



# Ki-67 proliferative index in immunoglobulin A nephropathy; A pilot study

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## Abstract

**Introduction:** IgA nephropathy (IgAN) regarded as the main type of primary glomerulonephritis globally. It has a diverse clinical course and unpredictable prognosis. Traditionally, clinical and pathological features are conducted to prognosticate the outcome of the disease. Recently, attention has been directed towards other indices that may have prognostic implications for IgAN progression. The Ki-67 index have been extensively detected as a proliferation marker of malignant cells for several years.

**Objectives:** In this study, we aimed to determine the prognostic impact of Ki-67 expression in IgAN biopsies.

**Patients and Methods:** This cross-sectional study was conducted on 18 biopsy-proven IgAN patients. IgAN was diagnosed by significant IgA deposits and according to the morphologic variables of Oxford (MEST) classification for this disease. The expression of Ki-67 in the glomeruli, interstitium and tubules was described as the intensity percentage of the immunohistochemical staining.

**Results:** In all of the eighteen cases with biopsy-proven IgAN, the mean  $\pm$  SD of age, degree of proteinuria and serum creatinine were  $36.3 \pm 17$  years,  $1600 \pm 733$  mg/day and  $1.2 \pm 0.4$  mg/dL, respectively. In this study, we found a significant correlation between glomerular Ki-67 immunohistochemical staining and MEST scores ( $r: 0.780, P < 0.001$ ). We also detected a significant correlation between interstitial Ki-67 staining and MEST scores ( $r: 0.700, P = 0.001$ ). Moreover, Ki-67 immunohistochemical staining of the interstitium had a significant relationship with IgM deposits ( $r = 0.544, P = 0.02$ ).

**Conclusion:** In this pilot study, we found that interstitial and glomerular Ki-67 immunohistochemical staining had positive and significant associations with MEST scores. Our data also showed that interstitial Ki-67 staining had a significant relationship with IgM deposits. These preliminary results require further investigation in large-scale studies.



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## Introduction

IgA nephropathy (IgAN) is regarded as the main type of primary glomerulonephritis globally (1). IgAN mainly presents in the second and third decade of life and usually leads to end-stage renal disease (ESRD) after 20 years from diagnosis (2). Numerous investigations showed a nearly 2:1 male-to-female preponderance in the Western European and North American populations; however, there is an equal prevalence for both genders in the Eastern Asia region. (3). Pathologically, IgAN is

diagnosed by predominant or co-dominant IgA deposition in the mesangial area of the glomeruli (4), which is commonly associated with the hypercellularity of the mesangial and endocapillary regions (5). The clinical predictors of IgAN including decreased estimated glomerular filtration rate (eGFR), hypertension and proteinuria at the time of the diagnosis are well known (6). Studies regarding the prognostic value of morphologic lesions showed that hypercellularity of the mesangium, segmental glomerulosclerosis, endocapillary hypercellularity, crescents, and

**Key point**

IgA nephropathy (IgAN) is the most common type of primary glomerulonephritis globally. It has a diverse clinical course and unpredictable prognosis. In a cross-sectional study on 18 biopsy-proven IgAN patients, we found a significant correlation between glomerular Ki-67 immunohistochemical staining and the Oxford classification of IgAN (MEST-C) scores. We also found a significant correlation between interstitial Ki-67 immunohistochemical staining and MEST scores. Moreover, interstitial Ki-67 immunohistochemical staining had a significant relationship with IgM deposits. Our results suggest the possible predictive and prognostic roles of Ki-67 immunostaining in patients with IgAN.

finally, interstitial fibrosis/tubular atrophy (IFTA) have prognostic implications (5). Recently, more attention has been directed towards other indices with prognostic implications in the progression of IgAN. Ki-67 (antigen KI-67) is a protein that is encoded by the *MKI67* gene (antigen identified by monoclonal antibody Ki-67) (7). The Ki-67 protein has been extensively used as a proliferation marker of malignant cells for several years (8). Immunohistochemically, an elevated Ki-67 proliferative index is correlated with cellular proliferation (9).

More recently, its use has been extended to some non-malignant diseases that are associated with cellular proliferation and inflammation (9). Several studies have shown that elevated Ki-67 indices are correlated with cellular proliferation and clinical findings in patients with lupus nephritis (9). However, studies regarding the possible diagnostic or prognostic usefulness of Ki-67 expression in IgAN are scarce.

**Objectives**

In this study, we aimed to determine the prognostic impact of Ki-67 expression in biopsy-proven IgAN cases.

**Patients and Methods****Patients and specimens**

This cross-sectional study was conducted on biopsy-proven IgAN patients between Jan 2020 and Jan 2021. A total of eighteen IgAN patients were enrolled in the study.

**Definition of immunoglobulin A nephropathy**

The morphologic diagnosis of IgAN was based on diffuse and global mesangial IgA deposits of more than 2+ intensity detected by immunofluorescence microscopy accompanied by the lack of significant C1q deposits (10).

**Histologic data**

During kidney biopsy, two specimens were taken for light and direct immunofluorescence microscopic studies. One fragment was fixed in 10% formalin for histologic evaluation and was stained with periodic acid-Schiff (PAS), Jones methenamine silver, Masson's trichrome, and hematoxylin and eosin (H&E). The specimen for immunofluorescence was snap-frozen in liquid nitrogen, to

stain for IgA, IgM, IgG, C1q and C3. Immunofluorescence slides were reported on a magnitude of 0 to 3+ intensity (10).

**Immunohistochemical analysis for Ki-67**

For Ki-67 immunohistochemical staining, 4  $\mu$ m thick sections were stained with rabbit monoclonal antibodies (Zytomed Systems GmbH, Berlin, Germany). The expression of Ki-67 in the glomeruli (gKi-67), interstitium (iKi-67) and tubules (tKi-67) was expressed as the percentage of intensely stained nuclei on the immunohistochemical staining. We also analyzed the total score of Ki-67 in the glomeruli, interstitium, and tubules (igtKi-67).

**Definitions of morphologic variables of MEST (Oxford) classification**

In 2009, the MEST-C score or Oxford classification of IgAN, which includes various morphologic features and independently predicts the clinical outcome, was published. These features included mesangial hypercellularity (M), endocapillary hypercellularity (E), segmental glomerulosclerosis (S), tubular atrophy/interstitial fibrosis (T) and crescents (C) (11).

**Statistical analysis**

Statistical analysis was conducted by the SPSS software, version 24. The correlations between quantitative variables were tested through Pearson's or Spearman's correlation tests. We assessed the relationships between qualitative variables by chi-square test. Differences between quantitative means of variables were investigated through independent t-test or one-way ANOVA. The level of significance was defined as less than 0.05 (P value <0.05).

**Results**

This investigation comprised eighteen cases of biopsy-proven IgAN that were stained with Ki-67 immunostaining. Of eighteen patients, ten were male. The mean  $\pm$  SD of age, quantity of proteinuria, and serum creatinine in all patients were 36.3 $\pm$ 17 years, 1600 $\pm$ 733 mg/d and 1.2 $\pm$ 0.4 mg/dL, respectively. Of eighteen patients, 55.5% had mesangial proliferation (M1), 27.8% had endocapillary hypercellularity (E1) and 50% had segmental sclerosis (S1) while 33.3% of the patients had crescents. T0 (*interstitial fibrosis/tubular atrophy*) was detected in 44.4%, T1 in 38.9% and T2 in 16.7% of the patients. Studies regarding the association between gKi-67 and the age, gender, level of proteinuria, and serum creatinine level were not significant ( $P>0.05$ ). Moreover, the study regarding the association between gKi-67 and interstitial fibrosis (percent) and the number of crescents was not significant ( $P >0.05$ ). The relationship of gKi-67 with global versus segmental involvement of the glomeruli was not significant ( $P >0.05$ ). Furthermore, the correlation of gKi-67 with IgA, IgG, IgM and C3 deposits was not significant ( $P >0.05$ ). However, we found a significant and positive correlation between

gKi-67 and MEST scores ( $r: 0.780, P < 0.001$ ).

The study regarding the association between iKi-67 and the age, gender, level of proteinuria and serum creatinine level was not significant ( $P > 0.05$ ). The association between iKi-67 and interstitial fibrosis and the number of crescents was also not significant ( $P > 0.05$ ). However, our results showed a significant correlation between iKi-67 and MEST scores ( $r: 0.700, P = 0.001$ ).

Likewise, the correlation between iKi-67 and IgA, IgG and C3 deposits was not significant ( $P > 0.05$ ); however, the correlation of iKi-67 with IgM deposits was significant ( $r = 0.544, P = 0.02$ ).

The associations between tKi-67 and the age, gender, level of proteinuria and serum creatinine were not significant ( $P > 0.05$ ). Similarly, the association between tKi-67 and interstitial fibrosis and the number of crescents was not significant too ( $P > 0.05$ ). Furthermore, the correlation between tKi-67 and IgA, IgG, IgM and C3 was not significant ( $P > 0.05$ ). Our study showed no significant association between igtKi-67 and the age, gender, level of proteinuria and serum creatinine level ( $P > 0.05$ ). Similarly, the correlation of igtKi-67 with interstitial fibrosis and the number of crescents was also not significant ( $P > 0.05$ ). In addition, the correlation between igtKi-67 and IgA, IgG, IgM and C3 deposits was not significant as well ( $P > 0.05$ ).

## Discussion

IgAN is one of the most prevalent types of glomerulonephritis and is considered an important cause of ESRD (12). The deposition of IgA (an important immunoglobulin) and the consequent proliferation of mesangial cells, inflammatory system activation and podocytopathy are the main pathophysiological features of the disease (13). Additionally, a rise in circulatory galactose-deficient IgA<sub>1</sub>, the generation of IgA and IgG anti-glycan antibodies and the stimulation of alternative complement systems are involved in the mechanisms of the disease development and progression (14). Clinically, patients with IgAN have proteinuria, hematuria, increased blood pressure and decreased GFR. Progressive kidney damage and ESRD occur in approximately a quarter of the patients (15, 16); in particular, in individuals with a low-baseline GFR, proteinuria ( $>1$  g/d) and hypertension. However, the lack of useful prognostic biomarkers of IgAN has hindered the exact evaluation of the loss of kidney function and further disease treatment. Additionally, the biomarkers which are reproducible and can predict the disease in the early stages are preferred to access available therapeutic options. In this context, molecular alterations in the mesangial cells, which play a crucial role in the deposition of IgA immune complexes and subsequent inflammatory responses, are the target of several studies regarding the identification of novel diagnostic markers.

Ki-67 is a monoclonal antibody that binds with a nuclear antigen (Ki-67 antigen) in all phases of the cell cycle except G<sub>0</sub> and has been applied for the detection of cell

proliferation and T-cell activation in common viral diseases (17, 18). This antigen as well as the cell surface transferrin receptor are abundantly found in the initial stages of renal tubulogenesis, but not in the progressive maturation phases (19). It has been shown that Ki-67 shows positive staining in glomerular crescents and proliferating mesangial cells (20). However, in our study, no association was observed between gKi-67 and interstitial fibrosis (percent) and the number of crescents. The expression of Ki-67 in the kidney samples of IgAN patients has been observed in close relation with positive staining of MHC class II antigens and the proliferation of endocapillary cells, but not in the samples of healthy subjects (21). Moreover, the gKi-67 has been shown in abundance during the acute phases of glomerulonephritis. However, endocapillary proliferation and cellular activation demonstrated by specific markers such as Ki-67 are only observed in severe cases of IgAN nephropathy. In our study, no significant association was found between gKi-67 and iKi-67 and the age, gender, level of proteinuria and serum creatinine, which might be due to the small number of cases (21).

In a study conducted by Park et al, the glomeruli were collected from biopsy samples of IgAN patients and healthy individuals to perform an RNA-sequencing (RNA-seq) analysis (22). In this study, primary mesangial cell cultivation and immunohistochemical labeling were employed to confirm the outputs of RNA-seq tests. The expression of Ki-67 in cultured mesangial cells was evaluated by immunofluorescence microscopy. According to their results, treatment of mesangial cells with patient-derived IgA<sub>1</sub> increased their proliferation, which was in line with the elevation of Ki-67 expression (22). In addition, the assessment of immunohistological features of IgAN in the context of cellular proliferation gives us more clues regarding the disease prognosis (23). The pivotal role of the proliferation and activation of mesangial cells in the development of glomerulonephritis has been shown in other studies (24-26). It is assumed that mesangial cells contribute to the disease progression through glomerular hemodynamics and mesangial matrix production and subsequently the induction of glomerular sclerosis (27). Both mesangial cells and monocytes infiltrating capillary loops might be Ki-67 positive in glomerulonephritis (28).

Jiang et al have reported substantial elevation of Ki-67 positive cells in IgAN samples compared to membranous nephropathy and diabetic glomerulosclerosis samples, as non-proliferative types of glomerulonephritis (29). This was in close correlation with the expression of  $\alpha$ -smooth muscle actin ( $\alpha$ -SMA) which is mainly expressed in different human glomerular diseases (30). Furthermore, the number of Ki-67 positive cells was more in patients with higher rates of proteinuria. The authors concluded that the assessment of the proliferative function of mesangial cells using Ki-67 expression in renal biopsies might be a valuable biomarker for the possible prediction of IgAN progression (29).

The Oxford classification of IgAN has been validated in several cohorts with comprehensive inclusion criteria and the important pathological variables have been summarized into the 'MEST-C' scores (11). In this context, M1 predicts a rapid loss of kidney function and the need for immunosuppressive therapy. E1 and C1-2 showed lower survival in the cases not receiving immunosuppression. Enhanced glomerular infiltration and activated inflammatory response are observed. Podocyte injury and segmental sclerosis should be reported in these patients. T1-2 is linked with plummeted GFR (31). The poor and scarce correlations between Ki-67 and MEST-C scores in our study may be due to the small number of biopsy samples and milder forms of the disease.

### Limitations of the study

The relatively small number of kidney biopsies is a limitation of our study. We conducted this study in the COVID-19 pandemic. The study is cross-sectional in its nature and follow-up data is not included. We suggest additional prospective studies on a large number of cases on this feature of IgAN patients.

### Authors' contribution

AB and NH conducted the investigation. MBA conducted the statistical analysis. MB, EE, MBad and ARM prepared the draft. MM edited the manuscript. ARM, EA and AB both are corresponding authors, which edited and finalized the paper equally. All authors read and signed the final paper.

### Conflicts of interest

The authors declare no conflict of interest.

### Ethical issues

This investigation was conducted in accordance with the Declaration of Helsinki. This study was conducted on paraffin-embedded blocks of kidney biopsies to assess the Ki-67 intensity in the glomerular, tubular, and interstitial areas. Written informed consents were obtained from the patients at the time of renal biopsy. The ethical board committee of the national institute for medical research development (NIMAD) approved this study (NIMAD; <http://nimad.ac.ir>, ethical code# IR.NIMAD.REC.1398.067). Besides, ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors.

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### References

- Saha MK, Julian BA, Novak J, Rizk DV. Secondary IgA nephropathy. *Kidney Int.* 2018;94:674-681. doi: 10.1016/j.kint.2018.02.030.
- Sallustio F, Curci C, Di Leo V, Gallone A, Pesce F, Gesualdo L. A New Vision of IgA Nephropathy: The Missing Link. *Int J Mol Sci.* 2019;21:189. doi: 10.3390/ijms21010189.
- Galla JH. IgA nephropathy. *Kidney Int.* 1995;47:377-87.
- Magistroni R, D'Agati VD, Appel GB, Kiryluk K. New developments in the genetics, pathogenesis, and therapy of IgA nephropathy. *Kidney Int.* 2015;88:974-89. doi: 10.1038/ki.2015.252.
- Maixnerova D, Reily C, Bian Q, Neprasova M, Novak J, Tesar V. Markers for the progression of IgA nephropathy. *J Nephrol.* 2016;29:535-41. doi: 10.1007/s40620-016-0299-0.
- Fabiano RC, Pinheiro SV, de Almeida Araújo S, Simões E Silva AC. Immunoglobulin a nephropathy: Pathological markers of renal survival in paediatric patients. *Nephrol (Carlton).* 2016;21:995-1002. doi: 10.1111/nep.12850.
- Sobecki M, Mrouj K, Camasses A, Parisi N, Nicolas E, Llères D, et al. The cell proliferation antigen Ki-67 organises heterochromatin. *Elife.* 2016 Mar 7;5:e13722. doi: 10.7554/eLife.13722.
- Sun X, Kaufman PD. Ki-67: more than a proliferation marker. *Chromosoma.* 2018;127:175-186. doi: 10.1007/s00412-018-0659-8.
- Rioux-Leclercq N, Turlin B, Bansard J-Y, Patard J-J, Manunta A, Moulinoux J-P, et al. Value of immunohistochemical Ki-67 and p53 determinations as predictive factors of outcome in renal cell carcinoma. *Urology.* 2000;55:501-5.
- Espinosa M, Ortega R, Gómez-Carrasco JM, López-Rubio F, López-Andreu M, López-Oliva MO, et al. Mesangial C4d deposition: a new prognostic factor in IgA nephropathy. *Nephrol Dial Transplant.* 2009;24:886-91.
- Trimarchi H, Barratt J, Cattran DC, Cook HT, Coppo R, Haas M, et al; IgAN Classification Working Group of the International IgA Nephropathy Network and the Renal Pathology Society; Conference Participants. Oxford Classification of IgA nephropathy 2016: an update from the IgA Nephropathy Classification Working Group. *Kidney Int.* 2017;91:1014-1021. doi: 10.1016/j.kint.2017.02.003.
- D'Amico G. The commonest glomerulonephritis in the world: IgA nephropathy. *QJM: Int J Med.* 1987;64:709-27.
- Suzuki H, Kiryluk K, Novak J, Moldoveanu Z, Herr AB, Renfrow MB, et al. The pathophysiology of IgA nephropathy. *J Am Soc Nephrol.* 2011;22:1795-803. doi: 10.1681/ASN.2011050464.
- Kiryluk K, Novak J. The genetics and immunobiology of IgA nephropathy. *J Clin Invest.* 2014;124:2325-32. doi: 10.1172/JCI74475.
- Lee H, Kim DK, Oh KH, Joo KW, Kim YS, Chae DW, et al. Mortality of IgA nephropathy patients: a single center experience over 30 years. *PLoS One.* 2012;7:e51225. doi: 10.1371/journal.pone.0051225.
- Park S, Baek CH, Park SK, Kang HG, Hyun HS, Park E, et al. Clinical Significance of Crescent Formation in IgA Nephropathy - a Multicenter Validation Study. *Kidney Blood Press Res.* 2019;44:22-32. doi: 10.1159/000497808.
- Scholzen T, Gerdes J. The Ki-67 protein: from the known and the unknown. *J Cell Physiol.* 2000;182:311-22.
- Jonat W, Arnold N. Is the Ki-67 labelling index ready for clinical use? *Ann Oncol.* 2011;22:500-502. doi: 10.1093/annonc/mdq732.
- Fleming S, Jones D. Immunocytochemical evidence for transferrin-dependent proliferation during renal tubulogenesis. *J Anat.* 1987;153:191.
- Hall P, Greenwood R, d'Ardenne A, Levison D. In situ demonstration of renal tubular regeneration using the monoclonal antibody Ki67. *Nephron.* 1988;49:122-5.
- Yokoyama H, Takaeda M, Wada T, Ogi M, Tomosugi N, Takabatake T, et al. Intraglomerular Expression of MHC Class II and Ki-67 Antigens and Serum  $\gamma$ -Interferon Levels in IgA Nephropathy. *Nephron.* 1992;62:169-75.
- Park S, Yang SH, Jeong CW, Moon KC, Kim DK, Joo KW, et al. RNA-Seq profiling of microdissected glomeruli identifies potential biomarkers for human IgA nephropathy. *Am J Physiol Renal Physiol.* 2020;319:F809-21. doi: 10.1152/ajprenal.00037.2020.
- Nabokov A, Waldherr R, Ritz E. Demonstration of the

- proliferation marker Ki-67 in renal biopsies: correlation to clinical findings. *Am J Kidney Dis.* 1997;30:87-97.
24. Floege J, Johnson R, Couser W. Mesangial cells in the pathogenesis of progressive glomerular disease in animal models. *Clin Investig.* 1992;70:857-64.
  25. Floege J, Alpers C, Burns M, Pritzl P, Gordon K, Couser W, et al. Glomerular cells, extracellular matrix accumulation, and the development of glomerulosclerosis in the remnant kidney model. *Lab Invest.* 1992;66:485-97.
  26. Floege J, Burns MW, Alpers CE, Yoshimura A, Pritzl P, Gordon K, et al. Glomerular cell proliferation and PDGF expression precede glomerulosclerosis in the remnant kidney model. *Kidney Intern.* 1992;41:297-309.
  27. Pichler RH, Bassuk JA, Hugo C, Reed MJ, Eng E, Gordon KL, et al. SPARC is expressed by mesangial cells in experimental mesangial proliferative nephritis and inhibits platelet-derived-growth-factor-mediated mesangial cell proliferation in vitro. *Am J Pathol.* 1996;148:1153.
  28. Kim O. Immunohistochemical study of the expression of alpha-smooth muscle actin and the proliferation marker Ki-67 of glomerulonephritis. *J Korean med sci.* 2001;16:455.
  29. Jiang W, Zhou D, Inui K, Watanabe S, Morita H, Yoshimura A. The Proliferation-associated Antigen Ki-67 is a Useful Marker for Evaluating Disease Activity in Human IgA Nephropathy. *Showa Univ J Med Sci.* 2006;18:117-24.
  30. Utsunomiya Y, Kawamura T, Abe A, Imai H, Hirano K, Maruyama N, et al. Significance of mesangial expression of  $\alpha$ -smooth muscle actin in the progression of IgA nephropathy. *Am J Kidney Dis.* 1999;34:902-10.
  31. Soares MF, Roberts IS. IgA nephropathy: an update. *Curr Opin Nephrol Hypertens.* 2017;26:165-71. doi: 10.1097/MNH.0000000000000312.