Kidney involvement in Takayasu arteritis

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ABSTRACT

Objective. *To evaluate whether the presence of glomerulonephritis is or is not associated with the extent of arterial wall inflammatory cell infiltrate in Takayasu arthritis (TA).*

Methods. *Retrospective chart and pathology review of large artery and kidney specimens of TA autopsy cases. Kidney specimens were classified, according to their histopathological findings, in those with specific glomerular entities and those with non-specific, ischemic and/or hypertensive, glomerular changes. A control group of autopsy kidney specimens was utilized for comparison. Morphometric analysis was used to assess the extent of the arterial inflammatory infiltrates; results were compared among the different groups with kidney lesions.*

Results. *We included 25 kidney specimens from 25 autopsies. Specific glomerular entities were present in 14 specimens; 10 (40%) were classified as diffuse mesangial proliferative glomerulonephritis (DMPG [Group A]), and 4 (16%) as other associated glomerulopathies (Group B). Non-specific changes were observed in 11 (44%) specimens (Group C). The arterial inflammatory infiltrate proportion was 9.4 % for group A, 1.4% for group B, and 2.7% for group C. Furthermore, a larger proportion of vascular inflammation was confirmed for group A when compared with the other groups ($p < 0.05$). Group A patients were younger than those in groups B and C ($p < 0.005$) and exhibited shorter disease duration.*

Conclusion. *The presence of DMPG was associated with a larger extent of vascular inflammatory cell infiltrate, suggesting a relationship between both phenomena.*

Introduction

The systemic manifestations and parenchymatous involvement of Takayasu arteritis (TA) have been emphasized (1). Hypertensive and ischemic changes secondary to renal artery stenosis usually characterize kidney involvement in TA. Occasionally, primary glomerular changes have been reported in TA, most commonly mesangial pro-liferative glomerulonephritis (2-4). An association between an active vascular inflammatory process and glomerulonephritis has been proposed on the basis of a variety of immunologic abnormalities observed in such cases (5, 6). However, whether this potential association is the result of an underlying systemic inflammatory process or it represents a coincidental event, remains to be elucidated.

TA has distinct patterns of histo-pathological changes at different stages of its evolution. There is transmural inflammation and patchy destruction of the medial muscle-elastic lamellae in the active stages; the cell infiltrate is predominantly lymphoplasmacytic, with variable numbers of giant cells. Inactive arteritis is indicated by intimal and adventitial fibrosis, with sparse lymphocytic infiltrate and extensive scarring of the media (7). A quantitative computer-based morphometry technique is used to assess the extent of inflammatory cell infiltrates in diverse tissue samples, including blood vessels (8).

These considerations led us to evaluate by morphometric analysis whether the presence of glomerulonephritis is or not associated with the extent of vascular inflammatory cell infiltrate in TA.

Materials and methods

All deaths registered among hospitalized TA patients from January 1960 to April 2003 were analyzed; only those patients in whom an autopsy was performed were included. Their clinical

records, aortographic studies, and autopsy findings were reviewed. The diagnosis was confirmed at autopsy in all instances; cases revealing other causes of aortitis were excluded (9).

Proteinuria was detected by enzyme-coated dipstick test, and results were semi-quantitatively expressed as negative (-), 0.3 g/L (+), 1 g/L (++), and 5 g/L (+++). Microscopic hematuria was diagnosed when > 5 red blood cells by field (40x) were detected. Reference values for serum creatinine ranged from 62 to 115 μmol/L (0.7 – 1.3 mg/dL) for males and 53 to 97 μmol/L (0.6 – 1.1 mg/dL) for females.

Kidney histopathology

Kidney specimens were evaluated by light microscopy after being fixed in a 10% PBF (phosphate-buffered formalin; pH 7.2) solution, embedded with pa-raffin, cut at 3μm, and stained with the following reagents: hematoxylin and eosin, periodic-acid Schiff's (PAS), Masson's trichrom, Jones' silver methenamine, and Verhoeff stains for elastic fibers. For a more precise definition of kidney abnormalities and to detect mesangial, subepithelial, and subendothelial dense deposits not seen by light microscopy, kidney specimens were post-fixed in glutaraldehyde, embedded in Epon, cut at 500 Å, contrasted with osmium tetroxide and lead citrate, and observed by electron microscopy. Frozen material was not available for immunofluorescence studies.

The World Health Organization histopathologic classification of glomerular diseases was used to categorize glomerular entities (10).

Two nephropathologists carried out blind and independent histological interpretations. When the observers did not fully agree, a consensual histopathological diagnosis was obtained.

Arterial histopathology and morphometric analysis

The aorta and large segments of its major branches (carotids, subclavians, intercostals, celiac trunk, hepatic, splenic, superior and inferior mesenterics, renals, and iliacs arteries) were fixed in a 10% PBF solution and embedded with paraffin. Hematoxylin and eosin-stained sections, cut at 4 μm, were observed by light microscopy.

Due to the patchy nature of the vascular inflammatory infiltrate and to ensure representative sampling, the aorta and its main branches were scrutinized for inflammatory cell infiltrates in all cases; those sections displaying the largest infiltrates were selected for morphometric analysis. The extent of the vascular inflammatory cell infiltrate was assessed on selected slides by an independent pathologist, without knowledge of kidney histopathology, through the use of the Zeiss interactive digital analysis system (ZIDAS) (Zeiss, Welwyn Garden City, Herts, UK). This automated morphometric analysis system incorporates a light microscope (10x objective) and a computer system. The computer's cursor was used to define the boundaries and to segment the areas of interest, such as the foci of inflammatory cell infiltrate observed on the selected microscope field. Morphometric analysis data were expressed as a ratio (percentage) of arterial wall infiltrated by inflammatory cells; comparisons among groups were performed.

Statistical analysis

Statistical analysis was performed with the Graph Pad Prism® 4.0 statistical package. Descriptive section included mean, standard deviation (SD), and proportions. Inferential section was supported by Fisher exact test, Chi square test to compare proportions, Mann-Whitney test for comparisons between groups, and Kruskal-Wallis method for comparisons of more than two groups. P values < 0.05 were considered statistically significant.

Results

A total of 29 deaths were registered among hospitalized TA patients; an autopsy study was performed in 25 of them, 15 female (60%) and 10 male (40%). The mean age at the time of death was 24.1 ± 8.9 years (range: 13-45) and the mean disease duration was 7.6 ± 7.1 years (range: 1-28). The ethnic background in all cases was Mexican-Mestizo.

Causes of death

Most TA patients died due to cardiovascular complications. The cause of death was congestive heart failure in eight cases (32%), stroke in five (20%), postoperative complications in four (16%), myocardial infarction in 3 (12%), pulmonary thromboembolism in two (8%), and infective endocarditis, amyloidosis, and mesenteric infarction in one case each (4%). In no case the renal disease was the cause of death.

Kidney histopathology

All cases displayed diverse degrees of non-specific changes associated with long-standing arterial hypertension and/or renal ischemia, such as arterial and arteriolosclerosis, parenchymal hypoperfusion and atrophy, interstitial fibrosis with lymphocytic irregular infiltration, tubular dilation and/or shrinkage, as

Table I. Kidney histopathological findings in Takayasu arteritis.

Kidney histopathology	Number of cases (%) n = 25
Group A	
• Diffuse mesangial proliferative glomerulonephritis	10 (40)
Group B	
• Other associated glomerulopathies:	4 (16)
Focal segmental glomerulosclerosis*	1
Global and focal glomerulosclerosis*	1
Segmental necrotizing and proliferative glomerulonephritis**	1
Amyloidosis***	1
Group C	
• Non specific changes	11 (44)

*Cases presenting with nephrotic syndrome.
**Henoch-Schönlein purpura associated.
***Tuberculosis associated.

well as sclerofibrotic glomerular obsolescence. Specific glomerulopathies were demonstrated in 14 kidney specimens, as shown in Table I. Of them, 10 cases (Group A) were classified as having diffuse mesangial proliferative glomerulonephritis (DM-PG), defined as a hypercellularity of mesangial axis with clusters of four or more nuclei per mesangial space, in at least 80% of the glomeruli; cell proliferation was usually accompanied by an increase in the mesangial matrix. Four cases were classified as having other associated glomerulopathies (Group B). The remaining 11 cases exhibited exclusively non-specific hypertensive and/or ischemic changes (Group C).

Proliferation of glomerular cells (endothelial, epithelial, and mesangial) from TA patients was compared with a control group of autopsy kidney specimens from 33 age- and sex-matched individuals who died from a variety of diseases, including infectious, inflammatory, metabolic, neoplastic, autoimmune, and congenital disorders (Table II). Mesangial hypercellularity was found in a larger proportion of TA kidney specimens (10 out of 25 48%), than in control kidneys, (5 out of 33 15.2%) ($p < 0.05$).

In no case, ultrastructural studies revealed neither basal membrane abnormalities of the capillary walls nor electron-dense immune deposits in the mesangium. The main clinical manifestations of renal dysfunction were similar among groups (Table III).

Table II. Proliferation of intrinsic glomerular cells (endothelial, epithelial and mesangial) in 33 control autopsy kidney specimens.

Age / Gender (Years)	Glomerular Proliferative Changes	Disease	Cause of Death
36/F	Positive	Diabetes mellitus	Sepsis
54/F	Negative	Hepatitis C, hepatic transplant	Hypovolemic shock
44/F	Negative	Acute pancreatitis	Septic shock
22/F	Positive	SLE	Pulmonary hemorrhage
37/F	Negative	Pneumonia	Sepsis
17/F	Positive	SLE	Pulmonary hemorrhage
53/M	Negative	Ulcerative colitis	Sepsis
45/M	Negative	Stroke	Cerebral infarction
54/F	Negative	Liver cirrhosis secondary to hepatitis C infection	Variceal hemorrhage
39/F	Negative	SLE, systemic histoplasmosis	Sepsis
37/F	Negative	Ovarian cancer	Pulmonary embolism
22/F	Negative	Massive hepatic necrosis	Hepatic failure
32/F	Negative	Hemophagocitic syndrome, disseminated candidiasis	Disseminated intravascular coagulation
41/F	Negative	Diabetes mellitus, bronchopneumonia	Septic shock
30/M	Negative	Necrotizing pancreatitis	Sepsis
34/F	Positive	Wegener's granulomatosis	Cerebral vasculitis
51/M	Negative	Hepatic cirrhosis, acute pyelonephritis	Sepsis
17/F	Negative	Hepatic necrosis	Multiple organ failure
31/F	Negative	Acute myelogenous leukemia	Pulmonary hemorrhage
24/F	Negative	Hepatic abscess	Sepsis
35/F	Negative	Infective endocarditis	Sepsis
36/F	Positive	Hepatitis C, hepatocellular carcinoma	Cerebral hemorrhage
8/F	Negative	Congenital heart disease	Ventricular septum defect
19/F	Negative	Infective endocarditis	Hypovolemic shock
13/M	Negative	Pulmonary valve replacement	Acute pulmonary edema
19/F	Negative	Inactive rheumatic fever	Congestive heart failure
11/M	Negative	Muscular dystrophy	Disseminated intravascular coagulation
14/M	Negative	Infective endocarditis	Septic shock
15/M	Negative	Inactive rheumatic fever	Acute respiratory distress
14/M	Negative	Patent ductus arteriosus	Transoperatory death
9/F	Negative	Tetralogy of Fallot	Hypovolemic shock
19/F	Negative	Spider bite	Disseminated intravascular coagulation
13/M	Negative	Left atrial myxoma	Hypovolemic shock

Arterial histopathology and morphometric analysis

Table IV summarizes morphometric findings and clinical characteristics according to groups. Arterial inflammatory infiltrate proportion was 9.4 % (± 8.8) for group A, 1.4 % for group B (± 1.9), and 2.7 % (± 4.7) for group C. A significantly larger proportion of vascular inflammation was confirmed for cases in group A, when compared with groups B and C ($p < 0.05$). The presence of glomerulonephritis, particularly DMPG, was associated with a larger proportion of vascular inflammatory cell infiltrate ($p < 0.05$).

Furthermore, patients in group A were younger at death than those in groups B and C ($p < 0.005$) and exhibited shorter disease duration.

Discussion

Patients with TA may occasionally display diverse specific glomerular entities, including mesangial proliferative glomerulonephritis (11), IgA nephropathy (6), membranous glomerulonephritis (12), membranoproliferative glomerulonephritis (13), crescentic glomerulonephritis (14, 15), renal amyloidosis (AA) (5), and focal glomerulosclerosis (16). Commonly, diverse non-specific renal changes associated with long-standing arterial hypertension and/or renal ischemia, such as arterial and arteriolosclerosis, parenchymal hypoperfusion and atrophy, interstitial fibrosis with scattered lymphocytic infiltration, tubular dilation and/or shrinkage, as well as sclerofibrotic glomerular obsolescence are observed. Furthermore, peculiar lesions such as intraglomerular microaneurysms have also been described (17).

This autopsy study identified diverse glomerulopathies in a large proportion of TA patients; among them, DMPG was the commonest. Furthermore, all cases displayed diverse degrees of non-

Table III. Renal manifestations in Takayasu arteritis.

	Group A (n = 10)	Group B (n = 4)	Group C (n = 11)	*P* value
Proteinuria (g/L)	0.9 ± 1.5	2.6 ± 2.8	0.3 ± 0.4	NS
Serum creatinine (μmol/L)	167 ± 101	253 ± 93	158 ± 61	NS
Hematuria (present/absent, %)	4/6 (40)	2/2 (50)	6/5 (55)	NS
Arterial hypertension (present/absent, %)	7/3 (70)	2/2 (50)	9/2 (82)	NS

Table IV. Morphometric findings and clinical characteristics.

Kidney histopathology groups	Group A	Group B	Group C	*P* value
n	10	4	11	
Gender F/M	5/5	1/3	9/2	NS
Age at death (years), mean (± SD)	18.6 ± 5	22.5 ± 8.3	29.7 ± 8.9	< 0.005*
Disease duration (years), mean (± SD)	5 ± 4.9	7.2 ± 5.3	10.1 ± 8.7	NS
Morphometry, mean (± SD)	9.4 ± 8.8	1.4 ± 1.9	2.7 ± 4.7	< 0.05*

*Group A vs group B, and group A vs group C.

specific renal changes associated with long-standing arterial hypertension and/or renal ischemia. Neither proteinuria: serum creatinine nor hematuria has shown significant differences among groups. Due to the retrolective data collection applied, this is not the most appropriate study to make clinical correlation; thus, the clinical manifestations of renal involvement in TA deserve further investigation.

The presence of a larger proportion of proliferative glomerular changes in TA patients than in the control group allowed us to confirm that this finding is prevalent in TA. The high frequency of glomerulopathies, particularly DMPG, which has been found in this study, is further explained by several circumstances. Some patients died in the sixties; in those days, therapeutic interventions were limited to control hypertension and heart failure, corticosteroids and immunosuppressive drugs were not in use to treat TA. Furthermore, in our series, 7 out of 25 cases (28%) were children, and they usually suffer an aggressive, often lethal illness, since they frequently present a severe multi-systemic inflammatory involvement. In one series, renal abnormalities have been found in up to 46% of TA children (18). Finally, being an autopsy study, the spectrum of the disease is clearly biased towards the most aggressive cases; in addition, our hospital is a referral center, thus, being likely to admit severely ill patients.

The mechanisms involved in the pathogenesis of the primary glomerulopathies are not clear; nevertheless, several authors have proposed an association of TA disease activity with glomerular injury in the setting of an autoimmune milieu (6). The proposed mechanisms include: the presence of circulating anti-aortic antibodies (19), anti-endothelial cell antibodies (20), circulating immune complexes trapped by the kidney (13, 21), immunoglobulin deposition in the glomerulus and mesangium (17), low serum complement levels, hypergammaglobulinemia (2), AA type reactive amyloidosis (5), antiphospholipid antibodies (22), and anti-neutrophil cytoplasmic antibodies (ANCA) (6, 23). Nevertheless, ANCA presence has not been demonstrated in a large series of cases (24).

The glomeruli react in a limited fashion to a myriad of renal injuries, immune or non-immune in nature. The mesangial cell seems to be the most reactive cell in the glomerulus, and mesangial hypercellularity is probably the most common change or abnormality found in injured glomeruli. Mesangial proliferation is a non-specific change that can be seen as the only histological abnormality in patients with diverse clinical conditions, ranging from mild isolated microscopic hematuria to overt nephritic syndrome, or as a component of more severe inflammatory glomerulonephritis that may accompany several systemic idiopathic and autoimmune diseases. Among the diverse types of associated glomerulopathies occurring in TA, mesangial proliferative glomerulonephritis is the most common (1-2). In TA, the ability to assess and monitor inflammatory activity by clinical manifestations, acute-phase reactants, and/or serial arteriographic studies is inadequate. On the other hand, definitive proof of vascular inflammation by tissue biopsy is rarely possible due to the risk involved in large vessel biopsies. In this regard, our post-mortem study is unique insofar as it included a series of cases, which in life had the diagnosis of TA. Furthermore, it allowed us to examine and to compare the extent of the inflammatory infiltrate in vessels, with the different types of glomerular lesions.

The presence of DMPG was associated with a larger extent of vascular inflammatory cell infiltrate, suggesting a relationship between both phenomena. Our findings concur with the notion that there is an early inflammatory phase in TA in which the kidney may also be involved, and that in late stages of the disease, renal ischemic and atrophic changes predominate.

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