Steroid effect on serum leptin in Libyan children with minimal change nephritic syndrome

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Abstract: Few studies have looked at the status of leptin in renal diseases especially nephrotic syndrome. The aim of this study was to investigate the effect of steroid therapy on serum leptin, anthropometric and appetite in patients with minimal change nephrotic syndrome (MCNS). Twenty children were recruited for this prospective study. Group I included ten children aged between 2 - 12 years with MCNS without any associated complications as impaired renal functions, hematuria, hypertension and others. Group II included ten healthy children with matching age, sex and BMI as a control group. Body weight, height, nutritional history including appetite, quantity of food, clinical examination, blood pressure and blood for kidney function parameter and serum leptin were taken before and after 3 days and after 2 weeks following prednisolone dose of 2 mg/kg/day. The same parameters were also performed for group II. Serum leptin was higher in group II than group I (1.575 ± 1.07 vs 0.575 ± 0.76 ng/ml $p < 0.01$). After 3 days and after 2 weeks of 2 mg/kg of prednisolone, serum leptin increased significantly in group II ($t = 4.65$, $p < 0.014$) without significant difference as it compared with group I ($t = 1.65$, $p = 0.08$). A highly significant increase in serum leptin concentration was detected in 2 weeks after prednisone therapy compared to the base line levels before therapy, compared to 3 days after therapy and to the control group ($t = 5.69$, $p < 0.001$, $t = 3.95$, $p < 0.001$ and $t = 8.96$, $p < 0.001$, respectively). In group I, serum leptin was higher in females than males (4.8 ± 2.8, $p < 0.001$). BMI was also higher in females compared to males. Patients' appetite improved after prednisolone for food quantity as recorded by the recall methods. Blood urea and serum creatinine in groups I and II did change significant within group II after steroid and even the changes were not significantly different from the control. Serum albumin, total serum protein, serum cholesterol concentrations and proteinuria revealed a significant difference between the two groups ($t = 5.9$, 8.9, 8.98 & 16.33, respectively, $p < 0.01$, for all). Weight, BMI in group I before and 2 weeks after intake of prednisone did not revealed any significant differences in weight, BMI before and after 2 weeks of prednisone ($t = 0.99$ and 0.88.8, respectively) in spite of the increase in appetite. Blood pressure had not changed significantly after the steroid therapy in MCNS patients' group. Thus, serum leptin concentration and appetite were significantly higher especially in females without significant change in anthropometric, blood pressure and renal function indices after steroid therapy in minimal change nephropathy.

Keywords: leptin, nephrotic syndrome, steroid, weight, Libya, children

Introduction

Leptin discovered in 1994 by Jeffrey M. Friedman and colleagues (1). Human leptin is a protein of 167 amino acids. Circulatingleptin blood level is directly proportional to the total amount of body fat. It produces primarily in the adipocytes of white adipose tissue. Other tissues as brown adipose tissue, placenta, ovaries, skeletal muscle, stomach fundus, mammary epithelial cells, bone marrow, pituitary and liver produce some leptin (2 - 4).

In human, leptin has a physiological role as an anti-obesity hormone and as integrator of adiposity to the satiety centers in the hypothalamus after binding to leptin-specific receptors causing a decrease in appetite and increase in energy expenditure (5). Leptinis circulating in the blood as free substance and is bounded with serum proteins (6). It metabolises in kidney tubules and then excreted in urine. Experimental studies in animals reported that bilateral nephrectomy induces rapid increase in plasma leptin concentrations (7). In chronic renal failure (CRF), glomerular filtration rate decreases, leading to reduction of leptin clearance that consequently causes an increase in serum leptin concentration. It is being reported that higher serum leptin concentration may contribute to appetite loss in patients with renal impairment and in patients on dialysis (8).

Leptin is secreted in pulses and in a rhythm pattern that has been well established in adults. Serum leptin concentration reaches a peak between midnight and 2 a.m. Factors controlling leptin secretion rhythm include sleep, stress and others (9). Glucocorticoids induce insulin resistance, and insulin by itself stimulates leptin production in vivo and in vitro. In vitro, cultures of human and rat adipocytes showed that glucocorticoed have stimulatory effect on leptin transcription that is potentiated by insulin (2 - 4, 9). Serum leptin increases during childhood and before puberty in girls and boys. After puberty, it tends to continue increasing in females while in males it decreases. It has been reported that women had a higher serum leptin than male at any given BMI. This difference has been attributed to proportionally larger adipose stores, distribution and different hormonal environment in females (10 - 12). There are evidences suggesting that androgens plays arole in serum leptin reduction in males during pubertal period. In contrast to the inhibitory effect of androgens, estrogen seems to stimulate leptin production (13). Glucocort-icoids increases ob gene expression and leptin production in vivo when administered at pharmacological doses in man, and in vitro in subcutaneous adipocyte cultures (14). Low concentration of glucocorticoid may stimulate the expression of the human of gene, the mechanisms of glucocorticoid stimulation of plasma leptin in humans remain unclear, but steroid direct effect on adipocytes and its central affects probably the possible mechanism (15). Other studies reported that combination of insulin and dexamethasone increases release of leptin from human subcutaneous adipocytes than either alone can do (16), however some reported an inhibitory effect of insulin on dexamethasone effect on leptin secretion from isolated adipocytes. Differences in incubation conditions are likely to explain these in vitro discrepancies. In vivo, however hyperinsulinemia after oral dexame-thasone did not decrease serum leptin (17).

Steroids appear that they have not direct effect on lepton secretion in absence of food intake. Steroids appear to potentiate the food intake that increases serum lepton. This synergism may be mediated by insulin and/or other factors associated with food ingestion as gastrointestinal hormones and/or glucose (18). The nephrotic syndrome (NS) is the most common chronic renal disease of childhood (19). It is clinical state characterized by heavy proteinuria, hypoalbuminemia associated with edema and hyperlipidemia. It can occur at some point in the course of many different glomerular diseases particularly in adulthood and aged patients (20). Estimation of annual overall incidence of the NS in children less than 16 years of age varies from 2 - 7 per 100.000 (21). The common age of MCNS occurrence in about half of the children is before fourth years of age (22, 23). About 60% of the children have MCNS between 2-6 years of age according to the international study of kidney disease in children (24). Incidence and prevalence of MCNS in children under 16 years of age are about 1.6 and 13 per 100.000, respectively (21). Minimal change disease found in about 75% of children with NS, with a predominance in early childhood (95% of NS aged 1 to 4 years and 75% of those aged 4-8 years have MCNS.

MCNS in children responds mostly well to steroid therapy (25). The role of the role of leptin in human physiology is becoming clearer but is still not fully understood. Leptin has a physiological role as an anti-obesity hormone by decreasing appetite and metabolic rate by affecting hypothatlamic satiety centers (26). The aim of this work is to study the relationship between serum leptin levels and appetite changes in a group of children had idiopathic-MCNS before and after short and longer-term administration of higher doses of corticosteroid.

Materials and methods

Patients: Twenty children enrolled in this prospective study. Ten patients (group I) diagnosed as minimal change nephropathy according to clinical and lab criteria of nephrotic syndrome diagnosis of International study of kidney disease in children (ISKDC). All cases were diagnosed between 2 - 6 years of their age and all they responded to prednisone therapy within one month. They were 6 males and 4 female, aged between 2 - 12 years (5.9 ± 3.5 years). Seven patients were suffering from the first attack of nephrotic syndrome. Three cases were having relapse and were off prednisone for more than 1 year. Control group of children (group II) were 10 children. They were normal children and were well matched with group I in age and body mass index (BMI), blood pressure, nutritional history and other parameters. All studied children enrolled in this study subjected to the followings:

I. Nutritional history including appetite, quality and quantity of food intake using recall method for the last three days.

II. Physical examination before and two weeks after corticosteroid therapy for height (ht) and weight (wt) to have body mass index (BMI).

III. Blood pressure (BP) estimated in resting supine posture from the right upper limb using mercury sphygmomanometer.

IV. Laboratory investigations: Random urine sample for protein to creatinine ratio, serum albumin, total serum protein, blood urea, serum creatinine concentration and fasting serum leptin were measured. Serum leptin was analyzed by enzyme linked immunosorbent assay (ELISA) technique.

Children in group II were treated with prednisone in 2 mg/kg/day for 3 - 4 weeks. After three days and at the end of two weeks of corticosteroid therapy. Random analysis of anthropometric assessment and serum leptin concentration were measured.

Statistical analysis: after data were collected, they were coded and transferred into Excel program of Microsoft office for analysis. Statistical analysis was done for arithmetic mean and standard deviation. To compare the mean of the parameters in the two groups, unpaired and paired t-test were used. A p value < 0.05 was considered significant.

Results

Age, sex, weight, height and BMI in group I and group II revealed no significant differences either between the two groups or within the group II after 3 days and 2 weeks on steroid (Table 1).

Table 1: Age, weight and body mass index of patients before treatment

	Group I				Group II			
	age (yrs)	wt (Kg)	ht (cm)	BMI (kg/m^2)	age (yrs)	wt (kg)	ht (cm)	MBI (kg/m^2)
Min.	**2**	**12.5**	**89**	**15.7**	**3.5**	**14.5**	**97**	**15.2**
Max.	**12**	**32.5**	**140**	**17.8**	**9**	**26**	**127**	**19**
Mean	**5.9**	**20.96**	**111.3**	**16.76**	**5.3**	**18.59**	**106.6**	**16.62**
SDM	**3.50**	**7.51**	**18.32**	**0.74**	**1.81**	**3.66**	**9.43**	**0.99**

Blood urea and serum creatinine in group I and group II did not show significant difference between both groups (t = 0.85 and 1.003). Serum albumin, total serum protein, serum cholesterol concentrations and proteinuria revealed a significant difference between the two groups (t = 5.9, 8.9, 8.98 & 16.33, respectively, p < 0.01, Table 2).

Table 2: Laboratory investigations of patients and controls

Parameters	**group I**			**group II**			t & p values
	range	mean	SD	range	mean	SD	
S. urea (mg/dl)	19 - 47	28.1	8.7	20 - 32	25.6	3.7	
S. creatinine (mg/dl)	0.2-0.6	0.36	0.15	0.2 - 0.6	0.4	0.11	
S. albumin (g/dl)	1.4	2.1	0.6	4 - 5	4.2	0.3	t = 5.9, p = 0.01*
S. total protein (g/dl)	3 - 6	4.5	0.9	6.2 - 8	6.9	0.6	t = 8.9, p = 0.01**
protein/creatinine ratio	5 - 16	7.5	1.8	0.05 - 0.1	0.08	0.01	

Serum leptin was significantly lower in group II than the control group (0.575 ± 0.76 vs 1.575 ± 1.07 ng/ml, p < 0.01). A significant increase in serum leptin concentration was revealed after 3 days on prednisone therapy than before treatment in group II (t = 4.65, p < 0.014) but there was no significant difference compared to group I (t = 1.65, p < 0.08). A significant increase in serum leptin was reported after 2 weeks on prednisone therapy compared to the base line levels before therapy and also after 3 days after therapy in comparison to the control group (t = 5.69, p < 0.001, t = 3.95, P = 0.001 and t = 8.96, p < 0.001, respectively, Table 3).

Table 3: Serum leptin before, 3 days and 2 weeks after prednisone

	group I			group II
	Before	After 3 days	After 2 wks	
Min	0	0	1.5	0
Max	2	5.5	11.25	3
Mean	0.575	1.8	3.975	1.575
S.D.	0.76	1.46	2.92	1.07
t_1, p_1		4.65, 0.014*	5.69, 0.001*	
t_2, p_2			3.95, 0.01*	
t_3, p_3	4.32, 0.01*	1.65	8.96, 0.001*	

t_1 and p_1 comparison between before and after treatment in patients group.
t_2 and p_2 comparison between serum leptin concentration 3 days and 2 weeks after treatment in patients group.
t_3 and p_3 comparison between controls and group I (in the three periods)

In group I, serum leptin was higher in females than males (4.8 ± 2.8, $p < 0.001$). BMI was also higher in females compared to males (Table 4). Patients' appetite improved after prednisolone for food quantity as recorded by the recall methods. Weight, BMI in group I before and 2 weeks after intake of prednisone. There were no significant difference in weight, BMI before and after 2 weeks of prednisone intake ($t = 0.99$ and 0.88.8 respectively $p > 0.05$, Table 5).

Blood pressure in group II before and after 2 weeks of prednisone, it did not show signify-cant difference in both systolic and diastolic blood pressures before and after steroid drug, and there were not significant differences between the two groups (Table 6).

Table 4: Anthropometric measurements and leptin in males and females in group I

2-week	**Female n = 4**	**Male n = 6**	
serum leptin (ng/ml)			
range	1.5 - 11.25	1.75 - 3.25	
mean	4.8	2.75	t = 6.25
S.D.	3.61	0.68	p < 0.001*
BMI (kg/m2)			
range	13.8 - 32	16.6 - 15.4	t = 2.33
mean	18.48	14.0	p < 0.05*
S.D.	6.766	13.7	

Table 5: Anthropometric measurements before and 2 weeks after the intake of prednisone in group I

	weight (kg)		MBI (kg/m²)	
	before	after	before	after
Min	12.5	12	15.7	14
Max	32.5	31.5	17.8	17
Mean	20.96	20.08	16.76	16.07
s.d.	7.513	7.39	0.738	0.89
t	0.99		0.88	
P	0.32		0.41	

Table 6: Blood pressure before and 2 weeks after intake of prednisone in group II

	Systolic blood pressure (mmHg)		Diastolic blood pressure (mmHg)	
	before treatment	after treatment	before treatment	after treatment
Min	90	90	60	65
Max	120	115	80	80
Mean	102	101.5	70.5	71
S.D.	10.593	9.144	7.246	6.146
t, p	0.52, 0.5		0.65, 0.5	

Discussion

Steroids increase gene expression of lipid deposition in the adipocyte including lipoprotein lipase (27, 28) steroids increase also leptin expression in rodents, in vivo and in vitro (29, 30). Steroids are potent stimulators of both ob gene expression and circulating leptin levels in rats, and they are powerful appetite stimulant in human (31). In this study, serum leptin was significantly lower in nephrotic patients than control group (0.575 ± 0.76 vs. 1.575 ± 1.07 ng/ml), with low total plasma protein and serum albumin. After starting 2 mg/kg prednisolone. In group I, serum leptin increased after 3 days and also after two weeks 1.8 ± 1.5, 4 ± 2.9 respectively.

The increase in serum leptin had associated with changes in anthropometric parameters, appetite, plasma protein and protein creatinine ratio in group I. These changes after steroid might be due to an improvement in glomerular self-repair. Occurred self-repair in the acutely damaged glomeruli increased the glomerular filtration rate and reduced albumin and leptin loss viathe glomeruli. Furthermore, nephrotic syndrome steroid-responder patients are usually loss weight after starting steroid or at least they do not gain weight during early stage, and that was happen in group I patients in this study. Steroids increase serum leptin concentration (32-35). The increased serum leptin in this study after steroid might be due to reduction in leptin loss via self-repaired glumeruli-basement membrane, increased lept-inbinding protein availability and/or decreased body fluid content after steroid. These factors might have a role in the changes occurred in leptin plasma concentration. Serum leptin increased in end-stage renal disease and chronic renal failure (36). Protein filtration increases in nephrotic syndrome, which could increase urinary leptin excretion, although Valle et al. reported in nephrotic syndrome protein and leptin urinary loss did not lead to low serum leptin (37) but it may even remain at its normal serum concentration (38). Increase appetite is well known side effect of steroid, and this usually leads to an increase in body weight.

In the present study, significant increase in body mass index (especially in girls) might be due to the mineralocorticoid effect of steroid rather than increased appetite. Hence, change of appetite after steroid seems to be due steroid effect that made the leptin appetite depressive effect ineffective in this study. Blood pressure monitoring did not revealed significant change in any of the patients after the steroid therapy. Because of water and sodium retention effect of steroid, it is expected, blood pressure should increase. The change in blood pressure did not happen in our patients. The absence of blood pressure change might be due to the reduction in fluid on board that retained before the start of steroid, plus the possible effect of leptin hormone on circulation or other unknown associated mechanisms that can affect blood pressure control. In comparison with the present study, there was no significant change in the anthropometric measurements before

versus after prednisone intake but serum leptin concentrations and BMI, were significantly higher in females than males, difference in body composition and body fat distribution between both sexes might be factors modulating the gender difference in serum leptin concentration (10, 12, 39).

In conclusion, in MCNS patients, serum leptin concentrations significantly increase after steroid, and associates with an increase of appetite and body mass index. To clarify the relationship between the increase in serum leptin in this group of patients and its effect on MCNP treatment and prognosis further larger studies should be done.

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