Serum levels of soluble urokinase plasminogen activator receptor as a new inflammatory marker in adolescent obesity

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Background & objectives: Obesity is known for low-grade inflammatory state with enhanced production of inflammatory mediators in children and adolescents. Soluble urokinase plasminogen activator receptor (suPAR) can be generated as a pro-inflammatory marker. This study was conducted to evaluate the role of suPAR, and its association with leptin, adiponectin, interleukin-6 (IL-6), high-sensitive C-reactive protein (hsCRP) and fibrinogen in adolescent obesity.

Methods: A total of 98 participants, 55 obese individuals and 43 healthy controls, aged between 10 and 17 yr, were included in the study. Serum suPAR, IL-6, leptin and adiponectin were measured using ELISA method.

Results: Serum suPAR, IL-6, fibrinogen, hsCRP and leptin levels in obese individuals were significantly higher than those of controls ($P<0.05$ & $P<0.001$). Serum adiponectin levels in obese individuals were significantly lower than those of controls ($P<0.01$).

Interpretation & conclusions: Our findings showed that suPAR, IL-6, fibrinogen, hsCRP and leptin were significantly higher in the obese individuals than those of controls. suPAR may be a good novel biomarker for systemic subclinical inflammation and immune activation linked to adolescent obesity.

Key words Fibrinogen - high-sensitive C-reactive protein - inflammation - obesity - soluble urokinase plasminogen activator receptor

Adolescent and childhood obesity is a rapidly increasing public health problem globally[1]. Obesity is associated with significant adverse effects on health, including metabolic syndrome (MS), insulin resistance (IR), type 2 diabetes mellitus (T2DM) and cardiovascular diseases (CVD)[2]. The major function of adipocyte is to regulate fat mass and nutrient homeostasis[3]. Adipose tissue is not only a passive site of energy storage, but also an active endocrine organ releasing a large number of bioactive mediators (adipokines), thereby modulating haemostasis, blood pressure, lipid and glucose metabolisms, inflammation and atherosclerosis[1,3]. Obesity is associated with chronic low-grade inflammation characterized by increased circulating free fatty acids (FFAs) and chemoattraction of immune cells such as macrophages in adipose tissue[4,5]. Obesity is also associated with altered production of adipokines by enhancing the production of leptin and resistin and by reducing the production of adiponectin[4].

Adiponectin, a protein of 30 kDa and exclusively expressed in adipose tissue, has antiatherogenic, antidiabetic and anti-inflammatory properties[1,6]. Leptin, a cytokine-like molecule, is secreted by adipocytes to regulate food intake at hypothalamic level[2]. Interleukin-6 (IL-6) is another cytokine with a role in inflammation, haematopoiesis, immune responses and tissue injury[1,7]. IL-6 production is increased in obese and insulin-resistant individuals[1,3]. Leptin and IL-6 levels are directly correlated with adiposity and body mass index (BMI)[6,7].

In obese individuals, hepatic biosynthesis and maintenance of circulating C-reactive protein (CRP) is a response to the increase in the secretion of cytokines of adipose tissue such as IL-1, IL-6 and tumour necrosis factor alpha (TNF-α)[8]. Circulating high-sensitive CRP (hsCRP) is a widely accepted marker of chronic low-grade systemic inflammation[9]. Fibrinogen is an acute-phase reactant protein synthesized in the liver and plays an important role in promoting atherogenesis and thrombogenesis[10].

Urokinase plasminogen activator receptor (uPAR) is present in several types of cells including neutrophils, lymphocytes and monocytes/macrophages[11]. After uPAR is cleaved from the cell surface in response to inflammatory stimulation[11], soluble uPAR (suPAR) is generated as a pro-inflammatory marker[12]. suPAR plays a role in numerous physiological pathways mainly involving immune activation, such as plasminogen-activating pathway, migration through interaction with vitronectin and integrin, cell adhesion, proliferation, tissue remodelling and systemic inflammation[12,13].

suPAR has not been evaluated as a biomarker for obesity in adolescent population, and the clinical significance of suPAR still remains uncertain as a marker of inflammation in adolescent obesity. Hence, this study was aimed to document the serum levels of suPAR as a marker for inflammation in adolescent obesity. Another aim was to reveal the relationships among hsCRP, suPAR, IL-6, fibrinogen, leptin and adiponectin levels in adolescent obesity.

Material & Methods

This study was performed on 55 obese adolescent individuals and 43 healthy controls. The two groups were randomly selected as obese patients and healthy controls at similar age in puberty. Controls were composed of adolescents with BMI between 25th and 74th percentiles according to BMI reference curves

for Turkish adolescents[14,15]. Obesity was defined as BMI ≥95th percentile for age and sex according to BMI reference curves for Turkish adolescents[14,15]. Weight-standard deviation scores (SDs), height-SDs, BMI-SDs and BMI percentile (BMI p) were calculated for all patients. Percentile and SDs of weight and height were assessed according to the standards of the reference curves for Turkish adolescents[14,15]. The height and weight scores of controls were between 3rd and 97th percentiles[14,15]. Pubertal stage was determined in both obese and control groups according to the Tanner criteria[16] and validated by plasma sex hormone concentrations[16].

The exclusion criteria included the presence of infection, DM, familial hyperlipidaemia, hypertension, congenital cardiac problems, chronic diseases, smoking, overweight/obese patients with hypothalamic obesity, genetic disorders, growth hormone deficiency, hypothyroidism and glucose intolerance. Any patient receiving pharmacological treatment was also excluded from the study. Obese adolescents were recruited consecutively from the outpatient clinic of Pediatric Endocrinology Department in Konya Education and Research Hospital, Konya, Turkey. The controls were selected from the adolescents of hospital staff and matched with obese adolescents by age and gender. Trained paediatricians performed the clinical examinations of both obese and control groups according to standardized methods[14-16]. The study was performed between November 2014 and May 2015. The study protocol was approved by the local Ethics Committee of Meram Medical School, Necmettin Erbakan University. All parents were informed about the study design, and written consents were obtained.

Anthropometric measurements: Anthropometric parameters including height, weight and waist circumference (WC) were measured. Height was measured to the nearest 0.1 cm using a rigid stadiometer. Weight was measured to the nearest 0.1 kg using a weighing scale (Sınbo, Turkey). BMI was calculated by dividing weight to height as square metre (kg/m²). WC was measured to the nearest 0.5 cm at the midline diameter between the iliac crest and the lower rib during minimal respiration. Adolescents with WC >90th percentile for age and sex were evaluated as obese according to normal values for WC of Turkish adolescents[17]. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured after 20 min rest in supine position. The average of two measurements taken on the right arm was recorded.

Hypertension was defined as average SBP and/or DBP, that is, 95th percentile for gender, age and height[18].

Analytical methods: The homeostasis model assessment of IR (HOMA-IR) was calculated as fasting serum insulin (μU/ml) × fasting plasma glucose (mmol/l)/22.5 and used as an index of IR[1]. HOMA-IR scores ≥2.5 show IR[1]. Blood samples were obtained after an overnight (12 h) fasting between 0800 and 0900 h into empty vacuum tubes and in tubes containing 3.2 per cent of Na-citrate. After blood sampling, serum samples for leptin, adiponectin, IL-6 and suPAR were stored at -80°C until assayed. An oral glucose tolerance test was performed (1.75 g of glucose/1 kg; glucose/insulin measured at baseline, 1st and 2nd h) in obese individuals. Detailed characteristics of the study population are shown in Table I.

Analyses of other analytes: Serum total cholesterol (TC), triglycerides (TGs), high-density lipoprotein-cholesterol (HDL-C), low-density lipoprotein-cholesterol (LDL-C), glucose and alanine aminotransferase (ALT) were measured by commercially available kits (Abbott, USA) based on the Architect C 8000 System (Abbott Laboratories, Abbott Park, Illinois, USA). Serum insulin was determined by routine chemiluminescence method on E170 analyzer (Roche Diagnostics, Mannheim, Germany). hsCRP was measured by a highly sensitive immunonephelometric assay using Cardio-Phase hsCRP on BN-II Dade Behring analyzer (Dade Behring Marburg GmBH; Marburg, Germany). Fibrinogen levels were measured using commercially available kits (Siemens, Germany) based on routine methods on Siemens BCS System (Global Siemens Healthcare, Germany).

The analyses of serum adiponectin, IL-6 and leptin levels were performed with an enzyme immunoassay method using commercial kits (Avibion Human Elisa Kits, Orgenium Laboratories Business Unit FIN-01720 Vanta, Finland). The levels of suPAR were detected in serum samples using Human uPAR ELISA Kit (Sun Red, Shanghai Sunred Biological

Table I. Clinical and demographic characteristics of obese and non-obese adolescents			
Clinical and demographic characteristics	Obese adolescents (n=55)	Non-obese adolescents (n=43)	*P*
Age (yr)	14.29 (11.10-17)	14.26 (10.40-17)	0.94
Gender (male/female)	19/36	18/25	0.112
Waist circumference (cm)	99.23±9.88	66.40±6.10	<0.001
Height SDs	-0.08±0.88	-0.41±1.39	0.20
BMI p	98.61±2.17	31.83±29.59	<0.001
BMI SDs	2.62±0.72	-0.65±1.11	<0.001
SBP (mmHg)	127.02±24.44	100.88±9.53	<0.001
DBP (mmHg)	77.98±14.13	61.25±6.38	<0.001
Fasting glucose (mg/dl)	89.19±6.96	82.35±12.26	<0.01
Fasting insulin (μIU/ml)	16.61±8.36	10.54±4.93	<0.001
Second hour glucose (mg/dl)	107.47±25.68		
Second hour insulin (μIU/ml)	66.00±36.84		
HOMA-IR	3.29±1.73	2.31±1.16	<0.01
Puberty	4.32±1.07	4.05±1.13	0.26
TG (mg/dl)	117.15 (36-398)	87.06 (33-260)	<0.05
TC (mg/dl)	168.98±31.71	157.92±42.10	0.19
HDL-C (mg/dl)	44.72±9.71	50.66±8.88	<0.01
LDL-C (mg/dl)	100.69±27.57	89.85±39.94	0.16
ALT (U/l)	20.85 (6-55)	13.16 (7-27)	<0.01

All values are mean±SD and median (range). SDs, standard deviation score; BMI p, body mass index per cent; SBP, systolic blood pressure; DBP, diastolic blood pressure; HOMA-IR, homeostasis model assessment of insulin resistance; TC, total cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; ALT, alanine aminotransferase

Technology, Pelobiotech GmbH-Am Klopferspitz 19, 82152, Planegg, Germany) in accordance with the manufacturer's guidelines. The absorbance of all samples was measured at 450 nm on an ELx800 Absorbance Microplate Reader (Biotek, Winooski, VT, USA). This assay employed a quantitative sandwich enzyme immunoassay technique to measure suPAR. The unknown sample concentration was determined from the standard curve, and concentration values were reported in ng/ml.

Statistical analysis: All data were expressed as mean±SDs. Statistical analyses were performed using SPSS software for Windows Version 16.0 (SPSS Inc., IL, USA). To compare the ratio of categorical variables, Chi-square test was used [sex (female/male)]. The normality of the variables was evaluated using the one-sample Kolmogorov–Smirnov test. For the independent samples, the Student's *t* test and Mann–Whitney U-test were used for comparing mean and median values, respectively. Correlations between the variables were determined using Pearson's correlation coefficient. Values of hsCRP, suPAR, IL-6 and fibrinogen were analyzed using receiving operating characteristic (ROC) curve analysis. When a significant cut-off value was observed, the sensitivity and specificity were presented. While evaluating the area under the curve (AUC), a 5 per cent Type-I error level was used to accept a significant predictive value of the test variables.

Results

This study was performed on 55 (19 males, 36 females) obese adolescents between the ages of 10 and 17 yr (14.29±1.70 yr) and 43 (18 males, 25 females) controls between the ages of 10 and 17 yr (14.26±1.80 yr). Anthropometric and analytical characteristics of the obese individuals and controls are shown in Table I. BMI ($P<0.001$), HOMA-IR ($P<0.01$) and fasting glucose ($P<0.01$) values were higher, compared with those of controls ($P<0.01$). WC, weight, SBP and DBP, BMI p, BMI-SDs, fasting insulin and ALT were significantly higher than those of the control group. While TG was significantly higher than those of controls ($P<0.05$), HDL-C levels of the obese were lower than those of controls ($P<0.01$). No difference was found in terms of height, age-SDs, height-SDs, TC and LDL-C between the obese and control groups. The number of obese individuals with hypertension was 18.

Serum levels of inflammatory biomarkers in the obese individuals and controls are shown in Table II. suPAR ($P<0.05$), IL-6 ($P<0.001$), leptin ($P<0.001$), hsCRP ($P<0.01$) and fibrinogen ($P<0.001$) values of the obese individuals were significantly higher than those of the controls. Adiponectin ($P<0.01$) values of the obese individuals were significantly lower than those of the control group.

ROC analysis was used to compare the values of suPAR, IL-6, hsCRP, HOMA-IR and fibrinogen levels in the obese adolescents. It was tested whether the predictive value of suPAR was equal or superior to IL-6, hsCRP and fibrinogen using ROC curves. suPAR value was found to be an AUC of 0.626 (cut-off value, 3.495 ng/ml; sensitivity, 50% and specificity, 78%); HOMA-IR value to be an AUC of 0.685 (cut-off value, 2.874 ng/ml; sensitivity, 56% and specificity, 73%); IL-6 value to be an AUC of 0.697 (cut-off value, 26.60 pg/ml; sensitivity, 41% and specificity, 91%), hsCRP value to be an AUC of 0.883 (cut-off value, 0.685 mg/l; sensitivity, 82% and specificity, 78%) and fibrinogen value to be an AUC of 0.879 (cut-off value, 312.90 g/l; sensitivity, 80% and specificity, 88%). IL-6 showed superiority, compared to suPAR, fibrinogen and hsCRP in predicting obesity in adolescents (Figure).

Table II. Serum biomarkers of obese and non-obese adolescents			
Serum biomarkers	Obese adolescents (n=55)	Non-obese adolescents (n=43)	*P*
suPAR (ng/ml)	5.68 (2.27-25.15)	3.46 (2.12-13.46)	<0.01
Adiponectin (µg/ml)	15.79±4.70	19.42±5.85	<0.01
IL-6 (pg/ml)	29.58 (21.60-130.50)	23.80 (19.40-35.40)	<0.001
Leptin (ng/ml)	14.63±6.43	4.83±3.77	<0.001
hsCRP (mg/l)	4.37 (0.23-24.00)	0.56 (0.16-3.75)	<0.01
Fibrinogen (g/l)	371.13±88.17	262.98±46.82	<0.001
All values are presented as mean±SD and median (range). suPAR, soluble urokinase plasminogen activator receptor; IL-6, interleukin-6; hsCRP, high-sensitive C-reactive protein; SD, standard deviation			
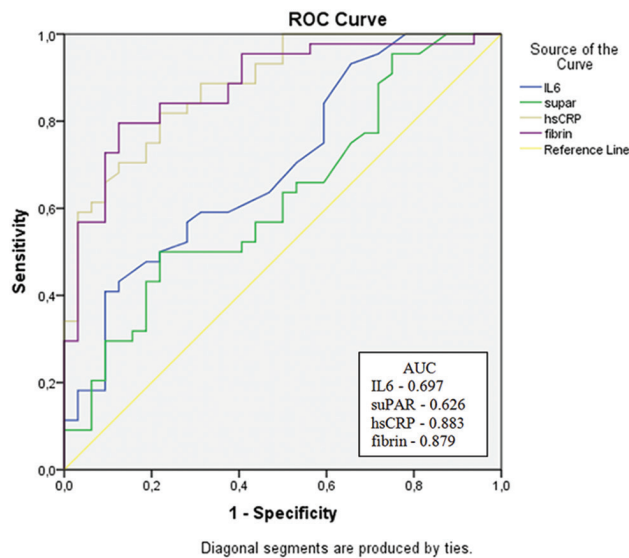
Figure. ROC curve of serum biomarkers in adolescent obesity. AUC, area under the curve; suPAR, soluble urokinase plasminogen activator receptor; IL-6, interleukin-6; hsCRP, high-sensitive C-reactive protein.

According to the multiple linear regression analysis, BMI and gender had no effect on suPAR (P=0.05 and P=0.123), while obesity had a significant effect on suPAR (P=0.004). The overall significance of model was 0.01 with R^2=0.126. The model for boys was not significant, and both obesity and BMI had no individual effect on suPAR, while the model for girls was significant (P=0.009). However, obesity was observed to be significant on girls (P=0.004), whereas BMI could not be found as significant (P=0.06).

Spearman's Rho correlation analysis was performed to investigate the association between measures of serum suPAR and IL-6, hsCRP, leptin, fibrinogen, BMI, HOMA-IR, fasting glucose, WC, weight, SBP and DBP, BMI p, BMI-SDs and fasting insulin. No poor or moderate correlation was found between suPAR and other parameters in obese adolescents.

Discussion

In the present study, serum suPAR, IL-6, leptin, hsCRP and plasma fibrinogen levels were significantly higher and adiponectin concentration significantly lower in the obese group, compared to those found in the controls. Serum leptin levels are elevated in obese adolescents due to decreased hypothalamic sensitivity and leptin resistance and may play a role in the complications of obesity[1]. The levels of leptin detected in our obese adolescents were consistent with the findings reported in studies by other investigators[2,19]. Adiponectin decreases in obesity and obesity-related diseases and may be involved in CVDs and IR, a risk for the development of T2DM and dyslipidaemia[6]. Winer *et al*[20] found that adiponectin levels were decreased in obese children and adolescents, whereas the markers of inflammation and pro-inflammatory cytokines were higher. Adiponectin has been described to have insulin-sensitizing effects through multiple mechanisms by increasing fatty acid oxidation, inhibiting hepatic gluconeogenesis, stimulating glucose uptake in adipocytes, protecting cardiovascular effects by diminishing the expression of adhesion molecules, suppressing the transformation of macrophages into foam cells and decreasing the production of inflammatory cytokines[1,5]. Increased adiposity is associated with an adverse cardiovascular risk profile, characterized by elevated TG, LDL-C and reduced HDL-C[21]. In our study, adiponectin and HDL-C values of the obese were significantly lower and TG was significantly higher than those of the controls. A previous study related to obese adolescents showed that serum levels of TC, LDL-C, TG, insulin, leptin and TNF-α were higher, whereas HDL-C and adiponectin levels were lower in the obese group, compared with the controls[22]. Shin *et al*[23] showed that while serum adiponectin level was decreased, serum CRP and TNF-α levels were increased in obese children. In another study, adiponectin serum concentrations were reported to be significantly lower although serum leptin concentrations were greater in the obese, compared to the non-obese controls[24]. Our results were consistent with those reported in these studies.

In obese patients, an increase in IL-6, CRP and TNF-α levels and a decrease in adiponectin and IL-10 induce pro-inflammatory stage, contributing to IR and endothelial dysfunction vascular injury[1]. IL-6 stimulates lipolysis, increases free fatty acids (FFAs) concentrations and whole body fat oxidation and reduces insulin-dependent hepatic glycogen synthesis, glucose uptake in adipocytes and the expression and secretion of adiponectin in human adipocytes[3,7]. hsCRP may be a useful marker in childhood obesity and a predictor of future occurrence of MS[25]. Fibrinogen promotes activities, such as platelet aggregation and increased blood viscosity[26]. Excess body fat may lead to increased production or decreased clearance of fibrinogen, an independent risk factor for CVDs[10]. Consistent with the findings in other studies, we found that IL-6, hsCRP and fibrinogen levels of obese adolescents were significantly higher than those of the controls. Kapiotis *et al*[9] found that obese

children had significantly higher levels of hsCRP and IL-6 than healthy controls. Hiura *et al*[25] reported that compared with the non-obese counterparts, the obese children had significantly higher hsCRP and LDL-C and lower HDL-C levels. In another study, the obese children had significantly higher concentrations of LDL-C, fibrinogen, IL-6 and TNF-α levels and lower concentrations of HDL-C than non-obese controls[27]. Balagopal *et al*[10] showed that plasma fibrinogen was significantly higher in obese female adolescents, compared with the lean group. Mauras *et al*[28] found that the obese with IR without MS comorbidities had about 10 times higher hsCRP concentrations and higher fibrinogen and IL-6 than the controls. IL-6 and fibrinogen were shown to be significantly increased in the obese children, compared to the controls[29].

Existing evidence also suggests that suPAR in the obese patients may be correlated with macrophage accumulation in adipose tissue and is strongly influenced by adiposity[12,13]. suPAR is produced in sites of inflammation by activated neutrophils and macrophages, and plays a role in extracellular matrix degradation for macrophage migration, leading to immune activation, and under certain conditions, systemic subclinical inflammation and atherosclerosis, independently of other known inflammatory biomarkers such as hsCRP[12,13]. Hence, plasma suPAR levels in the obese individuals could be correlated with macrophage accumulation in their subcutaneous adipose tissue[13]. Baseline mean suPAR levels were higher in obese individuals than the overweight and lean individuals, and higher suPAR levels were also linked to higher risk of T2DM in non-smokers and overweight participants[12]. Cancello *et al*[13] found that the total amount of suPAR protein was significantly higher in obese individuals, compared to lean controls. suPAR was significantly more expressed in white adipose tissue of obese individuals, compared to lean controls. Lyngbæk *et al*[30] reported that CRP was positively associated with anthropometric measures, whereas suPAR was linked to endothelial dysfunction and atherosclerosis.

In the ROC curve IL-6 showed superiority, compared to suPAR, fibrinogen and hsCRP in predicting obesity in adolescents. However, the area of suPAR was close to that of IL-6. Perhaps the combined evaluation of suPAR and other parameters would be a better therapeutic aid.

In conclusion our findings indicate that suPAR may be a good novel biomarker for systemic subclinical inflammation and immune activation linked to obesity in adolescents. suPAR is involved in the pathogenesis of inflammation in obesity. Effective prevention and treatment processes can be achieved better by understanding the pathogenesis of obesity. The exact pathophysiological mechanisms linking suPAR and subclinical inflammation related to obesity still remain unclear. In our study the sample size was limited. Additional studies are needed to enlighten the role of suPAR in obesity seen in adolescents.

Acknowledgment

Conflicts of Interest: None.

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