ORIGINAL PAPERS

Serum TGF-β1 and TNF-α concentrations in children with reflux and obstructive nephropathy

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SUMMARY

Serum TGF-β1 and TNF-α concentrations in children with reflux and obstructive nephropathy

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Progressive renal parenchyma scarring, developing secondary to urinary tract infections (UTI) in patients with vesicoureteral-reflux (reflux nephropathy) and patients with an obstacle in the urine outflow (obstructive nephropathy), is the result of many factors. In recent years, researchers pay special attention to the role of immune mechanisms in the development and progression of inflammatory nephropathy. The most important in the activation and progression of renal scarring attributed to transforming growth factor (TGF-β) and tumor necrosis factor α (TNF-α), which took part in the general and local inflammatory response may contribute to the development and progression of fibrosis secondary to respiratory infection tract.

***The aim of this study** was to evaluate the concentration of TGF-β1 and TNF-α in serum of children with confirmed inflammatory renoscyntygraficznie nephropathy.*

***Materials and methods.** The study included 70 children (24 boys and 46 girls) aged 1-17 years. All patients had a history of UTI, which had at least one form of acute pyelonephritis. Imaging studies showed in 85.7% (60/70) children, vesicoureteral reflux, and in the remaining 14.3% (10/70) unilateral ureteropelvic/vesicoureteric junction or ureteral stricture. The concentration of TGF-β1 and TNF-α in blood serum was determined by ELISA 1-3 months after cure UTI.*

***Results.** Mean serum concentrations of TGF-β1, both in children with reflux nephropathy and in children with obstructive nephropathy was significantly lower compared with the control group ($p = 0.00009$ and $p = 0.02$). There were no major differences in serum concentrations of TNF-α in both groups compared to the control group ($p = 0.93$ and $p = 0.88$).*

***Conclusions.** Lower serum concentration of TGF-β1 in patients with reflux nephropathy and obstructive nephropathy may be related to greater use in the kidney of circulating TGF-β1 and/or an*

STRESZCZENIE

Stężenie TGF-β1 i TNF-α w surowicy dzieci chorych na nefropatię odpływową i zaporową

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Postępujące bliznowacenie miąższu nerek, rozwijające się wtórnie do zakażeń układu moczowego (ZUM) u pacjentów z odpływem pęcherzowo-moczowodowym (nefropatia odpływowa) oraz u pacjentów z przeszkodą w odpływie moczu (nefropatia zaporowa), jest wypadkową działania wielu czynników. W ostatnich latach badacze zwracają szczególną uwagę na rolę mechanizmów immunologicznych w rozwoju i progresji nefropatii pozapalnej. Największe znaczenie w uaktywnieniu i progresji bliznowacenia nerek przypisuje się transformującemu czynnikowi wzrostu (TGF-β) i czynnikowi martwicy guza α (TNF-α), które biorąc udział w ogólnej i miejscowej odpowiedzi zapalnej mogą przyczyniać się do rozwoju i progresji włóknienia wtórnego do infekcji dróg moczowych.

***Celem pracy** była ocena stężenia TGF-β1 i TNF-α w surowicy dzieci z potwierdzoną renoscyntygraficznie nefropatią pozapalną.*

***Materiał i metody.** Badaniem objęto 70 dzieci (24 chłopców i 46 dziewcząt) w wieku od 1-17 lat. U wszystkich pacjentów w wywiadzie występowały ZUM, z których przynajmniej jedno miało postać ostrego odmiedniczkowego zapalenia nerek. Badania obrazowe wykazały u 85,7% (60/70) dzieci odpływy pęcherzowo-moczowodowe, a u pozostałych 14,3% (10/70) jednostronne podmiedniczkowe lub przypęcherzowe zwężenie moczowodu. Stężenie TGF-β1 i TNF-α w surowicy krwi oznaczono testem ELISA 1-3 mies. po wyleczeniu ZUM.*

***Wyniki.** Średnie surowicze stężenie TGF-β1 zarówno grupie dzieci z nefropatią odpływową jak i w grupie dzieci z nefropatią zaporową było istotnie niższe w porównaniu z grupą kontrolną (odpowiednio p=0,00009 i p=0,02). Nie wykazano natomiast istotnych różnic surowiczego stężenia TNF-α w obu grupach w porównaniu do grupy kontrolnej (odpowiednio p=0,93 i p=0,88).*

***Wnioski.** Niższe surowicze stężenie TGF-β1 u pacjentów z nefropatią odpływową i nefropatią zaporową może mieć związek z* *increased urinary excretion of the secondary to the ongoing inflammatory process. Confirmation of the role of these factors in the pathogenesis of reflux and obstructive nephropathy calls for the continuation of research.*

Key words: *transforming growth factor (TGF-β), tumor necrosis factor α (TNF-α), reflux nephropathy, obstructive nephropathy, children*

większym wykorzystaniem w nerkach krążącego TGF-β1 i/lub ze zwiększonego jego wydalania z moczem wtórnie do toczącego się przewlekłego procesu zapalnego. Potwierdzenie roli tych czynników w patogenezie nefropatii odpływowej i zaporowej wymaga kontynuacji badań.

Słowa kluczowe: *transformującemu czynnikowi wzrostu (TGF-β) i czynnikowi martwicy guza α (TNF-α), nefropatia refluksowa, zaporowa nefropatia, dzieci*

Progressive renal scarring being secondary to urinary system infections develops in patients with vesicoureteral reflux (reflux nephropathy) and in patients with urinary obstruction (obstructive nephropathy). Numerous factors are thought to be implicated in the pathogenesis of this process. In the recent years, the role of immune and molecular mechanisms in the pathogenesis of renal tubular atrophy and interstitial fibrosis with subsequent scarring of renal parenchyma and glomerulosclerosis has been a subject of intensive studies. Nowadays, there is a large body of evidence for the concept that TGF-β (transforming growth factor β) and TNF-α (tumor necrosis factor α) are one of the most important factors in the pathogenesis of renal scarring. .

TGF-β is released during renal interstitial inflamation. It influences cell cycle, cell differentiation and apoptosis [1]. TGF-β increases renal extracellular matrix proteins synthesis (collagen, proteoglicans and fibronectin), integrin receptors synthesis, activates inhibitors of enzymes degrading renal extracellular matrix proteins (metalloproteinases), and transforms renal tubular cells into myofibroblasts. It is also chemoattractant for monocytes and fibroblasts [2, 3]. TGF-β induces apoptosis of renal tubular cells. This results in renal tubular atrophy, perivascular inflammation and fibrosis [4-6]. In chronic renal diseases, TGF-β induces apoptosis of endothelial cells. This causes atrophy of peritubular and glomerular capillaries. TGF-β probably inhibits synthesis of endothelial growth factor [7].

Progressive scarring of kidneys results in gradual lost of their function [2, 4]. In humans, TGF-β_1, on of five isoforms of TGF-β family, is thought to be a crucial factor in the pathogenesis of renal sparring in patients with vesicoureteral reflux and urinary obstruction [8, 9].

In addition to TGF-β1 TNF-α is also implicated in the pathogenesis of renal scarring. TNF-α is released by leukocytes, mesangial cells and renal tubular cells during acute phase of inflammation. There is a large body of evidence that TNF-α is an important factor in the pathogenesis of obstructive nephropathy. In urinary obstruction, the release of TNF-α is stimulated by angiotensin II [9].

The aim of the study were to assess serum TGF-β_1 and TNF-α level in children with reflux nephropathy and obstructive nephropathy, to compare serum TGF-β_1 and TNF-α level between children with unilateral and bilateral vesicoureteral reflux, vesicoureteral reflux of high and low grade, and to compare serum TGF-β_1 and TNF-α level between children with renal scarring and with and without hypertension, with and without proteinuria. Serum TGF-β_1 and TNF-α level in children with renal scarring in relation to their age and sex was also analyzed.

MATERIAL AND METHODS

The study comprised 70 children (24 boys and 46 girls) aged 1-17 years (mean 9.8 ± 4.8 years) with renal scarring diagnosed scintigraphically. Children aged below 5 years constituted 21.4% (15/70) and children aged 6-17 years – 78.6% (55/70). All patients had a history of recurrent urinary system infections including at least one episode of acute pyelonephritis. In 14 (14/70, 20%) children, the first episode of urinary system infection occurred in infancy. In all patients blood pressure was measured by routine sphigmomanometric method. In those suspected of arterial hypertension, blood pressure was assessed again by ambulatory blood pressure measurement method. Arterial hypertension was diagnosed in 12 children (12/70, 17.2%). During study all children were afebrile and free from urinary system infection.

In all patients, serum C-reactive protein level was normal (below 0.5 mg/dl). Proteinuria, microscopic hematuria and proteinuria with microscopic hematuria were observed in 18 (18/70, 25.7%), 4 (4/70, 5.7%) and 2 (2/70, 2.9%) children, respectively. In all patients radiologic evaluation was performed including ultrasonography, voiding cystourethrography and renal scintigraphy. In addition, urography and urodynamic examination were performed in those with clinical indications.

In 60 children unilateral or bilateral vesicoureteral reflux was diagnosed by voiding cystourethrography. Primary vesicoureteral reflux was detected in 30 (30/60, 50%) patients. In children with secondary vesicoureteral reflux, congenital obstrution of the bladder outlet (strictura of urethra, posterior urethral valves) (19/60, 31.7%) and urodynamic abnormalities (11/60, 18.3%) were detected. Unilateral and bilateral vesicoureteral refluxes were diagnosed in 23 (23/60, 38.3%) and 37 (37/60, 61.7%) children, respectively. Severe (grade III-V) and mild (grade I-II) vesico-ureteral refluxes were found in 46 (46/60, 76.7%) and 14 (14/60, 23.3%) patients, respectively. 11 (11/60, 18.3%) children had additional congenital urinary system abnormalities including renal duplication, horseshoe kidney and unilateral renal agenesis.

In 10 children, unilateral urinary obstruction was diagnosed. 8 (8/10, 80%) patients had ureteropelvic junction obstruction. In the remaining 2 patients (2/10, 20%), vesicoureteric junction obstruction was diagnosed.

Renal scintigraphy (^{99} Tc EC or ^{99m} Tc DMSA) was performed 1-3 months after cure of the last episode of urinary system infection. Bilateral renal scarring was detected in 36 (36/60, 60%) children with bilateral vesicoureteral reflux. Unilateral renal scarring was found in the remaining 24 (24/60, 40%) children with unilateral (23 patients) and bilateral (1 patient) vesicoureterl reflux. In all children with urinary obstruction, unilateral renal scarring was diagnosed.

36 healthy children (12 boys and 24 girls) aged 2-16 years (mean 8.6 ± 3.5 years) with no positive history of urinary system infections were controls. In all controls, serum C-reactive protein level, urinalysis, and urine culture were normal. There were no statistically significant differences in age and sex between studied children and controls. Serum TGF-β_1 and TNF-α levels were measured by ELISA (BenderMedSystems) 1-3 months after cure of the last episode of urinary system infection.

Statistical analysis were performed by Mann-Whitney U and Spearman tests. We considered P values below 0.05 to be statistically significant.

RESULTS

The mean serum TGF-β_1/TNF-α levels in children with reflux nephropathy as compared to controls and the mean serum TGF-β_1/TNF-α levels in children with obstructive nephropathy as compared to controls are presented in table 1 and table 2, respectively.

The mean serum TGF-β_1 levels in children with reflux nephropathy and in children with obstructive nephropathy were significantly lower than in controls (p=0.00009 and p=0.02, respectively). There were no statistically significant differences in the mean serum TNF-α levels between children with reflux/obstructive nephropathy as compared to controls.

The mean serum TGF-β_1 level in girls was lower than in boys (83.8 ng/ml and 107.1 ng/ml, respectively, p=0.06). The mean serum TNF-α levels in girls and boys did not differ significantly (47.0 pg/ml and 41.8 pg/ml, respectively, p>0.05).

Table 1. Serum TGF-β1 concentrations in children with reflux nephropathy (RN) and obstructive nephropathy (ON) as compared to controls (C)

Cytokine	Type of nephropathy	n	Range	Median	Statystical analysis
TGF-β1 (ng/ml)	RN	60	35,96-149,62	89,2	RN/C p=0,00009
	ON	10	32,77-137,14	89,2	ON/C p=0,02
	controls	36	58,82-169,62	126,2	RN/ON NS

n – number of patients
NS – statisically not significant

Table 2. Serum TNF-α concentrations in children with reflux nephropathy (RN) and obstructive nephropathy (ON) as compared to controls (C)

Cytokine	Type of nephropathy	n	Range	Median	Statistical analysis
TNF-α (pg/ml)	RN	60	21,81-291,1	48,86	RN/C NS
	ON	10	24,41-222,2	41,21	ON/C NS
	controls	36	28,94-96,78	50,74	RN/ON NS

n – number of patients
NS – statistically not significant

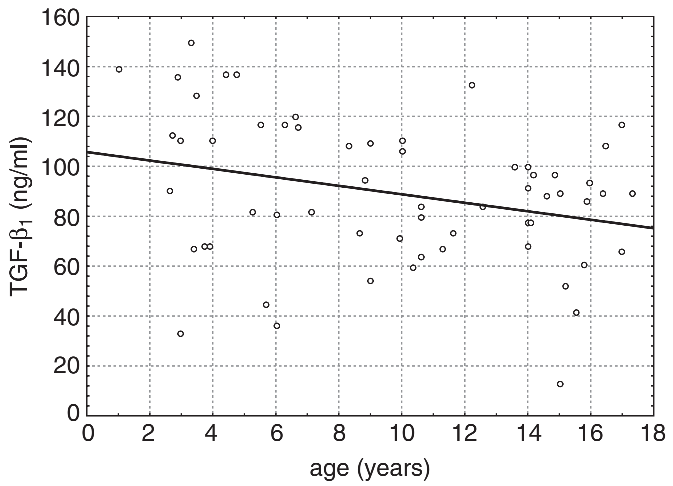

Fig. 1. Serum TGF-β_1 concentration in relation to age of patients

Statistically significant negative correlation between serum TGF-β_1 level and patients' age was observed (r = - 0.24, p = 0.04) (fig. 1.). There was no statistically significant correlation between serum TNF-α level and patients' age.

There were no statistically significant differences in the mean serum TGF-β_1 and TNF-α levels between children with unilateral vesicoureteral reflux (TGF-β_1 – 94.1 ng/dl, TNF-α – 42.6 pg/ml) and children with bilateral vesicoureteral reflux (TGF-β_1 – 89.2 ng/ml, TNF-α – 57.5 pg/ml). There were also no statistically significant differences in the mean serum TGF-β_1 and TNF-α levels between children with vesicoureteral reflux of high grade (TGF-β_1 – 90.9 ng/ml, TNF-α – 52.3 pg/ml) and children with vesicoureteral reflux of low grade (TGF-β_1 – 92.8 ng/ml, TNF-α – 43.4 pg/ml).

There were no statistically significant differences in the mean serum TGF-β_1 and TNF-α levels between children with arterial hypertension (TGF-β_1 – 90.9 ng/ml, TNF-α – 41.4 pg/ml) and the remaining patients (TGF-β_1 – 89.2 ng/ml, TNF-α – 48.65 pg/ml).

In children with proteinuria the mean serum TGF-β_1 level (73.4 ng/ml) was significantly lower than in the remaining patients (90.5 ng/ml, p = 0.03). There was no statistically significant difference in the mean serum TNF-α levels between children with proteinuria and the remaining patients (41.8 pg/ml and 48.7 pg/ml, respectively, p>0.05).

DISCUSSION

Renal scarring being secondary to urinary system infections in children with vesicoureteral reflux or urinary obstruction is still an important clinical problem, because it is common cause of end-stage renal disease [10].

In the recent years, the role of immune mechanisms in the pathogenesis of renal scarring has been the subject of numerous studies. TGF-β is thought to be a crucial factor in the pathogenesis of renal scarring. *Khalil* et al. [11] demonstrated an increase in the expression of TGF-β m RNA in kidneys of mice in the course of infection with *Escherichia coli*. *Malik* et al. [12] revealed that *Escherichia coli* stimulates renal tubular cells to TGF-β_1 production. *Sermetis* et al. [13] shown an increase in the expression of TGF-β mRNA in renal interstitial tissue in patients with ureteropelvic junction obstruction [13]. The importance of TGF-β in progression of renal scarring was confirmed in the studies by *Zhou* et al. [14] and *Fukasawa* et al. [15]. The authors demonstrated an improvement in renal function and inhibition of renal interstitial fibrosis in rats with chronic nephropathies treated with inhibitors of TGF-β expression. Circulating serum TGF-β may also contribute to the development and progression of renal scarring. Experimental studies by *Sanderson* et al. [6] in transgenic mice revealed that an increased serum TGF-β level due to its increased hepatic production resulted in renal extracellular matrix accumulation, interstitial fibrosis, and mesangial cells proliferation.

Some investigators demonstrated profibrogenic activity of TGF-β_1 in the course of glomerulopathies. *Goumenos* et al. [16] shown that in the course of the different types of glomerulopathies, urinary TGF-β_1 excretion is increased [16]. *Murakami* et al. [17] demonstrated that in patients with glomerulopathies, urinary TGF-β_1 excretion is positively correlated with intensity of renal interstitial fibrosis [17]. An increase in serum and urinary TGF-β_1 levels in patients with glomerulopaties and proteinuria was also observed in the study by *Pstrusińska* et al. [18]. In our studies, serum TGF-β_1 level in children with renal scarring and proteinuria was lower as compared to the remaining patients. This might be a result of increased influx of serum TGF-β_1 into renal parenchyma.

The results of the studies concerning serum and urinary TGF-β_1 levels in patients with urinary system infection or renal scarring are sparse and conflicting. Solari et al. [8] demonstrated that there was no significant difference in serum TGF-β_1 level between patients with reflux nephropathy and controls. The authors also revealed that polymorphism of the TGF-β_1 gene is of great importance in the development of renal scarring. Similar observations were made by other authors [19]. *Junkier* et al. [20] determined serum TGF-β_1 level in patients with different renal diseases including acute pyelonephritis, end-stage renal disease and renal carcinoma. Significantly higher serum TGF-β_1 level was observed only in patients with renal carcinoma.

In our studies, serum TGF-β_1 level was significantly lower in children with reflux nephropathy than in controls. This might be a result of increased urinary TGF-β_1 excretion due to tubular damage associated with nephropathy. This hypothesis is in agreement with the results of other studies. *Murakami* et al. [17] and *Pstrusińska* et al. [18] demonstrated positive correlation between urinary TGF-β_1 excretion and intensity of renal scarring. Different results were obtained by *Farmami* et al. [21]. The authors assessed urinary TGF-β_1 excretion in relation to the results of renal scintigraphy ^{99m} Tc DMSA in children with urinary system infection. The authors found that in patients with normal renal scintigraphy ^{99m} Tc DMSA, urinary TGF-β_1 excretion was significantly increased.

Some authors suggest that there is a positive correlation between urinary TGF-β_1 excretion and the grade of vesicoureteral reflux. *Smółko* et al. [22] demonstrated that urinary TGF-β_1 excretion is higher in children with vesicoureteral reflux of high grade than in those with vesicoureteral reflux of low grade. In our studies, in children with reflux nephropathy, there was no correlation between the grade of vesicoureteral reflux and serum TGF-β_1 level.

In our studies, serum TGF-β_1 level in patients with obstructive nephropathy was significantly lower than in controls. In the majority of studies concerning the role TGF-β_1 in the pathogenesis of renal scarring in patients with urinary obstruction only urinary TGF-β_1 level was assessed. In children with ureteral obstruction, an increased urinary TGF-β_1 excretion was observed [23]. This phenomenon might be a cause of a decreased serum TGF-β_1 level observed in our studies. In our series, there was no statistically significant differences in serum TGF-β_1 level between children with obstructive nephropathy and those with reflux nephropathy. This might result from participation of TGF-β_1 in the pathogenesis of both types of nephropathies.

In summary, the low serum TGF-β_1 level in children with renal scarring seems to be a result of increased influx of serum TGF-β_1 into renal parenchyma and/or increased urinary TGF-β_1 excretion due to tubular damage associated with nephropathy.

There is a large body of evidence for the concept that TNF-α is also one of the most important factors in the pathogenesis of renal scarring. It was documented that TNF-α stimulates synthesis of other cytokines including IL-1β and TGF-β. These cytokines participate in cascade of reactions leading to renal extracellular matrix accumulation and interstitial fibrosis [24]. In the majority of studies, the expression of TNF-α is assessed in kidneys because it is thought that only locally produced TNF-β is important in the pathogenesis of renal scarring. *Wuthrich* et al. [25] demonstrated an increase in the expression of TNF-α mRNA in tubular cells in mice after injection of bacterial lipopolysaccharide. *Kaneto* et al. [26] shown an increase in the expression of TNF-α in kidneys of mice with ureteral obstruction. This was due to an increased production of TNF-α by renal tubular cells. TNF-α induces renal tubular cells apoptosis and necrosis leading to their permanent damage [27, 28]. Experimental studies in TNF-α receptor gene knock-out mice with ureteropelvic junction obstruction revealed a decrease in the synthesis of collagen IV [29]. In experimental studies by *Roberts* et al. [30], an increased serum TNF-α level was demonstrated in the course of acute pyelonephritis.

Little is known about serum and urinary TNF-α levels in patients with urinary system infection. *Ko* et al. [31] demonstrated increased urinary TNF-α level in the course of urinary system infection. It was also demonstrated that in patients with acute pyelonephritis serum and urinary soluble TNF-α receptors level is increased [32], but correlation between serum/urinary soluble TNF-α receptors levels and the presence of renal scarring was not observed [33].

CONCLUSIONS

In our studies, there was no statistically significant difference in serum TNF-α levels between patients with renal scarring and controls. This might be a result of its local production in renal parenchyma. There were also no statistically significant differences in serum TNF-α levels between children with unilateral and bilateral vesicoureteral reflux and between children with vesicoureteral reflux of high and low grade. Further investigations are required to elucidate the role of TNF-α in the development and progression of renal scarring.

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