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See commentary on page 210

Correlation of immunostaining findings with demographic data and variables of Oxford classification in IgA nephropathy

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ABSTRACT

Background: Oxford classification for IgA nephropathy (IgAN) did not include pattern of immunostaining in the analysis.
Objective: The aim of this study is to determine the potential correlation between the immunostaining data and morphologic variables of Oxford classification (MEST) and various clinical and demographic data of patients with IgAN.
Patients and Methods: The pathologic diagnosis of IgAN requires the demonstration of IgA-dominant mesangial or mesangio-capillary immune deposits through immunofluorescence (IF) microscopy. The immune deposits were semiquantitatively assessed as 0 to 3+ positive bright. These were correlated with various clinical, demographic and histological variables of Oxford classification.
Results: A total of 114 biopsies were enrolled to the study (70.2% were male). Mean age of the patients was 37.7 ± 13.6 years. This study showed that, only C3 deposits had a significant correlation with serum creatinine. Other antibodies (IgA, IgM and IgG) had no significant association with serum creatinine. This study also showed that IgA deposition score had significant positive association with endocapillary proliferation (E) and segmental glomerulosclerosis (S) variables of Oxford classification. Moreover, IgM deposition score had positive association with S variable. There was no significant association of IgG deposition score with four morphologic variables of Oxford classification. There was significant association of C3 deposition score with S and E variables too.
Conclusions: The significant relationships of IgA and C3 deposits with some of the Oxford variables need more attention. We propose to further investigate this aspect of IgAN disease.

Original Article

Implication for health policy/practice/research/medical education:
Immunoglobulin A nephropathy (IgAN) is the most common primary glomerular disease. The relationship of immunoglobulin depositions with clinical and histological findings and also responses and resistance to therapy, and disease prognosis still remains largely unclear. In a study on 114 biopsies of IgAN patients, significant relationships of IgA and C3 deposits with some of the Oxford variables was observed. We propose to further investigate this aspect of IgAN disease.

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1. Background

The Oxford classification of IgA nephropathy (IgAN) identified four histological features, consisting of mesangial cellularity (M), endocapillary proliferation (E), segmental sclerosis (S) and tubular atrophy/interstitial fibrosis (T), which are predictors of clinical outcome and characterized as MEST morphologic variables (1). However, Oxford classification did not include pattern of immunostaining in the analysis (1, 2). IgA deposits are typically found in mesangial area, although capillary wall deposits of IgA are seen in around one-third of cases (3). Furthermore, glomerular IgG or IgM, in addition to IgA, is also a frequent finding. Recent studies have shown that deposits of IgG or IgM may be associated with adverse clinical outcome (3). However, beyond the morphologic variables of Oxford classification or other histological features, it is still unclear whether the immunostaining findings are of predictive value. Indeed after publication of Oxford-MEST classification for IgAN in 2009, various studies suggested the significance of some other morphological lesions or immunostaining findings to include and complete this classification.

2. Objectives

Studies concerning correlation of immunostaining findings with morphologic findings of Oxford classification and clinical data are quite scarce. In this study we sought to investigate the association of immunostaining data with four morphologic variables of Oxford classification and also with various clinical and demographic data in a group of primary IgAN patients.

3. Patients and Methods

The pathologic diagnosis of IgAN requires the demonstration of IgA-dominant mesangial or mesangio-capillary immune deposits through immunofluorescence (IF) microscopy (1-3). The immune deposits were semiquantitatively assessed as 0 to 3+ positive bright. The standard definition of IgAN was used for inclusion of the cases (1-3). Patients with systemic diseases such as diabetes mellitus, collagen diseases, abnormal hypergammaglobulinemia and chronic liver diseases were excluded from this study.

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 IF 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 (6 μm in thickness) were stained for IF study with fluorescein isothiocyanate-conjugated antibodies specific for human IgG, IgM, IgA, C1q, C3 and fibrin (DAKO, Produktionsvej 42, DK-2600 Glostrup, Denmark). IF slides were reported in a scale of 0 to 3+ positive bright. The blinded IF slides were reported by a single nephropathologist (HN). After IF diagnosis of IgAN, histopathology glass slides were reviewed to assess the morphologic vari-

ables, which were applied in Oxford-MEST classification system. Other important morphologic lesions were also assessed, 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. 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 and proteinuria (based on a 24-hour 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.

4. Results

This is an observational study, conducted on IgAN patients. 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). Mesangial proliferation as M variable (mesangial proliferation in more than 50% of glomeruli) was found in 64% of the patients. However mesangial proliferation determined as more than 3 cells/mesangial area was seen in 86.8% of the patients. The morphologic variables of Oxford-MEST classification are summarized in table 1. In this study scores of 3+ and 2+ of IgA deposits were in 76 and 47 cases, respectively. Study regarding the IgG deposits showed that, 29 cases had score 1+ ,10 had 2+ and one case 3+. This study also showed, IgM was deposited in 33 cases with a score of 1+ and in 7 with score of 2+. Frequency distribution of IgA, IgG, IgM and C3 deposits is shown in table 2. In this study, a positive association between IgA and IgM ($r= 0.251$, $P=0.007$) and C3 ($r= 0.458$, $P<0.001$) deposits was seen. There was no correlation of IgG with IgA, IgM and C3 deposits ($P>0.05$). There was a positive association of IgM with C3 deposits ($P=0.01$). There was no correlation of IgA, IgG, IgM and C3 deposits with age or proteinuria ($P>0.05$). In this study, only C3 deposits had a significant correlation with serum creatinine ($r=0.22$, $p=0.017$), other antibodies consisting IgA, IgM and IgG had no significant association with serum creatinine ($P>0.05$). In this study, no significant association of sex with IgA, IgM, IgG and C3 was found ($P>0.05$). There was no significant association of IgA, IgG and C3 with age below and more than 40 years ($P>0.05$). However there was a weak relationship of IgM with age. Mann–Whitney U-test showed that, patients of below 40 years age had little IgM deposition score than age of more than 40 years. Study regarding the correlation of deposited antibody brightness scores with Oxford variables showed that IgA deposition scores had significant positive association with E and S variables ($p=0.025$ for two correlations). IgM deposition score had positive association with S variant ($p=0.017$). There was no significant association of IgG deposition score with four morphologic variables of Oxford classification. Findings regarding association of antibody deposition scores with four morphologic variables of MEST classification are summarized in table 3. In summary, there was significant association of IgA, IgM and C3 deposition scores with S variable, only IgA and C3 deposits had significant association with E variable. In the present study only IgA and C3

deposition score had significant correlation with mesangial widening and thickening of Bowman's capsule. Among mentioned deposited antibodies, only C3 had significant positive association with globally sclerosed glomeruli (p=0.031).Tables 4 and 5, summarize the association of deposited antibodies brightness scores with other morphologic lesions and with crescents.

Table 1. 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

Table 2. Frequency of deposited antibody intensity scores.

Antibody deposition intensity				
N=114	0	+	++	+++
IgA	0	0	47	67
IgG	74	29	10	1
IgM	74	33	7	0
C3	27	44	29	14

Table 3. Association of deposited antibody intensity scores with morphologic variables of MEST-classification (Oxford).

N=114	M	E	S	T
IgA	P=N.S	P=0.025	P=0.025	P=N.S
IgG	P=N.S	P=N.S	P=N.S	P=N.S
IgM	P=N.S	P=N.S	P=0.017	P=N.S
C3	P=0.010	P=0.004	P=0.003	P=N.S

Table 4. Association of deposited antibody intensity scores with various morphologic lesions.

N=114	Globally sclerosed glomeruli	Peri-glomerular fibrosis	Mesangial widening	Thickening of Bowman's capsule
IgA	P=N.S	P=N.S	P=0.049	P=0.049
IgG	P=N.S	P=N.S	P=N.S	P=N.S
IgM	P=N.S	P=N.S	P=N.S	P=N.S
C3	P=0.031	P=N.S	P=0.001	P=0.001

Table 5. Association of deposited antibody intensity scores with crescents.

N=114	Total number of crescents	Cellular crescents	Fibrous crescents
IgA	P=0.005	P=0.008	P=0.050
IgG	P=N.S	P=N.S	P=N.S
IgM	P=N.S	P=N.S	P=N.S
C3	P=0.002	P=0.046	P=0.035

5. Discussion

In this study no correlation between IgA, IgG, IgM and C3 deposits with age or proteinuria was found. This study showed that, only C3 deposits had a significant correlation with serum creatinine. Other antibodies (IgA, IgM and IgG) had no significant association with serum creatinine. In this study also, no significant association of sex with IgA, IgM, IgG and C3 was found.

There was no significant association of IgA, IgG and C3 with age below and more than 40 years.

Present study showed that IgA deposition score had significant positive association with E and S variables. IgM deposition score had positive association with S variable. There was no significant association of IgG deposition score with four morphologic variables of Oxford classification.

There was significant association of IgA, IgM and C3 deposition scores with S variable, and only IgA and C3 deposits had significant association with E variable.

Numerous prior studies identified various potential clinical or morphological predictors of progression, consisting of degree of renal impairment at diagnosis, histologic findings mostly morphologic variables of Oxford classification and degree of proteinuria. These factors appear to contribute independently to the risk of progression in multivariate models. However, there may be other factors predicting progression and outcome of IgAN. Among them, immunostain-

ing findings need more investigation. In the study conducted by Bellur et al on a group of IgAN patients, association of IgG staining with the presence of endocapillary proliferation and a higher mesangial cellularity score was found (3). These findings were not supported by our study. In contrast, we showed positive association of IgA and C3 deposits with endocapillary proliferation. In the study of Bellur et al, the presence of IgG was not associated with the presence of crescents, focal and segmental glomerulosclerosis, interstitial or vascular lesions. Tables 3 and 5 of our study, show the same results. They also found, no significant association between the presence of IgG and urine protein excretion and eGFR at the time of diagnosis. This finding is also in agreement with our investigation. However we found that, C3 deposits had a significant correlation with serum creatinine. In an observational cohort study, Kim et al. identified that mesangial C3 deposition was an independent risk factors for progression of IgAN, suggesting that complement activation may play a pathogenic role in patients with IgAN (4). Ohsawa et al. found that IgAN patients who have extraglomerular (for example in Bowman's capsule) C3 deposits have worse clinical outcomes (5). Strong significant positive association of C3 deposition score with thickening of Bowman's capsule was also observed in our study (P=0.001). Wada et al. conducted a retrospective study on 57 IgAN patients, who were divided into two groups of IgA+IgG deposition (IgA-IgG) group and IgA deposition alone (IgA) group(6). They found that mesangial IgG deposition is associated with more severe clinical features in patients with IgAN (6). Tang et al. also noted that mesangial IgM deposition had correlation with crescent formation (7), a finding which was not observed in our study (table 5).

In this study, we found the correlation of immunostaining data with some variables of Oxford classification. However, there are still unresolved questions which need further evaluation (8). Of these, the correlation of immunostaining findings with clinical and morphologic variables of IgAN patients has special importance (8-10).

6. Conclusions

The important question is whether the immunostaining findings have independent prognostic significance and whether to include them to Oxford classification or not? However, the relationship of immunoglobulin depositions with clinical and histological findings and also responses and resistance to therapy, and disease prognosis still remains largely unclear. Therefore, we propose to further investigate this aspect of IgAN disease.

Authors' contributions

HN, SS and AA designed the study. SM, AM, YM and AEN wrote some parts of the paper. HN completed the final draft.

Conflicts of interest

The authors declared no competing interests.

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