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Original Article

Leptin and Soluble Leptin Receptor in Serum and Urine of Children with Primary Nephrotic Syndrome Before and After Steroid Therapy

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JI13STRACT

Background: Previous data revealed a urinary Ieptin loss in prepubertal and early pubertal children suffering from active nephrotic syndrome with proteinuria greater than 1 g/m ⁻.

Objective: We evaluated leptin and soluble leptin receptor (sOB-R) in serum and urine of children with primary nephrotic syndrome before and after one month steroid therapy.

Patients & Methods: This case control prospective study included 35 primary nephrotic syndrome children and 20 age and sex matched healthy children. Leptin and sOB-R levels in serum and urine were detected at beginning of the study and after one month corticosteroid **therapy.**

Results: In comparison to control group, there were significantly higher urinary leptin level & serum sOB-R level and significantly lower free leptin index in nephrotic syndrome children ($p < 0.01$), these diftërences attenuated and became non significant with remission of proteinuria ($p > 0.05$) and remained significant only in corticosteroid non responsive nephrotic children ($p < 0.05$). Urinary leptin level and serum sOB-R showed strong positive correlations with proteinuria ($p < 0.01$), whereas they showed strong negative correlations with serum albumin levels ($p < 0.01$). Urinary leptin level and serum sOB-R level did not show significant correlation with age or lipid profile component parameters ($p > 0.05$).

Conclusion: Serum soluble leptin receptor level was elevated and associated with the increase of leptin loss in urine in primary nephrotic syndrome. Both of them correlated positively with proteinuria and decrease with clinical and laboratory remission of the disease.

INTRODUCTION

Leptin, a **167-amino** acid polypeptide with a **molecular** size of 16 klla, is mainly synthesized in adipose tissuet [1.2)] Other tissues, such as the placenta[(')], the gastro-intestinal tract[t4-51] and neuronal tissuesc[6t] synthesize leptin. Ceptin plays an important role in the regulation of appetite and food intake in mice and humanst[5 7]. Mutations of the leptin gene or its receptor gene lead to obesity in mice and humanst[l] . In human blood, leptin is bound to a high affinity binding protein, which is the soluble leptin receptor (sOB-R), modulating the effects of its ligand[9"] . The sOB-R represents the major . leptin binding protein in the circulation. The presumed biologically active form of leptin is determined by the free leptin index (FL!), the ratio between serum leptin and serum sOB-R levelst91 H' Recent data provide further insight into multiple system effects of leptin, ts.human

congenital leptin deficiency is associated with multiple hormonal dcfects[12] Circulating leptin, which is partly cleared by the kidney, has been reported to increase in chronic renal failuret[13], but is not changed in the nephrotic syndrome (NS)t[14]. It is not known whether elevated leptin levels contribute to uremic anorexia, weight loss and changes in body composition[15].

In children, the most common cause of NS is idiopathic NS, also called nephrosis[16]. Previous data revealed urinary lëptin loss in prepubertal and early pubertal children suffering from active NS with proteinuria greater than 1 g/m[2][17].

AIM OF THE WORK

We tried to evaluate leptin and soluble leptin receptor in serum and urine of children with primary nephrotic syndrome before and after one month steroid therapy.

SUBJECTS AND MEiHHODS

Our study was performed during the period from August 2008 to November 2009 on 55 children in Nephrology Unit in Pediatric Department and Clinical Pathology Department, Zagazig University Hospital and Outpatient Clinics in the same hospital. Parental consent was taken to be eligible for enrollment into the study. The study protocol was approved by the Institutional Ethical Committee. Children under the study were classified into 2 groups:

Group {1} included 35 children presented with clinical and laboratory data supporting diagnosis of primary NS. NS was diagnosed as heavy proteinuria, (24 hr urine protein > 3.5 g/24 hr), hypoalburninemia (serum albumin less than 2.5% gm/dl), hyperlipidemia and edema.

Group {2} included 20 healthy age and sex matched children, without previous illness or previous medication.

We excluded cases of secondary NS, cases of previous corticosteroid therapy, congenital renal anomalies, hypertension, hematuria or renal dysfunction.

Patients were treated by standard oral steroids (prednisolone) for at least 4 weeks, 60 mg/m^2/d (2 mg/kg/d). If the patients achieved remission steroids were tapered and withdrawn over the next two months.

The following was **done for patients and control group:**

1-Full history taking and thorough clinical examinations were done with stress on general examinations (blood pressure, edema, body weight and eye examinations to monitor any complications), chest and heart examination (for pleural effusion and pericardial effusion) and abdominal examination (for ascites and renal mass).

2- Routine laboratory investigations including urinalysis, stool analysis, complete blood count, ESR and C-reactive protein.

3-Urine culture, 24 hour urinary protein execration, serum albumin, serum C3 level, lipid profile, renal function tests and abdominal ultrasonography.

4-Antinuclear antibodies, HBsAg, hepatitis-C antibodies and serum electrolytes (sodium and potassium) were performed for patients to exclude secondary NS and monitor any complications.

5-Estimation of leptin and sOB-R levels in serum and urine were performed initially for all children under the study and

repeated after one month of steroid therapy for NS cases. Free leptin index (FLI) was determined by the ratio between serum leptin and serum sOB-R levels [9-11].

6 - Creatinine analysis in urine was done and associated with estimation of leptin in urine.

Laboratory Measurements:

After an overnight fasting, blood samples were withdrawn for measurement of blood chemistry including total protein/ albumin, triglyceride (TG), cholesterol (Cho), high density and low density lipoprotein levels. 24-hour urine samples wore collected for detection of proteinuria, leptin and sOB-R levels in patients and control children.

Creatinine analysis in urine: Urinary creatinine concentrations were measured by the Jaffe reaction using the Creatinine Parameter Assay (R&D Systems, Minneapolis, MN).

Determination of plasma and urine leptin: After centrifugation of blood and urine samples, which were all taken between 10 am & 12 am, serum and urine samples were kept frozen at - 20°C and were analyzed when all specimens had been obtained.

Leptin levels in serum and urine were determined by quantitative sandwich enzym innufloassay (Ray Biotech., Inc., Minmapolis, MN, USA) according to the manufacture's instructions. Lower detection limit was 0.006 ng/m1. To account for the variable dilution of urine samples, urine leptin concentrations were normalized to urine creatinine (Cr).

Dcttrmination of the s0B-R level in serum and urine: sOB-R was measured by ELISA technique (R&D Systems, Minneapolis, MN) with a sensitivity of 0.06 ng/ml.

The free leptin index (FLI): ratio between leptin and sOB-R levels was calculated[9,11,18]

Statistical analysis:

The collected data were computed and statistically analyzed by using Chi-square test, Student's t-test and one-way ANOVA test. When ANOVA test was statistically significant, LSD test was done to detect site of significances in the three groups. Correlation between variables was performed. Data were expressed as mean + standard deviation (normally distributed data). $P < 0.05$ was considered significant. Computations were performed using statistical software package SPSS version 11 for windows (SPSS Inc., Chicago, Illinois, USA).

RESULTS

There were no significant differences in age, sex and body mass index (BMI) between NS cases and control group (Table 1). There were significant differences in serum albumin, lipid parameters and 24-hour proteinuria levels between NS cases and control group and that consistent with disease activity (Table 1).

Comparison between NS cases and control group showed that there were significantly higher urinary leptin level and serum sOB-R level in children presented with NS ($p < 0.01$) (Table **1).** On the other hand, there was no significant difference between NS cases and control group as regards serum leptin level and urinary sOB-R ($p > 0.05$) (Table **1).** The ratio between serum leptin levels and serum sOB-R (FLI)

was significantly lower in the NS group than in control group ($p < 0.01$) (Table 1).

Elevated urinary leptin and serum sOB-R levels decreased after one month steroid therapy in corticosteroid responsive NS cases, as there were no significant differences in comparison to healthy control group ($p > 0.05$) (Table 2). On the other hand, urinary leptin and serum sOB-R levels were significantly higher in corticosteroid non responsive NS cases than in corticosteroid responsive NS cases and control group ($p < 0.05$) (Table 2). Meanwhile, no significant changes in serum leptin level and urinary sOB-R were observed ($p > 0.05$) (Table 2).

Serum sOB-R showed significant positive correlation with urinary leptin level ($r = 0.593$, $p = 0.0001$) (Figure 1).

Urinary leptin level and serum sOB-R showed significant positive correlations with proteinuria ($p < 0.01$), whereas they showed significant negative correlation with serum albumin levels ($p < 0.01$) (Table 3).

Urinary leptin level and serum sOB-R level did not show significant correlation with age or lipid profile component parameters ($p > 0.05$) (Table 3).

Table 1: Clinical and laboratory data of patients and controls before treatment.

	Children with NS (n = 35)	Healthy controls (n = 20)	p value
Age (year)	6.78 ± 1.33	7.00 ± 1.25	> 0.05
Sex (male/female)	19/16	11/9	> 0.05
BMI (kg/m^2)	17.25 ± 2.1	16.95 ± 2.5	> 0.05
Albumin (gm/dl)	2.11 ± 0.48	4.68 ± 0.55	< 0.01
Triglyceride (mg/dl)	190.50 ± 75.44	86.47 ± 35.23	< 0.01
Cholesterol (mg/dl)	335.40 ± 75.45	140.44 ± 45.21	< 0.01
HDL-C (mg/dl)	52.14 ± 10.40	49.58 ± 17.50	> 0.05
LDL-C (mg/dl)	201.41 ± 31.24	79.25 ± 25.15	< 0.01
VLDL-C (mg/dl)	52.74 ± 12.45	18.23 ± 3.54	< 0.01
Proteinuria (g/24 hr)	8.11 ± 1.47	0.06 ± 0.02	< 0.01
Serum C_3 (mg/dl)	89.75 ± 18.75	90.84 ± 16.65	> 0.05
Serum leptin (ng/ml)	3.54 ± 0.65	3.60 ± 0.78	> 0.05
Urine leptin (ug/g creatinine)	2.68 ± 0.031	0.074 ± 0.02	< 0.01
Serum sOB-R (ng/ml)	70.2 ± 13.4	32.7 ± 5.6	< 0.01
Urine sOB-R (ng/ml)	4.0 ± 0.5	3.98 ± 0.4	> 0.05
Free leptin index	0.049 ± 0.003	0.11 ± 0.02	< 0.01

NS = nephrotic syndrome, $p < 0.01$ is highly significant, $p < 0.05$ is significant, $p > 0.05$ is not significant.

Table 2: Laboratory data in patients and controls after one month steroid therapy.

	Steroid responders (n = 28)	Steroid non responders (n = 7)	Healthy control patients (n = 20)	p value
Serum albumin (g/dl)	4.28 ± 0.48	2.15 ± 0.54 $^{p^*, p^{**}}$	4.68 ± 0.55	< 0.01
Triglyceride (mg/dl)	98.36 ± 25.69	200.60 ± 85.44 $^{p^*, p^{**}}$	86.47 ± 35.23	< 0.01
Cholesterol (mg/dl)	145.54 ± 41.25	337.70 ± 76.45 $^{p^*, p^{**}}$	140.44 ± 45.21	< 0.01
HDL-C (mg/dl)	48.45 ± 14.80	50.25 ± 13.25	49.58 ± 17.50	> 0.05
LDL-C (mg/dl)	80.56 ± 26.98	217.72 ± 42.57 $^{p^*, p^{**}}$	79.25 ± 25.15	< 0.01
VLDL-C (mg/dl)	19.45 ± 2.36	49.87 ± 11.25 $^{p^*, p^{**}}$	18.23 ± 3.54	< 0.01
Proteinuria (g/24 hr)	0.07 ± 00.03	7.87 ± 2.45 $^{p^*, p^{**}}$	0.06 ± 00.02	< 0.01
Serum leptin (ng/ml)	3.62 ± 0.79	3.56 ± 0.65	3.60 ± 0.78	> 0.05
Urine leptin (ug/g creatinine)	0.069 ± 0.03	2.61 ± 0.031 $^{p^*, p^{**}}$	0.074 ± 0.02	< 0.01
Serum sOB-R (ng/ml)	30.99 ± 6.8	69.1 ± 11.4 $^{p^*, p^{**}}$	32.7 ± 5.6	< 0.01
Urine sOB-R (ng/ml)	3.92 ± 0.6	3.93 ± 0.5	3.98 ± 0.4	> 0.05
Free leptin index	0.116 ± 0.01	0.0515 ± 0.004 $^{p^*, p^{**}}$	0.110 ± 0.02	< 0.01

$p < 0.01$ is highly significant, $p < 0.05$ is significant, $p > 0.05$ is not significant.

p: Comparison between the three groups by ANOVA test.

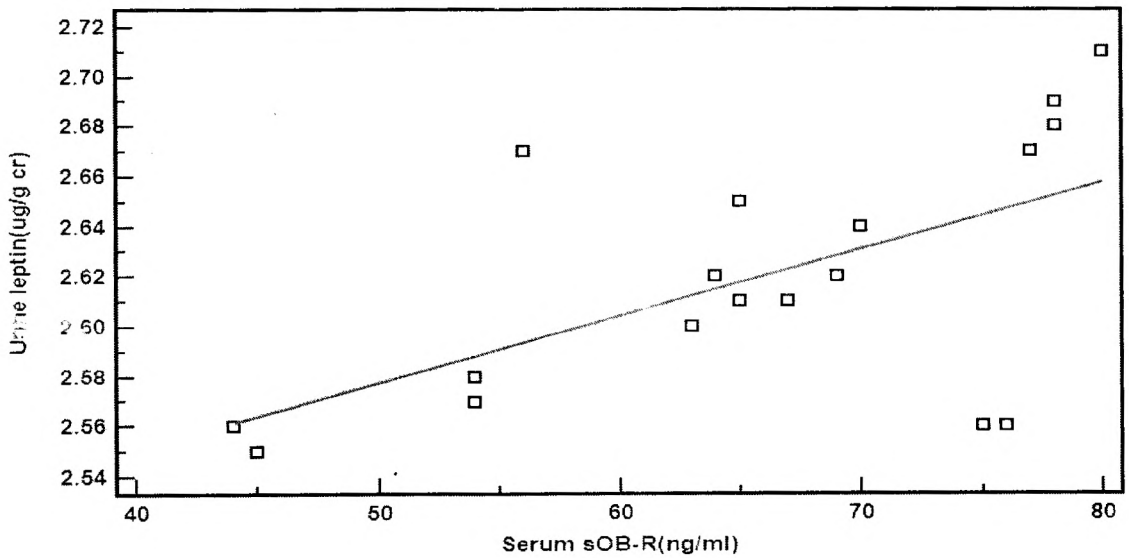
p*: Significant difference between steroid responsive & non responsive NS cases.

p**: Significant difference between healthy control and steroid non responsive NS cases.

Table 3: Urinary leptin and serum sOB-R correlations with clinico-laboratory parameters.

Variables	Urinary leptin (ug/g creatinine)		Serum sOB-R (ng/ml)	
	r	p	r	p
Age (year)	0.152	> 0.05	0.120	> 0.05
Body mass index (kg/m^2)	0.241	> 0.05	0.301	> 0.05
Serum albumin (g/dl)	- 0.798	**< 0.01**	- 0.788	**< 0.01**
Triglyceride (mg/dl)	0.197	> 0.05	0.218	> 0.05
Cholesterol (mg/dl)	0.148	> 0.05	0.167	> 0.05
HDL-C (mg/dl)	0.183	> 0.05	0.138	> 0.05
LDL-C (mg/dl)	0.215	> 0.05	0.181	> 0.05
VLDL-C (mg/dl)	0.287	> 0.05	0.215	> 0.05
Proteinuria (g/24 hr)	0.870	**< 0.01**	0.756	**< 0.01**

$p < 0.01$ is highly significant, $p < 0.05$ is significant, $p > 0.05$ is not significant.



(r = 0.593, p = 0.0001)

Fig. 1: Correlation between serum sOB-R and urinary leptin level.

DISCUSSION

Leptin is now implicated as a regulatory molecule in lipid metabolism. 1:-Iyperlipidemia is one of the most striking manifestations of NS. However, little is known about the alteration of leptin concentrations in NSt[19].

In our study, children with NS had significantly higher urinary leptin level and serum sOB-R level than healthy control group. Schroth et a1.[17] found that urinary leptin excretion in proteinuric patients was significantly higher than in nonproteinuric patients with and without NS and in healthy controls. Proteinuria may be associated with urinary leptin wasting in renal protein-losing diseases[17]. Schroth et a1.[201], in another study, detected that sOB-R level in serum of patients with NS was significantly higher during proteinuria (61.0 н 17.8 ng/ml) than those in remission or in controls (36.7 + 7.0 ng/ml, 36.6 H 12.0 ng/ml, respectively). The ratio between serum leptin levels and the sOB-R (free leptin index) was significantly lower in the proteinuric group (0.012 + 0.005 *vs.* 0.06 H 0.03 and 0.07 + 0.03 in remission and control group, respectively).

In our study, there were no significant differences between children with NS and control group as regards serum leptin level and urinary sOB-R level. Circulating leptin, which is partly cleared by the kidney, has been reported to increase in chronic renal failure[13], but is not changed in NS[14]. In previous studyl[171], despite an up to 100-fold increment in leptin excretion in proteinuria, serum leptin levels were similar in both proteinuric and nonproteinuric children. These findings suggest that the renal loss of leptin is counterregulated. Renal loss of leptin is compensated for by a substantial increase in leptin production. The mechanisms that cause the up-regulation of leptin synthesis remain unclear[21-25]. Elevated secretion of stored leptin, stimulated mRNA synthesis, or an increased fraction of protein-bound leptin appear possible[ʌьı]. To answer the question as to what degree of glomerular protein leakage allows for the loss of leptin, excretion of leptin was related to albumin and IgG in urine. Schroth et al.[ʌ17] data showed that there was no significant difference in urinary leptin excretion in proteinuric patients with an IgG/albumin ratio lower or higher than 1. Consequently, glomerular albumin loss was associated with the loss of leptin. According to these data, they proposed a glomerular loss of the 16 kDa peptide leptin in patients with proteinuria that is independent of the molecular size[17].

In our study, the ratio between serum leptin levels and the sOB-R (FLI) was significantly lower in the NS group than in control group ($p < 0.01$).

The counteracting pathway in case of leptin loss in parallel to severe proteinuria in NS is the up-regulation of its soluble binding protein in serum, which can keep total serum leptin levels constant[201]. Delayed clearance of leptin from circulation could be due to binding of leptin to its soluble receptor. Solub e receptor is up-regulated and an over expression of the s0B-R results in an increase of circulating leptin. Increase in serum sOB-R compensates the urinary loss of leptin from the circulating blood to keep serum leptin levels stable[261].

In our **study, urine leptin level and** scrum sOB-R level decreased significantly in corticosteroid responders who showed **non significant difference compared to the healthy controls. In contrary, corticosteroid non responders remained with significant high urine leptin level and serum sOB-R level. Other studies stated that urinary leptin loss disappeared after** remission of proteinuria in NS[17,20].

In our study, urinary leptin level and serum sOB-R level showed significant positive correlations with proteinuria, whereas they showed significant negative correlations with serum albumin levels. That coincides with Schroth et al[17] who found that **Urine leptin positively correlated with urine albumin concentration in NS** cases.

In our study, urinary **leptin level and serum sOB-R level did not show significant correlations with age or lipid parameters (p > 0.05).**

Ozata et al. [9] did not establish a relation between leptin and plasma triglycerides or other lipid parameters in NS subjects. Thus, they suggested that plasma leptin levels are normal in the NS and do not play a role in the dyslipidemia observed in this syndrome.

Leptin **promotes renal growth and fibrogenesis in vivo and in vitro [27]. Proliferation** of endothelial cells stimulated **by leptin may contribute to a progression of renal damage and, in consequence, may promote glomerulosclerosis. Leptin was shown to stimulate the expression of transforming growth factor [3l (TGF-f31), a major modulator of renal fibrosis, in vitro** and in vivo. This may activate the transcription of extracellular matrix proteins such as collagen type IV, which may eventually lead to damage of renal glomerular endothelial cells[27]. It is well known that renal injury leads to an increased generation of angiotensin II, which induces an up-regulation of the TOF-31 **gene. As a consequence,** tubular cells show hypertrophy which, together **with** increased synthesis of type IV collagen, leads ultimately to fibrogenesis and renal scarring[28]. Thus, grossly elevated glomerular and urinary leptin levels might accelerate the proliferation of endothelial and **possibly tubular cells in renal glomeruli, leading to an aggravation of** glomerulosclerosis and tubular damage. In consequence, **continuous leptin excretion might be an additional mechanism in the progression of renal failure(17).**

CONCLUSION

- **Serum soluble leptin receptor was elevated and associated with the increase of leptin loss in urine in primary nephrotic syndrome. Both of them correlated positively with proteinuria and decrease with clinical and laboratory remission of the disease.**
- **Further studies with long-term follow up of** children with steroid non responsive **NS are recommended to assess progression of renal failure in those with severe leptinuria. Furthermore, the effect of leptin on renal endothelial and tubular cell proliferation should be examined in vivo and in vitro.**

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