

Changes in the Dickkopf-1 and tartrate-resistant acid phosphatase 5b serum levels in preschool children with nephrotic syndrome

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Abstract. The aim of the present study was to investigate the changes in the serum Dickkopf-1 (DKK-1) and tartrate-resistant acid phosphatase 5b (TRACP-5b) levels in preschoolers with nephrotic syndrome (NS). A total of 50 preschoolers (3-5 years old) with NS and 20 healthy preschoolers (control group) were enrolled in the prospective single-center study. The patients with NS received glucocorticoid treatment and the control group received no treatment. The levels of serum calcium, phosphorus, TRACP-5b, DKK-1 and 25-hydroxyvitamin D3 were measured at baseline and at 3 and 6 months in all the subjects. The levels of DKK-1 and TRACP-5b were significantly higher in the NS group prior to treatment when compared to the control group ($P < 0.05$), but did not differ significantly between the two groups following treatment ($P > 0.05$). Therefore, DKK-1 and TRACP-5b can be used as biomarkers of bone formation and bone resorption, respectively, in the early evaluation of bone metabolism.

Introduction

Nephrotic syndrome (NS) is a common chronic glomerular disease in children (1). The typical clinical manifestations are significant proteinuria, edema, low concentrations of serum albumin and hypercholesterolemia. However, other symptoms can be present, including hypocalcemia, hyperparathyroidism, osteoporotic fractures and other metabolic abnormalities. Glucocorticoid is the preferred treatment for NS. However, the

long-term administration of large quantities of glucocorticoids may result in glucocorticoid-induced osteoporosis by inhibiting the formation of osteoblasts, stimulating bone resorption and contributing to a negative calcium balance (2-4).

In recent years, several studies have suggested that Dickkopf-1 (DKK-1) inhibits bone formation by its inhibition of the WNT signaling pathway (5). It is a key factor in maintaining the imbalance between bone resorption and replacement. DKK-1 inhibits WNT signaling in cells by binding to the DKK-1 receptors, thus preventing bone marrow stromal cells from differentiating into bone cells, whereas it also induces the differentiation and development of osteoclasts (6). DKK-1 expression has been used as a biomarker of bone metabolism disorders in adults (7). The role of DKK-1 in diseases of bone loss and the treatment of certain diseases by inhibiting DKK-1 have recently become topical (8). However, there has been limited research into secondary osteoporosis caused by kidney disease.

Tartrate-resistant acid phosphatase 5b (TRACP-5b) is an iron-containing glycoprotein (9). Osteoclasts predominantly secrete TRACP-5b into serum, implying that serum TRACP-5b has a unique specificity as a biomarker of osteoclast activity, and it is therefore a useful marker of bone resorption (10). Based on this, serum TRACP-5b is used clinically as a serum marker for the diagnosis of osteoporosis. However, there has been limited research into TRACP-5b levels in preschoolers with NS.

Therefore, 50 preschoolers with NS and 20 healthy preschooler children (control group) were the research subjects in the present study. The serum levels of DKK-1 and TRACP-5b were investigated prior to treatment and 3 and 6 months after treatment, and these proteins were identified as novel biomarkers of abnormal bone metabolism in NS patients.

Materials and methods

Patients and data collection. All the subjects provided written informed consent, and the study was approved by the Ethics Committee of The First Affiliated Hospital of Zhengzhou University (Zhengzhou, Henan, China). The study sample comprised a consecutive series of children who were admitted to the Department of Pediatrics, The

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Table I. General and biochemical characteristics of the study groups.

Variable	NS group	Control group	t/χ^2	P-value
Total, n	50	20		
Gender, n (male/female)	34/16	11/9	1.052	0.305
Age, years	3.19±0.98	3.02±0.78	0.825	0.414
Serum calcium, mmol/l	1.94±0.17	2.34±0.62	-8.454	0.000
Serum phosphorus, mmol/l	1.65±0.40	1.38±0.47	0.259	0.797
25-hydroxyvitamin D3, ng/ml	6.81±5.03	43.05±3.16	-27.743	0.000
Serum albumin, g/l	18.9±5.0	42.82±4.83	-15.929	0.000
Total urine protein, g/day	1.80±0.81			

Values are expressed as the mean ± standard deviation. NS, nephrotic syndrome.

Table II. Biochemical characteristics of the NS group after treatment for 3 months.

Variable	NS group	Control group	t	P-value
Serum calcium, mmol/l	2.38±0.17	2.34±0.62	-0.368	0.714
Serum phosphorus, mmol/l	1.67±0.19	1.38±0.47	0.573	0.569
25-hydroxyvitamin D3, ng/ml	24.86±3.05	43.05±3.16	-6.186	0.000
Serum albumin, g/l	42.8±5.74	42.82±4.83	1.258	0.214
Total urine protein, g/day	0.12±0.06			

Values are expressed as the mean ± standard deviation. NS, nephrotic syndrome.

First Affiliated Hospital of Zhengzhou University between January 2012 and December 2013. In total, 50 preschool (<5 years old) children (34 males, 16 females) who were diagnosed with primary NS and 20 healthy preschool children (control group) were enrolled. The patients were diagnosed with primary NS according to the criteria of the International Study of Kidney Disease in Children (11), and had no history of prior treatment with glucocorticoids or other immunosuppressive agents. The exclusion criteria of all subjects included the previous use of glucocorticoids or other immunosuppressive agents, hyperparathyroidism, chronic hepatitis, congenital bone disease, multiple myeloma, chronic diarrhea, bone metastasis of a malignant tumor, diabetes and thyroid disease.

Table III. Biochemical characteristics of the NS group after treatment for 6 months.

Variable	NS group	Control group	t	P-value
Serum calcium, mmol/l	2.30±0.17	2.34±0.62	-0.903	0.371
Serum phosphorus, mmol/l	1.60±0.18	1.38±0.47	-0.324	0.748
25-hydroxyvitamin D3, ng/ml	30.45±4.91	43.05±3.16	-7.047	0.000
Serum albumin, g/l	41.86±4.51	42.82±4.83	0.720	0.475
Total urine protein, g/day	0.10±0.04			

Values are expressed as the mean ± standard deviation. NS, nephrotic syndrome.

The control group received no drug treatment. All the NS patients received a sufficient amount of glucocorticoid to induce remission and consolidation. To induce remission, the patients were treated with 2 mg/kg/day of prednisone, to a maximum dose of 80 mg/day, in divided doses. The patients were administered prednisone every morning when their urine protein was negative and the treatment was continued for 6 weeks. The dose of prednisone was decreased by 1.5 mg/kg for the next day for 6 weeks and was subsequently reduced gradually, so that in general, the treatment was discontinued with 9-12 months. The children were followed-up for 6 months from the time of diagnosis.

The serum levels of calcium, phosphorus, TRACP-5b, DKK-1, 25-hydroxyvitamin D3 [25(OH)D3] and albumin were measured prior to treatment and at 3 and 6 months after treatment in the NS group, and were measured at enrolment in the control group. The gender and ages of all the patients were recorded.

Measurements. Venous blood (3 ml) was collected from the NS patients prior to treatment and at 3 and 6 months after treatment. Venous blood samples were also collected from the healthy controls in the same way. All samples were centrifuged at 1,000 rpm for 15 min to remove the cellular components, and the supernatants were frozen in aliquots at -80°C until analysis.

The serum levels of DKK-1 (Ameko Medical Industries, Shanghai, China) and TRACP-5b (Cusabio Biotech Co., Ltd., Wuhan, China) were measured with enzyme-linked immunosorbent assays in accordance with the manufacturer's protocol. The lowest limit of DKK-1 detected with this assay was 1.25 pg/ml. The intra-assay precision was <9% and the inter-assay precision was <15%. The lowest limit of TRACP-5b detected with this assay was 0.078 U/l. The intra-assay precision was <8% and the inter-assay precision was <10%.

Statistical analysis. SPSS 20.0 (IBM, Corp., Armonk, NY, USA) was used for all statistical analyses. Continuous data are expressed as the mean ± standard deviation or median

Table IV. Comparison of the DKK-1 and TRACP-5b levels among the NS and control groups at different times.

Treatment	DKK-1, pg/ml		t	P-value	TRACP-5b, U/l		t	P-value
	NS group	Control group			NS group	Control group		
Prior to	21.7±9.39	15.43±2.36	2.918	0.005	5.56±2.99	2.01±0.34	5.220	0.000
After 3 months	18.4±8.34	15.43±2.36	1.577	0.121	2.35±1.47	2.01±0.34	0.932	0.356
After 6 months	15.1±5.25	15.43±2.36	-0.297	0.768	2.38±1.59	2.01±0.34	0.951	0.347

Values are expressed as the mean ± standard deviation. NS, nephrotic syndrome; DKK-1, Dickkopf-1; TRACP-5b, tartrate-resistant acid phosphatase 5b.

Table V. Pearson correlation analysis among the levels of serum DKK-1, TRACP-5b and other biochemical parameters in the nephrotic syndrome group prior to treatment.

Variable	DKK-1		TRACP-5b	
	r	P-value	r	P-value
Serum calcium, mmol/l	-0.100	0.955	0.418	0.019
Serum phosphorus, mmol/l	0.163	0.382	0.230	0.214
Serum albumin, g/l	-0.042	0.821	0.151	0.417
Total urine protein, g/day	0.451	0.011	0.850	0.000
25-hydroxyvitamin D3, ng/ml	0.177	0.342	0.050	0.790

DKK-1, Dickkopf-1; TRACP-5b, tartrate-resistant acid phosphatase 5b.

(interquartile range), as appropriate. The normally distributed continuous variables of the two groups were compared with Student's t-test. When the variables were not normally distributed, the Mann-Whitney and Kruskal-Wallis tests were used. Dichotomous data were assessed using χ^2 test. In all tests, $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Characteristics and biochemical parameters. The characteristics and biochemical parameters of the patients are listed in Table I. In total, 50 NS patients and 20 healthy children were enrolled. There were no significant differences in the age or gender distributions between the two groups ($P > 0.05$). The serum calcium, albumin and 25(OH)D3 levels were higher in the control group compared to the NS group prior to starting treatment ($P < 0.05$).

As shown in Tables II and III, there was no significant difference between the levels of serum calcium and phosphorus in the two groups after the NS group had been treated for 3 or 6 months ($P > 0.05$).

Table IV shows the levels of serum DKK-1 in the NS group prior to treatment and 3 and 6 months after treatment.

Prior to treatment, the levels of serum DKK-1 and TRAP-5b were higher in the NS group compared to the control group ($P < 0.05$), whereas after 3 and 6 months treatment, there was no significant difference between the two groups ($P > 0.05$).

Table V compares the correlation between the level of serum DKK-1, TRACP-5b and other biochemical parameters in the NS group prior to treatment. Pearson correlation analysis showed that the level of serum DKK-1 prior to treatment was positively correlated with 24-h urinary protein excretion ($r = 0.451$, $P = 0.011$ and $P < 0.05$). There were no correlations between the level of DKK-1 and calcium, phosphorus, serum albumin and 25(OH)D3 ($P > 0.05$). Pearson correlation analysis showed that the level of serum TRACP-5b prior to treatment was positively correlated with 24-h urinary protein excretion and serum calcium ($r = 0.850$, $P = 0.000$; $r = 0.418$, $P = 0.019$, respectively). There were no correlations between the level of TRACP-5b and phosphorus, serum albumin and 25(OH)D3 ($P > 0.05$).

Discussion

Several previous studies have demonstrated that children with primary NS are at risk of metabolic bone disease (12). Long-term treatment with large quantities of glucocorticoids may result in glucocorticoid-induced osteoporosis. Children are particularly susceptible to bone metabolic disorders, and even osteoporosis, during their growth stages (13). Several studies have reported the involvement of DKK-1 and TRACP-5b in primary and secondary osteoporosis (14,15). However, their levels have not been analyzed in children with NS. The gold standard for assessing osteoporosis is the measurement of bone mineral density (16), and although these measurements can be used to analyze the condition of the human bone mass, variations in bone morphology are not evident in the early stages of bone metabolic disorders. The measurement of bone density also involves certain exposure to radioactivity, and parents are reluctant to condone radioactive testing. Therefore, this technique is not used to assess early osteoporosis or in following up osteoporosis in children. Therefore, it is important to identify an effective biomarker of bone metabolism. A previous study has indicated that patients with higher DKK-1 values showed higher sublesional bone mineral density loss in spinal cord injury (17). Further research is required to determine whether DKK-1 and/or TRACP-5b have the utility as biomarkers of bone metabolism.

In the present study, the levels of DKK-1 and TRACP-5b were higher and 25(OH)D3 was lower in the NS patients

compared to the control group, suggesting that bone metabolic abnormalities are present in the early stage of NS. Under normal circumstances, the blood 25(OH)D3 exists in the human body by combination with a carrier protein. The level of 25(OH)D3 decreased as the urinary protein excretion increased in children with NS. The intestinal absorption of calcium decreased in NS patients resulting in hypocalcemia and hyperparathyroidism. These cause bone demineralization and osteoporosis. Therefore, DKK-1 and TRACP-5b were elevated and 25(OH)D3 was reduced early in NS patients. Furthermore, the long-term administration of glucocorticoids may increase these abnormalities as glucocorticoids can increase bone resorption by inhibiting factors that protect the bone (18,19), and they reduce the expression of a calcium ion transporter and calcium-binding protein, thus inhibiting the intestinal reabsorption of calcium (11,20). These factors can reduce bone mineral density. Additionally, a glucocorticoid receptor is expressed on the surfaces of osteoblasts, so long-term glucocorticoid therapy can promote osteoblast apoptosis and reduce the formation of new bone (21). In the present study, DKK-1 and TRACP-5b levels in the NS patients and the control group were not significantly different after the NS patients had been treated with glucocorticoid for 3 and 6 months, indicating that the levels of DKK-1 and TRACP-5b can recover to normal in the NS remission time. This may be due to the glucocorticoid reduction in the dosage and the total reduction of urine protein leakage.

The present study also identified that the level of serum DKK-1 prior to treatment was positively correlated with 24-h urinary protein excretion and the level of serum TRACP-5b prior to treatment was positively correlated with 24-h urinary protein excretion and serum calcium. This may also explain the phenomenon that the levels of DKK-1 and TRACP-5b were higher compared to the control group in the early stage of NS. However, there were no correlations between the level of DKK-1 and 25(OH)D3. There were also no correlations between the levels of TRACP-5b and 25(OH)D3. This may be associated with the small number of cases.

In conclusion, DKK-1 and TRACP-5b can be used as novel biomarkers of bone metabolism in children with NS. Bone metabolic abnormalities exist in patients in the early stage of NS, prior to initiating treatment with glucocorticoid. However, in the present study, only preschool children with NS in northern China were evaluated. Further studies of school-age children and adolescents are required. These findings should improve the clinical management of abnormal bone metabolism in children with NS.

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