Annals of Diagnostic Pathology 18 (2014) 104–108

Contents lists available at ScienceDirect

Annals of Diagnostic Pathology

C3d deposition in the media of renal arterioles is a useful marker for arteriolosclerosis in IgA nephropathy

Rui Zhang, MD [a,b], Jiansong Lin, MD [a], Lijuan Qu, MD [a], Feng Zheng, MD [c], Zhiyong Zheng, MD [a,*]

[a] Department of Pathology, Dongfang Hospital, Fujian Medical University, Fuzhou, China
[b] Department of Pathology, The Second Hospital of Xiamen, Xiamen, China
[c] Department of Nephrology, Dongfang Hospital, Fujian Medical University, Fuzhou, China

ARTICLE INFO

Keywords:
C3d
Arteriolosclerosis
Arterioles
Arteries
IgA nephropathy

ABSTRACT

C3d deposition in peritubular capillaries has been demonstrated to indicate antibody-mediated alloresponse during renal transplantation. C3d deposition in renal arterioles in IgA nephropathy (IgAN), however, is poorly documented. Especially, its significance to the pathology of primary glomerulonephritis remains unclear. This retrospective study included 340 patients with IgAN who underwent renal biopsy at our center. C3d strongly positive deposition in arterioles was observed in 123 (36.2%) of the 340 cases, and weakly positive deposition of C3d was observed in 217 cases (63.8%). In the weakly positive group, C3d mainly deposited in the intima of arterioles. In the strongly positive group, C3d deposited in the intima and the media of arterioles, presenting as the medial thickening and sclerosis of varying severities. The prognosis was worse in the C3d strongly positive group than in the weakly positive group during a 2-year follow-up ($P = .027$). The predictive value of C3d deposition in the media of arterioles in patients with IgAN may be a useful marker for arteriolosclerosis indicating unfavorable clinical outcomes.

1. Introduction

IgA nephropathy (IgAN) is a type of primary glomerulonephritis commonly observed in clinical patients, and it is also the most important cause of terminal renal failure. Histologic features such as glomerulosclerosis, mesangial hypercellularity, and interstitial fibrosis, and so on, are identified as independent predictors of renal failure. It is important to identify other risk factors associated with a poor progression of disease to better evaluate the prognostic significance of IgAN. The complement activation and expression could be an important risk factor in addition to histologic and clinical characteristics.

Some previous studies have shown that renal vascular lesion in IgAN is significantly associated with clinical parameters including blood pressure, urine protein, serum creatinine (SCr), and so on, and pathological features including glomerulosclerosis, interstitial fibrosis, mesangial hypercellularity, and so on. Prior studies showed that renal vascular lesion could be used as an important pathological prognostic indicator.[1–5] Other studies focus on the complement expression in the glomerular in patients with IgAN. Nakagawa H et al[6] found that the levels of glomerular C3d deposition in patients with IgAN were significantly higher than the levels observed in mesangial proliferative glomerulonephritis (MsPGN), suggesting that deposition of C3d in the mesangium may play an important role in chronic pathological effects observed in patients, and glomerular C3d deposition may be a useful marker for the degree of inflammatory activity in IgAN. In addition, the study by Gherghiceanu et al[7] had shown that C3d deposition in peritubular capillaries in IgAN is considered an indicator for unfavorable outcome.

C3d is an end-product of the 3 complement activation pathways. It is a stable marker that binds covalently to cell surfaces, and it is much easier to be detected in tissues. Presently, immunohistochemical staining of complement is universally considered as a useful diagnostic procedure in the assessment of renal biopsies, where C3d has shown to be a marker of humoral rejection. Although C3d may be potentially used for clinical analysis tool, the significance of C3d deposition in vessels, especially the sclerotic arterioles in IgAN, is not well documented. The purpose of the study was to assess the relationship between C3d deposition in arterioles and arteriolosclerosis in IgAN.

2. Materials and methods

2.1. Patient selection

The subjects in the present study consisted of 541 patients who received renal biopsy between January 2008 and November 2009 in our kidney treatment center. They included 340 patients with IgAN, 31 with MsPGN without IgA deposition, 33 with membranous nephropathy (MN), 26 with focal segmental glomerulosclerosis

* Corresponding author. Department of Pathology, Dongfang Hospital, Xierhuanbei Rd 156, Fuzhou, Fujian, 350025 China. Tel./fax: +86 59183717703.
E-mail address: fzghpd2013@163.com (Z. Zheng).

1092-9134/$ – see front matter © 2014 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.anndiagpath.2014.01.001

(FSGS), 35 with lupus nephritis (LN), 32 with diabetic nephropathy (DN), and 12 with hypertensive nephroslerosis (HN) as disease controls. These diagnoses were based on clinical findings and renal biopsies. Thirty-two cases of normal kidney (NK) from zero allograft biopsy tissue comprise the normal control group, and ethical considerations were followed.

2.2. Immunohistochemistry

Three-micrometer formaldehyde-fixed sections underwent immunohistochemical staining of C3d, IgG, IgM, IgA, C1q, fibrinogen (Fib), and complement receptor 2 (CD21). The details of the procedures were published previously.[8]

To assess the distribution of C3d deposition in arterioles, immunohistochemical double-label staining was performed. Smooth muscle actin (SMA) and C3d staining was accomplished by immersion in a 3% hydrogen peroxide solution for 10 minutes, incubation with anti-SMA antibodies at room temperature for 1 hour; treatment with a polymer enhancer at room temperature for 5 to 10 minutes, and a final treatment with an alkaline phosphatase-labeled secondary antibody incubated at room temperature for 20 minutes. Positive staining was then revealed by immersing the sections in an alkaline phosphatase chromogen substrate solution consisting of 5-bromo-4-chloro-3-indolyl phosphate and p-nitroblue tetrazolium chloride and then performed with C3d staining.

A semiquantitative grading of the intensity of C3d and other antibodies staining was performed. A score of 0 to 3 was defined as follows: 0, no staining (negative); 1, less than 25% of arterioles stained (+, weak); 2, 25% to 50% arterioles stained (2+, moderate); and 3, more than 50% arterioles stained (3+, strong).[9]

2.3. Pathological criteria

Microscopic observation of the pathological features (mesangial hypercellularity, segmental glomerulosclerosis, endocapillary hypercellularity, tubular atrophy/interstitial fibrosis) of tissue samples from 340 patients with IgAN was performed according to the 2009 Oxford classification of IgAN system and Lee's pathological grades.[10,11] The diagnostic and scored criteria of arteriolosclerosis were based on the criteria by Katafuchi et al[12]: arteriole wall thickening was semiquantitatively estimated based on the cross-sectional ratio of luminal diameter to outer diameter. *Thickened arteriole wall* was defined as the ratio of less than 0.5 and no arteriole lesions in any arterial cross sections throughout the specimen. A *score of 1* was defined as arteriole lesions involving 1% to 25% of the arteriole cross sections; a *score of 2*, arteriole cross sections lesions involving 26% to 50%; and *a score of 3*, arteriole cross sections lesions involving more than 50%.

2.4. Clinical data

The study was conducted using retrospective data, with each patient's clinical data collected at the time of renal biopsy. Only complete patient data sets were used. Patient's age, sex, hematuria, SCr, blood urea nitrogen (BUN), mean arterial pressure (MAP), and an amount of 24-hour urine protein were documented. This study was approved by the hospital ethics committee prior to clinical data retrieval and analysis.

2.5. Study end points

Sixty-two patients with IgAN having Lee's pathological grade of IV had 2-year follow-up. The end point was the development of end-stage renal disease (ESRD) including the initiation of chronic dialysis or renal transplantation.[13]

2.6. Statistical analysis

Independent-sample tests or univariate data analysis of variance was used to compare different groups. Values were expressed as mean ± SD. A level of $P < .05$ was accepted as statistically significant. One-way analysis of variance was performed to evaluate the impact of C3d staining on renal survival using the Kaplan-Meier analysis. Calculations were performed using SPSS statistical software version 16.0 (SPSS, Chicago, Illinois).

3. Results

Clinical characteristics of the patients at the time of renal biopsy in the IgAN and control groups are shown in Table 1.

3.1. Immunohistochemistry findings

C3d staining was strongly positive in arterioles in 123 (36.2%) of the total 340 IgAN patient tissue samples (score ≥ 2). A total of 217 cases (63.8%) expressed weakly positive results (score = 1), and C3d negative (score=0) was not found in all the cases. In the weakly positive group, C3d was deposited in the absence of significantly thickened intima (Fig. 1A). In the strongly positive group, C3d deposition was found in the intima and the interstitium between the smooth muscle cells in the media of the thickened and sclerotic arterioles, but was not found in the adventitia (Fig. 1B, C).

A total of 116 of the 340 cases exhibit interlobular arteries. They had no C3d deposition or weak and irregular deposition in interlobular arteries. In the C3d strongly positive group, 65 cases showed vasa recta, of which 16 (24.6%) of cases showed that C3d deposits were well distributed in the vasa recta (Fig. 1D), whereas 96 cases showed vasa recta in the weakly positive group, of which only 7 (7.3%) of cases exhibited that C3d deposits were well distributed in the vasa recta. The difference was statistically significant between the 2 groups ($P < .05$). In addition, 14 (4.1%) of the total 340 cases observed showed signs of IgM deposition, although IgG, IgA, C1q, and Fib were not observed.

C3d deposition was also found in arterioles in the control groups (MsPGN, MN, FSGS, LN) and the NK group. C3d expression patterns were similar to those of patients with IgAN, although these were a small number of C3d strongly positive cases (Fig. 1E). The intensity of C3d was

Table 1
Clinical characteristics of the patients at the time of renal biopsy in the IgAN and control groups

Clinical characteristics	IgAN (n = 340)	Control group (n = 169)					
		MsPGN (n = 31)	MN (n = 33)	FSGS (n = 26)	LN (n = 35)	DN (n = 32)	HN (n = 12)
Age (y)	32.08 ± 10.87	32.19 ± 14.77	45.50 ± 14.82	28.00 ± 14.72	27.17 ± 12.12	52.22 ± 10.17	44.7 ± 14.1
Sex, male (%)	46.8	58.1	51.5	57.7	8.6	68.8	41.7
MAP (mm Hg)	91.11 ± 12.17	88.52 ± 11.15	94.45 ± 10.00	94.11 ± 11.66	94.82 ± 15.05	104.57 ± 10.36	122.4 ± 25.0
Hematuria (%)	15.6	3.2	0	0	2.8	0	8.3
Proteinuria (g/d)	2.05 ± 2.39	2.90 ± 2.14	5.80 ± 3.15	3.15 ± 1.83	3.38 ± 3.92	4.48 ± 1.65	1.4 ± 1.2
SCr (μmol/L)	113.10 ± 73.14	76.30 ± 33.76	74.89 ± 26.42	91.13 ± 43.24	81.05 ± 36.93	116.18 ± 52.17	212.5 ± 158.2
BUN (mmol/L)	6.25 ± 3.71	5.75 ± 3.17	5.27 ± 1.59	6.38 ± 3.59	7.48 ± 3.99	7.18 ± 2.83	10.1 ± 4.2