The Importance of Immunofluorescence Staining in Patients with Primary FSGS

Primer FSGS Hastalarında İmmunfloresan Boyamanın Önemi

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ABSTRACT

OBJECTIVE: In patients with primary FSGS, the degree of proteinuria, serum creatinine levels, histologic findings, and response to therapy are important prognostic factors. In this study, the importance of immunofluorescence staining on response to therapy and progression to chronic renal failure were retrospectively evaluated in patients with primary FSGS.

MATERIAL and METHODS: Pathologic, clinical and laboratory features, follow-up outpatient/clinical records of 60 patients with a pathologic diagnosis of FSGS by renal biopsy in GATA Nephrology Clinic between 2000-2012 were retrospectively evaluated.

RESULTS: In this retrospective study conducted on the effect of the immunofluorescence staining on renal surveillance in patients with primary FSGS, accumulation of fibrin was found to have a relationship with renal surveillance. Progression was more rapid in patients with fibrin accumulation, but this result did not reach a statistical significance. No statistical relationship was found between other parameters and renal surveillance. For complete understanding of the possible negative impact of the accumulation of fibrin, further studies on larger patient groups are needed.

CONCLUSION: The studies examining many parameters are in advance in order to estimate response to treatment in patients with primary FSGS. New parameters to predict renal surveillance is still needed while steroid or other immunosuppressives are been initiating in patients presenting with nephrotic range proteinuria.

KEY WORDS: Primary focal segmental glomerulosclerosis, Immunfluorescence staining, Proteinuria

ÖZ

AMAÇ: Primer FSGS hastalarında, proteinürinin derecesi, serum kreatinin düzeyi, histolojik bulgular ve tedaviye yanıt önemli prognostik faktörlerdir. Bu çalışmada, primer FSGS hastalarında immünfloresan boyamanın tedaviye yanıtta ve kronik böbrek hastalığına progresyonda önemi retrospektif olarak değerlendirilmiştir.

GEREÇ ve YÖNTEMLER: GATA Nefroloji kliniği tarafından 2000-2012 yılları arasında takip edilen 60 primer FSGS olgusunun patolojik, klinik ve laboratuvar özellikleri retrospektif olarak değerlendirilmiştir.

BULGULAR: Bu retrospektif çalışmada, immünfloresan boyamanın renal fonksiyonlarla ilişkisi değerlendirilmiştir. Fibrin dışında diğer parametrelerle bir ilişki kurulamamıştır. Fibrin birikimi olan olgular daha progresif seyretmiş fakat bu istatistiksel bir değere ulaşmamıştır. Fibrin birikiminin negatif ilişkisini anlamak için daha fazla hasta içeren çalışmalara ihtiyaç vardır.

SONUÇ: Primer FSGS olgularında tedaviye yanıtı belirlemek için birçok parametre çalışmalarda değerlendirilmiştir. Nefrotik düzeyde proteinüri ile seyreden hastalara steroid veya diğer immünsupresif tedavilere başlama kararı verirken yeni parametrelere ihtiyaç vardır.

ANAHTAR SÖZCÜKLER: Primer fokal segmental glomerüloskleroz, İmmünfloresan boyama, Proteinüri

Received : 27.08.2013
Accepted : 21.10.2013

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INTRODUCTION

Focal segmental glomerulosclerosis (FSGS) is a clinicopathologic syndrome with nephrotic range proteinuria and may present with focal and segmental glomerulosclerotic lesions usually accompanied by loss of foot processes (1). FSGS is an important cause of nephrotic syndrome in adults. In the United States, FSGS was responsible for 35% of all patients and 50% of blacks undergoing renal biopsy because of idiopathic nephrotic syndrome between 1995 and 1997 (2). Socioeconomic, environmental and genetic factors in blacks may cause these high rates (3). In 1980, its frequency was only 0.2% compared to 2.3% in 2000, and it has increased more than 11-fold. This increase consisted mainly of black and female patients (4). In the United States, idiopathic FSGS is the most common primary glomerular disease detected on renal biopsy that leads to ESRD in all races (5). The results of 536 renal biopsies performed between 2011-2011 in Ankara Numune Education and Research Hospital were evaluated and membranous nephropathy was the diagnosis in most nephrotic sydrome patients (28).

There is usually no cause in primary or idiopathic cases, while there is an underlying cause in secondary cases. To call it as primary, it is important to reach the full medical record and history of the patient and exclude other renal, systemic or familial conditions completely. At the same time, minimal change disease and early-onset cases of FSGS are also very difficult to differentiate (6). Most cases of primary FSGS present with nephrotic range proteinuria, hypoalbuminemia, and peripheral edema. However, patients with a secondary FSGS have an indolent clinical course and the level of proteinuria does not reach nephrotic range, and accordingly hypoalbuminemia and peripheral edema are rare findings (7). Electron microscopy findings may help to distinguish these cases. Patients with primary FSGS represent diffuse fusion of foot processes, while these abnormalities are limited to sclerotic areas in secondary cases (8,9). FSGS is examined in five subgroups histologically; 1) Classic FSGS, also called FSGS NOS (Not Otherwise Specified). 2) Collapsing variant 3) Tip variant 4) Perihilar variant and 5) Cellular variant (10). This classification suggests the exclusion of FSGS caused by glomerular scarring seen in the clinical course of other idiopathic glomerular diseases. The presence of sclerosis is not necessary for the diagnosis of FSGS as sclerosis is often absent, especially in the tip and the collapsing variants. It has not been proven if the different variants reflect different diseases (with different causes and differences in pathogenesis). The different variants may just be a result of different stages of FSGS, dependent on the activity and time of onset of the disease (11).

All of these differentiations are important in the treatment and decision-making of patients. Primary cases respond to immunosuppressive (IS) agents while secondary FSGS cases do not respond to IS treatment. These cases can mostly benefit from ACEI/ARB therapies reducing intraglomerular pressure, but these may not provide a full treatment if the underlying cause is not fixed. Factors affecting treatment of patients with primary FSGS are the degree of proteinuria, serum creatinine level, histologic findings, and response to treatment (12, 13, 14).

In this study, we aimed to retrospectively evaluate whether there is an effect of immunofluorescence staining of primary FSGS patients on renal surveillance.

MATERIAL and METHODS

Pathologic, clinical and laboratory features, and follow-up outpatient/clinical records of 60 patients with a pathologic diagnosis of FSGS by renal biopsy in GATA Nephrology Clinic between 2000 and 2012 were retrospectively evaluated.

The diagnosis of primary FSGS was made with these criteria: (I) a lesion involving some of the glomeruli in the biopsy with other glomeruli remaining uninvolved, (II) the involved glomeruli having a part that have collapse of capillaries with obliteration of capillary lumina with or without adhesions, and (III) no clinical or pathological condition of primary disease that might lead to secondary FSGS.

The reduction of proteinuria to <300 mg/day with a stable serum creatinine concentration was considered as a complete remission. Partial remission was accepted as proteinuria from 300 mg/day to 3 g/day, or reduction of proteinuria by 50% from baseline, and a stable serum creatinine concentration.

Patients with a serum creatinine 1.5 mg dl and above at the time of diagnosis, those with glomeruli less than 10 on the biopsy specimens, those with interstitial fibrosis and atrophy in pathology reports, ones in follow-up of lower than 6 months, and family members of patients with proteinuria and a detected familial FSGS were not taken into consideration. Patients with a primary FSGS, lacking a systemic disease such as diabetes, hypertension or a connective tissue disease such as SLE-RA, ones with a negative ANA, hepatitis and HIV serology, ones that does not have any secondary cause such as cyclosporine or antihypertensive drug usage or obesity were recruited in the study.

Cases were not considered as familial with the age of onset out of childhood age, lack of family history and absence of gene mutations such as NPHS1, NPHS2, alpha-actinin 4 found in familial forms.

Statistical Analysis

SPSS 15.0 (Statistical Package for the Social Sciences ver. 15.0, SPSS Inc, Chicago, Illinois, USA) was used for the statistical analyses. Quantitative variables were expressed as mean ± standard deviation. The Kolmogorov Smirnov test was used to determine the distribution characteristics of variables and Levene's test was used to determine the equality of variance. Differences between groups were studied for significance by independent samples *t*-test or MannWhitney-U test as

appropriate. Categorical variables were compared with the Chi-square test.

RESULTS

The aim of our study was to evaluate the prognostic value of clinical, laboratory, and morphological indicators at the onset of treatment of primer FSGS. The baseline demographic, clinical and laboratory features of the patients are summarized in Table I. In this retrospective study conducted on the effect of the immunofluorescence staining on renal surveillance in patients with primary FSGS, accumulation of fibrin was found to have a relationship with renal surveillance. The pathologic appearance in the renal biopsy specimen and associated situation of treatment response are summarized in Table II.

Progression was more rapid in patients with fibrin accumulation, but this result did not reach a statistical significance (nephrotic level of proteinuria was present in three patients with positive fibrin staining and there was subnephrotic level of proteinuria in other patients). No statistical relationship was found between other parameters and renal surveillance. The association between remission rates and medications of the patients are summarized Table III.

For complete understanding of the possible negative impact of the accumulation of 129 fibrin, further studies on larger patient groups are needed.

DISCUSSION

FSGS patients with nephrotic syndrome is characterized by a poor prognosis. U.S. studies of spontaneous remission rates of 4-6%, while a rate of 16% was found in studies from Europe. The reason for the high rate in Europe may be increased ACEI/ARB use and tight control of blood pressure. Approximately 50% of untreated patients will need dialysis in 8 years (15). Factors that affect treatment in patients with known primary FSGS are the degree of proteinuria, serum creatinine level, histological findings, and response to treatment (12,13,14). Response to

Table I: Clinicopathologic findings of the patients.

Number of patients (n)	60
Age (years)	26.12±8.64(18-34)
Gender (male)	51 (%85)
eGFR (ml/min/1.72 m^2 MDRD)	93.57±28.81(60.45-121.8)
24 h urinary protein excretion (mg/day)	2574.5±2333.8(492-15200)
Serum albumin (g/dl)	3.89 ±0.67 (1.1-4.56)
Treatment duration (months)	18.25 ± 22.59 (6-120)

Table II: The pathologic appearance in the renal biopsy specimen and associated situation of treatment response: treatment failure (no), partial remission (partial), and complete remission (yes).

Pathology		**Remission**			**Total**
		No	**Partial**	**Yes**	
Tubuluinterstitial fibrosis	n(%)	0 (0)	0 (0)	0 (0)	0 (0)
Crescent	n (%)	0 (0)	2 (3.3)	2 (3.3)	4 (6.7)
Lambda	n (%)	8 (13.3)	2 (3.3)	2 (3.3)	12 (20.0)
Kappa	n (%)	7 (11.7)	2 (3.3)	2 (3.3)	11 (18.3)
C1q	n (%)	3 (5.0)	3 (5.0)	2 (3.3)	8 (13.3)
Fibrin	n (%)	1 (1.7)	3 (5.0)	3 (5.0)	7 (11.7)
C3	n (%)	16 (26.7)	8 (13.3)	8 (13.3)	32 (53.3)
IgM	n (%)	12 (20.0)	10 (16.7)	6 (10.0)	28 (46.7)
IgG	n (%)	8 (13.3)	4 (6.7)	2 (3.3)	14 (23.3)
IgA	n (%)	10 (16.7)	5 (8.3)	4 (6.7)	19 (31.7)
Total	n (%)	29 (48.3)	21 (35.0)	10 (16.7)	60 (100)

Table III: The association between remission rates and medications of the patients.

Medication	Number of patients (%)	Remission			Total
		No	Partial	Yes	
ACEI	n (%)	25 (41.7)	16 (26.7)	8 (13.3)	49 (81.7)
ACEI + Steroids	n (%)	0 (0)	4 (6.7)	1 (1.7)	5 (8.3)
ACEI+ Steroids+ Immunsupressives	n (%)	4 (6.7)	1 (1.7)	1 (1.7)	6 (10.0)
Total	**n (%)**	**29 (48.3)**	**21 (35.0)**	**10 (16.7)**	**60 (100)**

initial therapy is the most important predictor of outcome in patients with primary FSGS. IS treatment should be initiated to all cases of primary FSGS nephrotic proteinuria. The judgement to provide immunosuppression to patients with significantly decreased kidney function (eg, GFR <25 to 35 mL/min per 1,73 m2) should take into consideration the acuity of the renal failure (suggesting more reversible disease) findings on renal biopsy (e.g., presence of significant tubulointerstitial fibrosis and glomerulosclerosis, which suggest less reversible disease) and the individual patient's risk for adverse consequences related to immunosuppression (24). Rate of deterioration of renal function increases with increasing degree of proteinuria. While most of the patients with more than 10gr/day proteinuria reach end stage renal failure in 5 years follow up, 80% of patients with non-nephrotic proteinuria have better renal prognosis in 10-year follow up (18,19,20). Bazzi et al. reported that a fractional excretion (FE) of IgG <0.14% was associated with a high remission rate after immunosuppressive therapy. However, patients with an FE of IgG >0.14% had a dismal prognosis even with immunosuppressive therapy (21). In another study, the value of FE IgG divided into two groups as the top and bottom of 0,14 and response to therapy, spontaneous remission rates were compared. However, the results were in contradiction with the results of Bazzi et al. There was no difference between the two arms (22).

The condition is divided into 5 separate histologic types according to light microscopy as classic, collapsing, tip, perihilar and cellular variants. Histologic type may be an important indicator for prognosis. While the most common form is the classic form, the worst prognosis is seen in the collapsing form. Rojas et al. evaluated the prognostic factors in 44 patients diagnosed with primary FSGS. After a mean follow-up of 21.6 ± 27.8 months, the renal function decreased in 18.2% of patients. 37.8% and 32.4% of the patients reached partial and complete proteinuria remission respectively. Treatment with steroids, antihypertensive therapy and IgM and C3 glomerular deposits were associated with a high renal survival. The treatment with steroids was the only factor that forecasted high renal survival and proteinuria remission. In general, high blood pressure, renal failure, cellular variant and interstitial fibrosis were prognostic factors for not achieving proteinuria remission (23).

An additional prognostic factor in patients with FSGS was the serum creatinine level at the beginning of the disease. According to Rydel et al., patients with serum creatinine >1.3 mg/dL (114.92 µmol/L) at the beginning of the disease have a significantly poorer prognosis than those whose creatinine is <1.3 mg/dL (15). Diana Tahiri et al. assessed the impact of surveillance of renal histopathologic findings of 50 patients diagnosed with primary FSGS. Presence of a statistically significantly lower response to treatment was determined in patients with mesangial hypercellularity and hyalinosis at a average 35-month follow-up (25).

Jadranka et al. determined high initial scores at IS as well as the high ratio of tubulointerstitial infiltration , hyaline arteriolosclerosis and interstitial fibrosis in patients with primary FSGS. Given all the covariates in this study of 60 patients, injury score has emerged as the most reliable parameter. IS>0.84 was more often found in patients who did not reach remission, whereas IS <0.34 was more frequent in patients who did reach remission (P=0.002). Serum creatinine and creatinine clearance correlated with IS (P<0.001), but proteinuria showed no correlation (26). The studies examining many parameters are continuing in order to estimate response to treatment in patients with primary FSGS. New parameters to predict renal surveillance are still needed while steroid or other immunosuppressives are been initiating in patients presenting with nephrotic range proteinuria.

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