

## The incidence of possible causes of membranoproliferative glomerulonephritis: a single-center experience

Pavinic J, Miglinas M

Nephrology Center of Vilnius University, Vilnius, Lithuania

### Abstract

**Background:** Diagnosis of membranoproliferative glomerulonephritis (MPGN) is based on kidney biopsy findings: unique glomerular injury pattern and characteristic changes on light, electron microscopy and immunohistochemical analysis. The purpose of this study was to identify possible etiology and incidence of glomerular injury among patients with a diagnosed MPGN.

**Materials and Methods:** A retrospective analysis (years 2000-2014) of 81 clinical cases with a diagnosis of MPGN based on biopsy results was performed. Records were examined, and data about viral, bacterial infections, autoimmune and hematological diseases was collected. Test results of blood C3 and C4 factors of the complement system, and results of kidney biopsy immunohistochemical analysis were investigated. Statistical analysis was performed using Statistical Package for the Social Sciences and p-value less than 0.05 was considered statistically significant.

**Results:** Study population consisted of 55 males (67.9%) and 26 females (32.1%). The average patients' age was 48.53 (standard deviation  $\pm$  16.67) years. The identified etiology of MPGN was: idiopathic in 26 cases (32.10%), bacterial infections in 20 cases (24.69%), viral hepatitis in 16 cases (19.75%), autoimmune diseases in 11 cases (13.58%), and hematological diseases in eight cases (9.88%). Changes of the concentration of complement component C3 as well as component C4 were found; their concentration was decreased in 26 (32.1%) and 17 (20.99%) patients' respectively while concentration was within the normal range in 11 (13.58%) and 19 (23.46%) patients respectively. Immunohistochemistry results revealed immunoglobulin (Ig) deposits: C3+/Ig+ was found in 47 (58.02%) cases, C3-/Ig+ was found in 16 (19.75%) cases and in six (7.41%) cases test was not performed. The total number of immunoglobulin positive biopsies (C3+/Ig+ and C3-/Ig+, also called immune-complex mediated MPGN) was 63 (77.78%). Complement-mediated MPGN (C3+/Ig-) was less common and was diagnosed only in seven cases (8.64%). C3-/Ig- was found in five cases (6.17%).

**Conclusions:** The leading cause of MPGN was idiopathic as well as bacterial infections. Complement component C3 concentration was mostly decreased. The incidence of normal and decreased concentration of the complement component C4 was almost equal. Most immunohistochemical deposits in kidney biopsy appeared to be C3/Ig positive, and it was observed in more than half of the cases of each MPGN etiological group. Hippokratia 2015; 19 (4): 314-318.

**Keywords:** Membranoproliferative glomerulonephritis, immunohistochemistry, complement system, kidney biopsy

**Corresponding author:** Pavinic J, Nephrology Center of Vilnius University, Santariskiu st. 2, LT-08661, Vilnius, Lithuania, e-mail: jelena.pavinic@gmail.com

### Introduction

Diagnosis of membranoproliferative glomerulonephritis (MPGN) is based on kidney biopsy findings, unique glomerular injury pattern and characteristic changes on light microscopy, immunohistochemistry and electron microscopy<sup>1-4</sup>.

MPGN clinical classification divides cases of the disease into idiopathic (primary) and secondary types. In case of secondary MPGN the cause of disease could be identified (infections, hematological or autoimmune diseases) while in primary MPGN no such direct etiology is present. Secondary type is more common and is diagnosed by reviewing clinical features, laboratory test results, and renal histopathological injury<sup>1-8</sup>.

Over the past decades, improved understanding of

the pathogenesis of glomerular diseases has led to the progress in MPGN classification based on immunofluorescence microscopy. MPGN may be either immune complex or complement-mediated<sup>2-6</sup>.

The most common type of glomerular injury in MPGN is immune complex-mediated. The pathogenesis involves persistent antigenemia, which causes the deposition of circulating immune complexes in the glomeruli<sup>1-5</sup>. Chronic antigenemia (or circulating immune complexes) is a result of chronic bacterial or viral infections<sup>1-5</sup>, autoimmune diseases or monoclonal gammopathies<sup>9-21</sup>. Immunofluorescence staining reveals the type of immunoglobulin (Ig) and complement component.

Immunohistochemical analysis showing dominant C3 deposition without Ig points to the complement-mediated

MPGN, also called C3 glomerulopathy. C3 glomerulopathy is divided into two main histopathological groups: dense deposit disease (DDD) and C3 glomerulonephritis (C3GN). The difference between these disorders is determined by characteristic changes on electron microscopy.

If immunofluorescence staining is negative, chronic thrombotic microangiopathy may be the cause of MPGN<sup>3-4</sup>.

The ratio of MPGN among biopsy-confirmed glomerulonephritis (GN) varies from country to country. It is the third most frequent form of GN in Lithuania and it has been found in 12.5% of analysed renal biopsies<sup>22-25</sup>. The highest rates have been reported in Romania, Nepal and Saudi Arabia<sup>26-29</sup>. Lower rates of MPGN have been reported in Iran (11.5%), France (10%), Czech Republic (5.8%), China (5.8%) and Japan (2.4%)<sup>30-34</sup>.

Even though the incidence of MPGN in Lithuania has decreased in the last decades (from 16.8% in 1994-1999 to 7.5% in 2007-2012), this entity still remains one of the four most common primary glomerulopathies in Lithuania<sup>22-25</sup>. As the new classification of MPGN based on immunofluorescence findings was proposed only several years ago, there are still very few studies analyzing MPGN data according to this classification. In our study, we present the incidence and clinical data of MPGN according to the new immunofluorescence findings-based classification.

### Materials and Methods

The research was performed with the approval of Vilnius Regional Biomedical Research Ethics Committee (Nr. 158200-14-744-261). We retrospectively analyzed 81 clinical cases with renal biopsies performed from 2000 to 2014 in the Nephrology Department of Vilnius University Hospital Santariskiu Klinikos. Biopsies were evaluated at the National Center of Pathology, where the diagnosis of MPGN was confirmed according to histological and immunofluorescence findings. Data regarding the incidence of viral infections, probable acute or persistent bacterial infections, autoimmune diseases and hematological diseases were collected. Test results of C3 and C4 factors of the complement system, results of kidney biopsy immunohistochemistry were also thoroughly investigated. Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) version 20.0 software (IBM, Armonk, New York, USA), Student's t-test was used for continuous variables and the chi-square test was used for the categorical data. A p-value less than 0.05 was considered significant.

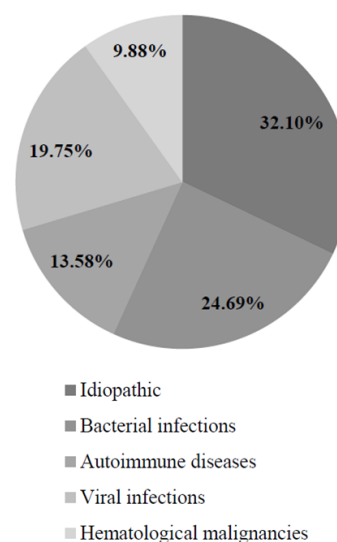
### Results

There were 81 patients during the study period. The number of MPGN diagnosis per year varied from three to nine. There were 55 males (67.9%) and 26 females (32.1%). The age of patients ranged from 19 to 83 years, and the mean age was 48.53 (standard deviation  $\pm 16.67$  years) years.

The analysis of clinical features and laboratory

data showed that the most common type of MPGN appeared to be idiopathic which was present in 26 cases (32.10%). Twenty cases (24.69%) were caused by various bacterial infections: the most frequent of them was tonsillitis (n = 6, 7.79%), followed by pneumonia (n = 4, 5.19%), tuberculosis and skin infections (n = 3, 3.90% in each group). Less common bacterial infections were dental infection and endocarditis that appeared each in the same number of cases (n = 2, 2.60%). Viral infections manifested as viral hepatitis (n = 16, 19.75%); hepatitis B (HBV) and hepatitis C (HCV) serology was positive in eight patients (9.88%) each. Autoimmune diseases were associated with MPGN in 11 cases (13.58%); eight (9.88%) of which were due to systemic lupus erythematosus (SLE) while the diagnosis of three cases (3.70%) was less specific and it was summarized as systemic vasculitis. Hematological malignancies were found in eight cases (9.88%), B-cell and T-cell lymphomas were generally diagnosed in three cases (3.70%), myelodysplastic syndrome occurred in two cases (2.47%), and multiple myeloma, hemolytic uremic syndrome and monoclonal gammopathy presented in one case (1.23%) each. All this data is summarized in Figure 1.

Decreased complement components C3 and C4 levels were found in 26 (21.06%) and 17 patients (13.77%) respectively. In patients with MPGN caused by bacterial infection, we found significantly lower complement component C3 concentration (p = 0.002) and normal C4 complement concentration (p = 0.004). In the group of autoimmune diseases, all patients have had significantly lower complement components C3 and C4 concentration (p = 0.04 and p = 0.004 respectively).



**Figure 1:** Main etiological causes of membranoproliferative glomerulonephritis in the 81 clinical cases with renal biopsies performed from 2000 to 2014; the most common type appeared to be idiopathic and due to bacterial infections.

Renal biopsy immunohistochemistry was performed in 75 cases and revealed that the most frequent immunohistochemical finding was C3+/Ig+, which was present in 58.02% of cases (n =47). C3-/Ig+ appeared in 19.75% of cases (n =16). According to this data the total number of immunoglobulin positive biopsies (C3+/Ig+ and C3-/Ig+) was 77.78% (n =63) (Figure 2).

C3/Ig positive immunohistochemical results manifested in more than half of each MPGN etiological group. Relatively the most frequent incidence of C3 and Ig deposition in glomeruli was found in the group of autoimmune diseases, in 90.90% of cases (n =10). In the patients with hematological diseases and in the group of bacterial infections these positive immunohistochemical results appeared in 62.50% (n =5) and 60% (n =12) of cases respectively.

Complement-mediated MPGN C3+/Ig- findings were less common and were diagnosed in seven patients

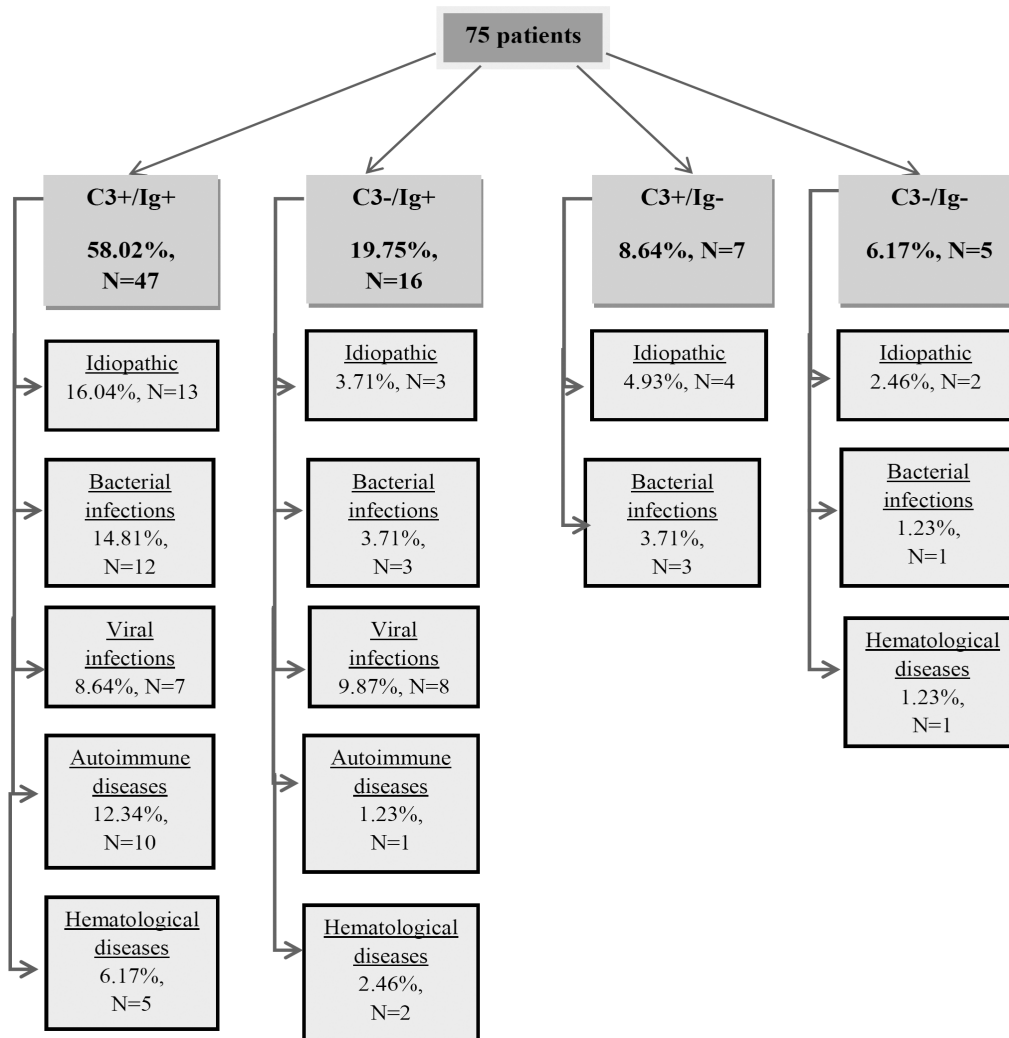
(8.64%). The remaining five cases (6.17%) were C3-/Ig-.

The comparison of MPGN types in male and female groups did not revealed any significant difference.

Patients were divided into three groups according to their age: patients aged 19-40 years were included in the young patient group, 41-60 years in the middle-aged group, and 61-83 years in the elderly group (Table 1). There were more MPGN caused by autoimmune diseases in the young group (63.64%, n =7) and more malignancy associated MPGN in the group of elderly patients (50%, n =4), the difference between these groups was statistically significant (p =0.004).

**Discussion**

The most common type of MPGN appears to be the idiopathic. However, in some cases, this diagnosis could be revised and specified, as the understanding of the dis-



**Figure 2:** Renal biopsy immunohistochemistry was performed in 75 cases. Findings were categorised into four groups according to staining pattern of C3 and immunoglobulins. The most frequent immunohistochemical finding was C3+/Ig+. According to this data the total number of immunoglobulin positive biopsies (C3+/Ig+ and C3-/Ig+) was 77.78% (n =63). Ig: immunoglobulin, N: number of patients.

ease and the diagnostics improve over time. Current data considers MPGN as a characteristic pattern of glomerular injury but not a diagnosis itself. It is recognized that immunofluorescence findings in MPGN better reflect pathogenesis of the damage comparing to electron microscopy findings. Moreover, a better understanding of etiology and pathogenesis of the disease guides to a more appropriate diagnosis and treatment. Therefore, the diagnosis of real idiopathic MPGN becomes very rare.

In our study, HBV or HCV serology and auto-anti-

MPGN, C4 levels are usually normal, and C3 are low<sup>2-6</sup>. In most cases in our study C3 complement component concentration was decreased or normal. Lower complement component C4 concentration was found more often in patients with autoimmune diseases.

Cryoglobulinemia may also occur in MPGN, because of the chronic immune system stimulation, which presents in persistent bacterial or viral infections such as HCV or HBV. Furthermore, hematological malignancies may have the same stimulating effect on patient's

**Table 1:** The distribution of causes of membranoproliferative glomerulonephritis in the age groups. Patients were divided into three groups according to their age: patients aged 19-40 years were defined as the young group, 41-60 years as the middle-aged group, and 61-83 years as the elderly group. There were more MPGN caused by autoimmune diseases in the young group and more malignancy associated MPGN in the group of elderly patients, the difference between these groups was statistically significant ( $p=0.004$ ).

Age \ Cause	Idiopathic	Bacterial infection	Viral infection	Autoimmune diseases	Hematologic malignancy	Total
Young	N =8 30.77%	N =5 25%	N =4 25%	N =7 63.64%	N =1 12.5%	N =25 30.86%
Middle-aged	N =10 38.46%	N =11 55%	N =7 43.75%	N =3 27.27%	N =3 37.5%	N =34 41.98%
Elderly	N =8 30.77%	N =4 20%	N =5 31.25%	N =1 9.09%	N =4 50%	N =22 27.16%
Total	N =26 100%	N =20 100%	N =16 100%	N =11 100%	N =8 100%	N =81 100%

N: number of patients, MPGN: membranoproliferative glomerulonephritis.

bodies or complement factor mutations were not investigated in all patients. Routine dentist examination was missing in some patients. Thus, the diagnosis of idiopathic MPGN in some cases could be due to undiagnosed bacterial, viral infections or autoimmune diseases.

The biopsy findings demonstrated a characteristic pathogenesis of MPGN, which in most cases presented with an antigenemia and deposition of Ig or immune complexes, causing activation of the complement system and, as a result, damage of glomeruli. We found five cases (6.17%) with C3-/Ig-. According to other studies<sup>3-4</sup>, if immunofluorescence staining is negative, chronic thrombotic microangiopathy may be the cause of MPGN. In our study, only one patient was diagnosed with hemolytic uremic syndrome.

Complement-mediated MPGN C3+/Ig- was diagnosed in seven patients (8.64%). Isolated C3 deposition is characteristic for C3 glomerulopathy. This new disease entity includes rare types of GN, such as DDD and C3GN<sup>35</sup>. However, electron microscopy was not performed in all cases, so we could not differentiate this type of MPGN in more detail.

The complement cascade activation affects serum complement factor concentration, therefore, hypocomplementemia is common in all types of MPGN<sup>1-8</sup>. Usually immune complex-mediated MPGN manifests with low serum C3 and C4 concentration, but normal C3 rate does not exclude this type of MPGN. In complement-mediated

immune system<sup>8-15</sup>. Some data supports the link between the HCV infection and kidney injury<sup>15-21</sup>. Cryoglobulinemia has not been tested in all cases in our study. The association between cryoglobulins and MPGN could be analyzed more thoroughly in further prospective studies. However, we found MPGN-type kidney injury in patients with HCV or HBV hepatitis. This finding supports other data about viral infections in MPGN<sup>15-21</sup>.

Our data shows that younger patients with a diagnosis of MPGN should be investigated for autoimmune diseases, and elderly patients with MPGN should be thoroughly examined for hematological malignancies.

## Conclusions

The main cause of the MPGN in the study appeared to be idiopathic and due to bacterial infections. In patients with MPGN caused by autoimmune disease, lower complement component C4 concentration was found more often. The most frequent immunohistochemical findings in the kidney biopsy were both C3 and Ig positivity. The most frequent incidence of C3 and Ig deposition in glomeruli was in the group of autoimmune diseases. Ig positive biopsy results (C3+/Ig+ and C3-/Ig+) were diagnosed in 77.78% of cases, and these results could be defined as immune-complex mediated MPGN type. Complement-mediated MPGN C3+/Ig- findings were less common and were diagnosed only in 8.64% of cases.

**Conflict of interest**

The authors declare no conflict of interest.

**References:**

- Alchi B, Jayne D. Membranoproliferative glomerulonephritis. *Pediatr Nephrol.* 2010; 25: 1409-1418.
- Sethi S, Fervenza FC. Membranoproliferative glomerulonephritis—a new look at an old entity. *N Engl J Med.* 2012; 366: 1119-1131.
- Masani N, Jhaveri KD, Fishbane S. Update on Membranoproliferative GN. *Clin J Am Soc Nephrol.* 2014; 9: 600-608.
- Fervenza FC, Sethi S, Glassock RJ. Idiopathic membranoproliferative glomerulonephritis: does it exist? *Nephrol Dial Transplant.* 2012; 27: 4288-4294.
- Bomback AS, Appel GB. Pathogenesis of the C3 glomerulopathies and reclassification of MPGN. *Nat Rev Nephrol.* 2012; 8: 634-642.
- Rabasco-Ruiz C, Huerta-Arroyo A, Caro-Espada J, Gutiérrez-Martínez E, Praga-Terente M. C3 Glomerulopathies. A new perspective on glomerular diseases. *Nefrologia.* 2013; 33: 164-170.
- Smith RJ, Harris CL, Pickering MC. Dense deposit disease. *Mol Immunol.* 2011; 48: 1604-1610.
- Sethi S, Gamez JD, Vrana JA, Theis JD, Bergen HR 3rd, Zipfel PF, et al. Glomeruli of Dense Deposit Disease contain components of the alternative and terminal complement pathway. *Kidney Int.* 2009; 75: 952-960.
- Ferri C. Mixed cryoglobulinemia. *Orphanet J Rare Dis.* 2008; 3: 25.
- Hiramatsu R, Hoshino J, Suwabe T, Sumida K, Hasegawa E, Yamanouchi M, et al. Membranoproliferative glomerulonephritis and circulating cryoglobulins. *Clin Exp Nephrol.* 2014; 18: 88-94.
- Nasr SH, Satoskar A, Markowitz GS, Valeri AM, Appel GB, Stokes MB, et al. Proliferative glomerulonephritis with monoclonal IgG deposits. *J Am Soc Nephrol.* 2009; 20: 2055-2064.
- Sethi S, Zand L, Leung N, Smith RJ, Jevremovic D, Herrmann SS, et al. Membranoproliferative glomerulonephritis secondary to monoclonal gammopathy. *Clin J Am Soc Nephrol.* 2010; 5: 770-782.
- Bridoux F, Desport E, Frémeaux-Bacchi V, Chong CF, Gombert JM, Lacombe C, et al. Glomerulonephritis with isolated C3 deposits and monoclonal gammopathy: a fortuitous association? *Clin J Am Soc Nephrol.* 2011; 6: 2165-2174.
- Subramanian M, Kilara N, Manjunath R, Mysorekar V. Membranoproliferative glomerulonephritis secondary to chronic myeloid leukemia. *Saudi J Kidney Dis Transpl.* 2010; 21: 738-741.
- Garini G, Allegri L, Lannuzzella F, Vaglio A, Buzio C. HCV-related cryoglobulinemic glomerulonephritis: implications of antiviral and immunosuppressive therapies. *Acta Biomed.* 2007; 78: 51-59.
- Fabrizi F. Hepatitis C virus, cryoglobulinemia, and kidney: novel evidence. *Scientifica (Cairo).* 2012; 2012: 128382.
- Saddadi F, Attari F, Najafi I, Gangi MR, Hakemi M, Amini M. Renal involvement in patients with hepatitis C virus infection. *Iran J Kidney Dis.* 2010; 4: 123-127.
- Barsoum RS. Hepatitis C virus: from entry to renal injury—facts and potentials. *Nephrol Dial Transplant.* 2007; 22: 1840-1848.
- Khedmat H, Taheri S. Associations of various histological morphologies of renal involvement in hepatitis B infection: analysis of 118 subjects. *Saudi J Kidney Dis Transpl.* 2010; 21: 964-966.
- Ko HM, Hernandez-Prera JC, Zhu H, Dikman SH, Sidhu HK, Ward SC, et al. Morphologic features of extrahepatic manifestations of hepatitis C virus infection. *Clin Dev Immunol.* 2012; 2012: 740138.
- Latt N, Alachkar N, Gurakar A. Hepatitis C virus and its renal manifestations. *Gastroenterol Hepatol (N Y).* 2012; 8: 434-445.
- Brazdziute E, Miglinas M, Gruodyte E, Priluckiene J, Tamosaitis A, Bumblyte IA, et al. Nationwide renal biopsy data in Lithuania 1994–2012. *Int Urol Nephrol.* 2015; 47: 655-662.
- Razukeviciene L, Bumblyte IA, Kuzminskis V, Laurinavicius A. Membranoproliferative glomerulonephritis is still the most frequent glomerulonephritis in Lithuania. *Clin Nephrol.* 2006; 65: 87-90.
- Laurinavicius A, Gruodyte E, Priluckiene J, Razukeviciene L, Supranaviciene L, Salkus G. Potential causes of antigenemia in the patients with an immune-complex mediated membranoproliferative glomerulonephritis in Lithuania. *Medicina (Kaunas).* 2003; 39: 28-31.
- Beitnaraitė S, Kovaliūnas E, Laurinavicius A. [Primary glomerulopathies in Lithuania: a retrospective analysis of renal biopsy cases (2000-2006)]. *Medicina (Kaunas).* 2007; 43 Suppl 1: 6-10.
- Covic A, Schiller A, Volovat C, Gluhovschi G, Gusbeth-Tatomir P, Petrica L, et al. Epidemiology of renal disease in Romania: a 10 year review of two regional renal biopsy databases. *Nephrol Dial Transpl.* 2006; 21: 419-424.
- Volovăt C1, Căruntu I, Costin C, Stefan A, Popa R, Volovăt S, et al. Changes in the histological spectrum of glomerular diseases in the past 16 years in the North-Eastern region of Romania. *BMC Nephrol.* 2013; 14: 148.
- Garyal, Kafle RK. Hisopathological spectrum of glomerular disease in nepal: a seven-year retrospective study. *Nepal Med Coll J.* 2008; 10: 126-128.
- Huraib S, Al Khader A, Shaheen FA, Abu Aisha H, Souqiyeh MZ, Al Mohana F, et al. The spectrum of glomerulonephritis in Saudi Arabia: the results of the Saudi registry. *Saudi J Kidney Dis Transpl.* 2000; 11: 434-441.
- Naini AE, Harandi AA, Ossareh S, Ghods A, Bastani B. Prevalence and clinical findings of biopsy-proven glomerulonephritis in Iran. *Saudi J Kidney Dis Transpl.* 2007; 18: 556-564.
- Hanko JB, Mullan RM, O'Rourke DM, McNamee PT, Maxwell AP, Courtney AE. The changing pattern of adult primary glomerular disease. *Nephrol Dial Transplant.* 2009; 24: 3050-3054.
- Maixnerova D, Jancova E, Skibova J, Rysava R, Rychlik I, Viklicky O, et al. Nationwide biopsy survey of renal diseases in the Czech Republic during the years 1994–2011. *J Nephrol.* 2015; 28: 39-49.
- Wu YQ, Wang Z, Xu HF, Jin XM, Zhou HZ. Frequency of primary glomerular disease in northeastern China. *Brazilian J Med Bio Res.* 2011; 44: 810-813.
- Sugiyama H, Yokoyama H, Sato H, Saito T, Kohda Y, Nishi S, et al; Committee for Standardization of Renal Pathological Diagnosis; Committee for Kidney Disease Registry; Japanese Society of Nephrology. Japan Renal Biopsy Registry and Japan Kidney Disease Registry: Committee Report for 2009 and 2010. *Clin Exp Nephrol.* 2013; 17: 155-173.
- Barbour TD, Pickering MC, Cook HT. Recent insight into C3 glomerulopathy. *Nephrol Dial Transplant.* 2013; 28: 1685-1693.