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Fibronectin in reflux nephropathy; Is it a marker of reflux grade?

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Abstract

Background and Objective: Vesicoureteral reflux (VUR) is one of the most common urinary tract abnormalities in patients with urinary tract infection. Nowadays noninvasive diagnostic methods are suggested to recognize VUR and its severity.

Methods: We measured urinary and serum fibronectin in 51 children with VUR.

Results: The mean serum fibronectin was 318.3±112.1 in children with low grade VUR versus 356.1±189.9 in children with high grade VUR ($p>0.05$). The mean urinary fibronectin was also 31.5±12.9 in low grade VUR and 25.9±14.2 in high grade VUR ($p>0.05$). Thus we found no association between the severity of VUR and the amount of fibronectin in serum and urine of patients. We also f found no relationship between dimercaptosuccinic acid (DMSA) changes at the acute phase of unitary tract infection (UTI) and serum and urine fibronectin.

Conclusion: Contrary to some previous studies, we showed the serum and urinary fibronectin cannot preclude the severity and grade of VUR and hence it is not suitable surrogate marker for imaging techniques for VUR diagnosis.

Keywords: Vesicoureteral reflux (VUR), Fibronectin, Children, Unitary tract infection (UTI)

Introduction

Vesicoureteral reflux (VUR) is associated with varied degrees of renal damage. Dilating VUR as an important risk factor for pyelonephritis is one the most important causes of chronic renal failure in children (1). Vesicoureteral reflux can induce renal damage by several mechanisms. Inflammatory response induced by urinary tract infection can stimulate cytokines release such as transforming growth factor (TGF) and platelet growth factor. These cytokines especially TGF-Beta attract and stimulate proliferation of fibroblasts. These cells produce matrix proteins such as collagen type I, III and fibronectin. Fibronectin is released in all biological fluids including serum and urine. In our study we measured the concentration of serum and urinary fibronectin in children with reflux nephropathy. We determined the association between the degree of VUR and the amount of serum and urinary fibronectin.

Methods

Fifty one children aged between 3 months and 12 years were recruited. Fourteen patients (27.5%) were male. Patients were divided into subgroups based on the degree of reflux in voiding cystourography (VCUG) or radionuclide cystograpghy. These subgroups include patients with mild VUR, and with moderate VUR and severe VUR. Patients with VUR grades 1 and 2 in VCUG were considered to have mild VUR. VUR grade 3 was considered moderate and VUR grade 4 and 5 were as severe. The serum and urinary fibronectin were measured in these patients. All patients who had urinary tract infection at the time of sample taking were excluded. Patients who had bilateral VUR, the highest grade of VUR was considered for their classification.

Acute phase dimercaptosuccinic acid (DMSA) scan of patients was also assessed blindly by our nuclear medicine specialist without any knowledge about the VUR status and the amount of serum and/or urinary fibronectin. Renal dam-

Table 1. The serum and urine fibronectin in patients with low, moderate and severe VUR.

	Low grade VUR	Moderate grade VUR	Severe grade VUR	p value
Serum Fibronectin	318.3±112.1	279.6±160	356.1±189.9	0.39
Urine Fibronectin	31.5±12.9	23.54±11.03	25.9±14.2	0.17
Urine Fibronectin/cr ratio	1.2±0.33	0.78±0.25	1.64±0.69	0.45

ages in DMSA scan were classified into 8 grades as follows.

Grade 0: normal kidney, grade 1: decreased uptake in one pole with intact border, grade 2: decreased uptake in two poles with intact borders, grade 3: diffuse decreased uptake with intact border, grade 4: decreased uptake in one pole with scar, grade 5: decreased uptake in two poles with scar, grade 6: multiple scars, grade 7: diffuse decreased uptake with one pole scar, grade 8: diffuse decreased uptake with multiple scars.

We had data of acute phase DMSA scan only in 26 patients. The highest grade of DMSA scan was considered for DMSA classification.

Statistical analysis: The data were analyzed using SPSS ver.13 software. Values are expressed as mean (±SD) or mean (SEM). In order to examine differences, the independent sample t-test and one way ANOVA test were employed. $P<0.05$ was considered to be statistically significant.

Results

Fifty one children with vesicoureteral reflux were included in this study. The mean age of patients was 4.24±2.98. Twenty three patients (45.1%) had unilateral VUR and 28 (54.9%) had bilateral VUR. Low vesicoureteral reflux was reported in 19 patients (37.3%), moderate VUR in 15 patients (29.4%) and severe VUR in 16 patients (31.4%). The mean of serum fibronectin was 320.3 ± 153.7 and the mean of urine fibronectin was 27±13 in all patients irrespective of the grade of VUR. The mean of the urine fibronectin to creatinine ratio was also 1.19 ± 1.86. The association between the serum fibronectin concentration and the grade of vesicoureteral reflux was not significant (ANOVA test, p=0.39). We also found no association between urine fibronectin or urine fibronectin to creatinine ratio and the severity of VUR (ANOVA test, p value for urine fibronectin: 0.17, for urine fibronectin to creatinine ratio: 0.45) (Table 1). The mean of urinary fibronectin was 29.36±12.86 in patients with unilateral VUR and 25.2±11.45 in patients with bilateral VUR (p=0.25). The mean of serum fibronectin was 350.09±96.27 in unilateral VUR and 306.2±183.2 in bilateral VUR (p=0.28). The mean of urinary fibronectin to creatinine ratio was 1.2±0.28 in unilateral VUR and 1.2±0.43 in bilateral VUR (p=0.99).

We also assessed the association between urine fibronectin and DMSA changes at the acute phase. We also found no association between urine fibronectin or urine fibronectin to creatinine ratio and the grade of DMSA changes (ANOVA test, p value for urine fibronectin: 0.12, for urine fibronectin to creatinine ratio: 0.46). We also found no association between serum fibronectin and DMSA grades.

Discussion

Fibrosis of the glomerulus (glomerulosclerosis) and/or fibrosis of tubulointerestitum (tubulointerestitial nephritis) are the end period of any kidney damage. When renal injury occurs, TGF-Beta production is increased. Severe and repeated injuries result in continuous overproduction of TGF-B and continuous extracellular matrix proteins production (1-4).

TGF-B, and fibroblast growth factor 2 (FGF-2) are potent autocrine inducer of fibroblast proliferation and finally renal scarring (5-9). Fibroblasts and myofibroblasts are considered to be the key cells to produce fibrosis in kidney (2,3,8). These cells synthesize many extra-cellular matrix components like collagen type I, III and fibronectin. In renal fibrosis, the interestitium is substituted by collagen type I and III and fibronectin at an early phase which are followed by the accumulation of proteoglycans and decorin (DCN) (10,11).

The varied types of renal diseases may be ended to renal fibrosis such as pyelonephritis, reflux nephropathy, SLE, IgA nephropathy, drug nephropathy etc. There are some fibroblast markers to determine the activity of these cells in tissues such as fibroblast specific protein 1 (FSP-1) (6,7), serum beta fibroblast growth factor (9,10), and the urinary amino-terminal propeptide of type III procollagen (PIIINP) (11-13).

Fibronectin as the major extracellular protein is secreted by fibroblasts. This protein is the scaffold for deposition of other proteins. Fibronectin also induces the conversion of fibroblasts to myofibroblasts (14). TGF-B released during sustained renal injuries induces the fibronectin production which is mediated by cconnective tissue growth factor (CTGF) (15-19). Fibronectin is commonly

found in biological fluids such as plasma and urine. Serum fibronectin usually originates from liver, but sometimes other cells release fibronectin into the plasma (20-23). Some researchers believe that the urinary fibronectin is originated from the kidney not originated from the plasma (16).

Increased expression of fibronectin and/or fibronectin accumulation in glomeruli were reported in some renal disorders by some authors (24,25). Kozlovskaia et al showed a correlation between the amount of urinary excretion of fibronectin and the severity of renal fibrosis in patients with lupus nephritis and chronic glomerulonephritis. In this study, the highest amount of urinary fibronectin was found in patients with renal dysfunction (17). The correlation between urinary fibronectin and pathologic changes was also shown in diabetic patients (18). In a study in children, the urinary fibronectin was higher significantly in patients with IgA nephropathy and Henoch Schonlein in comparison with control group, but there were not significant relationship between grade of disease in pathology and the urinary fibronectin levels (19). Increased accumulation of fibronectin in renal biopsies obtained from the patients with reflux nephropathy was reported by some researchers (26,27). Sabasinka et al measured serum and urinary fibronectin in children with vesicoureteral reflux. They concluded that significant higher serum fibronectin is found in patients with VUR in comparison with control group. The urinary fibronectin level was significantly higher in patients with high grade VUR. They also found a negative correlation between urine osmolality and urinary fibronectin level (28).

In contrast we found that the serum and urinary fibronectin concentration were not associated with the grade of VUR or grade of DMSA changes. Thus we cannot use serum and urine fibronectin as a marker of VUR diagnosis.

Conflicts of interest: None declared.

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30.