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Short Communication

Evaluation of cases of membranoproliferative glomerulonephritis according to newer classification: A retrospective record-based study

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ABSTRACT

Background: Membranoproliferative glomerulonephritis (MPGN) has traditionally been classified on electron microscopy (EM) into different types based on the location of the immune complexes. Sethi et al. subsequently suggested a more relevant etiology-based and clinically useful classification based on immunofluorescence.

Methods: In this retrospective study, 18 diagnosed cases of MPGN over a one-year period for which direct immunofluorescence (DIF) study results were available, were selected. Cases without archived records of immunofluorescence photographs/reports were excluded. Histological diagnosis of MPGN was confirmed and DIF results were analyzed with reference to antibodies to IgG, IgA, IgM, C3, C1q, kappa, and lambda light chains.

Results: Evaluation of cases revealed 8 males and 10 females with age range from 11 to 66 years. Fifteen cases presented with nephrotic syndrome. On evaluation, 88.89% cases (16/18) were immune complex mediated while two (11.11%) were of complement mediated type of MPGN. Among immune complex-mediated cases, a single case of monoclonal gammopathy associated or light chain mediated MPGN was present.

Conclusion: The classification described by Sethi et al. is easy to use since it relies on DIF instead of EM which is not readily available. Most of the cases were immune complex mediated whereas incidence of complement mediated MPGN, that is, C3 glomerulopathy was low (11.11%). Application of the new classification allows more relevant categorization of cases based on etiology and without the requirement of EM.

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Introduction

Membranoproliferative glomerulonephritis (MPGN) is a heterogeneous disease entity defined by the morphological pattern of injury that can be seen in various disease conditions sharing common pathogenic mechanisms. It constitutes an important cause of nephrotic syndrome. The exact incidence in the Indian context is not available due to the absence of renal registries. However, individual institution-based studies show incidence rate between 3.7% and 17.9% of all renal biopsies which are comparable to those of western literature.^{1,2} The traditional classification of MPGN divides it into three categories (Types I, II, and III) based on morphology and electron microscopic demonstration of deposits at specific locations. However, the actual etiology of the disease, which can guide proper management of the patient, is not specified using electron microscopy alone. Recently, Sethi et al.³ described an etiology-based newer classification which divides MPGN into immune complex mediated and complement mediated type of MPGN which can be demonstrated using direct immunofluorescence (DIF) studies.^{3,4} Newer classification emphasizes the underlying etiopathogenesis rather than just providing morphological details unlike the older classification. However, the exact prognostic implications are not known.

We carried out a retrospective study to categorize known cases of MPGN into the newer classification and to investigate the incidence of C3 glomerulopathies, that is, complement mediated MPGN.

Methods and material

This was a retrospective record-based study in which we re-evaluated the renal biopsies of MPGN cases received at the department of pathology of a tertiary care hospital over a period of one year. Relevant clinical data was also retrieved from the archives. All cases diagnosed as MPGN and for whom DIF study results (as reports/archived images) were available, were included in the study. Cases without archival records of immunofluorescence reports or photographs were excluded. The total sample size was 18 cases. In all existing slides, renal biopsy had been obtained in two tissue cores, one for light microscopy and one for immunofluorescence. The routine formalin-fixed paraffin embedded tissue was used for light microscopy. Three-micron thick sections were cut and stained with Hematoxylin and Eosin (H&E), Periodic acid Schiff, Masson Trichrome, and Periodic acid Schiff methanamine silver. Tissue

cores for immunofluorescence were stained with antibodies to IgG, IgA, IgM, C3, C1q, kappa, and lambda light chains. Since electron microscopic evaluation was not available, cases were classified as MPGN only. No subclassification based on location of deposits at ultrastructural level was done. Histopathology slides (H&E and all special stains) as well as archival images were reviewed and analysis was done in the following steps:

1. Confirmation of MPGN on light microscopy.
2. Evaluation of DIF images and slides.
3. Reclassification into immune complex mediated and complement mediated MPGN as per Sethi et al.

The histological criteria used to establish the MPGN pattern were as suggested by Zhou et al.⁵ After reviewing the pathological findings and DIF patterns, we classified all the cases into two categories – that is, immune complex mediated and complement mediated MPGN (C3 glomerulopathy) according to newer classification by Sethi et al.³

The cases showing immunoglobulin positivity as well as complement positivity were classified as immune complex mediated while cases showing exclusive C3 complement staining were classified as complement mediated MPGN. There was no follow-up data available. Diagnosis of both the types of classification was compared with each other and with clinical findings.

Results

Of the 18 cases, 10 were female while 8 were male with M:F ratio of 0.8:1. The mean age of all cases was 35 years with range of 11–66 years. In the clinical presentation, 15 cases presented with features of nephrotic syndrome while one case each presented with nephritic syndrome, nephritic-nephrotic picture, and subnephrotic proteinuria (Table 1). Four cases presented with associated hypertension. A single case was hepatitis B positive. Other markers such as anti-hepatitis C antibody (HCV Ab), antinuclear antibodies and anti-dsDNA were absent in all of the cases.

On reclassification by the new system, out of the total of 18 cases, 2 were reclassified as C3 glomerulopathy (11.11%) based on review of immunofluorescence images. The remaining 16 cases were reclassified as MPGN, immune complex mediated (Fig. 1). One of these cases showed Kappa restriction on DIF and was subclassified as proliferative glomerulonephritis with monoclonal immunoglobulin deposits. Further workup in this case established the diagnosis of plasma cell myeloma.

Table 1 – Comparison of presentation of cases in both traditional and newer classification of MPGN.

	Nephrotic syndrome	Nephritic syndrome	Mixed nephrotic and nephritic picture	Subnephrotic proteinuria	Total cases
Older classification MPGN	15	01	01	01	18
Newer classification					
Immune complex mediated MPGN	13	01	01	01	16
Complement mediated MPGN	02	00	00	00	02

Fig. 1 – (A) Photomicrograph of kidney biopsy at scanner magnification showing multiple enlarged, cellular glomeruli (PAS, 10×). **(B)** Photomicrograph of an enlarged glomerulus with accentuated lobulation showing endocapillary proliferation (horizontal arrow) and splitting of capillary walls (vertical arrow) (PAS, 40×). **(C)** Photomicrograph of glomerulus stained with antibody to IgG showing coarse granular deposition in glomerular capillary walls (horizontal arrow), and mesangium (vertical arrow) (DIF, IgG, 40×). **(D)** Photomicrograph of glomerulus stained with antibody to C3 showing coarse granular deposition in glomerular capillary walls (horizontal arrow), and mesangium (vertical arrow) (DIF, C3, 40×).

Discussion

The field of Pathology mainly deals with evaluation of diseases at gross, microscopic, ultrastructural, and molecular levels to establish a firm diagnosis which would allow appropriate therapy for the patient. A “classification” provides ease and increases the comfort level of a pathologist in such evaluation of a tissue since he or she is able to “categorize” or “slot” the disease into a fixed category. The therapeutic response for the disease, the long term outcome and prognosis, would to a large extent now be based on the place it occupies in the classification system. This of course is an “ideal” situation where the classification system takes into account all aspects of the biology of the disease in order to dictate therapy and to prognosticate.

A classification system, which is useful for both clinicians and pathologists, is well accepted. The traditional older classification of MPGN was solely based on ultrastructural findings of electron dense deposits. Type I (primary/idiopathic) MPGN was defined by the presence of subendothelial deposits whereas Type III (secondary) MPGN showed both subendothelial as well as subepithelial deposits. Type II MPGN included dense deposit disease having intramembranous electron dense deposits. However, this classification failed to pinpoint the exact underlying etiology or the mechanism of injury leading to these patterns. Sethi et al.³ reclassified MPGN into

immune complex mediated and complement mediated types based on underlying mechanism of injury. Immune complex mediated injury can be due to various etiologies such as infections, especially hepatitis virus, autoimmune diseases including systemic lupus erythematosus, rheumatoid arthritis, Sjögren's syndrome, etc. and monoclonal gammopathies including monoclonal gammopathy of undetermined significance (MGUS) (the most common condition), multiple myeloma, low-grade B-cell lymphoma, chronic lymphocytic leukemia, and lymphoplasmacytic lymphoma.⁶ In immune complex mediated MPGN, immune complexes or immunoglobulins deposits formed as result of above-mentioned conditions, in mesangium or subendothelial sites leading to injury. This involves classical complement pathways. Thus, renal biopsies show typically both immunoglobulins as well as complement on DIF studies. While in complement mediated MPGN, dysregulation of alternate complement pathway forming activated products such as complement C3b and terminal complement factors is the major cause of injury. These activated products are deposited over endothelial surface leading to glomerular injury and subsequently MPGN. These events do not directly involve the immunoglobulins; thus, this type shows exclusive complement positivity on immunofluorescence studies.⁴

C3 glomerulopathies form a major part of the complement mediated MPGN. The concept of C3 glomerulopathy was first introduced by Servais et al.⁷ C3 glomerulopathies are rare

disorders encompassing various entities such as dense deposit disease, idiopathic C3 glomerulonephritis, MPGN Type I with isolated subendothelial C3 complement deposits, familial MPGN Type III, CFHR5 nephropathy (familial C3 glomerulonephritis associated with heterozygous mutation in CFHR5).^{7,8}

Our study showed incidence of complement mediated MPGN among all MPGN cases as 11.11%. Similar Indian study conducted by Himamani⁹ showed incidence of 14% among all MPGN cases. While the study conducted by Woo et al.¹⁰ showed incidence of 4.3% which was significantly lower than our study. However, overall incidence of complement mediated MPGN was not calculated among all renal biopsies received for histopathological diagnosis. Just to mention incidence of complement mediated MPGN cases, Viswanathan et al.¹¹ and Mathur et al.¹² calculated incidences of complement mediated MPGN in all renal biopsies which were 0.7% and 1.16% respectively.

The major advantage of the newer classification is that it gives better insight into the management of the cases of MPGN. It utilizes DIF studies with routine markers, which are readily available in nephrology/nephropathology centers. Classifying cases based on immunofluorescence results is much easier than using electron microscopy, which is not available in most centers. The role of electron microscopy today appears to be considerably reduced in this category of disorders except for the diagnosis of dense deposit disease where it plays an important part.

Cases with immune complex mediated MPGN are further investigated for underlying chronic inflammatory condition, monoclonal gammopathies as well as autoimmune disease.⁴ Sethi et al.⁶ further subclassified MPGN into monoclonal gammopathy associated MPGN based on underlying etiology. Cases with complement mediated MPGN should be investigated for abnormalities of the alternate complement pathway. Tests such as serum membrane attack complex levels, an alternate pathway functional assay, hemolytic assay as well as tests for autoantibodies and mutations to complement-regulating proteins should be carried out.⁴ In therapeutics, complement mediated type is important as therapies can be developed targeting complement as potential disease-modifying agents. Mainly, C3 convertase and C5 are considered as potential targets. Eculizumab is one of the available anti-complement therapies, which is a monoclonal antibody that prevents activation of C5 by creating block in complement cascade and can be useful in these disorders. Bomback et al.¹³ demonstrated promising results using eculizumab in C3 glomerulopathies in native and allograft biopsies.¹³ Hence, this newer classification can guide appropriate management.

The major limitation of the study was that no electron microscopy was available to classify cases according to older classification. Sample size was limited with no follow-up data to prognosticate two types of MPGN. No work-up regarding evaluation of alternate pathway abnormalities was available, as it is not done routinely in this lab. However, incidence of

11.11% of C3 nephropathy among all diagnosed cases of MPGN was noted. To conclude, the usefulness of this newer classification should be further investigated with additional prognostic factors and novel therapeutic approaches.

Conflicts of interest

The authors have none to declare.

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