Expression of Podocyte Related Proteins and TGF-β1, WT-1 in Minimal Change Disease and Focal Segmental Glomerulosclerosis

Minimal Değişiklik Hastalığı ve Fokal Segmental Glomerulosklerozda Podosit İlişkili Proteinler ve TGF-β1, WT-1 Ekspresyonu

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ABSTRACT

OBJECTIVE: Focal segmental glomerulosclerosis and minimal change disease are primary renal diseases representing with proteinuria. Following the identification of the role of podocyte-related molecules in the glomerular filtration barrier and the detection of the pivotal role of nephrin in the development of congenital nephrotic syndrome, the assessment and expression profiles of these proteins in acquired nephrotic syndrome have also come into question.

MATERIAL and METHODS: Renal biopsies from 74 patients with diagnoses of MCD, FSGS and nonspecific mesangial proliferation were included in this study. The light microscopic sections were re-examined and the definitive diagnoses were recorded. Nephrin, podocin, synaptopodin, WT-1 and TGF-β1 distribution were examined by immunohistochemistry. The histopathological parameters examined were correlated with clinical parameters.

RESULTS: The predominant staining pattern for all three podocyte-related proteins in FSGS was coarse granular and it was statistically significant between groups. FSGS cases showed a statistically significant loss in the glomerular expression of WT-1 compared to the other two groups. TGF-β1 expression was considerably higher in FSGS and it was correlated to the degree of interstitial fibrosis, inflammation and tubular atrophy.

CONCLUSION: The staining patterns of podocyte-related proteins, WT-1 and TGF-β1 expressions are definitely different in FSGS. This may reflect just a distributional change but a possible renal pathogenetic role has to be elucidated.

KEY WORDS: Podocyte, Nephrin, FSGS, MCD

ÖZ

AMAÇ: Fokal segmental glomerüloskleroz (FSGS) ve minimal değişiklik hastalığı (MCD), nefrotik düzeyde proteinüriye neden olan primer renal parankim hastalıklarıdır. Her ikisi de glomerüler epitel hücre hasarından kaynaklanır ve "podositopati" olarak da isimlendirilir. Glomerüler filtrasyon bariyerinde podositle ilişkili moleküllerin rolünün tanımlanması ve konjenital nefrotik sendromun gelişiminde nefrin rolünün saptanmasının ardından, bu proteinlerin edinilmiş nefrotik sendromdaki ekspresyon profilleri de sorgulanmıştır.

GEREÇ ve YÖNTEMLER: Bu çalışmada renal biyopsi örnekleri ile MCD, FSGS ve nonspesifik mezenşiyal proliferasyon tanıları alan 74 hasta çalışmaya dahil edildi. Işık mikroskobik kesitleri; interstisyel inflamasyon, tübüler atrofi, fokal ve global glomerüloskleroz bulguları için tekrar derecelendirildi. Nefrin, podosin, sinaptopodin, WT-1 ve TGF-β1 dağılımı immünohistokimya ile incelendi ve boyama paternleri ve yoğunlukları kaydedildi. İncelenen histopatolojik parametreler, klinik parametreler ile ilişkilendirildi.

BULGULAR: FSGS'de tüm üç podosit ile ilişkili proteinler kaba granüler baskın boyanma paterni gösterdi ve diğer gruplar arası istatistiksel anlamlı farklılık izlendi. Benzer şekilde, FSGS vakaları diğer iki gruba kıyasla WT-1'in glomerüler ekspresyonunda istatistiksel olarak anlamlı bir kayıp gösterdi. FSGS'de TGF-β1 ekspresyonu oldukça yüksekti ve interstisyel fibrozis, inflamasyon ve tübüler atrofinin derecesi ile korele idi.

SONUÇ: Bulgular, podosit ile ilişkili proteinlerin, WT-1 ve TGF-β1 boyanma paternlerinin FSGS'de farklı olduğunu göstermektedir. Bu sadece bir dağılım değişikliğini yansıttığı gibi olası bir renal patojenetik rolü de ifade ediyor olabilir.

ANAHTAR SÖZCÜKLER: Podosit, Nefrin, FSGS, MCD

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INTRODUCTION

Focal segmental glomerulosclerosis (FSGS) is a clinicopathologic syndrome characterized by proteinuria, usually of nephrotic range, associated with lesions of focal and segmental glomerular sclerosis (1). Minimal change disease (MCD) has a very similar clinical presentation but it is distinguished from FSGS by its responsiveness to the empirical immunosuppressive treatment including corticosteroids, a mostly non-progressive clinical course, and a very low risk of recurrence in renal allograft (2, 3). It is important to differentiate these two diseases, but some cases still pose a diagnostic problem for nephropathologists because of the presence of some histologically indistinguishable and borderline lesions (4, 5). A mild degree of mesangial hypercellularity with negative immunofluorescence can be considered to fall either between the boundary of MCD and FSGS or within the spectrum of MCD.

Both FSGS and MCD have in common the ultrastructural evidence of injury to glomerular epithelial cell (podocyte) and therefore they are classified as "podocytopathies" (6). Together with the glomerular endothelial cells and the glomerular basement membrane (GBM), podocytes constitute the glomerular filtration barrier (GFB) and act to prevent the filtration of plasma proteins into the urine (7). Podocytes have been a subject of many studies during the last 10 years and identification of mutations in podocyte-related proteins, the examples of which are nephrin, podocin, CD2-associated protein (CD2AP), α-actinin IV and TRPC6, in various congenital nephrotic syndromes has improved the understanding of podocyte biology and the key role of the podocyte for the pathophysiology of proteinuria (8-11).

We have limited knowledge about the process of the formation of structural changes in the barrier that leads to nephrotic syndrome. However, a number of different structural components are required to maintain the integrity of the filtration barrier (12). Nephrin is a type-1 transmembrane protein of the immunoglobulin family and it has a role in cell-to-cell adhesion and signalling functions (13). Immunoelectron microscopy has showed that nephrin is localized at the slit diaphragm area (14) and it is synthesized by the glomerular podocytes (10). Kestila et al. demonstrated that mutations in NPHS1 gene encoding for nephrin lead to a rare autosomal recessive disorder with proteinuria already in utero and severe nephrotic syndrome soon after birth, "the congenital nephrotic syndrome of the Finnish type (CNF)" (10). Following this major discovery, it was suggested that nephrin plays a pivotal role in the maintenance of the glomerular filtration barrier (15).

Podocin is a hairpin-shaped integral membrane protein. It is located solely in the slit-diaphragm region and interacts with the intracellular domains of nephrin and Neph1 and with CD2AP (8, 16,17). As a NPHS2 gene product, mutations in podocin causes an autosomal recessive, steroid resistant nephrotic syndrome in some patients (8). It was also shown that severe proteinuria develops in podocin-knockout mice which die within a few days after birth (18). Thus, ıt was suggested that podocin may act as a scaffolding protein which has a role in the structural organization of the slit diaphragm and regulation of its filtration function (19).

Synaptopodin has a role in the actin-based shape and motility of podocyte foot processes. It represents a proline-rich actin-associated protein of telencephalic dendrites and glomerular podocytes (20). The expression of this protein coincides with the formation of foot processes since its first appearance is at the capillary loop stage during nephrogenesis in rat models (21).

The Wilms' tumor (WT) 1 is a zinc finger transcription factor, which is expressed by glomerular progenitor cells during the early glomerular development but becomes restricted to podocytes as the glomerulus matures (22, 23). In patients with Denys-Drash syndrome, characterized by the association of early-onset nephrotic syndrome, male pseudo hermaphroditism, and nephroblastoma, the glomerular lesions exhibit diffuse mesangial sclerosis and WT-1 expression was found to be abnormal (24, 25). Therefore, it may also be important in the maintenance of mature podocyte phenotype (26).

TGF-β is a cytokine that accumulates in injured kidneys in experimental animal models and most of the chronic renal diseases in humans. Although exaggerated TGF- ^{2} signaling is considered a major profibrotic stimulus in mesangial injury and expansion, little is known about its role of podocyte injury pathogenesis. However, podocytes was shown to undergo apoptosis and precedes mesangial expansion in TGF- ^{2}1 transgenic mice, which has been proposed as a new pathogenetic mechanism for podocyte depletion in progressive glomerulosclerosis (27).

This study was designed to analyze and compare the glomerular expression profiles of the above mentioned podocyte associated proteins, nephrin, podocin, synaptopodin, WT-1 and TGF- β1 in a number of kidney biopsies from patients with proteinuric glomerulopathies whose light microscopic diagnoses were FSGS, MCD or nonspecific mesangial proliferation with negative immunofluorescence staining. Our aim was to identify whether FSGS and MCD show any differences in terms of the expression of these proteins and to determine that can we separate the borderline cases in between FSGS and MCD from the expression data of these markers.

MATERIAL and METHODS

We studied renal biopsies from 74 patients with primary FSGS, MCD and nonspecific mesangial proliferation histopathologically diagnosed in our department from January 2002 to January 2008. Clinical information of 37 cases regarding the level of proteinuria, blood levels of urea and creatinine, blood albumin level and creatinine clearance at the time of diagnosis were obtained from the hospital database.

Patients

A total of 74 cases were selected from the archives of the pathology department at Gazi University Medical School, including 37 cases of FSGS, 25 cases of nonspecific mesangial proliferation, 12 cases of MCD. Normal renal parenchyma from nephrectomy specimens performed for surgical reasons of the 10 patients were used as controls for antibodies used immunohistochemistry. Forty-six patients (54.8%) were male and 38 (45.2%) were female with a mean age of 29±18.16 (range varied from 1 to 66). Patients under the age of 15 made up 29.8% (n: 25) of all cases.

Light Microscopy

The three μm-thick serial sections of all patients, stained with hematoxylin and eosin, periodic acid-Schiff and trichrome were re-examined by an expert nephropathologist (LM). In total, 1036 glomerular cross-sections from 74 cases (14 cross sections per one case) were investigated. Focal segmental and global glomerular sclerosis as well as interstitial inflammation, interstitial fibrosis and tubular atrophy were evaluated. Sclerosis was defined as an expansile scar of the glomerular tuft characterized by increased matrix, with or without associated hyalinosis and adhesion to Bowman's capsule. For segmental and global glomerular sclerosis, the percentage of affected glomeruli per total number of glomeruli in each biopsy was recorded. The other parameters including interstitial fibrosis, tubular atrophy and interstitial inflammation were evaluated semi quantitatively as mild, moderate and severe. The variant of FSGS was also identified as follows: FSGS (NOS), perihilar variant, cellular variant, tip lesion and collapsing variant.

Immunohistochemistry

To characterize the expression profiles of podocyte-related proteins, 3 μm-thick sections of formalin-fixed paraffin-embedded tissues from each case were studied by indirect immunoperoxidase staining. The sections were rehydrated in a graded series of alcohol, blocked with non-immune blocking solution. Antigen unmasking was performed by microwaving the sections in a 10 mM citrate buffer, pH 6.0 for 20 minutes. The sections then were incubated with the following individual primary antibodies for 2 hours at 37°C; nephrin (dilution; 1:500, mouse polyclonal antibody, Life scan Bioscience), podocin (dilution; 1:500, rabbit polyclonal antibody, ABCAM), synaptopodin (dilution; 1:250, rabbit polyclonal antibody, ABCAM), WT-1 (1:100, rabbit polyclonal antibody, ABCAM) and TGF-β1 (dilution; 1:500, mouse monoclonal antibody, ABCAM). After washing, the sections were incubated with secondary antibody (multi-species ultra streptavidine detection system-HRP, Zymed, Massachusetts, USA) and streptavidine-biotin complex (Zymed, Massachusetts, USA) for 20 minutes per each at room temperature respectively. Immunoreaction was developed with diaminobenzidine (diaminobenzidinetetrachloride, Zymed, USA) as chromogen.

Normal kidney tissue for nephrin, podocin, synaptopodin and WT-1 and tonsil for TGF-β1 were used as positive controls.

The staining pattern, staining intensity and loss of expression were examined for nephrin, podocin and synaptopodin. The staining patterns along the glomerular basement membranes were divided into following 3 groups: linear, fine but granular and coarse granular. If more than one pattern was present for a single biopsy, the predominant one was recorded. The staining intensity was compared with the control group and graded as mild (+), moderate (++), severe (+++). Both the percentage of glomeruli and the percentage of the glomerular area with decreased or loss of expression of three markers (nephrin, podocin and synaptopodin) were calculated and recorded.

The positive staining cells per glomerulus and the percentage of glomeruli showing staining in each biopsy were counted for TGF-β1. For WT-1 expression, the percentage of podocytes with nuclear expression was calculated

RESULTS

Of the 84 cases (74 with parenchyma injury, 10 controls) included in the study, 54.8% (N = 46) were male and 45.2% (N = 38) were female. The mean age was 29 (range 1-66). Pediatric patients under 15 years made up 29.8% of the cases (N = 25) and patients aged over 15 years made up 70.2% (n = 59) . There was no significant difference between the diagnostic groups in terms of age below 15 years and above (p=0,35). Of the total of 37 FSGS cases, 24 were NOS, 11 were perihilar, 1 type and 1 were cellular variants.

All ten cases of control group showed only linear staining along the glomerular basement membrane with nephrin, podocin and synaptopodin. Table I shows the distribution of staining patterns of nephrin, podocin and synaptopodin in study and control groups. As shown in the table, the granularity of staining was increased from MCD to FSGS. MCD cases only showed linear and fine granular staining along the GBM for all three proteins, for which FSGS cases showed predominant coarse granular staining (Figure 1A-D). When groups were compared to each other in terms of staining patterns of nephrin, podocin and synaptopodin, FSGS was the only group that showed significant difference ($P<0.05$). No clear-cut distinction could be made between MCD and cases with mesangial proliferation for staining patterns of podocin, synaptopodin and nephrin (p:0.16). Among the clinical variables included in this study, the level of proteinuria was statistically correlated with the increased coarse granular staining of nephrin along the glomerular basement membranes ($p\geq0.05$).

The staining intensities of podocin, nephrin and synaptopodin were compared between groups and the results are presented in Table 2. When nephrin, podocin and synaptopodin staining intensity were compared, there was no statistically significant difference between the groups of the disease and the control

Table I: The distribution of staining patterns of nephrin, podocin and synaptopodin in the control and study groups.

	Control group, n=10			MCD, n=12			Nonspecific mesangial proliferation, n=25			FSGS, n=37		
	Nephrin	Podocin	Synaptopodin	Nephrin	Podocin	Synaptopodin	Nephrin	Podocin	Synaptopodin	Nephrin	Podocin	Synaptopodin
Linear along the GBM	10 (100%)	10 (100%)	10 (100%)	3 (25%)	6 (50%)	0 (0%)	10 (40%)	5 (20%)	6 (24%)	1 (2.7%)	1 (2.7%)	3 (8.1%)
Fine granular along the GBM	0 (0%)	0 (0%)	0 (0%)	9 (75%)	6 (50%)	7 (58.3%)	14 (56%)	18 (72%)	14 (56%)	9 (24.3%)	8 (21.6%)	7 (18.9%)
Coarse granular along the GBM	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	5 (41.7%)	1 (4%)	2 (8%)	5 (20%)	27 (73%)	28 (75.7%)	27 (73%)

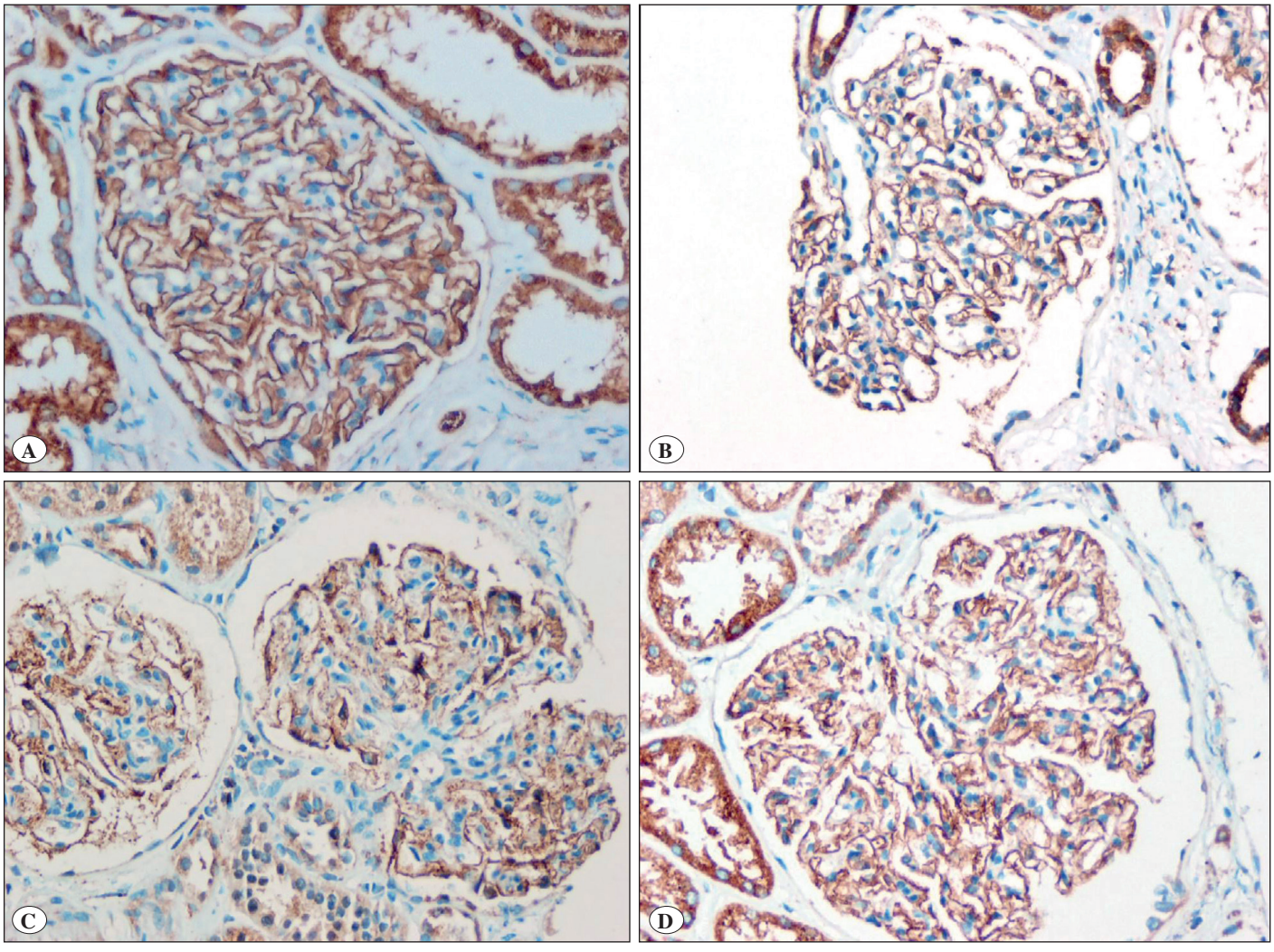

Figure 1: *The staining patterns of nephrin along the glomerular basement membranes in control, MCD, mesangial proliferation and FSGS cases.* ***A)*** *linear expression pattern in control group Note that the staining profile is changing from linear to coarse granular as the lesion progressed to MCD* ***(B)*** *to FSGS* ***(C)****. Granular but fine staining was observed in most cases of mesangial proliferation* ***(D)****, streptavidin-biotin peroxidaseX400.*

Table II: The staining intensities of podocin. nephrin and synaptopodin between the control and study groups

	Diagnosis	Staining intensity (%)		
		(+)	(++)	(+++)
Nephrin	FSGS	6 (20.7)	10 (34.5)	13 (44.8)
	Mesangial proliferation	1 (4.8)	12 (57.1)	8 (38.1)
	MCD	2 (16.7)	6 (50.0)	4 (33.3)
	Control	0 (0)	2 (20.0)	8 (80.0)
Podocin	FSGS	4 (14.8)	15 (55.6)	8 (29.6)
	Mesangial proliferation	2 (10.0)	11 (55.0)	7 (35.0)
	MCD	1 (9.1)	8 (72.7)	2 (18.2)
	Control	0 (0)	2 (20.0)	8 (80.0)
Synaptopodin	FSGS	6 (21.4)	13 (46.4)	9 (32.1)
	Mesangial proliferation	5 (25.0)	10 (50.0)	5 (25.0)
	MCD	3 (25.0)	7 (58.3)	2 (16.7)
	Control	0 (0)	3 (30.0)	7 (70.0)

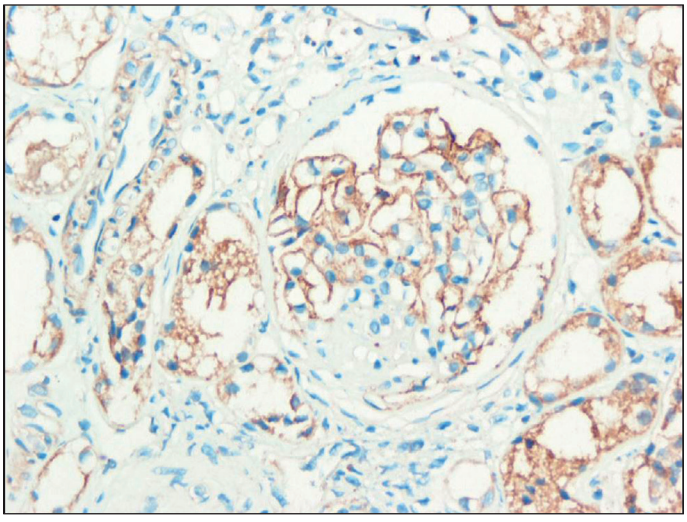

Figure 2: *Loss of nephrin expression in segmental sclerosis, streptavidin-biotin peroxidaseX200.*

group in terms of nephrin staining (all p values were ≥0.05). There was a statistically significant difference between the FSGS group and the control group in terms of podocin staining intensity. (p=0.007). A significant reduction in the intensity of synaptopodin staining was found in FSGS, MP and MCD compared to the control groups (p=0.008, p=0.004 and p=0.003 respectively). However, there was no significant difference in the intensity of staining between disease groups.

When glomerulus percentages showing loss of expression of nephrin, podocin and synaptopodin were evaluated, a significant difference was found only in terms of loss of nephrin expression between the groups (p=0.009), (Figure 2). The difference in loss of nephrin expression was associated with a significant decrease in FSGS cases compared to MCD, MP, and control groups (p values are 0.01, 0.01, 0.00 respectively). There was a significant positive correlation between the loss of nephrin expression and the serum creatinine when loss of expression of nephrin, podocin and synaptopodin were correlated with the albuminuria level, serum creatinine, estimated glomerular filtration rate (eGFR), albumin and BUN (r=0.78, p=0.00). In addition, there was a moderate positive correlation between the loss of nephrin expression and BUN (r = 0.419, p = 0.026) and a significant negative correlation with eGFR (r = -0.416, p = 0.026). A significant positive correlation was found between podocin expression loss and serum creatinine (r=0.694, p=0.000).

In the FSGS cases, a statistically significant increase was observed in the mean number of TGF beta 1 expressing cells compared to the MCD cases and the control group (p values are 0.002 and 0.001 respectively) Figure 3A-C. When the mean number of glomerular TGF-beta expressing cells was correlated with histological findings, a moderately significant positive correlation was found between TGF beta 1 expressing cells and interstitial inflammation, fibrosis, tubular atrophy, segmental and global sclerosis (all p values were ≤0.05). When nuclear WT1 expression in podocytes was compared, there was no statistically significant difference between the groups (p=0.93). In FSGS cases there was a decreased expression of WT-1 but it was not statistically significant when compared with other groups (Figure 4A,B). In some FSGS cases, decreased WT-1 expression was observed in large, rounded podocytes, which showed grave stones around the segmental sclerosis.

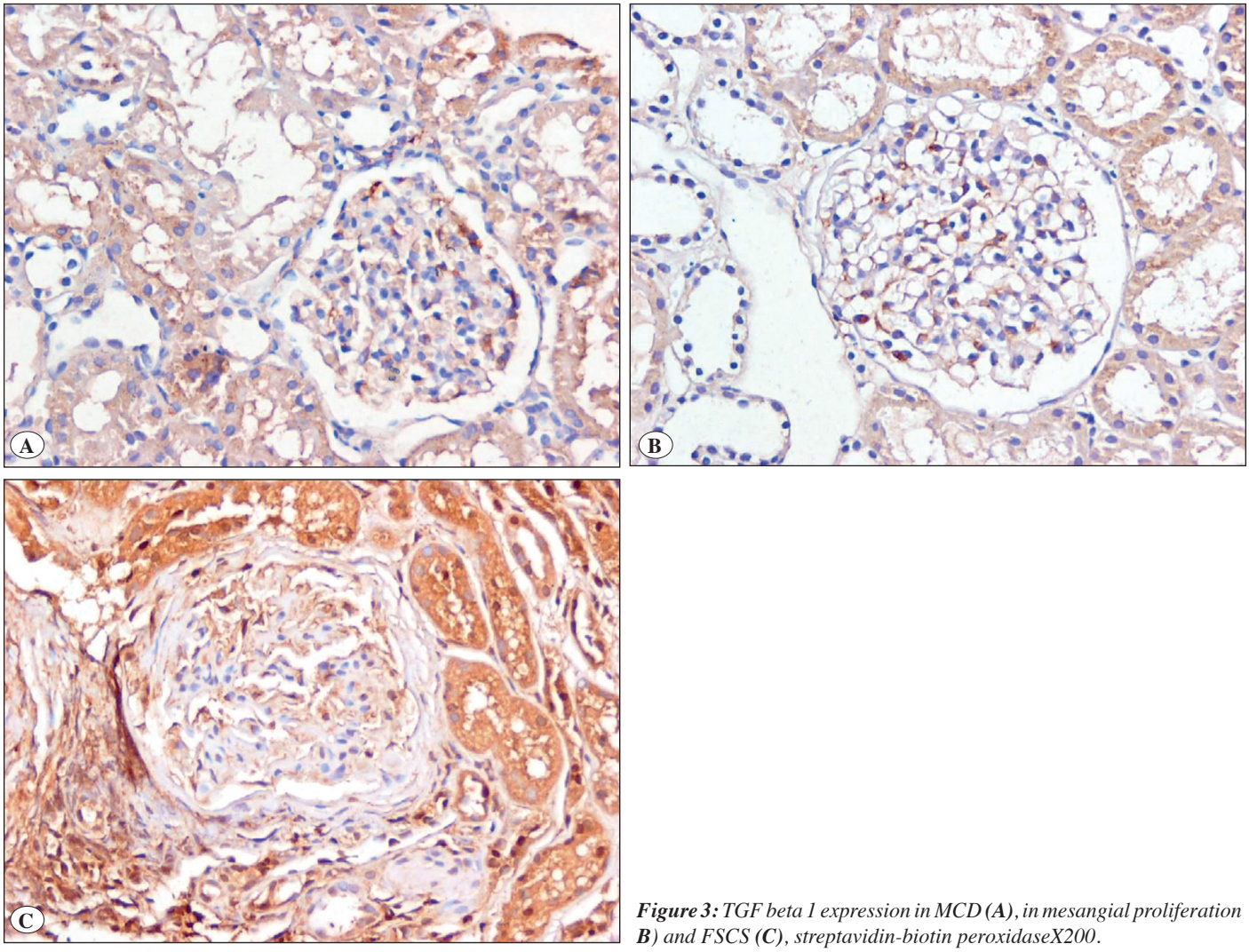

Figure 3: *TGF beta 1 expression in MCD* ***(A)****, in mesangial proliferation* ***B****) and FSCS* ***(C)****, streptavidin-biotin peroxidaseX200.*

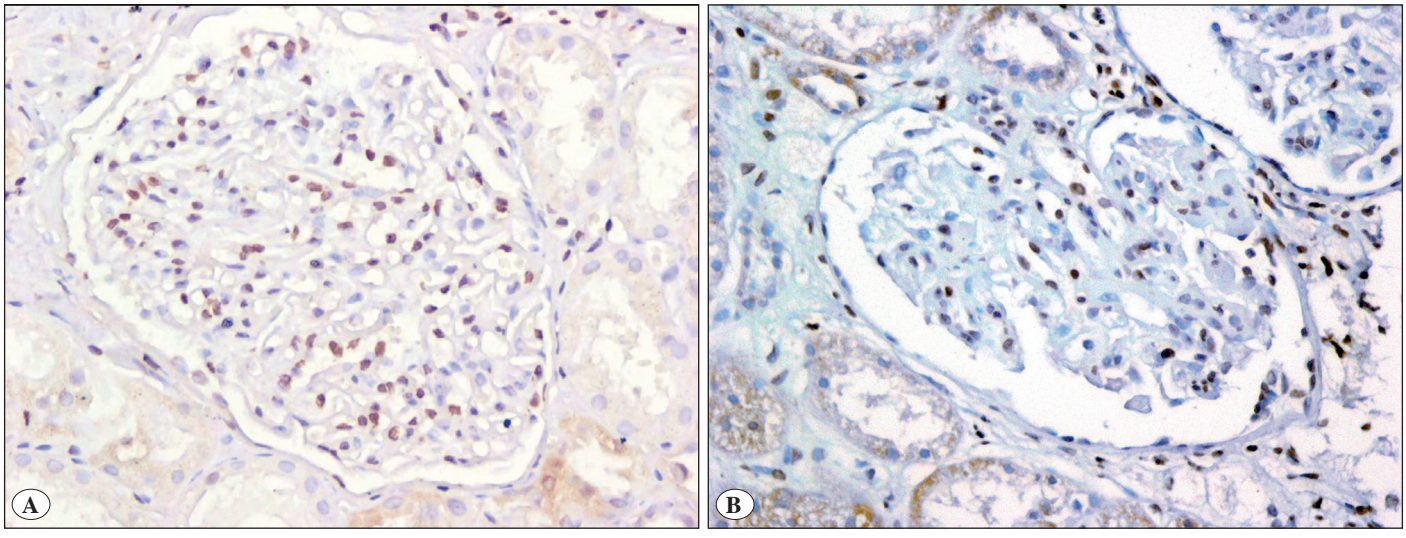

Figure 4: *WT1 expression in MCD* ***(A)****, and FSGS* ***(B)****, streptavidin-biotin peroxidaseX400.*

DISCUSSION

As a cause of "Finnish type congenital nephritic syndrome", NPHS1 gene mutation encoding for nephrin initiates effacement in the foot processes and results in proteinuria (10, 28). Since foot process effacement and variable levels of proteinuria may also be seen in acquired glomerular diseases and glomerulonephritis models in animals, the assessment of nephrin expression in renal diseases has been a hotspot for many studies. Both functional and structural defects in this slit diaphragm localized protein can have a critical role in proteinuria (29). MCD and FSGS are the most common causes of acquired nephrotic syndrome. Previous studies concerning nephrin expression in MCD and FSGS have revealed inconsistent results. Patrakka et al. did not show any difference in the immunohistochemical expression of nephrin in 56 of their cases, which included MCD, FSGS, membranous nephropathy (MN), Henoch-Schonlein nephritis, IgA nephropathy and membranoproliferative glomerulonephritis (MPGN), when compared with the control group (30) .They observed a linear glomerular capillary staining pattern for nephrin in their cases similar to the control group. They figured the loss of staining in the areas of cellular crescents and sclerotic foci as secondary to the degradation of the normal microanatomy of the glomeruli, instead not to proteinuria. On the other hand, Furness et al. determined that nephrin mRNA was decreased in their 1 case of MN and 3 cases of MDH compared to the control group by PCR amplification (31). Similarly, Doublier et al. found an extensive loss of nephrin staining and a shift from a podocyte-staining pattern to a granular pattern by immunofluorescence in patients with nephrotic syndrome, independent of the primary disease (32). Decreased expression of nephrin was also reported in animal models of nephrotic syndrome (33). As a cellular adhesion molecule, nephrin has both extracellular and intracellular connections. Therefore, it has an important role in the signal transduction. Accordingly, pathological processes preventing the functions of the nephrin and other slit diaphragm components can cause proteinuria, in spite of the presence of intact proteins (34). Immunoelectron microscopic studies showed that gold particles binding to anti-nephrin polyclonal antibody were localized to the podocyte cytoplasm, submembranous areas in the cytoplasm and spaces in between the foot processes in normal kidneys (35). They found that the expression of nephrin in glomerulonephritis cases involving MCD, membranous GN, MPGN, IgA and lupus nephritis was decreased in regions where the foot processes were effaced when compared with normal controls where the foot process were preserved (35). Therefore, they suggested that nephrin may have a role in the pathogenesis of proteinuria in acquired human glomerular diseases. However, ultrastructural semi-quantification showed the amount of nephrin to be reduced both in areas with and without foot process effacement compared with the control specimens in another study, in which immunohistochemical nephrin expression pattern was shown to be granular in biopsies from patients with MCD and correlated with the degree of foot process effacement (36). The linearity or granularity of the staining pattern was explained by the distance between the slits that determines the distance between individual antibody complexes binding to the slit diaphragm. The foot processes should be severely affected in order to observe differences in nephrin expression between normal and glomerulonephritic kidneys. However, Wernerson et al. found that, the linear nephrin pattern was replaced by a granular pattern in patients with recent onset severe proteinuria. There was a mix of linear and granular pattern in patients with milder proteinuria and patients undergoing treatment (36). Our study also confirmed the results that coarse granular staining along the GBM was statistically correlated with the severity of proteinuria. Agrawal et al. showed that the reduced glomerular podocin expression in MCD and FSGS was related to the amount of proteinuria. They suggest that alteration in podocyte phenotype may reflect the degree of podocyte injury in primary nephrotic syndrome rather than a primary event (37). In a study by Shankar et al. evaluating podocin and dystroglycan expression in patients with proteinuria, they found no correlation between primary and secondary podocytopathies in terms of expression of these proteins. There was also no significant difference between patients with steroid-sensitive or steroid-resistant FSGS and MCD (38).

Kim et al. pointed out a fine granular staining pattern in MDH and more scattered and coarse granular staining pattern in FSGS with monoclonal extracellular nephrin antibody immunohistochemically (39) Doublier et al. have suggested that the nephrin redistribution mechanism that occurs in podocytes during primer-induced nephrotic syndrome causes proteinuria (32). In the study consisting of 13 membranous GN, 10 MDH, 7 FSGS and 16 controls, the indirect immunofluorescence method showed that there was widespread expression loss in the disease groups compared to the control group and the linear pattern turned into a coarsely granular pattern. Activation in the cytoskeleton can cause nephrin to alter expression, displacing it from the plasma membrane into the extracellular space. In acquired proteinuria, loss of podocyte protein expression may also be a consequence of secondary podocyte injury to stimuli such as antibody, immunocomplex, cytokine, and complement component. Another research topic on the pathogenesis of FSGS and MDH has been T-cell associated glomerular injury and T-cell associated chemokines, cytokines due to increased cytokine levels in blood.

Strehlau et al. analyzed the expression of TGF beta 1, interleukin 2 (IL 2), and IL 4 intrarenal gene by the PCR method in the renal biopsies of 53 pediatric patients with proteinuria at the nephrotic level (40). Significantly higher TGF beta 1 and cytotoxic T lymphocyte effectors Fas-ligand, granzyme B and perforin transcripts were detected in FSGS cases compared to MCD. Kanai et al. found an increase in urinary TGF beta 1 excretion in FSGS cases (41). TGF-beta, which has many functions such as proliferation, differentiation, apoptosis,

and immunoreaction, has recently gained in importance for glomerular and tubular epithelial cell apoptosis and epithelial-mesenchymal transdifferentiation in progressive renal disease (42). Another indicator of progressive renal disease is glomerular and peritubular microvascularisation loss. TGF beta and thrombospondin 1 are effective in the loss of microvascular endothelial cell apoptosis and peritubular capillary (43). Kim et al. evaluated the expression of TGF beta 1, thrombospondin 1 and TGF beta type 2 receptors immunohistochemically in 15 idiopathic FSGS and 6 control biopsies, and found that the expression increased significantly compared to the control group. In FSGS cases, only a few podocytes have been stained in nonsclerotic glomeruli, whereas in podocytes surrounding sclerotic segments, TGF expression has been detected at a moderate or severe level. Increased TGF beta 1 and TGF beta 2 receptor mRNA levels have also been demonstrated by in situ hybridization. They also noted that there was no WT 1 nuclear expression, a podocyte marker, in glomerular epithelial cells surrounding sclerotic segments (44). The loss of staining with podocyte-specific markers WT1 and GLEPP-1 was based on the hypothesis that glomerular epithelial lesions initiated the TGF beta signal cascade.

In conclusion, FSGS is a common pathway in the progression of toxic, metabolic, immunologic, hemodynamic diseases and structural or functional changes leading to loss of podocytes and phenotype change and nephron loss (45, 46). The above-mentioned predictors are important in that they can reflect podocyte injury or loss, give information about the progression or spread of the disease, and explain the pathogenesis

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