



A prospective study of serum concentrations of leptin, homocysteine and insulin resistance in children with steroid-sensitive nephrotic syndrome

Ipek Guney Varal¹ · Mahmut Civilibal² · Nilgun Selcuk Duru¹ · Murat Elevli¹

¹Department of Pediatrics, Haseki Teaching Hospital, University of Health Sciences, Istanbul, Turkey

²Department of Pediatrics, Division of Nephrology, Haseki Teaching Hospital, University of Health Sciences, Istanbul, Turkey

ABSTRACT

Aim: To measure serum leptin, homocysteine concentrations and insulin resistance in active and remission stages of children with nephrotic syndrome (NS) and to investigate their role in NS pathogenesis.

Methods: A total of 70 children were included in the study, 40 patients who had been diagnosed with NS and 30 healthy patients were control. Changes in plasma concentration of the serum homocysteine, leptin, and insulin were measured and compared with the other parameters in the groups.

Results: Serum leptin concentrations in active phase were lower than the remission phase (1.48 ± 0.09 ng/dl, 1.84 ± 1.64 ng/ml, $p < 0.05$). Also, serum homocysteine concentrations in NS group during the active phase were lower than the remission phase and the control group (6.45 ± 2.54 ng/dl, 9.35 ± 2.99 ng/ml, 7.76 ± 1.97 ng/ml, $p < 0.05$). The serum fasting insulin concentrations and homeostatic model assessment for insulin resistance (HOMA-IR) values of remission phase were significantly higher than those of active phase ($p < 0.05$). A positive relationship was found between the homocysteine concentrations and the body mass index of the patient; whereas, a negative relationship was detected between erythrocyte sedimentation rate (ESR), and the LDL-cholesterol concentrations ($p < 0.05$). ESR was found as the only factor associated with lower concentrations of homocysteine during the active phase ($r: -0.592$, $p < 0.05$).

Conclusion: In this study, we demonstrated that serum leptin and homocysteine concentrations decreased in active phase and increased in remission phase in children with NS. Insulin resistance could also develop as a result of steroid use in a short period of time in these patients.

Keywords: Nephrotic syndrome, leptin, homocysteine, insulin resistance, proteinuria.

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✉ Dr. Ipek Guney Varal,

Istanbul Haseki Teaching Hospital,

Department of Pediatrics, Istanbul, Turkey

E-mail: ipekguneyvaral@gmail.com

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Introduction

Nephrotic syndrome (NS) is a clinical condition characterized by heavy proteinuria, hypoalbuminemia, edema and hyperlipidemia. Approximately 80% of the nephrotic

syndromes seen in children present as minimal lesions that respond to steroid treatment [1]. Though NS is frequently encountered, its etiopathogenesis is not precisely clarified; however, genetic factors and immunology are suspected [2]. Today, genetic and metabolic studies aimed at explaining the development of the disease have been vigorously undertaken. It is known that patients given steroid treatment have a tendency of insulin resistance, obesity and endothelial dysfunction particularly in children [3].

Leptin, which is a 167-amino acid polypeptide, is synthesized in tissues like the adipose tissue, the placenta, the gastrointestinal tract and neuronal tissues, but the activity mainly occurs in the adipose tissue [4,5]. Body fat and serum leptin concentrations are directly proportional therefore, leptin concentrations are increased for obese person. Insulin resistant is determined in rodents which has leptin resistance and deficiency [6]. Leptin effects the 24-hour urinary protein amounts in children and is associated with the child's body weight and the severity of the disease [7].

Homocysteine, a thiol-containing amino acid, is generated by intracellular demethylation of dietary methionine, which is catabolized to form either cystathionine or cysteine [8]. Hyperhomocysteinemia is an independent risk factor for coronary heart disease, and display endothelial dysfunction. [9]. Renal function influences plasma homocysteine concentrations, and various reports have shown that plasma homocysteine concentrations may be lowered or elevated in children with NS compared to healthy controls [10].

We hypothesized that leptin and homocysteine metabolism is impaired in children with idiopathic nephrotic syndrome and during steroid treatment. The present study was designed to assess the changes in plasma

concentration of serum homocysteine, leptin and insulin in patients with nephrotic syndrome in active and remission phase after steroid treatment.

Materials and Methods

This study was conducted in children for the first time diagnosed with NS at our Pediatric Nephrology. This study was approved by the Medical Ethics Committee of University of Health Sciences, Istanbul Haseki Teaching Hospital (Approval date and number: 29.05.09/43). Furthermore, study was conducted in accordance with the revised Helsinki Declaration. The parents of the children in the patient and the control groups, as well as all children over 12 years of age, were informed in detail about the study, and informed consent with signature was received. The power calculation for the present study based on an effect size of 0.5 for leptin (ng/ml), a standard deviation of 2 (ng/ml) and an alpha level set at 0.05. Required sample size to get a power of 0.8 according to these assumptions was 30 patients for each group. Number of 10 patients were added as extra cases in case of withdrawal or drop out possibility. Therefore, at the end of study number of patients were 40 while control were 30.

The inclusion criteria to for the study were as follows: patients who were first diagnosed with NS between the ages of two and sixteen, responded to steroid treatment, did not have an additional disease. Blood samples were obtained first on admission as active phase (proteinuria), second at the end of treatment as remission phase (non-proteinuria). In the first step of the study, patients who had proteinuria above 40 mg/m²/hour, serum albumin concentrations below 2.5 gr/dl and hyperlipidemia were primarily evaluated during the active phase, or proteinuria phase,

prior to the start of steroid treatment. Then, these patients were given 2 mg/kg/day prednisolone as stated in the steroid treatment protocol. To assess the responsiveness to the steroid treatment, it was accepted that proteinuria in urine measured with a dipstick should be found in trace amounts, negative or under 4 mg/m²/hour, and that serum albumin concentrations should be over 3.5 g/l for three consecutive days for four weeks. Afterward, the same dosage of steroids was kept on every other day for the following four weeks. The steroid treatment was then gradually decreased and carried on fulfilling five months. Remission phase blood samples were obtained at the end of treatment. The systemic examinations of patients were carried out after their detailed anamneses and medical histories were obtained in both phases. As control group, age-sex matched thirty healthy children were chosen to determine normal leptin and homocysteine concentrations.

Blood samples were obtained after 12 hours fasting from patients who were included in the study and centrifuged at 1300 rpm for half an hour. Serum samples were taken into fine tubes and stored at -20°C. Routine biochemical examinations were practiced by Abbott C-16000 chemistry analyzer, and standard conventional methods were performed. Leptin concentrations were determined by enzyme-linked quantitative immunological measurement technique, using a Leptin ELISA kit (BioSource LEPTIN EASIA®, Nivelles Belgium). Homocysteine concentrations, however, were determined by use of an IMMULITE 2000® homocysteine kit and a competitive immunity measurement method. Insulin concentrations was measured by a chemiluminescent immunoassay method (ADVIA Centaur analyzer; Bayer Diagnostics) on fasting blood samples. HOMA-IR was

calculated as [fasting glucose (mg/dl) x fasting insulin (IU/ml)/405] [11]. Intra assay and inter-assay variations for the concentrations of leptin, homocysteine and insulin variables were calculated with the formula (CV: Standard Deviation/Mean).

Statistical analysis

Statistical analyses of the data were performed using the Statistical Package for the Social Sciences (SPSS), version 19, program (SPSS Inc., Chicago, IL, USA). All continuous values were presented as mean ± standard deviation, where suitable. The categorical values were presented as the frequency and percentage. Categorical variables were compared using Pearson's chi-squared test. Independent samples t-test was used for comparing two groups. Paired data were analyzed using paired samples t-test when data were normally distributed. General linear model was used for adjusting the effect of BMI confounder. The Spearman correlation coefficient was calculated to evaluate the correlation between the continuous variables. The values determined as p<0.05 were accepted as statistically significant

Results

Demographics, anthropometric variables and arterial blood pressures of the study groups are reported in Table 1. When the biochemical parameters of the patients with nephrotic syndromes in their active (proteinuria) phases and remission phases (non-proteinuria) were compared, as expected, total protein, albumin, cholesterol, LDL-cholesterol, triglyceride and IgM concentrations of patients in their active phases were found to be significantly different than those concentrations found during their in remission phases (Table 2). The serum fasting insulin concentrations and HOMA-IR values of

Table 1. Demographic, anthropometric variables and arterial blood pressures of the study groups.

Parameters	Active phase (n=40)	Remission (n=40)	Control (n=30)	P
Age (year)	7.45 ± 4.86	7.73 ± 4.58	8.28 ± 3.25	NS
Gender (M/F)	28/12	28/12	21/9	NS
BMI (kg/m ²)	18.9 ± 3.4	17.8 ± 3.9	15.9 ± 1.8	< 0.001
SBP (mmHg)	101.2 ± 10.6	96.2 ± 8.2	100.2 ± 8.8	NS
DBP (mmHg)	64.2 ± 8.7	60.2 ± 6.9	63.8 ± 6.9	NS

Data are reported as mean ± standard deviation (SD) or number and percentage. BMI: Body mass index, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, NS: Not significant.

Table 2. Biochemical values of the patients with nephrotic syndrome (active phase and remission) and healthy children.

Parameters	Active phase (n=40)	Remission (n=40)	Control (n=30)	P*	P**	P***
Glucose(mg/dl)	85.9 ± 14.1	86.5 ± 6.6	89.7 ± 10.9	NS	NS	NS
Urea (mg/dl)	23.4 ± 9.2	23.7 ± 6.3	27.6 ± 7.5	NS	0.04	NS
Creatinine (mg/dl)	0.42 ± 0.13	0.44 ± 0.13	0.53 ± 0.09	NS	0.002	0.01
Total protein (g/dl)	4.25 ± 0.75	6.54 ± 0.63	7.16 ± 0.51	< 0.001	< 0.001	0.001
Albumin (g/dl)	2.00 ± 0.72	4.08 ± 0.51	4.46 ± 0.37	< 0.001	< 0.001	0.003
Leucocyte count (/mm ³)	8155 ± 4937	7990 ± 2588	8120 ± 2475	NS	NS	NS
Hemoglobin (g/dl)	12.7 ± 1.6	12.5 ± 0.8	12.0 ± 0.8	NS	0.03	NS
Thrombocyte (x1000/mm ³)	438 ± 249	328 ± 100	305 ± 86	NS	0.01	NS
CRP (mg/l)	0.10 ± 0.12	0.12 ± 0.15	0.24 ± 0.38	NS	NS	NS
Cholesterol (mg/dl)	368.7 ± 168.2	196.9 ± 82.3	150.2 ± 19.7	< 0.001	< 0.001	0.02
LDL (mg/dl)	254.0 ± 143.4	111.6 ± 54.4	80.6 ± 19.9	< 0.001	< 0.001	0.02
HDL (mg/dl)	51.7 ± 21.4	52.0 ± 13.8	45.1 ± 10.2	NS	NS	NS
Triglyceride (mg/dl)	295.7 ± 216.3	111.7 ± 55.6	118.1 ± 65.6	< 0.001	< 0.001	NS
Insulin (μIU/ml)	7.09 ± 3.36	12.8 ± 8.8	9.69 ± 6.91	0.006	NS	NS
HOMA-IR	1.50 ± 0.75	2.76 ± 1.90	2.20 ± 1.64	0.007	NS	NS
ESR (mm/h)	59.4 ± 32.8	18.0 ± 13.4	15.5 ± 9.2	NS	NS	NS
IgG (g/dl)	403.1 ± 290.2	830.0 ± 251.9	1026 ± 317	NS	NS	0.006
IgA (g/dl)	112.4 ± 45.1	115.4 ± 53.9	114.5 ± 55.8	NS	NS	NS
IgM (g/dl)	255.5 ± 208.1	149.3 ± 159.8	114.8 ± 49.2	0.046	0.05	NS
IgE (g/dl)	380.4 ± 926.5	205.0 ± 299.4	129.8 ± 243.1	NS	NS	NS
C3 (g/l)	124.7 ± 25.1	116.4 ± 24.4	123.8 ± 55.9	NS	NS	NS
C4 (g/l)	24.9 ± 5.5	24.4 ± 7.2	19.8 ± 5.1	NS	0.001	0.01

Data are reported as mean ± standard deviation (SD) or number and percentage. CRP=C-reactive protein, HOMA-IR= Homeostatic model assessment of insulin resistance. P*: Active phase vs remission of the patients with nephrotic syndrome, P**: Active phase vs control, P***: Remission vs control, NS: Not significant.

remission phase were significantly higher than those of active phase ($p < 0.05$).

The active and remission phases of the patients and the leptin and homocysteine values of the control group are shown in Table 3. When we analysed leptin and homocysteine concentrations, we considered BMI as a confounder which was a significant different between the study and the control group. The serum leptin concentrations in active phase were lower than the remission phase (1.48 ± 0.09 ng/dl, 1.84 ± 1.64 ng/ml, $p < 0.05$). Also, serum homocysteine concentrations in NS

phase” (Table 3). Effective demographical, clinical and laboratory values on the reduction of serum homocysteine concentrations during the active phases of patients with NS were assessed.

A positive relationship was determined between homocysteine concentrations, the body mass index (BMI) of patients and their serum IgG concentrations; whereas, a negative relationship was detected between the erythrocyte sedimentation rate, and the total cholesterol and LDL-cholesterol concentrations (Table 4).

Table 3. Comparison of serum concentrations of leptin and homocysteine concentrations in study groups and BMI as a confounder.

Parameters	Active phase (n=40)	Remission (n=40)	Control (n=30)	P*	P**	P***
Leptin (ng/ml)	1.48 ± 0.09	1.84 ± 1.64	1.57 ± 0.90	0.004	NS	NS
Homocysteine (µmol/l)	6.45 ± 2.54	9.35 ± 2.99	7.76 ± 1.97	0.045	0.001	0.014

Data are reported as mean ± standard deviation (SD) or number and percentage. P*: Active phase vs remission of the patients with nephrotic syndrome, P**: Active phase vs control, P***: Remission vs control, NS: Not significant.

Table 4. The factors correlated with homocysteine concentrations of the patients in the active phase of the nephrotic syndrome (only significant correlations show).

Homocysteine					
	BMI	ESR	Cholesterol	LDL-cholesterol	IgG
r	0,38	-0,59	-0,42	-0,47	0,39
P	0,049	0,003	0,032	0,017	0,044

BMI= body mass index; ESR: erythrocyte sedimentation rate.

group during the active phase were lower than the remission phase and the control group (6.45 ± 2.54 ng/dl, 9.35 ± 2.99 ng/ml, 7.76 ± 1.97 ng/ml, $p < 0.05$). In brief, the average homocysteine concentrations of the study groups can be formulated in ascending order as “active phase < control group < remission

The factors causing low concentrations of homocysteine that were revealed via the Spearman correlation analysis were then tested to determine the independent predictor by implementing a Stepwise Linear Logistic Regression analysis. BMI, serum IgG concentrations, ESR, cholesterol, LDL-

cholesterol concentrations, which are some factors that may affect homocysteine concentrations, were included in the model. As a result of the administration of this model, an increase in sedimentation rate was found as the only factor associated with lower concentrations of homocysteine during the active phase of patients with NS.

Discussion

The etiopathogenesis of steroid-sensitive NS has not been clearly identified. In the literature, it has been revealed that there are many factors stimulating the disease, and as a consequence of these factors, the illness commences after a series of immunological events [2,3,10]. Today, there are studies proceeding that will be able to shed light on NS's etiopathogenesis. It is not yet clearly known whether the changes in leptin and homocysteine concentrations occur as a result of protein loss due via urine, the main cause of the illness, or whether there are other factors that contribute to the formation of NS [12-16]. We targeted to determine the leptin and serum homocysteine concentrations and the related factors in our patient group. In addition, we aimed to observe the effect of steroids on the insulin metabolism in the treatment of NS.

Glomerular dysfunction develops in NS, and heavy proteinuria is thus observed. Some studies have shown that leptin excretion through urine increases in children with NS during the proteinuria phase. Parallel to this, the serum leptin concentrations of these children were reduced and both urine and serum leptin concentrations returned to normal during the remission phase of the disease [12,13]. In other studies, however, it has been noted that though patients' leptin excretion increased during the proteinuria phase, there was no alteration in their serum leptin concentrations [12-14]. Therefore, the condition of the serum leptin

concentrations in children with NS and the relationship between these concentrations and the disease's pathogenesis remain unclear and controversial. Although the serum leptin concentrations of our patients during the proteinuria phase were determined to be lower than during the non-proteinuria phase and lower than the concentrations in the healthy children of the control group. The systemic elimination of leptin that is circulating in the blood happens through the kidneys. Leptin is not metabolized by the kidneys and is thus excreted as unimpaired proteins [17]. Therefore, in parallel with the daily urine decrease in children with chronic renal failure, the amount of leptin excreted via urine also diminishes and the serum leptin concentrations increase [3,15,16]. In NS, however, increased protein filtration causes leptin excretion via urine to increase. Though urinary leptin concentrations were not studied in our study, it was determined that leptin excretion with urine increased and the serum leptin concentrations decrease significantly. In a similar study, urinary leptin excretion is found as increased while serum leptin concentration is decreased. Serum leptin concentration plays an important role in the pathophysiology of NS (18)

Homocysteine, not involved in the 20 amino acids among the structural elements of proteins, is an amino acid involving thiol. Homocysteine, synthesized in the liver, muscle and other tissues, is excreted via urine from the kidneys after being metabolized with remethylation and transsulphuration reactions [19]. It has been shown that plasma homocysteine concentrations are inversely correlated with creatinine clearance, and hyperhomocysteinemia is often seen in patients with renal failure [20]. Moreover, homocysteine plays a significant role in endothelial damage and in the formation of

atherosclerosis developed as a consequence of this damage. It has been reported that homocysteine prominently increases in patients with chronic renal failure, diabetes, obesity, hypertension and metabolic syndrome prominently and leads to endothelial dysfunction [21,22]. Our study found that the homocysteine concentrations of patients during their acute phases were low, attributed to the increase in its excretion through urine. Consequently, this suggested that homocysteine alteration occurred in patients as a result of NS and was not responsible for the pathogenesis of the disease.

In the literature, homocysteine concentrations during the proteinuria phase were found to be high compared to the concentrations in healthy children; however, the reason for this could not be clarified [23,24]. In other most studies, though, it has been reported that serum homocysteine concentrations decreased depending on urinary excretion during the proteinuria phase and returned to normal during remission [25]. Homocysteine levels predicted damage accrual independently and is considered as proinflammatory marker in SLE patients [26,27]. In our patients, the decreased serum homocysteine concentrations during the proteinuria phase were higher than the values of those in the control group. Our findings were compatible with the recent study conducted by Tkaczyk et al. [28]. Their study further revealed that while the serum homocysteine concentrations were low during the proteinuria phase, they began to increase two weeks later and were considerably higher than those of the control group after eight weeks. Tkaczyk et al. [29] also found that the administration of cyclosporine A caused a significant increase in homocysteine and cysteine concentrations. However, we can infer that this abnormal increase may have been a reactional increase

related to steroid use to treat NS, similar to the treatment of polymyalgia rheumatica [30].

After the use of vitamin B6, B12 and folic acid in the same patient group, the homocysteine concentrations decreased again [30,31]. Although another study showed that homocysteine concentrations were low during the proteinuria phase, they normalized during remission at 12 weeks and 1 year. [32]. Therefore, when patients went into remission, these vitamin concentrations could be seen to decline and the homocysteine concentrations to increase. Elevated homocysteine concentrations during steroid treatment were associated with endothelium dysfunction and atherosclerosis; although vitamin concentrations were not measured in our study, vitamin supplementation is suggested based on estimation of low vitamin concentrations. Modulation of endothelial dysfunction in children with NS may be considered a therapeutic strategy to decrease the risk of future adverse cardiovascular events [33]. Indeed, if serial homocysteine concentrations had been determined at certain intervals after steroid treatment was ceased, this hypothesis would have been supported.

A positive relationship was determined between the homocysteine concentrations, the body mass index (BMI) and the serum total IgG concentrations alongside an established negative relationship between the erythrocyte sedimentation rates (ESR) and the total cholesterol and LDL-cholesterol concentrations. As BMI can be deceptive owing to the edematous period in NS, the relationship between the homocysteine concentrations and BMI was ignored. Nevertheless, when other data was analyzed carefully, they were all observed to be associated with the activity of the disease. In order to determine the most important independent predictor that could lead

to low concentrations of homocysteine, multiple logistic regression was applied. In our study, elevated ESR was observed as single independent predictor when stepwise linear logistic regression was applied to factors that causes low homocysteine concentration. ESR is the index of inflammatory activity and gives an indication regarding the progress of the disease and response to treatment. Therefore, this finding states that low homocysteine concentration could be another parameter in addition to ESR which indicates severity and response to treatment as a surrogate marker. The long-term effects of alterations on the homocysteine concentrations in patients will be possible only through randomized prospective studies. The long-term effects of homocysteine in NS are unexplored issues for future studies. Another important finding of our study was being significant difference between serum insulin concentrations of patients and homeostasis model assessment of insulin resistance (HOMA-IR) values during the active and remission phases. Although only two patients (5%) had a high HOMA-IR value (≥ 2.5) during the active phase, HOMA-IR values were found as ≥ 2.5 in sixteen out of forty patients (40%) after steroid treatment in remission phase. Furthermore, the serum fasting insulin concentrations of remission phase were significantly higher than those of active phase. Thus, discovering both insulin concentrations and HOMA-IR values at high concentrations in patients receiving steroid treatment indicated the development of insulin resistance. As the therapeutic benefits of glucocorticoids continue to expand across medical specialties, the incidence of steroid-induced insulin resistance will continue to rise [34]. If these patients do not have to use steroids again, over time these values will partly or totally return to normal.

The major limitation of this study was the lack of urinary analyses. Unfortunately, we did not study the urinary homocysteine and leptin concentrations of the patients. Another important limitation was the lack of frequent measurement of the serum homocysteine, leptin concentrations and vitamin levels during the steroid treatment. Last limitation of the study was difference of BMI between the study and the control group, which was adjusted by considering as a confounder.

Conclusion

In this study, we demonstrated that serum leptin and homocysteine concentrations decreased in active phase and increased in remission phase in children with NS. This decrease was likely caused by excretion via urine and is an important parameter revealing the activity of the illness. In addition, temporary or permanent insulin resistance could develop as a result of steroid use in a short period of time for this patient group. These and future studies will be crucial for understanding the pathogenesis of NS and determining treatment approaches.

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ORCID iD of the author(s)

Ipek Guney Varal /0000-0002-3298-066X
Mahmut Civilibal /0000-0002-0381-8145
Nilgun Selcuk Duru /0000-0001-9105-0529
Murat Eevli /0000-0002-0510-965X

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