression of PLA2R should warrant necessary investigations for a secondary cause with close follow-up. To the best of the knowledge, this is the first study in Bangladesh about IHC expression of PLA2R in MGN. However being with a very small cohort many correlation with the histology as well as clinical features may not be representative of the country. A large study including both PLA2R and THSD7A might be a good future scope for the researcher of Bangladesh.

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