Association of Proteinuria with Various Clinical Findings and Morphologic Variables of Oxford Classification in Immunoglobulin A Nephropathy Patients

Hamid Nasri, Yahya Madihi[1,2], Alireza Merrikhi[1,2], Allaleh Gheissari[1,2], Azar Baradaran[3], Soleiman Kheiri[4], Mahmoud Rafieian-Kopaei[4]

Department of Nephrology, Division of Nephropathology, Isfahan University of Medical Sciences, Isfahan, Iran, [1]Child Growth and Development Research Center, Isfahan University of Medical Sciences, Isfahan, Iran, [2]Department of Pediatric Nephrology, Isfahan Kidney Diseases Research Center, Isfahan University of Medical Sciences, Isfahan, Iran, [3]Department of Pathology, Isfahan University of Medical Sciences, Isfahan, Iran, 4Medical Plants Research Center, Shahrekord University of Medical Sciences, Shahrekord, Iran

Correspondence to:
Prof. Mahmoud Rafieian-Kopaei,
Medical Plants Research Center,
Shahrekord University of Medical
Sciences, Sharekord, Iran.
E-mail: rafieian@yahoo.com

Original Article

ABSTRACT

Background: Immunoglobulin A nephropathy (IgAN) with nephrotic syndrome is an uncommon form of IgAN. Clinical and morphological characteristics of proteinuria in IgAN, especially when is in nephrotic range have not yet been fully examined. This study was aimed to correlate morphologic variables of the Oxford classification, and various clinical data with proteinuria in IgAN patients. We also aimed to demonstrate the significance of prevention of proteinuria as one of the important factors in progression of this disease.

Methods: In an observational study conducted on IgAN patients, total of 114 biopsies were entered in the study. IgAN was diagnosed by light and immunofluorescence study.

Results: Of 114 patients 70.2% were male. Mean age of patients was 37.7 ± 13.6 years. The mean of proteinuria was 1742 ± 1324 mg/day. Also mean of serum creatinine (Cr) was 1.6 ± 1.5 mg/dL. Of 114 patients, 11(9.6%) had nephrotic range proteinuria. In this study, there was a positive correlation between proteinuria and serum Cr, peri-glomerular fibrosis or interstitial fibrosis. There was a positive association between proteinuria and totally sclerotic glomeruli too. There was also a positive association between the amount of fibrous crescents and the level of proteinuria. Nephrotic proteinuria could just be seen in male patients. Also, nephrotic syndrome had a positive association with the number of crescents.

Conclusions: Our findings firstly support the prognostic value of crescent due to its association with proteinuria and secondly imply the importance of treatment of proteinuria to prevent progression of IgAN.

Keywords: Crescent, immunoglobulin A nephropathy, nephrotic syndrome, Oxford classification, proteinuria

INTRODUCTION

Immunoglobulin A nephropathy (IgAN) is an autoimmune disease and is very common form of primary glomerulonephritis.[1-4] It occurs worldwide with different frequency from 2% to 52% of

all renal diseases in different parts of the world.[1-9] Proteinuria >1.0 g/day, at diagnosis is a well-known indicator of progressive renal disease in patients with IgAN.[9-17] Indeed persistent proteinuria exceeding this amount is an independent predictor of subsequent renal progression in patients with IgAN who have been followed for 10 years or more.[17-21] Nephropathy of IgA with nephrotic syndrome is an uncommon form of IgAN.[3,5,18-20] The clinical and morphological characteristics of this condition have not yet been understood. In IgAN, urinary findings usually consist of mild-to-moderate proteinuria and hematuria, and it rarely presents with a proteinuria in nephrotic range. The prevalence of IgAN with nephrotic syndrome (nephrotic IgAN; nIgAN), varies from 5% to 20%[18-25] The Oxford classification, created in 2009, is a novel method for evaluation of histological findings of IgAN.[23-28] This classification has good reproducibility and can be used to evaluate prognosis and response to therapy. This study was aimed to correlate four morphologic variables of the Oxford classification, and the various clinical data with proteinuria in IgAN patients. We also aimed to demonstrate, the significance of prevention of proteinuria as one of the important factors in progression of this disease.

METHODS

Following popularization of Oxford classification of IgA N on July 2009,[26-28] we applied it in this study for the classification of IgAN.

Definition of IgAN

The pathologic diagnosis of IgAN requires demonstration of IgA-dominant mesangial or mesangial-capillary immune deposits through immune fluorescence(IF) microscopy while there is subdominant, or weak deposit for C_1q. The immune deposits were semi quantified from 0 to 3+positive bright. The definition of IgAN needs the presence of diffuse and global IgA deposits that were graded $\geq$2+ and weak C_1q deposition.[5,29-31] All renal biopsies, performed at various medical centers (University Hospitals or Private Centers) from July 2009 to July 2012, were sent to a reference laboratory. None of the patients was treated before the biopsy. Biopsies with less than eight glomeruli were excluded from the study. None of the patients was diagnosed as primary IgAN, having a history

of collagen vascular diseases, diabetes or liver cirrhosis based on a questionnaire filled at the time of biopsy admission, laboratory data in patients' records and a brief history provided by referee physicians at the time of biopsy admission.

Histologic data

All renal biopsies were prepared for light and direct immunofluorescence microscopy. Tissue was fixed in 10% formalin for histologic sectioning. Each kidney biopsy was prepared by cutting paraffin blocks into 3-μm sections and staining two slides with periodic acid Schiff, two slides for hematoxylin and eosin, one slide for Jones methenamine silver and one slide for trichrome. Each slide contained 2-3 sections and nap-frozen in liquid nitrogen was used for IF. Sections (Six-micron in thickness) were stained for immunofluorescence study with IgG, IgM, IgA, C1q, C3, and find 1q.[32-34] IF slides were reported in a scale of 0-3+ positive bright and by a nephropathologist. IF study was performed before reviewing the slides for light microscopy unaware of patients' data. After IF diagnosis of IgAN, histopathology glass slides were reviewed to assess the morphologic variables, which were applied in Oxford classification method. We also assessed the presence of extracapillary proliferation (cellular, fibro-cellular or fibrous crescents). After selection of biopsies having dominant deposition IgA by IF study and in the absence of exclusion criteria, glass slides were reported for the classification of Oxford-MEST.

Definitions of morphologic variables

Total glomeruli and the number of glomeruli with global sclerosis were recorded for each biopsy. The presence of (i) mesangial hypercellularity(M), (ii) endocapillary proliferation(E), (iii) segmental glomerulosclerosis (S) and (iv) the proportion of tubular atrophy and interstitial fibrosis; IF/TA(T) was estimated as published for Oxford classification.[29]

Clinical studies and laboratory data

The medical records of patients were reviewed to obtain various demographic, clinical and laboratory information at the time of biopsy and for follow-up activities. Data gathered at the time of biopsy included race, gender, age, serum creatinine (Cr) and proteinuria (based on a 24 h urine collection).

Statistical analysis

The mean values and standard deviations were calculated, and statistical significance of the differences between groups were evaluated using T and Likelihood Ratio tests. Because of high positive skewness of data, the Spearman's coefficient of correlation was used to check the correlation. A computer program (SPSS version 16.0, Chicago, IL, USA) was used for statistical analysis. $P < 0.05$ was considered as statistically significant.

RESULTS

This observational study was conducted on IgAN patients. A total of 114 biopsies were enrolled to the study. In this trial, out of 114 patients, 70.2% were male. Mean age of patients was 37.7 ± 13.6 years (median = 35 years) (39 ± 14.3 years for male and 35 ± 11.7 years for female patients). Morphologic variables of Oxford classification are summarized in Table 1. The mean of proteinuria was 1742 ± 1324 mg/day (median = 1500 mg/day). The mean number of glomeruli in all renal biopsies was 14.8 ± 7.2 (median = 13 number). In all biopsies, the mean of totally sclerotic glomeruli was 2.4 ± 2.9 (median = 1 number). Furthermore, the mean of serum Cr was 1.6 ± 1.5 mg/dL

**Table 1:** Clinical and histological findings of IgAN patients at the time of renal biopsy

Clinical findings	
Sex (male/female)	80/34
Age (years)	37.7±13.6 (total)
	(M: 39±14.3, F: 35±11.7)
S-Cr (mg/dl)	1.6±1.5 (total)
	(M: 1.8±1.7, F: 1.1±0.7)
Histological findings	
(Oxford classification)	
Mesangial hypercellularity	
M0/M1 (number)	41/73
Endocapillary hypercellularity	
E0/E1 (number)	79/35
Segmental glomerulosclerosis	
S0/S1 (number)	42/72
Interstitial fibrosis/	
Tubular atrophy (IF/TA)	
T0/T1/T2 (number)	59/35/20

IF= Interstitial fibrosis, TA= Tubular atrophy,
IgAN= Immunoglobulin A nephropathy

(median = 1.2 mg/dL). Of 114 patients, 11 (9.6%) had nephrotic range proteinuria. In this study, there was a positive correlation between proteinuria and serum Cr ($P < 0.001$), peri-glomerular fibrosis ($P = 0.013$) or interstitial fibrosis ($P = 0.037$ Figure 1). There was a positive association between proteinuria and totally sclerotic glomeruli ($P = 0.008$). Off our variables of Oxford classification, only M (mesangial proliferation ≥50% of glomeruli) ($P < 0.01$) and T(interstitial fibrosis and tubular atrophy; IF/TA) ($P < 0.007$) had a positive association with proteinuria. There was a positive correlation between the amount of fibrous crescents and the level of proteinuria ($P = 0.028$). None off our deposited immune reactants (IgA, IgG, IgM and C3) had a significant association with proteinuria ($P > 0.05$). Nephrotic range proteinuria was only seen in male patients ($P < 0.05$). Furthermore, nephrotic syndrome had a positive association with the number of crescents ($P < 0.05$).

DISCUSSION

After publication of IgAN classification (Oxford) on July 2009,[29-31] the various studies were conducted to better find the clinical significance of each of four variables. In this study, we aimed to correlate proteinuria, especially in the nephrotic range, with Oxford classification. The present investigation showed that off our variables of Oxford classification only M variable (mesangial proliferation ≥50% of glmeruli) and T (interstitial fibrosis and tubular atrophy; IF/TA) had a positive association with
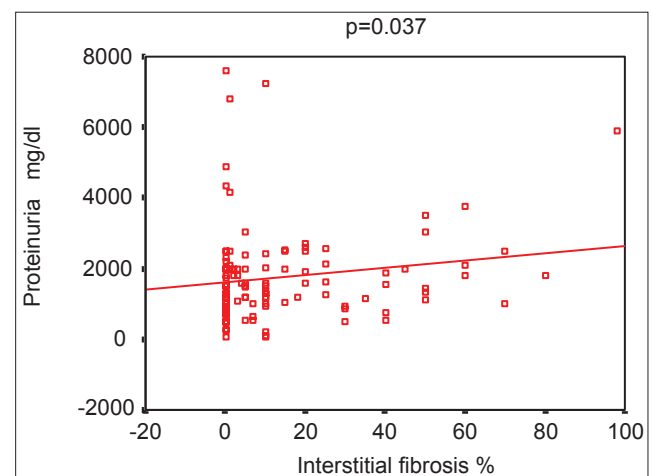
Figure 1: Positive association of proteinuria with interstitial fibrosis

proteinuria. There was not any significant association between four deposited immune reactants (IgA, IgG, IgM, and C3) and the level of proteinuria. Study on patients who had nephrotic range proteinuria showed that, this amount of proteinuria was only seen in male patients. We also found a positive association between nephrotic range proteinuria and T variable of Oxford classification. At the time of renal biopsy for diagnosis of glomerular disease, proteinuria is a well-known indicator of progressive renal disease invarious renal diseases[35-45] as well as in patients with IgAN.[14] In a study conducted by Donadio *et al.* on 154 IgAN patients, they found that 24-h urine protein was a good predictor for subsequent end-stage renal failure.[10] Kobayashi et al. also reported that persistent proteinuria more than 1 g/day was the most reliable, independent predictor of long-term prognosis in Japanese patients with IgAN followed for 10 years or more.[14] Our study was consistent with the results of these reports. Furthermore, it strengthens its importance because this study showed the highest correlation with various pathologic variables that to the best of our knowledge were not reported, yet. We found a positive association between proteinuria and peri-glomerular fibrosis or totally sclerotic glomeruli. We also found a positive correlation between proteinuria and the number of crescents. Specially, among cellular, fibro-cellular and fibrous crescents, only fibrous crescents had an association with proteinuria. In clinical point of view, we found nephrotic range proteinuria (nephrotic syndrome) in only male patients, which further support that, male gender is a risk factor for IgAN.[3-5] Similarly, Hwang *et al.* in a study on 125 biopsy-proven primary IgAN patients who had more than 1.0 g/day proteinuria at the first assessment and underwent anti-proteinuric treatment, found that achievement of less than 1.0 g/day proteinuria was important for limiting the loss of renal function, and relapse of proteinuria should be closely monitored in proteinuric IgAN.[46] The various studies have described that the natural courses and prognostic factors for IgAN patients are progressed to end-stage renal disease.[17,46-50] In these studies, the worst prognosis for nephropathy of IgA was overt proteinuria, renal impairment and high grades of histological classification. However, of these prognostic factors, proteinuria is the most important factor requiring the treatment in IgAN patients.[17,46-50] This finding suggests that reducing these verity of proteinuria in the longterm seems to be the most

important for limiting the loss of renal function in proteinuric IgAN.[17-21,51-55] Kim *et al.* conducted amulti center observational study on 1076 patients with biopsy-proven IgAN from four medical centers in Korea. Of 1076 patients, 100 presented with nephrotic syndrome. They showed that the prognosis of nephrotic syndrome in IgAN was not favorable.[56] In the present study, we showed a positive association between proteinuria and M as well as T variables of the Oxford classification in IgAN. Furthermore, out off our variables, we also showed a positive association between proteinuria and the number of crescents especially fibrous crescent.[57-61] Our findings firstly support the prognostic value of crescent due to its association with proteinuria and secondly imply the importance of treatment of proteinuria to prevent progression of IgAN.

Limitation of the study
We cannot follow the patients.

ACKNOWLEDGMENTS

REFERENCES

1. Chandrika BK. IgA nephropathy in Kerala, India: A retrospective study. Indian J Pathol Microbiol 2009;52:14-6.
2. Solati M, Mahboobi HR. Paraoxonase enzyme activity and dyslipidemia in chronic renal failure patients. J Nephropathology 2012;1:123-5.
3. Mubarak M. Oxford classification of IgA nephropathy: Broadening the scope of the classification. J Nephropathology 2012;1:13-6.
4. Julian BA, Waldo FB, Rifai A, Mesteckey J. IgA nephropathy, the most common glomerulonephritis worldwide: A neglected disease in the United States? Am J Med 1988;84:129-32.
5. Nasri H, Mortazavi M, Ghorbani A, Shahbazian H, Kheiri S, Baradaran A, *et al.* Oxford-MEST classification in IgA nephropathy patients: A report from Iran. J Nephropathology 2012;1:31-42.
6. Kim SM, Moon KC, Oh KH, Kwon Joo KW, Kim YS, Ahn C, *et al.* Clinicopathologic characteristics of IgA nephropathy with steroid-responsive nephrotic syndrome. J Korean Med Sci 2009;24:S44-9.
7. Assadi F. The epidemic of pediatric chronic kidney disease the danger of skepticism. J Nephropathology 2012;1:61-4.
8. Sahni N, Gupta KL. Dietary antioxidents and

oxidative stress in predialysis chronic kidney patients. J Nephropathology 2012;1:134-42.

9. Mubarak M. The prevalence of IgA nephropathy in Pakistan: Only a tip of the iceberg. J Pak Med Assoc 2009;59:733.

10. Tayebi Khosroshahi H. Short history about renal transplantation program in Iran and the world: Special focus on world kidney day 2012. J Nephropathology 2012;1:5-10.

11. Mubarak M. IgA nephropathy: An update on pathogenesis and classification. J Coll Physicians Surg Pak 2011;21:230-3.

12. Donadio JV, Bergstralh EJ, Grande JP, Rademcher DM. Proteinuria patterns and their association with subsequent end-stage renal disease in IgA nephropathy. Nephrol Dial Transplant 2002;17:1197-203.

13. Nasri H. Hypertension and renal failure with right arm pulse weakness in a 65 years old man. J Nephropathology 2012;1:130-3.

14. Kobayashi Y, Hiki Y, Sano T, Hashizume K, Matsuo T, Nakamura I, *et al*. Prognostic significance of persistent massive proteinuria in IgA nephropathy: 10-year follow-up study of 366 cases. Nephrology 2001;6:A23-4.

15. D'amico G. The most commonest glomerulonephritis in the world: IgA nephropathy. Q J Med 1987*;* 64: 709–27.

16. Maisonneuve P, Agodoa L, Gellert R, Stewart JH, Buccianti G, Lowenfels AB, *et al*. Distribution of primary renal diseases leading to end-stage renal failure in the United States, Europe, and Australia/New Zealand: Results from an international comparative study. Am J Kidney Dis 2000;35:157-65.

17. Koyama A, Igarashi M, Kobayashi M. Natural history and risk factors for immunoglobulin A nephropathy in Japan. research group on progressive renal diseases. Am J Kidney Dis 1997;29:526-32.

18. Berthoux FC, Mohey H, Afiani A. Natural history of primary IgA nephropathy. Semin Nephrol 2008;28:4-9.

19. D'amico G. Natural history of idiopathic IgA nephropathy and factors predictive of disease outcome. Semin Nephrol 2004;24:179-96.

20. Lv JC, Zhang H, Zhou Y, Li GT, Zou WZ, Wang HY. Natural history of immunoglobulin A nephropathy and predictive factors of prognosis: A long-term follow up of 204 cases in China. Nephrology (Carlton) 2008;13:242-6.

21. Alamartine E, Sabatier JC, Guerin C, Berliet JM, Berthoux F. Prognostic factors in mesangial IgA glomerulonephritis: An extensive study with univariate and multivariate analyses. Am J Kidney Dis 1991;18:12-9.

22. D'amico G. Natural history of idiopathic IgA nephropathy: Role of clinical and histological prognostic factors. Am J Kidney Dis 2000;35:157-65.

23. Bartosik LP, Lajoie G, Sugar L, Cattran DC. Predicting progression in IgA nephropathy. Am J Kidney Dis 2001;38:728-35.

24. Woo KT, Lau YK, Wong KS, Chiang GS. ACEI/ ATRA therapy decreases proteinuria by improving glomerular permselectivity in IgA nephritis. Kidney Int 2000;58:2485-91.

25. Praga M, Gutiérrez E, González E, Morales E, Hernández E. Treatment of IgA nephropathy with ACE inhibitors: A randomized and controlled trial. J Am Soc Nephrol 2003;14:1578-83.

26. Dillon JJ. Angiotensin-converting enzyme inhibitors and angiotensin receptor blockers for IgA nephropathy. Semin Nephrol 2004;24:218-24.

27. Ballardie FW, Cowley RD. Prognostic indices and therapy in IgA nephropathy: Toward a solution. Kidney Int 2008;73:249-51.

28. Barratt J, Feehally J. IgA Nephropathy. J Am Soc Nephrol 2005;16:2088-97.

29. Working Group of the International IgA Nephropathy Network and the Renal Pathology Society, Cattran DC, Coppo R, Cook HT, Feehally J, Roberts IS, *et al*. The Oxford classification of IgA nephropathy: Rationale, clinicopathological correlations, and classification. Kidney Int 2009;76:534-45.

30. Working Group of the International IgA Nephropathy Network and the Renal Pathology Society, Coppo R, Troyanov S, Camilla R, Hogg RJ, Cattran DC, *et al*. The Oxford IgA nephropathy clinicopathological classification is valid for children as well as adults. Kidney Int 2010;77:921-7.

31. Working Group of the International IgA Nephropathy Network and the Renal Pathology Society, Roberts IS, Cook HT, Troyanov S, Alpers CE, Amore A, *et al*. The Oxford classification of IgA nephropathy: Pathology definitions, correlations, and reproducibility. Kidney Int 2009;76:546-56.

32. Mubarak M, Kazi JI, Kulsoom U, Ishaque M. Detection of immunoglobulins and complement components in formalin fixed and paraffin embedded renal biopsy material by immunoflourescence technique. J Nephropathology 2012;1:91-100.

33. Mubarak M. Collapsing focal segmental glomerulosclerosis: Increasing the awareness. J Nephropathology 2012;1:77-80.

34. Mohammadi Torbati P. Focal segmental glomerulosclerosis; collapsing variant. J Nephropathology 2012;1:87-90.

35. Working Group of the International IgA Nephropathy Network and the Renal Pathology Society, Coppo R, Troyanov S, Camilla R, Hogg RJ, Cattran DC, *et al*. The Oxford IgA nephropathy clinicopathological classification is valid for children as well as adults. Kidney Int 2010;77:921-7.

36. Karimifar M. Deep vein thrombosis in combination

with granulomatosis with polyangiitis (Wegener's). J Nephropathology 2012;1:57-8.

37. Tolou-Ghamari Z. Neurotoxicity, mechanisms of rejection: A review on Tacrolimus and Cyclosporin in organ transplantation. J Nephropathology 2012;1:23-30.

38. Ardalan MR, Samadifar Z, Vahedi A. Creatine monohydrate supplement induced interstitial nephritis. J Nephropathology 2012;1:117-20.

39. Gheissari A, Mehrasa P, Merrikhi A, Madihi Y. Acute kidney injury: A pediatric experience over 10 years at a tertiary care center. J Nephropathology 2012;1:101-8.

40. Mortazavi M, Nasri H. Granulomatosis with polyangiitis (Wegener's) presenting as the right ventricular masses: A case report and review of the literature. J Nephropathology 2012;1:49-56.

41. Galesic K, Ljubanovic D, Horvatic I. Treatment of renal manifestations of ANCA-associated vasculitis. J Nephropathology 2013;2:6-19.

42. Shakeel Sh, Mubarak M, Kazi JI, Jafry N, Ahmed E. Frequency and clinicopathological characteristics of variants of primary focal segmental glomerulosclerosis in adults presenting with nephrotic syndrome. J Nephropathology 2013;2:28-35.

43. Seif EI, Ibrahim EA, Elhefnawy NG, Salman MI. Histological patterns of idiopathic steroid resistant nephrotic syndrome in Egyptian children: A single centre study. J Nephropathology 2013;2:53-60.

44. Vanikar A. IgM nephropathy; can we still ignore it. J Nephropathology 2013;2:98-103.

45. Grcevska L, Ristovska V, Nikolov V, Petrusevska G, Milovanceva-Popovska M, Polenakovic M. The Oxford classification of IgA nephropathy: Single centre experience. Prilozi 2010;31:7-16.

46. Hwang HS, Kim BS, Shin YS, Yoon HE, Song JC, Choi BS, *et al*. Predictors for progression in Immunoglobulin A nephropathy with significant proteinuria. Nephrology (Carlton) 2010;15:236-41.

47. Kari J. Epidemiology of chronic kidney disease in children. J Nephropathology 2012;1:162-3.

48. Rahimi Z. ACE insertion/deletion (I/D) polymorphism and diabetic nephropathy. J Nephropathology 2012;1:143-51.

49. Gheissari A, Attarzadeh H, Sharif H, Pourhossein M, Merrikhi A. Steroid dependent and independent ocular findings in Iranian children with nephrotic syndrome. Int J Prev Med 2011;2:264-8.

50. Kam-Tao Li PK, Burdmann EA, Mehta RL. Acute kidney injury: Global health alert. J Nephropathology 2013;2:90-7.

51. Gheissari A, Hemmatzadeh S, Merrikhi A, Fadaei Tehrani S, Madihi Y. Chronic kidney disease in children: Report from a tertiary care center over 11 years. J Nephropathology 2012;1:177-82.

52. Sánchez-Niño MD, Ortiz A. Is it or is it not a pathogenic mutation? Is it or is it not the podocyte? J Nephropathology 2012;1:152-4.

53. Maghsoudi AR, Baradaran-Ghahfarokhi M, Ghaed-Amini F, Nasri H, Mobarakeh MD, Rafieian-Kopaei M. Renal failure and submental lymphadenopathy in a 68 years old woman. J Nephropathology 2012;1:198-201.

54. Nasri H. Sudden onset of acute renal failure requiring dialysis associated with large B-cell lymphoma of colon. J Nephropathology 2012;1:202-6.

55. Nickavar A, Sotoudeh K. Treatment and prophylaxis in pediatric urinary tract infection. Int J Prev Med 2011;2:4-9.

56. Kim JK, Kim JH, Lee SC, Kang EW, Chang TI, Moon SJ, *et al*. Clinical features and outcomes of IgA nephropathy with nephrotic syndrome. Clin J Am Soc Nephrol 2012;7:427-36.

57. Katafuchi R, Ninomiya T, Nagata M, Mitsuiki K, Hirakata H. Validation study of Oxford classification of IgA nephropathy: The significance of extracapillary proliferation. Clin J Am Soc Nephrol 2011;6:2806-13.

58. Kawamura T, Joh K, Okonogi H, Koike K, Utsunomiya Y, Miyazaki Y, *et al*. A histologic classification of IgA nephropathy for predicting long-term prognosis: Emphasis on end-stage renal disease. J Nephrol 2013;26:350-7.

59. Nasri H. Comment on: Clinical, histopathological and immunofluorescent findings of IgA nephropathy and author's reply. Iran J Immunol 2012;9:266-7.

60. Nasri H, Sajjadieh S, Mardani S, Momeni A, Merikhi A, Madihi Y, *et al*. Correlation of immunostaining findings with demographic data and variables of Oxford classification in IgA nephropathy. J Nephropathology 2013;2:190-5.

61. Mubarak M. Significance of immunohistochemical findings in Oxford classification of IgA nephropathy: The need for more validation studies. J Nephropathology 2013;2:210-3.