Urinary procollagen III aminoterminal propeptide/creatinine (PIIINP/Cr) ratio and transforming growth factor beta/creatinine (TGF- β /Cr) ratio in children with idiopathic nephrotic syndrome on corticosteroid and cyclosporine A treatment

N-końcowy propeptyd prokolagenu typu III/kreatynina (PIIINP/Cr) oraz transformujący czynnik wzrostu beta/kreatynina (TGF- β /Cr) w moczu u dzieci z idiopatycznym zespołem nerczycowym leczonych kortykosteroidami oraz cyklosporyną A

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Abstract

Idiopathic nephrotic syndrome is a common nephropathy in children basically treated with corticosteroids and, in some cases, with cyclosporin A. There are concerns that proteinuria itself and cyclosporin A could cause renal fibrosis via activation of transforming growth factor beta (TGF- β), which may stimulate deposition of collagen type III, synthesised as procollagen III amino terminal propeptide (PIIINP). **The aim of this study** was to assess urinary TGF- β and PIIINP to creatinine (Cr) ratio as markers of renal fibrosis in children with idiopathic nephrotic syndrome put on corticosteroid and cyclosporin A treatment. **Materials and methods:** The study comprised 20 children with idiopathic nephrotic syndrome in remission on prednisone, aged 6.87 years [interquartile range (IQR): 6.37]; 21 children with idiopathic nephrotic syndrome in remission on cyclosporin A, aged 9.66 years (IQR: 5.0); and 21 healthy subjects, aged 7.16 years (IQR: 9.0). First morning urinary PIIINP/Cr and TGF- β /Cr were obtained and compared with Mann–Whitney *U* test, with the level of significance at $p \leq 0.05$. **Results:** PIIINP/Cr ratio was significantly elevated in the cyclosporin A group [3.44 (2.5) vs. 1.12 (0.96) $\mu\text{g}/\text{mmol}$], $p = 0.000$, as well as in the prednisone group when compared to controls [1.33 (1.58) vs. 1.12 (0.96) $\mu\text{g}/\text{mmol}$], $p = 0.042$. The TGF- β /Cr ratio was significantly higher only in the cyclosporin A group when compared to controls [4.03 (3.25) vs. 2.33 (1.62) $\mu\text{g}/\text{mmol}$], $p = 0.004$. **Conclusions:** Increased urinary PIIINP/Cr ratio in children with idiopathic nephrotic syndrome either on steroid or on cyclosporin A therapy may suggest that renal fibrosis is a process generally involved in idiopathic nephrotic syndrome. Elevated urinary TGF- β /Cr and PIIINP/Cr ratios in patients with idiopathic nephrotic syndrome on cyclosporin A may reflect nephrotoxicity of this drug.

Keywords: idiopathic nephrotic syndrome, interstitial fibrosis, cyclosporine A, transforming growth factor beta, procollagen type III aminoterminal propeptide

Streszczenie

Idiopatyczny zespół nerczycowy jest najczęstszą nefropatią wieku rozwojowego, standardowo leczoną glikokortykosteroidami, a w określonych sytuacjach – cyklosporyną A. Istnieją obawy, że zarówno białkomocz towarzyszący chorobie, jak i terapia cyklosporyną A, przy współdziale transformującego czynnika wzrostu beta (TGF- β), mogą prowadzić do depozycji kolagenu typu III, syntetyzowanego jako prokolagen zawierający N-końcowy propeptyd (PIIINP), w obrębie śródmiąższu nerek. **Celem pracy** była ocena stężeń TGF- β oraz PIIINP indeksowanych do stężenia kreatyniny (Cr) w moczu dzieci z idiopatycznym zespołem nerczycowym w okresie remisji, leczonych kortykosteroidami oraz cyklosporyną A. **Materiał i metoda:** W badaniu uczestniczyło 20 dzieci z idiopatycznym zespołem nerczycowym w fazie remisji leczonych prednizonem w wieku 6,87 [rozstęp międzykwartyłowy (IQR): 6,37] roku, 21 dzieci z idiopatycznym zespołem nerczycowym w fazie remisji leczonych cyklosporyną A w wieku 9,66 (IQR: 5,0) roku oraz 21 zdrowych dzieci z grupy kontrolnej w wieku 7,16 (IQR: 9,0) roku. W pierwszej porannej porcji moczu oznaczano wskaźniki: TGF- β /Cr, PIIINP/Cr, uzyskane wyniki porównywano za pomocą testu *U* Manna–Whitneya przy poziomie istotności $p \leq 0,05$. **Wyniki:** Wskaźnik PIIINP/Cr był istotnie wyższy u dzieci leczonych cyklosporyną A w porównaniu z grupą kontrolną [3,44 (2,5) vs 1,12 (0,96) $\mu\text{g}/\text{mmol}$], $p = 0,000$, jak również u dzieci leczonych prednizonem w odniesieniu do grupy kontrolnej [1,33 (1,58) vs 1,12 (0,96) $\mu\text{g}/\text{mmol}$], $p = 0,042$. Wskaźnik TGF- β /Cr był istotnie wyższy jedynie u dzieci leczonych cyklosporyną A w porównaniu z grupą kontrolną [4,03 (3,25) vs 2,33 (1,62) $\mu\text{g}/\text{mmol}$], $p = 0,004$. **Wnioski:** Podwyższona wartość wskaźnika PIIINP/Cr w moczu

dzieci leczonych zarówno kortykosteroidami, jak i cyklosporyną A może wskazywać na obecność włóknienia w idiopatycznym zespole nerczycowym. Podwyższone wskaźniki TGF-β/Cr i PIIINP/Cr w moczu u dzieci z idiopatycznym zespołem nerczycowym leczonych cyklosporyną A mogą być odzwierciedleniem nefrotoksyczności leku.

Słowa kluczowe: idiopatyczny zespół nerczycowy (IZN), włóknienie śródmiąższowe, cyklosporyna A (CsA), transformujący czynnik wzrostu beta (TGF-β), N-końcowy propeptyd prokolagenu typu III (PIIINP)

INTRODUCTION

Nephrotic syndrome (NS) is a clinical condition characterised by proteinuria, hypoalbuminaemia, hyperlipidaemia and oedema^(1,2). Idiopathic nephrotic syndrome (INS) is considered to be the most frequent cause of NS in children^(1,2). The International Study of Kidney Disease in Children (ISKDC), in the late 1970s, reported a study on kidney biopsies in children with NS, proving that most patients presented with minimal change nephrotic syndrome (MCNS)^(3,4). The absence of distinctive glomerular abnormalities on light microscopy accompanied by diffuse foot processes effacement on electron microscopy points to structural modifications in glomerular filtration barrier as an explanation for the mechanisms of proteinuria in INS⁽⁵⁾. According to the hypothesis of Shalhoub (1974), INS may be mediated by circulating permeability factors promoting molecular and functional changes in podocytes⁽⁶⁾. However, many factors, some of T-lymphocyte origin, have been postulated to play a role in INS, and defining a single putative factor remains elusive^(7,8). But the theory of possibly functional glomerular filtration membrane rearrangements in INS has a strong impact on treatment strategies as well as on INS final outcome. The therapeutic approach to INS has been established^(9,10). It is based on corticosteroids as a first-line treatment^(9,10). ISKDC showed steroid sensitivity in 93–98% of patients with MCNS⁽³⁾. Childhood INS is mostly characterised by periods of remission and relapses^(1–4). In frequent relapsers and/or steroid-dependent children, cyclosporin A (CsA) is recommended as an alternative treatment^(9,10). Therapy regimens used in INS show probably both: immunosuppressive potential diminishing the activity of permeability factors, as well as local antiproteinuric potential resulting in filtration membrane stabilisation⁽¹¹⁾. Due to its more functional rather than morphological pathogenesis and periods of remission free from proteinuria, childhood INS is generally considered as a benign entity with good prognosis^(11,12). However, there might be concerns that recurrent phases of proteinuria during relapses as well as CsA treatment itself could possibly cause irreversible changes in renal tissue in patients with INS. There is literature evidence that the passage of excess plasma proteins through the glomerular filtration barrier can lead to podocyte damage and, finally, glomerulosclerosis, as

well as proximal tubular cell overload, interstitial inflammation and fibrosis⁽¹³⁾. CsA is known for its nephrotoxicity resulting from both: vasoconstriction of the afferent glomerular arterioles, which leads to hemodynamic changes and renin–angiotensin system (RAS) activation, and from a direct toxic effect on tubular epithelial cells causing tubular atrophy and interstitial fibrosis^(14–16). The most accurate diagnostic tool to assess fibrosis is kidney biopsy. Nevertheless, the study focused on identifying biomarkers of renal fibrosis to allow for a non-invasive assessment are worth mentioning. The usefulness of measuring urinary transforming growth factor beta (TGF-β) has been postulated^(17–20). TGF-β shows a strong ability to stimulate extracellular matrix formation and collagen deposition^(13,21). In the case of proteinuria, protein overload in proximal tubules may result in a release of TGF-β, while CsA treatment leads to up-regulation of TGF-β, which is stimulated directly by the drug itself and indirectly by RAS activation^(13,21). Renal interstitial fibrosis progresses due to the increased deposition of extracellular matrix including collagen type III being synthesised as a procollagen with aminoterminal propeptides (PIIINP)^(13,20,22). Studies showing that the measurement of urinary PIIINP can be used as a non-invasive marker of renal fibrosis have been published^(22–25).

The aim of our study was to assess urinary TGF-β and PIIINP to creatinine (Cr) ratio as markers of renal fibrosis in children with INS in remission on corticosteroid and CsA treatment compared to healthy peers.

MATERIALS AND METHODS

The study was conducted in the Division of Paediatric Nephrology, Department of Paediatrics, Faculty of Medical Sciences in Zabrze, Medical University of Silesia, Katowice, Poland, between July 2018 and June 2019. The study included children who underwent diagnosis and treatment of INS according to the recommendations of the Polish Society of Paediatric Nephrology⁽⁹⁾. Exclusion criteria were as follows: congenital NS, secondary NS, steroid-resistant INS, INS on treatment other than corticosteroids or CsA. The control group consisted of healthy children screened for non-specific complaints after exclusion of organic illnesses, with no history of chronic medication. Aiming to describe the course of INS in the study participants according to the recommendations of the Polish Society of Paediatric

Nephrology, the following definitions have been used: remission – 3 consecutive days of trace or absent proteinuria; steroid sensitivity – remission obtained within 6–8 weeks of corticosteroid therapy; steroid resistance – lack of remission after 6–8 weeks of corticosteroid therapy; relapse – 3 consecutive days with proteinuria; frequent relapses – 2 or more relapses within 6 months or 4 or more relapses within a year; steroid dependence – relapse on prednisone dose reduction or relapse within 2 weeks from prednisone cessation⁽⁹⁾.

The initial treatment of steroid-sensitive patients with INS enrolled in the study was based on prednisone at a dose of 60 mg/m²/day for a period of 4–6 weeks followed by prednisone at 40 mg/m²/48 h for 4–6 weeks, with subsequent reduction of prednisone dose until 6 months of illness. Every relapse was treated with prednisone at a dose of 60 mg/m²/day until remission was reached, followed by prednisone at 40 mg/m²/48 h for 4 weeks, with subsequent reduction of prednisone dose within 4 weeks. Frequent relapsers and steroid-dependent patients were offered CsA treatment at a dose of 4–6 mg/kg/day under plasma drug concentration monitoring. Relapses in children on CsA were treated with prednisone according to relapse treatment regimen⁽⁹⁾.

The study included 20 children with INS on prednisone, aged 6.87 years [interquartile range (IQR): 6.37] and 21 children with INS with a history of prednisone treatment switched to CsA due to frequent relapses or steroid dependence, aged 9.66 years (IQR: 5.0). The control group consisted of 21 healthy subjects aged 7.16 years (IQR: 9.0). All participants had a negative medical history for any acute infectious disease or major trauma; moreover, participants with INS were in remission for 2 weeks prior to enrolment. The study protocol was approved by the Bioethical Committee of Medical University of Silesia, Katowice, Poland, and all the participants' parents as well as participants aged 16 years and older provided informed consent. Relevant clinical and biological data were collected for all subjects. Routine screening measurements of serum levels of Cr, uric acid, sodium, potassium, and magnesium were obtained. The estimated glomerular filtration rate (eGFR) was calculated according to Schwartz formula⁽²⁶⁾. Urine samples (first urine in the morning) were collected and stored at –70°C until analysis.

Urine levels of PIIINP were measured using ELISA kit (Cloud-Clone Corp., Houston, TX, USA) with an inter-assay variation below 12% and intra-assay variation below 10%, and with the sensitivity of 0.129 ng/mL. Urine levels of TGF-β were measured using ELISA kit (650.010.096, Diaclone SAS, France) with an inter-assay variation below 4.9% and intra-assay variation below 3.2%, and with the detection limit of 8.6 pg/mL. Urine Cr levels were measured using the modified kinetic Jaffe method, and urinary PIIINP and TGF-β levels were normalised for urinary Cr level (PIIINP/Cr, TGF-β/Cr).

Statistical analysis

Statistical analysis were performed with STATISTICA (version 12, StatSoft, Inc.). Distributions of quantitative variables were checked with the Shapiro–Wilk test. As not all data appeared to be normally distributed, median and IQR were used as distribution indicators for all parameters. A comparison between the group with INS on steroids, the group with INS on CsA and the control group was performed. Comparison of normally distributed parameters was performed with the two-tailed Student *t*-test. Data not-normally distributed were compared with the Mann–Whitney *U* test. The level of significance was set at $p \leq 0.05$.

RESULTS

Baseline and laboratory characteristics of study samples are summarised in Tab. 1. Study groups did not differ significantly in terms of age at the time of sampling. None of the study groups showed signs of glomerular filtration impairment. Moreover, eGFR was significantly higher in the steroid group [134 (IQR: 21) mL/min/1.73 m²] when compared with CsA group [115 (IQR: 35) mL/min/1.73 m²] and the control group [111 (IQR: 34) mL/min/1.73 m²], $p = 0.044$ and $p = 0.007$, respectively. There was a significant increase in serum uric acid levels in the CsA group [345.0 (IQR: 103.0) μmol/L] in comparison with the steroid group [255.0 (IQR: 86.5) μmol/L] and the control group [291.0 (IQR: 73.0) μmol/L], $p = 0.001$ and $p = 0.024$, respectively. There were significantly lower serum magnesium levels in the CsA group [0.77 (IQR: 0.06) mmol/L] when compared

Parameter	Control			INS on steroids			INS on CsA			<i>p</i>		
	<i>n</i>	Median	IQR	<i>n</i>	Median	IQR	<i>n</i>	Median	IQR	Control vs. steroids	Control vs. CsA	Steroids vs. CsA
Age [years]	21	7.16	9.0	20	6.87	6.37	21	9.66	5.0	0.389	0.473	0.072
GFR [mL/min/1.73 m ²]	21	111.0	34.0	20	134.0	21.0	21	115.0	35.0	0.007	0.606	0.044
Uric acid [μmol/L]	21	291.0	73.0	20	255.0	86.5	21	345.0	103.0	0.240	0.024	0.001
Na [mmol/L]	21	140.0	2.0	20	140.0	2.5	21	140.0	3.0	0.968	0.145	0.208
K [mmol/L]	21	4.38	0.26	20	4.38	0.34	21	4.63	0.38	0.602	0.151	0.109
Mg [mmol/L]	21	0.82	0.05	20	0.83	0.11	21	0.77	0.06	0.927	0.001	0.006

44 Tab. 1. Baseline and laboratory characteristics of study samples

Parameter	INS on steroids			INS on CsA			<i>p</i>
	<i>n</i>	Median	IQR	<i>n</i>	Median	IQR	Steroids vs. CsA
Age of INS onset [years]	20	3.37	1.66	21	3.58	1.50	0.593
Duration of INS [years]	20	2.12	3.37	21	5.50	5.58	0.005
Number of relapses	20	4.0	5.0	21	8.0	4.0	0.000

Tab. 2. Baseline characteristics of INS clinical course in study samples

Ratios	Control			INS on steroids			INS on CsA			<i>p</i>		
	<i>n</i>	Median	IQR	<i>n</i>	Median	IQR	<i>n</i>	Median	IQR	Control vs. steroids	Control vs. CsA	Steroids vs. CsA
TGF/Cr [μg/mmol]	21	2.33	1.62	20	2.69	3.39	21	4.03	3.25	0.134	0.004	0.434
PIIINP/Cr [μg/mmol]	21	1.12	0.96	20	1.33	1.58	21	3.44	2.50	0.042	0.000	0.028

Tab. 3. Urinary TGF-β/Cr, PIIINP/Cr ratios in study samples

with the steroid group [0.83 (IQR: 0.11) mmol/L] and controls [0.82 (IQR: 0.05) mmol/L], with $p = 0.006$ and $p = 0.001$, respectively. There was no significant difference in the age at INS onset between the steroid group [3.37 (IQR: 1.66) years] and the CsA group [3.58 (IQR: 1.5) years], but the duration of INS was significantly longer in the CsA group [5.5 (IQR: 5.58) years] when compared with the steroid group [2.12 (IQR: 3.37) years], $p = 0.005$. Also, the number of relapses in the CsA group [8.0 (IQR: 4.0)] was higher than in the steroid group [4.0 (IQR: 5.0)], $p = 0.000$ (Tab. 2). Tab. 3 shows urinary PIIINP and TGF-β to Cr ratio (PIIINP/Cr, TGF-β/Cr) in study groups. The PIIINP/Cr ratio was significantly elevated in the CsA group [3.44 (IQR: 2.5) vs. 1.12 (IQR: 0.96) μg/mmol], $p = 0.000$, and the prednisone group when compared to controls [1.33 (IQR: 1.58) vs. 1.12 (IQR: 0.96) μg/mmol], $p = 0.042$. Moreover, the PIIINP/Cr ratio in the CsA group was significantly higher than in the steroid group [3.44 (IQR: 2.5) vs. 1.33 (IQR: 1.58) μg/mmol], $p = 0.028$. The TGF-β/Cr ratio was significantly higher only in the CsA group when compared to controls [4.03 (IQR: 3.25) vs. 2.33 (IQR: 1.62) μg/mmol], $p = 0.004$.

DISCUSSION

This was a pilot study in a group of children with steroid-sensitive INS, assessing the utility of urinary screening for PIIINP and/or TGF-β, molecules tending to become biomarkers of renal fibrosis. Our study, which was based on a single urine sampling in the phase of remission to exclude the transient impact of gross proteinuria on the results, pointed to a significantly higher urinary PIIINP/Cr ratio in patients with INS treated either with steroids or with CsA when compared to healthy peers. The TGF-β/Cr ratio was significantly higher only in children on CsA compared to healthy controls. Our findings may be intriguing bearing in mind that INS is widely regarded as a benign clinical condition characterised by periods of relapses, but also periods of remissions free from proteinuria, which should prevent from chronic inflammation and tubulointerstitial injury^(12,13).

The potential utility of urinary PIIINP/Cr ratio as a biomarker of renal fibrosis has been described^(22,23,25). El Ghoul et al. demonstrated in their research a high correlation between urinary PIIINP/Cr and the extent of interstitial fibrosis in kidney biopsies. Teppo et al. showed a correlation between urinary PIIINP/Cr and interstitial fibrosis in transplanted kidneys in adults^(22,23,25). In our study, elevated urinary PIIINP/Cr ratios were found in both patients on steroid treatment with significantly shorter duration of INS and a lower number of relapses and patients treated with CsA with a higher number of relapses and significantly longer duration of INS, which suggests that renal fibrosis is associated with childhood INS regardless of the treatment used. However, significantly higher PIIINP/Cr ratio in CsA patients when compared with patients on steroids indicates the impact of either disease duration and the number of relapses or CsA itself on the intensity of fibrosis. The process of renal fibrosis is postulated to be linked with TGF-β activation⁽¹³⁾. TGF-β, a product of macrophages or proximal tubular cells exposed to proteinuria, stimulates collagen synthesis and tubulointerstitial fibrosis^(13,20). According to Susianti et al., urine TGF-β positively correlated with fibrosis on biopsy and was even able to diagnose fibrosis itself, while Honkanen et al. proved that urine TGF-β positively correlated with fibrosis confirmed in future biopsies^(17,18). In their meta-analysis, Mansour et al. showed that TGF-β is one of promising biomarkers for the detection of renal fibrosis and prediction of renal outcome⁽²⁵⁾. Moreover, Teppo et al. showed a correlation between urinary PIIINP/Cr and urinary TGF-β/Cr⁽²³⁾. In our steroid group, elevation of the urinary PIIINP/Cr ratio was not accompanied by a significant increase in urinary TGF-β/Cr index. However, significantly higher urinary PIIINP/Cr and urinary TGF-β/Cr ratios in children on CsA therapy may indicate the presence of renal fibrosis enhanced by CsA nephrotoxicity. CsA nephrotoxicity has been a well known phenomenon attributed to haemodynamic changes resulting from vasoconstriction of the afferent glomerular arterioles and to tubular dysfunction^(14–16). Vasoconstriction results in the

reduction of GFR and activation of renin-angiotensin system, while tubular dysfunction is characterised by decreased magnesium reabsorption and decreased uric acid excretion^(14,15). Patients on CsA treatment in our study presented with significantly higher plasma uric acid levels and lower plasma magnesium levels compared to children on corticosteroid treatment and healthy controls. Chronic CsA nephrotoxicity is defined by the presence of interstitial fibrosis and tubular atrophy^(14,16). CsA nephrotoxicity development is thought to be partially influenced by vasoconstriction, consequences of hypoxia/ischaemia and RAS activation with its potential to induce fibrosis, but also up-regulation of TGF- β , stimulating the production of extracellular matrix^(14,15,21). Significantly higher TGF- β /Cr ratio in the group on CsA therapy in our study may point to the crucial link between TGF- β and the drug.

CONCLUSIONS

Increased urinary PIIINP/Cr index in children with INS on steroid or CsA therapy may suggest that renal fibrosis is a process generally involved in INS. Elevated urinary TGF- β /Cr and PIIINP/Cr ratios in patients with INS on CsA treatment may reflect CsA nephrotoxicity. Nevertheless, establishing the role of urinary biomarkers of kidney fibrosis in childhood INS needs further investigations.

Conflict of interest

The author does not report any financial or personal affiliations to persons or organisations that could adversely affect the content of or claim to have rights to this publication.

Funding/Support and role of the sponsor

This work was supported by grant: SUM, KNW-1-065/N/8/K.

Acknowledgements

We thank Prof. Elżbieta Świętochowska for helping in laboratory assessment.

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