ORIGINAL ARTICLE

Type of immune and complement deposits and response of immunosuppressive treatment on Membranoproliferative Glomerulonephritis — a single centre experience

Salman Imtiaz, Murtaza Fakhruddin Dhrolia, Kiran Nasir, Beena Salman, Aasim Ahmad

Abstract

Objectives: To see the response of steroid and cyclophosphamide if membranoproliferative glomerulonephritis is classified by pattern of immune and complement deposits.

Methods: The retrospective study was conducted at The Kidney Centre, Karachi, and comprised patients treated for membranoproliferative glomerulonephritis between 1996 and 2013. Records of patients who were not treated with immunosuppressive medications were excluded. Patients were classified according to the types of immune deposits; one group had patients with only Complement factor 3 deposits, and the other with Complement factor 3 and immunoglobulin deposits. The effect of steroid alone and steroid with cyclophosphamide was observed on two histological patterns, according to the severity of kidney dysfunction and degree of interstitial fibrosis. SPSS 17 was used for statistical analysis.

Results: Of the 54 patients, 31(57%) were males and 23(42%) were females, with an overall mean age of 30.26±15.41 years. Group with Complement factor 3deposits had 17(31%) patients, while that with Complement factor 3 and immunoglobulin had 37(68%). Both groups were similar in terms of clinical and laboratory parameters (p>0.05). Both groups showed better response when treated with steroid and cyclophosphamide: 8/9(88.9%) vs. 3/8(37.5%) in Complement factor 3 only; and 10/15(66.7%) vs. 12/22(54.5%) in Complement factor 3 with immunoglobulin. Increasing severity of interstitial fibrosis (p=0.014) and presence of renal dysfunction (p=0.001) hampered the response. After adjusting the confounders, the odds ratio of response was 4.654(95%confidence interval: 0.957-22.63) in patients who received the treatment with steroid and cyclophosphamide compared to steroid alone.

Conclusion: Steroids and cyclophosphamide together have a beneficial role if treatment is initiated early in the course of the disease.

Keywords: Membranoproliferative glomerulonephritis, Glomerulonephritis, Nephroticsyndrome, Cyclophosphamide, Pakistan. (JPMA 65: 995; 2015)

Introduction

Idiopathic Membranoproliferative Glomerulonephritis (MPGN) is recognised for its high predisposition to developing end-stage renal disease (ESRD) regardless of treatment.[1,2] One important reason is the heterogeneity of underlying aetiological ailments and the other is variety of unrevealed, pathophysiological mechanisms leading to an identical histopathological pattern. Both classification and treatment of MPGN have controversies and there is alack of agreement. The frequency of MPGN is high in Pakistan compared to the western world.[3]

Traditionally MPGN was classified on the basis of the site of immune deposits into Type I, Type II and Type III. This classification was based solely on electron microscopic appearance of renal biopsy specimen, and it provided the diagnosis but discernment about the pathophysiology remained elusive.[4] Recently proposed a new classification has been proposed by two different studies, based mainly on the immune and complement deposits, which have classified the MPGN on pathophysiological basis.[5,6] This classification divides the MPGN into immunoglobulin-positive complement-positive MPGN, and immunoglobulin-negative complement-positive MPGN. The first one occurs when there is activation of classical pathway, while the second one occurs because of disorders associated with dysregulation of alternative complement pathway. Although this recent classification has its fair share of criticism,[7] but the classification seems more practical because it addresses the underlying disease mechanism which makes it easier for the physician to decide about the treatment according to fundamental pathophysiological process.[8] The evidence for the treatment of idiopathic MPGN is weak.[9] Earlier recommendation reserved immunosuppressive treatment only for those patients who had a high urinary protein excretion or deteriorating kidney function. Furthermore, the treatment was restricted to high-dose steroid therapy for 6 to 12 month for children and

The Kidney Centre Postgraduate Training Institute, Karachi.
Correspondence: Salman Imtiaz. Email: salman_imtiaz@hotmail.com

dipyrimidole or aspirin for adults.[9] Steroid alone was used in most of the trials[10-12] and steroid with other immunosuppressive agents likecyclophosphamide (CYP) and Cellcept were used in some studies.[13-15] Kidney disease improving global outcome (KDIGO) group recently recommended either steroid or steroid with CYP for those who had nephrotic syndrome (NS) and progressive decline of the kidney function over six months.[16]

Against this background, we planned the current study to review our patients on the basis of the type of deposits (Complement factor 3 [C3] only, and C3 with Immunoglobulin) and to measure the outcome at one year.

Patients and Methods

The retrospective study was conducted at The Kidney Centre, Karachi, and comprised records of native kidney biopsies of patients treated for MPGN between 1996 and 2013. Those selected had been treated with steroid or CYP and had a follow-up of at least 12 months. Patients who did not have complete data and those who were not treated with steroid or CYP were excluded. Patients were classified according to the newly-proposed classification[5,6] into those who had only C3 deposits(C-MPGN) and those who had C3 and immunoglobulin deposits(IC-MPGN), and then the effect of steroid only and steroid with CYP was observed on these two different patterns of histopathology (Figure). The two groups were compared for any differences in clinical and laboratory parameters. We also compared the factors in the two groups which influenced the outcome of the disease like treatment, degree of interstitial fibrosis on biopsy specimen, presence of kidney dysfunction and type of immune deposits.

Nephrotic range 3 as defined as proteinuria when urinary execration of protein was more than 3.5 grams/24 hours and non-nephrotic proteinuria when it was less than 3.5 grams/24 hours. NS was labelled when there was oedema, hypoalbuminemia and nephrotic range proteinuria. Kidney failure was decalredif serum creatinine was found to be more than 1.5mg/dl or estimated glomerular filtration rate (eGFR) calculated by Cockroft and Gault formula was less than 90 ml/min.

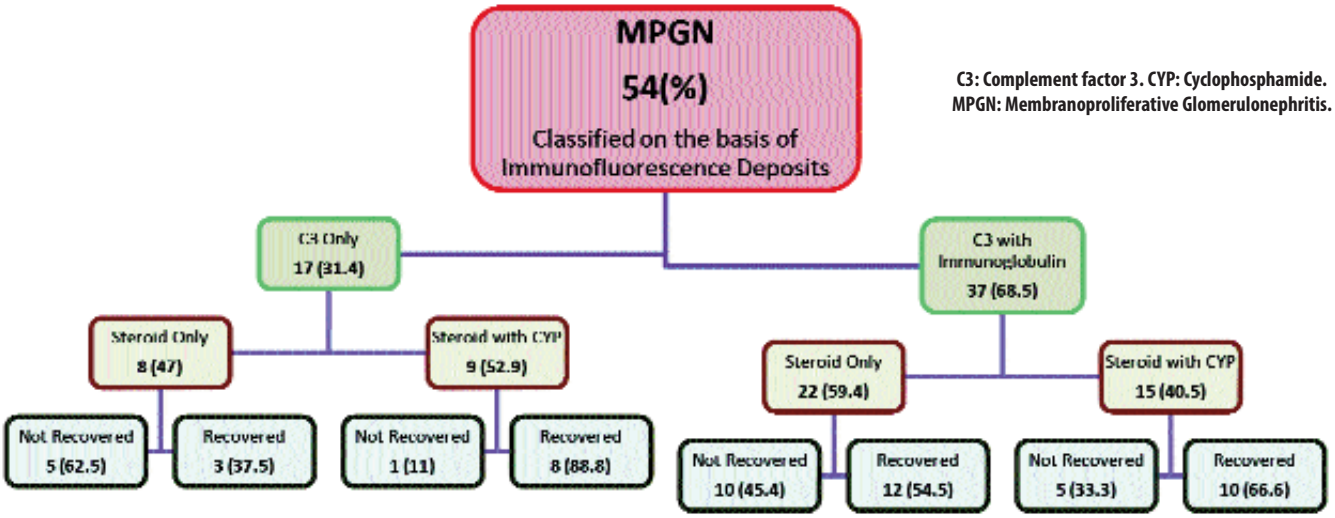
Haematuria was defined as macroscopic if visible to gross examination of urine, and microscopic if more than 4 cells were found on high-power field. Hypertension was labelled normal, moderate and severe with range of 120/80mm/Hg, 140/90mm/Hg, and 170/110mm/Hg respectively. Chronic kidney injury was defined as persistent proteinuria, haematuria or low eGFR in patients beyond a period of six months. On renal biopsy, mild, moderate and severe tubulo-interstitial fibrosis was labelled when less than 25%, more than 25% but less than 50%, and more than 50% of tubules and interstitum were involved respectively. We defined recovery or remission when patients achieved normal kidney function (serum creatinine <1.2 mg/dl) and absence of proteinuria.

The treatment of MPGN was based on physician discretion and due to retrospective nature of the data, it was not possible for us to evaluate the reason of prescribing a particular drug regimen for any individual patient. In general, most of the time CYP was reserved for progressive disease and steroid only for those who had nephrotic-range proteinuria. Steroid was mostly used with a dose of 1mg/kg of body weight, although few of the patients received alternate day steroid as well. The average duration of steroid used was six months. CYP was mostly added with the steroid if there was further deterioration of kidney function or increased in proteinuria and occasionally along with steroid as a primary therapy. CYP was also used for six months and stopped only in case of untoward side effects.

Data was analysed using SPSS 17. Mean ± standard deviation values were computed for quantitative variables. Categorical variables were described in terms of frequencies and percentages. Association of clinical symptoms with two immune deposits groups was checked by Chi-Square test, where cell counts were sufficient, on the other hand we used Fishers exact test where 20% of cells had expected count less than 5. Cross-tabulation was used to obtain the frequencies and percentages of these two groups related to the outcome in relation to different variables. Univariable logistic regression was run to see the effect of different variables on the outcome, and unadjusted odds ratios (OR) and 95%confidence intervals (CI) were obtained. At this stage, variables with p value at the most 0.2 was considered significant and included in multivariable logistic regression analysis. Significance of the results were set to be less than or equal to 0.05 in multivariable analysis. Confounders were adjusted by stratification and logistic regression analysis.

Results

During the period reviewed, 1891 native kidney biopsies were done and MPGN was found to have been done in 76(4%) patients. After leaving out those who met the exclusion criterion, record of 54(71%) patients comprised the study sample. Of them, 31(57%) were males and 23(42%) were females; their ratio being 1.35:1. The overall mean age was 30.26±15.41 years. The C-MPGN group had



C3: Complement factor 3. CYP: Cyclophosphamide. MPGN: Membranoproliferative Glomerulonephritis.

Figure: Patient distribution according to immune deposits and drug treatment

Table-1: Comparison of clinical and laboratory variables between C3 only and C3 with Immunoglobulin.

	Immune Deposits C-MPGN 17 (31.5%)	IC-MPGN 37 (68.5%)	p Value
Oedema	11 (64.7)	31 (83.8)	0.117
Macroscopic Haematuria	2 (11.8)	4 (10.8)	0.99
Oliguria	3 (17.6)	7 (18.9)	0.99
Blood pressure Systolic			*
Normal	6 (35.3)	18 (48.6)	
Hypertensive	2 (11.8)	5 (13.5)	
Severe Hypertensive	9 (52.9)	14 (37.8)	
Blood pressure Diastolic			*
Normal	8 (47.1)	16 (43.2)	
Hypertensive	4 (23.5)	10 (27.0)	
Severe Hypertensive	5 (29.4)	11 (29.7)	
Urinary red blood cells	14 (82.4)	33 (89.2)	0.665
InterstitialFibrosis			*
Mild	13 (76.5)	16 (43.2)	
Moderate	3 (17.6)	12 (32.4)	
Severe	1 (5.9)	9 (24.3)	
C4Deposits	1 (5.9)	11 (29.7)	0.078
SerumC3			0.581
Low	11 (64.7)	21 (56.8)	
Normal	6 (35.3)	16 (43.2)	
RenalDysfunction	11 (64.7)	20 (54.1)	0.462
Proteinuria			0.147
Nephrotic Range proteinuria	9 (52.9)	27 (73.0)	
Non-Nephrotic Proteinuria	8 (47.1)	10 (27.0)	
Presentation			0.534
Nephrotic Syndrome	12 (70.6)	29 (78.4)	
Acute GN	5 (29.4)	8 (21.6)	

MPGN: Membranoproliferative glomerulonephritis. C-MPFN C3: Complement factor 3.C4: Complement factor 4.GN: Glomerulonephritis. C-MPGN: Membranoproliferative glomerulonephritiswith C3 deposits. IC-MPGN: Membranoproliferative glomerulonephritis with C3 and Immunoglobulin deposits.

*p values were not valid for these variables due to more than two categories

17(31%) patients and 37(68%) were in the IC-MPGN groups. Both groups were similar in terms of clinical and laboratory parameters (p>0.05) (Table-1).

Cross-tabulation of the two groups with outcome stratified with the variables like renal dysfunction, interstitial fibrosis, treatment and immune deposit showed there were 5(83.3%)patients recovered in C-MPGN group when they had normal creatinine level compared to 6(54.5%) who had renal dysfunction. In the IC-MPGN group,16(94.1%) recovered when they had normal kidney function compared to those who had high creatinine 6(30%). Comparing the groups with outcome in relation to the degree of interstitial fibrosis showed that when patients with C-MPGN had mild interstitial fibrosis,10 out of 13(76.9%) recovered in comparison to those who had moderate interstitial fibrosis 1 out of 3(33.3%) and severe. 0 out of 1(0%). On the other hand, patients who had IC-MPGN 13 out of 16 (81.3%) recovered when they had mild fibrosis, while those who had moderate interstitial fibrosis, 6 out of 12(50%) and in severe 3 out of 9 (33.3%) patients recovered. The patients in C-MPGN group showed a better recovery when treated with both steroid and CYP, 8 out of 9 (88.9%) compared to those who were treated with steroid alone, 3 out of 8 (67.5%). On the other hand, the recovery in patients who had IC-MPGN when treated with steroid, 12 out of 22 (54.5%) patients recovered compared to 10 out of 15 (66.7%) when treated with both steroid and CYP (Table-2).

Univariable logistic regression analysis showed interstitial fibrosis (p=0.0140) and renal dysfunction (p=0.001) were statistically significant in relation to recovery (Table-3).

Multivariable binary logistic regression analysis showed

Table-2: Cross-Tabulation of immune deposits groups with outcome by stratification of the variables.

Immune Deposits				Outcome		
				Not Recovered	Recovered	Total
C-MPGN	Renal Dysfunction	No	Count (%)	1 (16.7)	5 (83.3)	6 (100)
		Yes		5 (45.5)	6 (54.5)	11 (100)
		Total		6 (35.3)	11 (64.7)	19 (100)
IC-MPGN	Renal Dysfunction	No	Count (%)	1 (5.9)	16 (94.1)	17 (100)
		Yes		14 (70)	6 (30)	20 (100)
		Total		15 (40.5)	22 (59.5)	37 (100)
C-MPGN	Interstitial Fibrosis	Mild	Count (%)	3 (23.1)	10 (76.9)	13 (100)
		Moderate		2 (66.7)	1 (33.3)	3 (100)
		Severe		1 (100)	0 (0)	1 (100)
		Total		6 (35.3)	11 (64.7)	17 (100)
IC-MPGN	Interstitial Fibrosis	Mild	Count (%)	3 (18.8)	13 (81.3)	16 (100)
		Moderate		6 (50)	6 (50)	12 (100)
		Severe		6 (66.7)	3 (33.3)	9 (100)
		Total		15 (40.5)	22 (59.5)	37 (100)
C-MPGN	Treatment	Steroid only	Count (%)	5 (62.5)	3 (67.5)	8 (100)
		Steroid with CYP		1 (11.1)	8 (88.9)	9 (100)
		Total		6 (35.3)	11 (64.7)	17 (100)
IC-MPGN	Treatment	Steroid only	Count (%)	10 (45.5)	12 (54.5)	22 (100)
		Steroid with CYP		5 (33.3)	10 (66.7)	15 (100)
		Total		15 (40.5)	22 (59.5)	37 (100)

MPGN: Membranoproliferative glomerulonephritis. C-MPGN: Membranoproliferative glomerulonephritiswith C3 deposits.
IC-MPGN: Membranoproliferative glomerulonephritis with C3 and immunoglobulin deposits. CYP: Cyclophosphamide.

Table-3: Univariate Logistic regression analysis showing the effect of interstitial fibrosis, immunedeposits, renaldysfunction and treatment.

Variables	Unadjusted Odds Ratios	95% CI of Odds Ratio		p Value
		Lower	Upper	
◆ Interstitial Fibrosis*			0.014	
◆ Interstitial Fibrosis (moderate)	0.228	0.59	0.885	0.033
◆ Interstitial Fibrosis (severe)	0.112	0.22	0.567	0.008
Immune deposits(IC-MPGN)+	0.8	0.243	2.634	0.714
Renal dysfunction(yes)	0.06	0.012	0.304	0.001
Treatment(steroid with CYP)#	3	0.932	9.653	0.065

*Mild interstitial fibrosis is referent category, +C3 only is referent category, # Steroid only is referent category.
IC-MPGN: Membranoproliferative glomerulonephritis with C3 and immunoglobulin deposits. MPGN: Membranoproliferative Glomerulonephritis.CYP: Cyclophosphamide. CI: Confidence interval.

Table-4: Multivariable Logistic Regression showing amount of effect of treatment, interstitial fibrosis, renal dysfunction and immune deposits on outcome of the patients.

Variables	Unadjusted Odds Ratios	95% CI of Odds Ratio		p Value
		Lower	Upper	
Treatment (Steroid with CYP) #	4.654	0.957	22.638	0.057
Interstitial Fibrosis (Mild)*				0.063
Interstitial Fibrosis (Moderate)	0.446	0.079	2.535	0.363
Interstitial Fibrosis (Severe)	0.051	0.004	0.615	0.019
Renal Dysfunction (Yes)	0.027	0.002	0.295	0.003
Immune Deposits (IC-MPGN)+	0.902	0.155	5.246	0.909

*Mild interstitial fibrosis is referent category. +C3 only is referent category.# Steroid only is referent category.
IC-MPGN: Membranoproliferative glomerulonephritis with C3 and immunoglobulin deposits. MPGN: Membranoproliferative glomerulonephritis. CYP: Cyclophosphamide.CI: Confidence interval

the amount of effect of different variables on outcome. Among patients who received steroid with CYP, the odds of recovery were 4.654(95% CI: 0.957-22.638) while adjusting for all other variables in the model. There was 4.654 times more recovery than those who were treated with steroid alone. Patients with moderate interstitial fibrosis had 55.4% (Adjusted OR: 0.446; 95%CI: 0.079-2.535) less chance of recovery compared to mild fibrosis by holding the effects of all other variables constant. Patients with severe interstitial fibrosis had 94.9% (Adjusted OR: 0.051; 95%CI: 0.004-0.615) less chance of recovery when compared with patients who had mild fibrosis (p=0.019) by holding the effect of all other variables in the model. Patients with renal dysfunction had 97.3% (Adjusted OR: 0.027; 95% CI: 0.002-0.295) less chance of recovery compared to no renal dysfunction (p=0.003) while adjusting for all other variables in the model. Patients in IC-MPGN group had 9.8% (Adjusted OR: 0.902; 95% CI: 0.155-5.246) less chance of recovery when compared to the C-MPGN group while holding the effects of all other variables constant (Table-4).

Discussions

The treatment of MPGN changed many times in the last decade.[8] It evolved from the use of dipyrimidole, aspirin and warfarin to steroids and CYP, and now to rituximab and possible role of anti-C5 monoclonal antibody. Recently, the KDIGO group recommended steroid and CYP together for the treatment of MPGN in both adult and paediatric groups.[16]

It is interesting to see the effect of immunosuppression on MPGN when it is classified according to the type of immunoglobulin deposits. It revealed that CYP has more pronounced effect on outcome in both groups when compared to steroid alone. Very few clinical trials have been performed to see the effect of immunosuppression on MPGN. A prospective study used an innovative regimen in 19 MPGN patients.[14] In the first phase it induced patients with intravenous (IV) methylprednisolone bolus along with oral CYP; in the second phase it maintained the patients on alternate day prednisolone and daily CYP; in the third phase it tapered down prednisolone alone; and in the fourth phase of discontinuation it omitted CYP and prednisolone was slowly tapered down as per its protocol. The treatment lasted for approximately 10 months and showed complete remission in 15 out of 19 patients, 8 patients had relapses that were treated with repeat cycles and remitted completely. In a prospective controlled trial[13] on 59 patients with confirmed MPGN (27 treatment and 32 control), all had proteinuria greater than 2 gm/day and corrected creatinine clearance less than 80 ml/min/1.73m^2. It found no difference in the outcome between treatment group (CYP, coumadin and dipyrimidole) and control group (no specific therapy) receiving treatment for 18 months. Steroid remains the mainstay of treatment and at least in children it showed benefits in various observational studies. One study used alternate day prednisolone for 41 months and showed a stable serum creatinine in 61% of the children compared to the placebo 12%, but the steroid side effects were enormous in this study.[10] Another study, after a prolonged follow-up of 76 children, showed a cumulative renal survival of 82% in the 10th year and 56% in the 20thyear after disease onset.[17] Our study also showed that isolated steroid gave benefits to those who had normal kidney function and stable proteinuria. We also analysed the variable that can affect outcome of treatment and found that the degree of interstitial damage and severity of kidney dysfunction changed the outcome. A study of 104 patients with a mixed population of all age groups found the presence of sclerosis a strong predictor of outcome.[18] Likewise, a retrospective analysis 1 of 220 case of MPGN, found that tubulo-interstitial changes were of decisive significance. It found that patients who had normal cortical interstitium and normal tubules maintained their normal kidney function even 10 year after biopsy, while the proportion reduced to 13% in the presence of interstitial fibrosis. In those cases he found all patients died within first 5 years after biopsy or reached end stage kidney disease. We also found a strong association of interstitial fibrosis on the outcome. We found that as the degree of fibrosis increased in severity, the outcome was hampered by the same rate in both groups.

The other important factor that affects the outcome was the presence or absence of kidney dysfunction. Patients who had reduced kidney function at the time of biopsy in both C-MPGN and IC-MPGN groups showed poor outcome. The effect was more pronounced in IC-MPGN. One study[1] found the same effect of level of serum creatinine on outcome. It found that when serum creatinine was more than 1.3 mg/dl at the time of kidney biopsy, there was marked tendency towards a further increase during the course of the disease. On the other hand, a study[12] found no significant difference in renal survival between those with renal impairment at presentation and those with normal renal function. However, in those with renal impairment at one year, 5-year renal survival was 87% (CI: 70-100%) compared to100% in those with normal eGFR at 1 year (p=0.037). It also found a mean survival of 11.3 years (CI: 8-14.7 years) in those with low eGFR at 1 year compared with 13 years if normal (p=0.065).

Our study has its limitations. Firstly, as electron

microscopy was not available at our centre, we were not able to traditionally classify MPGN. Secondly, due to the retrospective nature of our data-collection spanning 18 years, a uniformity of treatment was difficult to establish. We were, therefore, very stringent with our inclusion criteria and large numbers of patients were excluded and only 54 patients were selected.

Conclusion

MPGN had a poor outcome, especially if the patient presented with renal dysfunction and had chronic changes in kidney biopsy. Steroids and CYP had a beneficial role if treatment was initiated early in the course of the disease with less degree of interstitial fibrosis and normal kidney function. We found better response in those who had C-MPGN deposits when treated with steroids and CYP compared to those who were in the IC-MPGN group.

References

1. Schmitt H, Bohle A, Reineke. T, Eichberger DM, Vogal W. Long-Term Prognosis of Membranoproliferative Glomerulonephritis Type I. Nephron. 1990; 55: 242-50.
2. Pedersen RS. Long-Term Prognosis in Idiopathic Membranoproliferative Glomerulonephritis. Scan J. UrolNephrol. 1995; 29: 265-72.
3. Kazi JI, Mubarak M, Ahmed E, Akhter F, Naqvi SAA, Rizvi SAH. Spectrum of glomerulonephritides in adults with nephrotic syndrome in Pakistan. Clin Exp Nephrol 2009; 13: 38-43.
4. Fogo AB, Bruijn JA, Cohen AH, Colvin RB,Jennette JC, Alpers CE. Fundamental of Renal Pathology. Springer, 2008.
5. Fakhouri F1, Frémeaux-Bacchi V, Noël LH, Cook HT, Pickering MC. C3 Glomerulopathy: a new classification. Nat Rev Nephrol 2010; 6: 494-9.
6. Sethi S, Fervenza FC. Membranoproliferative Glomerulonephritis: PathogeneticHeterogenaity and Proposal for a new Classification. Semin Nephrol 2011; 31: 341-8.
7. Voskarides K, Pierides A, Deltas C. Letter to the Editor. Kidney Int. 2013; 83: 331-3.
8. Sethi S, Fervenza FC. Membranoproliferative Glomerulonephritis - A New Look at an Old Entity. N Engl J Med. 2012; 366: 1119-31.
9. Levin A. Management of membranoproliferative glomerulonephritis: Evidence-based recommendations. Kidney Intl. 1999; 55: S41-6.
10. Tarshish PN, Bernstein J, Tobin J, Edelmann CM. Treatment of mesangiocapillary glomerulonephritis with alternate-day prednisone - a report of The International Study of Kidney Disease in Children. Pediatr Nephrol. 1992; 6: 123-30.
11. Braun MC, West CK, Strife CF. Differences between Membranoproliferative Glomerulonephritis Type I and III in Long-Term Response to an Alternate-Day Prednisone Regimen. Am J Kidney Dis 1999; 34: 1022-32.
12. Cansick JC, Lennon R, Cummins CL, Howie AJ, McGraw ME, Saleem MA, et al Prognosis, treatment and outcome of childhood mesangiocapillary (Membranoproliferative) glomerulonephritis. Nephrol Dial Transplant. 2004; 19: 2769-77.
13. Cattran DC, Cardella CJ, Roscoe JM, Charron RC, Rance PC, Ritchie SM, et al. Results of a controlled drug trial in membranoproliferative glomerulonephritis. Kidney Intl. 1985;27: 436-41.
14. Faedda R, Satta A, Tanda F, Pirisi M, Bartoli E. Immunosuppressive treatment of membranoproliferative glomerulonephritis. Nephron. 1994; 67: 59-65.
15. Jones G, Juszczak M, Kingdon E, Harber M, Sweny P, Burns A. Treatment of idiopathic membranoproliferative glomerulonephritis with mycophenolatemofetil and steroids. Nephrol Dial Trans. 2004; 19: 3160-4.
16. Kidney Disease Improving Global Outcomes. [online] [cited2012 Dec 20]; Available from: URL:http://www.kidney-international.org
17. McEnery PT. Membranoproliferative Glomerulonephritis: The Cincinnati experience - cumulative renal survival from 1957 to 1989. J Pediatr 1990; S109-14.
18. Cameron JS, Turner DR, Heaton J: Idiopathic Mesangiocapillary Glomerulonephritis Comparison of Types I and II in Children and Adults and Long-Term Prognosis. Am J Med. 1993; 74: 175-92.