ARTÍCULO ORIGINAL

SIGNIFICANCE OF IgA DEPOSITS LOCATION (MESANGIOCAPILLARY VERSUS PURE MESANGIAL) IN IgA NEPHROPATHY AND ITS ASSOCIATION WITH MORPHOLOGIC VARIABLES OF OXFORD CLASSIFICATION AND VARIOUS DEMOGRAPHIC DATA

SIGNIFICADO DE LA UBICACIÓN DE LOS DEPÓSITOS DE IgA (MESANGIO CAPILARES VERSUS MESANGIALES PUROS) EN LA NEFROPATÍA IgA Y SU ASOCIACIÓN CON LAS VARIABLES MORFOLÓGICAS DE LA CLASIFICACIÓN DE OXFORD Y VARIOS DATOS DEMOGRÁFICOS

Germán T. Hernández,[1] Azar Baradaran,[2] Hamid Nasri[3]*

[1] Divisions of Nephrology & Hypertension and Biostatistics & Epidemiology, Paul L. Foster School of Medicine, Texas Tech University Health Sciences, El Paso, Texas USA.

[2] Department of Nephrology, Division of Nephropathology, Isfahan University of Medical Sciences, Isfahan, Iran.

[3] Department of Pathology, Isfahan University of Medical Sciences, Isfahan, Iran.

***Corresponding author:** Professor Hamid Nasri, Department of Nephrology, Division of Nephropathology, Isfahan University of Medical Sciences, Isfahan, Iran.

Nefrología, Diálisis y Trasplante 2013; 33 (2) Pag. 68 - 74

RESUMEN

Introducción: La nefropatía IgA se caracteriza por la presencia de depósitos glomerulares con predominio de IgA. Dentro de la descripción de la nefropatía IgA, existe una variación en la ubicación de los depósitos de inmunoglobulina A, desde el área mesangial hasta las paredes capilares. **Objetivo:** El objetivo de este estudio es determinar la posible correlación entre la ubicación de los depósitos de IgA y las variables morfológicas de la clasificación de Oxford (MEST, por sus siglas en inglés). Proliferación (hipercelularidad) mesangial (M) y endocapilar (E), glomeruloesclerosis (S) y atrofia tubular y fibrosis intersticial (T), y diversos datos clínicos de pacientes con nefropatía por inmunoglobulina. **Métodos:** El diagnóstico patológico de la nefropatía IgA requiere la demostración de depósitos inmunes con predominio de IgA con un patrón mesangial o mesangiocapilar a través de la microscopia por inmunofluorescencia (IF por su sigla en ingles). Los depósitos inmunes fueron semicuantificados con grados de fluorescencia de 0 a 3 cruces (+). La definición de la nefropatía por IgA requiere la presencia de depósitos de IgA difusos y globales con ≥ 2 + de fluorescencia y la ausencia de depósitos de C I q. Todas las biopsias renales realizadas entre julio del 2009 y julio de 2012, fueron enviadas a nuestro laboratorio de patología renal para analizarlas. Ninguno de los pacientes fue tratado antes de habérsele realizado la biopsia. Las biopsias con menos de 8 glomérulos fueron excluidas del estudio. Ninguno de los pacientes recibió un diagnóstico de nefropatía IgA, si había antecedentes de enfermedad vascular del colágeno o cirrosis hepática en los cuestionarios clínicos, los análisis de laboratorio o en el historial médico obtenidos al ingresar a los pacientes para realizarles la biopsia renal. **Resultados:** Un total de 114 biopsias fueron incluidas en el estudio. La edad media de los pacientes fue de 37,7 ± 13,6 años. Los pacientes se dividieron en dos grupos: depósitos puros mesangiales y depósitos mesangiocapilares. El número medio de glomérulos obtenidos por biopsia fue de 14,8 ± 7,2. El nivel medio de la proteinuria fue de 1742 ± 1324 mg /día (mediana = 1500 mg /día). En todas las biopsias, el número medio

de glomérulos totalmente esclerosados fue de 2,4 ± 2,9 (mediana =1 glomérulo). Asimismo, la media del nivel de creatinina sérica fue de 1,6 ± 1,5 mg/dl (mediana = 1,2 mg / dl). En este estudio, el 10,5 por ciento de las biopsias renales tenían depósito de IgA mesangiocapilares. No se encontró ninguna asociación significativa entre la proporción de glomérulos totalmente esclerosados, la proliferación extracapilar, el porcentaje de fibrosis peri-glomerular, el engrosamiento de la cápsula de Bowman, el porcentaje de fibrosis intersticial, la proliferación mesangial de cualquier grado, o el ensanchamiento mesangial con depósitos mesangiales puros o depósitos mesangiocapilares (p≤ 0,05). No hubo ninguna asociación significativa entre la edad, la creatinina sérica y los niveles de proteinuria con depósitos mesangiales puros o mesangiocapilares (p≥0,05). Entre las cuatro variables morfológicas de la clasificación MEST de Oxford, únicamente la variable E (proliferación endocapilar) tuvo asociación significativa con depósitos mesangiocapilares (p=0,04) **Conclusiones:** La asociación entre depósitos mesangiocapilares IgA y la proliferación endocapilar puede implicar una mayor gravedad de la enfermedad por nefropatía IgA. Por lo tanto, se recomienda que la ubicación y la intensidad de los depósitos de IgA se incluyan de forma sistemática en los informes de biopsia renal.

Palabras Clave: Nefropatía por IgA, inmunoglobulinas, depósitos inmunes, marcación por inmunofluorescencia, inmunomarcación.

ABSTRACT

Introduction: IgA nephropathy is characterized by the presence of IgA-dominant glomerular deposits. Within this description, there is variation in the location of this immunoglobulin, from mesangial area to capillary walls. **Objectives:** The aim of this study is to determine the potential correlation between the location of IgA deposits and morphologic variables of Oxford classification (MEST) and various clinical data of patients with immunoglobulin A nephropathy (IgAN). **Results:** A total of 114 biopsies were enrolled to the study. Mean age of patients was 37.7 ± 13.6 years. Patients were divided into two groups of pure mesagnial and mesangiocapillary deposits. In this study 10.5 percent of renal biopsies had mesangial-capillary IgA deposits. There was not significant association of proportion of totally sclerosed glomeruli, extracapillary, proliferation, percentage of peri-glomerular fibrosis, thickening of the Bowman's capsule, percent of interstitial fibrosis, mesangial proliferation in any degree and mesangial widening with pure mesangial or mesangial-capillary deposits (p>0.05).There was not significant association of age, serum creatinine and levels of proteinuria with pure mesangial or mesangiocapillary deposits (p>0.05).Among four morphologic variables of Oxford classification only E variable (endocapillary proliferation) had significant association with mesangiocapillary deposits (P=0.04). **Conclusion:** The association of mesangiocapillary IgA deposits with endocapillary proliferation may imply the severity of the disease. We recommend that the location and intensity of IgA is routinely included in the renal biopsy report.

Keywords: Immunoglobulin A nephropathy, immunoglobulins, Immune deposits, immunofluorescence, immunostaining, deposition.

INTRODUCTION:

Presence of IgA-dominant glomerular deposits is the definition of IgA nephropathy (1,2). However within this description, there is variation in the location of IgA and the presence of other immunoglobulins (2-4). This disease is the most common primary glomerular disease in the world and has diverse clinical manifestations, reflecting a wide range of morphologic lesions, from near normal appearance on light microscopy to severe necrotizing lesions with crescents (5-11). The recent Oxford classification for nephropathy of immunoglobulin IgA identified four morphologic features consisting, mesangial cellularity (M0/M1), endocapillary proliferation (E0/E1), segmental sclerosis (S0/S1) and tubular atrophy/interstitial fibrosis (T0/T1/T2), which are independent predictors of clinical outcome (1-3,12,13), however this classification was not include the pattern of immunostaining (1-3,12,13). In IgAN deposits were typically in mesangial area, however in around one-third of cases, there was capillary wall IgA too (4,6, 12-14). It seems that deposit of IgA in capillary wall have been associated with

an adverse clinical outcome (15 16).Whether the immunostaining findings in the capillary loops is of significant importance (15, 16), independent of other four morphologic variables of Oxford classification? Indeed studies concerning the association of location of deposited immunoglobulin IgA with various morphologic lesions especially with variables of Oxford classification and clinical data are quite scarce. Here, we investigate the association between location of IgA deposition with histology and some demographic data in a group of Iranian IgAN.

Patients and Methods

The pathologic diagnosis of IgAN requires the demonstration of IgA-dominant mesangial or mesangiocapillary immune deposits through immunofluorescence (IF) microscopy (1-3). The immune deposits were semiquantitied from 0 to 3+ positive bright. The definition of IgAN needs the presence of diffuse and global IgA deposits that were graded ≥ 2+ and the absence of C1q deposition (1-3, 12-14). All renal biopsies from July 2009 to July 2012 were sent to our renal pathology laboratory. None of the patients was treated before the biopsy. Biopsies with less than 8 glomeruli were also excluded from the study. None of the patients was diagnosed as IgAN, having history of collagen vascular diseases and liver cirrhosis based on 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.

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 2 µm sections and staining 2 slides with periodic acid Schiff, 2 slides for hematoxylin and eosin, 1 slide for Jones methenamine silver and 1 slide for trichrome. Each slide contained 2-3 sections. Materials used for IF were snap-frozen in liquid nitrogen. Sections (Six-micron in thickness) were stained for immunofluorescence study with fluorescein isothiocyanate-conjugated antibodies specific for human IgG, IgM, IgA, C1q, C3 and fibrin (DAKO, Produktionsvej 42, DK-2600 Glostrup, Denmark)(17,18). IF slides were reported in a scale of 0 to 3+ positive bright (1,2). The blinded IF slides were reported by a nephropathologist. After IF diagnosis of IgAN, histopathology glass slides were reviewed to assess the morphologic variables, which were applied in Oxford-MEST classification method. Other important morphologic lesions including proportion of totally sclerosed glomeruli, extracapillary proliferation (cellular, fibro-cellular or fibrous crescents), percentage of peri-glomerular fibrosis and thickening of the Bowman's capsule, percent of interstitial fibrosis, mesangial proliferation in any degree and mesangial widening was also assessed. After selection of biopsies having dominant IgA-deposition by IF study and in the absence of exclusion criteria, glass slides were reported for the classification of Oxford. 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 were included race, gender, age, serum creatinine and proteinuria (based on a 24hour urine collection).

Mean values and standard deviations were calculated, and statistical significance of the differences between groups were calculated using Mann-Whitney U-test and Chi-square tests. The Spearman's coefficient of correlation was used to check the correlations. A computer program (SPSS version 17.0, Chicago, IL, USA) was used for statistical analysis. $P < 0.05$ was considered statistically significant.

RESULTS

Population Characteristics

This is an observational study, conducted on IgAN patients.

Prevalence

A total of 114 biopsies were enrolled to the study. Of 114 patients, 70.2 % were male. Mean age of patients was 37.7 ± 13.6 years (for males and females were 39 ±14.3 and 35 ± 11.7 years, respectively).

Mean of glomeruli in all renal biopsies were 14.8 ±7.2 numbers. The mean of proteinuria was 1742±1324 mg/day (median=1500mg/day). Demographic data was summarized in **Table 1**. In all biopsies, the mean of totally sclerosed glomeruli were 2.4±2.9 number (median=1 number). Also mean of serum creatinine was 1.6 ±1.5mg/dL

(median=1.2mg/dL).

Table 1:
Demographic data of patients the time of renal biopsy

Clinical findings	
Sex (male/female)	80/34
Age (years)	37.7 = 13.6 (total)(M;39 = 14.3F; 35 = 11.7)
S-Cr (mg/dl)	1.6 = 1.5(total)(M;1.8 = 1.7.F; 1.1 = 0.7)

Oxford classification (MEST)
Mesangial proliferation as M variable (mesangial proliferation in more than 50% of glomeruli) was in 64% of the patients. However mesangial proliferation determined as more than 3 cells/mesangial area was in 86.8% of the patients. Frequency of morphologic variables of Oxford-MEST classification is summarized in **Table 2.**

Table 2:
Morphologic variables of oxford-mest classification.

Oxford-MEST variables N=114	**Number**	**Percent**
M (0/1)	**41/73**	**36/64**
E(0/1)	**79/35**	**69.3/30.7**
S(0/1)	**42/72**	**4(14.3%)**
T(0/1/2)	**59/35/20**	**51.8/30.7/17.5**

Immunostaining findings
In this study scores of 3+ and 2+ of IgA deposits were in 76% and 47 %, respectively. Study regarding the IgG deposits showed that, 29% had scored 2+ and 10% had score1+. This study also showed, IgM was deposited in 33% of patient with score of 1+ and 7% with score of 2+.In this study 10.5 percent of renal biopsies had mesangial-capillary IgA deposits.

Correlation of immunostaining of pure mesanial versus mesangial-capillary deposits with demographic data and morphologic lesions
We divided the patients into two groups of pure mesagnial and mangiocapillary deposits. There was not significant association of pure mesangial depositions with IgA , IgM, IgG and C3 depositions, in contrast , significant positive associations of mangiocapillary deposits with IgA , IgM, IgG and C3 depositions (p=0.041, p=0.002, p=0.006 and p=0.0041 respectively) was observed.
There was not significant association of proportion of totally sclerosed glomeruli, extracapillary proliferation (cellular, fibro-cellular or fibrous

crescents), percentage of peri-glomerular fibrosis, thickening of the Bowman's capsule, percent of interstitial fibrosis, mesangial proliferation in any degree and mesangial widening with pure mesangial or mesangial-capillary deposits was seen(p>0.05).

There was not significant association of age, serum creatinine and levels of proteinuria with pure mesangial or mesangiocapillary deposits was seen (p>0.05).Among four morphologic variables of Oxford classification only E variable had significant association with mesangiocapillary deposits (P=0.04). Association of morphologic variables of Oxford classification, various demographic data, crescent and other morphologic lesions with mesangial and mesangiocapillary deposits in IF study, was illustrated in **tables 3 and 4.**

Table3:
Association of morphologic variables of oxford classification, proportion of crescents and demograpgic data with mesangial and mesangial-capillary deposits in if study.

	Proteinuria	Age	Serum creatinine	M	E	S	T	Proportion of crescents
Mesangial-capillary deposits	P=0.39	P=0.59	P=0.16	P=0.14	P=0.04	P=0.20	P=0.17	P=0.29
Pure Mensangial deposits	P=0.92	P=0.26	P=0.67	P=0.55	P=0.92	P=0.55	P=0.85	P=0.35

Table 4:
Association of various morphologic lesions with mesangial and mesangial-capillary deposits in IF study.

	Percent of interstitial fibrosis	Bowman's capsule thickening	Mesangial proliferation	Mesangial widening	Peri-glomerular fibrosis	Fibro-cellular crescents	Fibrous crescents	Cellular crescents
Mesangial-capillary deposits	P=0.78	P=0.60	P=0.60	P=0.23	P=0.12	P=0.83	P=0.28	P=0.10
Pure Mensangial deposits	P=0.55	P=0.80	P=0.29	P=0.15	P=0.87	P=0.84	P=0.83	P=0.75

Discussion

Berger described the immunostaining of immunoglobulin A nephropathy as diffuse mesangial staining for IgA whose intensity of staining is either greater than or equal to that of other immunoglobulins present(19). Previous studies pointed out the significance of IgA deposits when extended from mesangial area to the peripheral capillary walls, which accompanied by significant adverse risk factor (15,16,19). The presence of glomerular capillary wall IgA deposits is reported to be associated with greater proteinuria and histological severity (20-22).In the study con ducted by Andreoli et al, found, children with capillary wall IgA deposition had higher urinary protein at diagnosis and more severe histological alterations, including more frequent crescent formation, segmental and global sclerosis, tubular atrophy and interstitial fibrosis, as compared to children with pure mesangial IgA deposition. Moreover, these children were more likely to show persistent proteinuria and progressive renal failure (23). Studies regarding the association

of location of deposited IgA with morphologic lesions of Oxford classification or demographic data are quite scarce and mainly limited to the recent study conducted by Bellur et al (16). In their study, of 175 IgAN patients, capillary wall IgA staining was noted in 15% of cases. In our study of 114 biopsies, 10.5% of IgAN patients had mesangiocapillary staining of IgA. Bellur et al. also found, the presence of capillary wall IgA deposits was associated with a higher mesangial cellularity score and endocapillary proliferation. We found that mesangiocapillary IgA deposits had significant association only with endocapillary proliferation. In the study of Bellur et al, there was no significant association between the location of IgA and rate of loss of renal function. Likewise, in our study, no significant association between location of IgA deposition and serum creatinine or level of proteinuria was seen. The correlation between immunostaining pattern and clinical outcome has not been observed in the study of Bellur et al.They concluded that, the location of glomerular IgA correlate with greater histological activity (16).They were not persuaded to include of immunostaining data to the Oxford classification at the present time.

The main question is still remained, whether the extension of deposited immunoglobulins from mesangial area to capillary walls have prognostic significance. The association of mesangiocapillary IgA deposits with endocapillary proliferation as E variant of Oxford classification in our study and the study of Bellur et al. may imply the severity of the disease. Indeed, in immunoglobulin A nephropathy as the most common primary glomerular disease (24-30), these findings need more attention. Therefore, validation of this finding is required in other cohorts. We recommend that the location and intensity of IgA is routinely included in the renal biopsy report.

Conflicts of interest

The authors declared no competing interests.

BIBLIOGRAFÍA.

1- 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(1):31-42.

2- Nasri H. Comment on: Clinical, Histopathological and Immunofluorescent Findings of IgA Nephropathy and author's reply. Iran J Immunol. 2012; 9(4):266-7.

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

4- Roberts ISD, Cook HT, Troyanov S, Alpers CE, Amore A, Barratt J, et al. The Oxford classification of IgA nephropathy: pathology definitions, correlations, and reproducibility. Kidney Int. 2009; 76(5):546-56.

5- Mubarak M. Oxford classification of IgA nephropathy: Broadening the scope of the classification. J Nephropathology. 2012; 1(1): 13-16.

6- Mubarak M. Immunostaining findings in IgA nephropathy: correlation with histology and clinical outcome in the Oxford Classification patient cohort. Nephrol Dial Transplant. 2012; 27(7):2998-9.

7- Assadi F. The epidemic of pediatric chronic kidney disease: the danger of skepticism. J Nephropathology. 2012; 1(2): 61-64.

8- Gheissari A, Hemmatzadeh S, Merrikhi A, Fadaei Tehrani S, Madihi Y. Chronic Kidney Disease in Children: A report from a tertiary care center over 11 years. J Nephropathology. 2012; 1(3): 177-182.

9- Kam-Tao Li PK, Burdmann EA, Mehta RL.Acute kidney injury: global health alert. J Nephropathology. 2013; 2(2): 90-97.

10- 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(2): 101-108.

11- Kari J. Epidemiology of chronic kidney disease in children. J Nephropathology. 2012; 1(3): 162-163.

12- 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(1): 53-60.

13- D'amico G. The most common glomerulonephritis in the world: IgA nephropathy. Q. J. Med. 1987; 245: 709–27.

14- 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(1): 28-35.

15- Cattran DC, Coppo R, Cook HT, Feehally J, Roberts ISD, Troyanov S, et al. The Oxford classification of IgA nephropathy: rationale, clinicopathological correlations, and classification. Kidney international. 2009; 76(5):534-45

16- Bellur SS, Troyanov S, Cook HT, Roberts IS. Immunostaining findings in IgA nephropathy: correlation with histology and clinical outcome in the Oxford classification patient cohort. Nephrol Dial Transplant. 2011; 26(8):2533-6.

17- 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(2): 91-100.
18- 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(1): 49-56.
19- Berger J, Hinglais N. Intercapillary deposits of IgA-IgG. J Urol Nephrol (Paris). 1968; 74(9):694-5.
20- IgA nephropathy in children: significance of glomerular basement membrane deposition of IgA. Am J Nephrol 1986; 6:28-33.
21- Abuelo JG, Esparza AR, Matarese RA, Endreny RG, Carvalho JS, Allegra SR. Crescentic IgA nephropathy. Medicine (Baltimore). 1984; 63(6):396-406.
22- Yoshimura M, Kida H, Abe T, Takeda S, Katagiri M, Hattori N.Significance of IgA deposits on the glomerular capillary walls in IgA nephropathy. Am J Kidney Dis. 1987; 9(5):404-9.
23- Andreoli SP, Yum MN, Bergstein JM.IgA nephropathy in children: significance of glomerular basement membrane deposition of IgA. Am J Nephrol. 1986; 6(1):28-33.
24- Barbour SJ, Reich HN. Risk stratification of patients with IgA nephropathy.Am J Kidney Dis. 2012; 59(6):865-73.
25- Nasri H. Hypertension and renal failure with right arm pulse weakness in a 65 years old man. J Nephropathology.2012; 1(3): 130-133.
26- Mohammadi Torbati P. Focal segmental glomerulosclerosis; collapsing variant. J Nephropathology. 2012; 1(2): 87-90.
27- Galesic K, Ljubanovic D, Horvatic I. Tratment of renal manifestations of ANCA-associated Vasculitis. J Nephropathology. 2013; 2(1): 6-19.
28- 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(1) 5-10.
29- Mubarak M, Collapsing focal segmental glomerulosclerosis: increasing the awareness. J Nephropathology. 2012; 1(2):77-80.
30- Wang T, Ye F, Meng H, Zhang L, Jin X. Comparison of clinicopathological features between children and adults with IgA nephropathy. Pediatr Nephrol. 2012; 27(8):1293-300.

Recibido en su forma original: 28 de Febrero de 2013
En su forma corregida: 16 de Abril de 2013
Aceptación final: 24 de Mayo de 2013
Dr. Germán T. Hernández
Divisions of Nephrology & Hypertension and Biostatistics & Epidemiology, Paul L. Foster School of Medicine, Texas Tech University Health Sciences, El Paso, Texas - USA
E-mail:hamidnasri@med.mui.ac.ir