Original Article

Serum Amyloid P and Endocrine Markers in a Cohort of Obese Children

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Abstract

Objectives: Obesity in children can lead to morbidity and mortality due to metabolic and inflammatory comorbidities. **Aims**: The objective of the study was to investigate the alterations in acute inflammatory markers, serum amyloid *P* (SAP) and cortisol, and endocrine markers, leptin and insulin, in obese children. **Materials and Methods:** Serum leptin, insulin, cortisol, and amyloid *P* concentrations were measured in obese (BMI percentile >85, n = 17) and nonobese (BMI percentile < 75, n = 20) children using ELISA and Bio-Plex Bead-based assay. **Statistical Analysis Used:** Serum concentrations of analytes were compared between normal and obese groups using 2-tailed student's *t*-test. **Results:** Mean leptin, insulin, and SAP serum concentrations were significantly higher in obese children as compared to the controls (97.19 vs. 4.06, $P < 0.05$; 21.31 vs 3.56, $P < 0.05$; 46.77 vs. 17.89, $P < 0.05$; respectively). No difference was found in mean serum cortisol levels of the two groups. However, cortisol values were higher in obese subjects compared to the control group (7.89 vs 6.30, $P = 0.15$). Leptin corelated with insulin ($r = 0.42$, $P = 0.043$) and cortisol ($r = 0.48$, $P = 0.025$) levels in the obese group. Furthermore, leptin, insulin, and SAP levels were corelated with BMI ($r = 0.80$, $P < 0.000$; $r = 0.67$, $P = 0.015$, respectively) and body weight ($r = 0.52$, $P = 0.01$; $r = 0.52$, $P = 0.002$; $r = 0.54$, $P = 0.01$, respectively) in the obese group but did not demonstrate a significant relationship in the nonobese group. **Conclusion:** Elevated SAP levels and increase in leptin and insulin indicated a preeminent disposition of morbidly obese children to the development of low-grade inflammation and metabolic syndrome.

Keywords: Cortisol, inflammation, insulin, leptin, metabolic syndrome, serum amyloid P

Introduction

Obesity, a disease with increasing pervasiveness, is a consequence of energy imbalance where the excess energy is stored as body fat in the form of adipose tissue. According to an estimate by World Health Organization (WHO), there are 35 million overweight/obese children in developing countries, compared with 8 million in developed countries.[1,2] Pakistan is one of the countries with double burden of disease, comprising both malnutrition and obesity.[3,4]

Obesity is governed by complex interplay of genetic and biochemical factors contributing to chronic cardiovascular metabolic disorders resulting in morbidity and mortality.[3] Obese children are more prone to complications than adults,[4,5] as their increasing body weight may result in sleep apnea, asthma, fractures, poor bone health, idiopathic hypertension, liver inflammation, insulin resistance, type 2 diabetes mellitus (T2DM), hypercortisolism, dyslipidemia, cardiovascular hyperdystrophy, and systemic inflammation.[6,7] Hence, it is very important to identify systemic and less invasive biochemical markers for predisposition of the risk factors contributing to these ailments.[8,9]

Leptin is a peptide hormone secreted by fat cells that helps in body weight regulation through satiety and its dysregulation can lead to congenital deficiency or hyperleptinemia.[10,11] Insulin is an important metabolic hormone primarily secreted by pancreatic islets in response to glucose levels in blood. Both leptin and insulin act through receptors present on the hypothalamic neurons and relay important feedback signals to the central nervous system, which are proportional to peripheral energy stores.[12,13]

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Obesity is considered as a low-grade inflammatory disease resulting in altered inflammatory state of the body, through secretion of adipokines (adipsin, visfatin, interferon-α [INF-α]), proinflammatory cytokines (interleukin 6 [IL-6], tumor necrosis factor (TNF-α), and hepatocytic acute phase proteins like SAP, hepatoglobulin, and C-reactive protein (CRP).[14] The pathogenicity of obesity has been in part associated to the acute and chronic state of inflammation, for example, increased levels of TNF-α lead to insulin resistance in obese and diabetic individuals. Also, CRP, an acute phase protein, have been directly associated with insulin and its resistance in children.[15-17]

Abnormal cortisol has been associated with physiologic, metabolic, or psychosocial stress both directly and indirectly.[18,19] Critical functions of cortisol include the regulation and mobilization of energy[20,21] through selection of substrates among the macronutrients. A few studies have also proposed that cortisol may directly influence food choices and intake through specific receptors in the hypothalamus. Corticotrophin releasing hormone (CRH), leptin, and neuropeptide Y (NPY) have also been reported to be affected by cortisol secretions. It also acts as antiinflammatory agent and potentially contributes to chronic inflammation.[22]

In addition to cardiovascular diseases, recent studies have also associated obesity, with increased amyloidosis.[23,24] For instance, serum amyloid A (SAA) has been investigated in obese and lean subjects and its serum levels were found to be higher in the obese group[25] whereas the potential role of SAP in obesity has not been explored. SAP belongs to protein family of pentraxins, is secreted by hepatocytes and plays key roles in immunity and inflammation. Tissue amyloid deposits due to systemic amyloidosis ubiquitously express SAP bound to amyloid fibrils and hence present themselves more abundantly compared to SAA.[26-28] Hence, SAP can potentially serve as a biomarker and a therapeutic target for obesity and associated amyloidosis.

The objective of this study is to investigate the alterations in serum leptin, insulin, and cortisol in obese children. We also identified the potential association of a novel inflammatory marker, SAP, with obesity in a local population of children.

Subjects and Methods

All the experimental procedures employed in the study were approved by the institutional ethical committee and were carried out in accordance with the principles of Declaration of Helsinki. Informed consents were acquired from the guardian of the children included in the study.

The study included 17 obese children (BMI >95th percentile) from Endocrine Unit of a local Children's Hospital. The subjects were compared to 20 age matched normal children (BMI <80th percentile). Complete medical history, anthropometric measurements (body weight and height), and physical examination were documented after informing the patients and their parents about the study. Patients with Cushing's syndrome, Down syndrome, Autism, among others, were excluded. BMI was calculated using the formula; body weight (BW)/height (m) ^{2}. BMI percentile was estimated using WHO growth charts, and z-score was calculated using WHO AnthroPlus software (Version 3.2.2, WHO, Geneva, Switzerland).[29-31] Nonfasting blood samples (3–4 ml) were drawn between 10 and 11 am in each case for biochemical estimations. Serum was aliquoted and stored at –80°C until used. Serum hormone concentrations were determined in duplicates using commercially available enzyme linked immunosorbent assay (ELISA) kits (leptin: DIAsource ImmunoAssays, Nivelles, Belgium; insulin and cortisol: Monobind Inc, Lake Forest, CA, USA) with an automated enzyme immunoassay (EIA) analyzer (BioRad Laboratories, Hercules, CA, USA). SAP concentrations were measured using Bio-Plex Pro Human Acute Phase Assay Panel (BioRad Laboratories, Hercules, CA, USA). All assays were carried out according to manufacturer's instructions.

The acquired data were analyzed for statistical differences using 2-tailed student's *t* test. Corelation between variables of interest was estimated using Pearson test. *P* value < 0.05 was considered statistically significant. All calculations were carried out using the statistical package for the social sciences software (SPSS, version 19, SPSS, Inc, Chicago, IL, USA).

Results

Mean BMI of nonobese (n = 20, mean age: 4.64 years) and obese children (n = 17, mean age: 7.46 years) was 18.18 and 28.71 kg/m^2, respectively. The mean weight and height of the nonobese subjects was 17.58 kg and 0.97 m compared to 46.31 kg and 1.23 m in the obese group [Table 1].

The mean serum leptin concentration was several folds higher in the obese group as compared to the controls (97.19 ± 14.12 vs 4.06 ± 0.612; $P < 0.05$). Leptin levels ranged from 1.00 to 8.40 (2.80) ng/ml and 24.41 to 235.40 (71.23) ng/ml in nonobese and obese children, respectively. Mean insulin levels in the obese group (21.31 ± 4.52) were significantly ($P < 0.05$) elevated as compared to the nonobese group (3.56 ± 0.43). Insulin levels ranged from 1.62 to 8.60 (3.08) μIU/ml in the lean subjects whereas 1.62 to 75.12 (18.50) μIU/ml in the obese children. Serum cortisol levels ranged between 2.40 and 16.00 (5.60) μg/dl and 3.8 and 18.00 (7.80) μg/dl in nonobese and obese children, respectively. Mean cortisol concentration in nonobese children (6.30 ± 0.82) was not different as compared

Table 1: Physical characteristics of children (0.5-10 years old). Data are expressed as mean±SEM (median)

	Non-obese	Obese	*P*
Weight (kg)	17.58±1.79 (15.75)	46.31±6.13 (45)	<0.05[a]
Height (m)	0.97±0.04 (0.98)	1.23±0.06 (1.30)	<0.05[a]
BMI (kg/m^2)	18.18±1.33 (16.97)	28.71±1.80 (26.63)	<0.05[a]
z-score (BMI-for-age)*	1.37±0.63 (1.19)	4.10±0.48 (3.25)	<0.05[a]

[a]Significantly different from non-obese group (Student's *t*-test); *(WHO AnthroPlus, 2009; Anthro, 2011)

to obese children (7.89 ± 0.73). Mean SAP level was threefold higher in the obese children (46.77 ± 6.43) compared to the control nonobese children (17.89 ± 3.03, $P = 0.00$). SAP in obese children ranged from 2.18 to 100.45 (49.22) mg/ml and 1.08 to 51.01 (15.06) mg/ml in nonobese children [Table 2].

Leptin levels in obese children were highly corelated with BMI ($r = 0.80$; $P < 0.000$). However, no relationship between leptin and BMI was found in nonobese children. In obese group, insulin significantly corelated with BMI ($r = 0.672$; $P = 0.015$), SAP ($r = 0.54$, $P = 0.01$), and leptin ($r = 0.428$; $P = 0.043$). Leptin corelated ($r = 0.481$; $P = 0.025$) with cortisol in the obese group [Figures 1 and 2].

Discussion

In the past decade, obesity and associated serious health problems in children have become increasingly prevalent.[30-32] Extreme obesity in children can lead to life threatening health hazards including metabolic and endocrine disorders.[6] The underlying genetic and physiologic factors leading to early onset obesity are known in only 3–6% of obese population.[33-36] In view of the above, the present study was carried out to evaluate the energy homeostasis-related endocrine and inflammatory markers in a group of children with idiopathic obesity.

Table 2: Mean serum leptin, insulin, cortisol and serum amyloid P concentration in non-obese and obese subjects (0.5-10 year old). Data are expressed as mean±SEM (median)

	Nonobese	Obese	*P*
Leptin (ng/ml)	4.06±0.612 (2.80)	97.19±14.12 (71.23)	<0.05[a]
Insulin (µIU/ml)	3.56±0.43 (3.08)	21.31±4.52 (18.50)	<0.05[a]
Cortisol (µg/dl)	6.30±0.82 (5.60)	7.89±0.73 (7.80)	0.15
SAP (mg/l)	17.89±3.03 (15.06)	46.77±6.43 (49.22)	<0.05[a]

[a]Significantly different from nonobese group (student's *t*-test)

The study subjects included in this investigation had a BMI percentile of greater than 95% compared to 50–80% in the control group. None of the subjects had syndromic obesity and had a normal growth pattern. Age and body height were highly corelated, and the rate of linear growth, as indicated by slope of cross-sectional body growth curves, of obese and control subjects was similar (0.065 vs 0.067, respectively).

The serum concentration of leptin (ng/ml) in all obese children was markedly higher than of the control group (97.19 ± 14.12 vs 4.06 ± 0.612; $P < 0.05$). Increased levels of leptin have also been reported in cases of adult obesity and T2DM and are ascribed to development of leptin resistance. Subjects with pathogenic mutations in the leptin receptor (LepR) or melanocortin 4 receptor (MC4R), are not only severely obese from childhood but also have raised leptin levels. On the other hand, children with congenital leptin deficiency (CLD), though very rare, but are phenotypically similar, have nondetectable or very low serum leptin concentration (<1.0 ng/ml).[37-41] As in all our obese children, leptin levels were found to be several fold-of the normal circulating levels, the presence of extreme obesity in these children due to CLD can be ruled out.

Insulin along with leptin has a major role in regulation of energy homeostasis and appetite.[11] The serum insulin concentration was above the normal values for this age group (21.31 ± 4.52 µIU/ml) and when compared to those of controls (3.56 ± 0.43 µIU/ml; $P < 0.05$). Insulin levels were raised above the normal cutoff values in 70% of obese children whereas leptin levels were raised above normal in all patients. This suggests the possibility of development of insulin

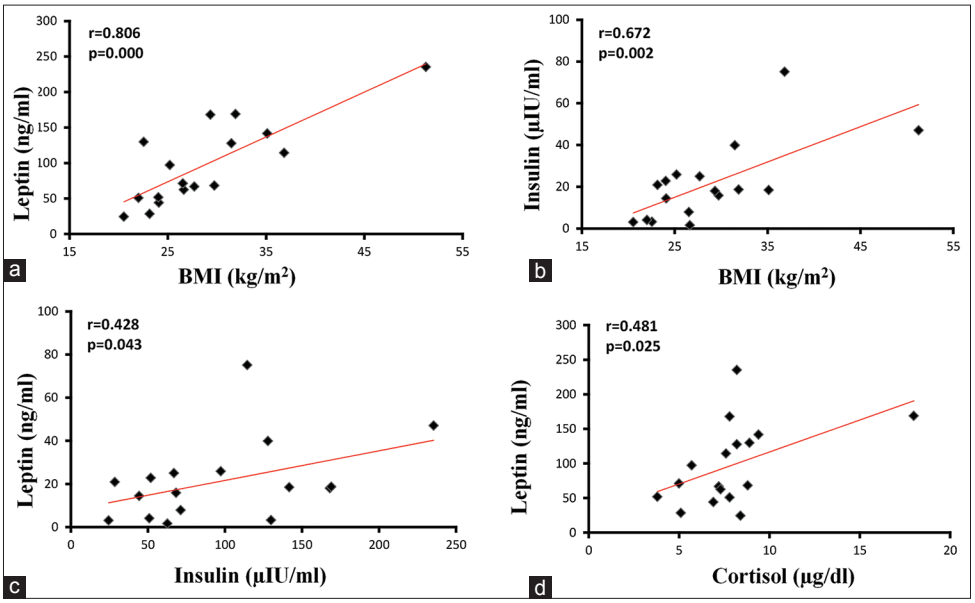

Figure 1: Pearson's corelation test showed significant corelation between (a)leptin and BMI, (b)insulin and BMI, (c)leptin and insulin, (d)leptin and cortisol values in obese group of children

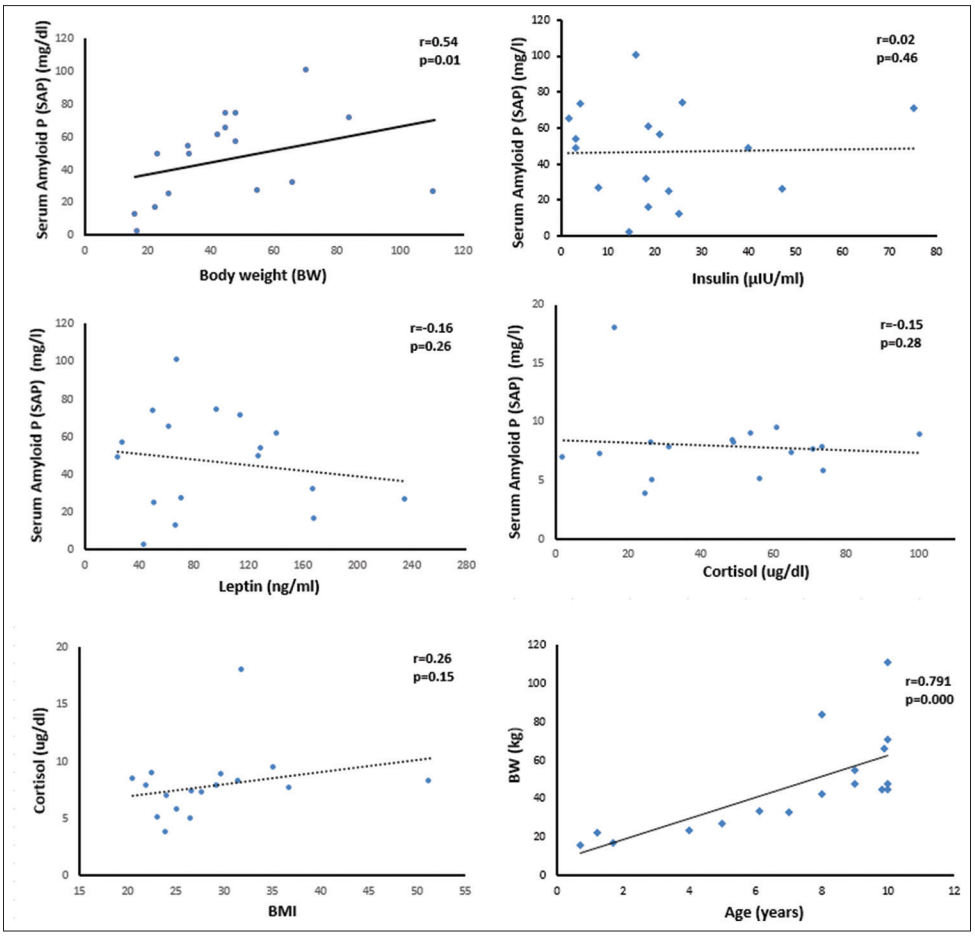

Figure 2: Scatter plot graphs showing Pearson's corelation of SAP with BW, insulin, leptin, cortisol, and corelation between cortisol, BMI and BW, age

resistance because of raised leptin levels and increased fat mass in obese children. In most forms of childhood and adult obesity, hyperleptinemia has been associated to hyperinsulinemia even before any other signs of T2DM make their appearance.[3,4] In the present study, leptin levels were not corelated with age, but a robust corelation was found between insulin and age of children. This suggests increased insulin resistance with advancement of age and it may be hypothesized that the obese children in our group are at a high risk of developing T2DM.

Increased fat mass results in production of IL-6 and TNF-α by the adipocytes, which in turn trigger the process of inflammation by stimulating acute phase proteins.[42-44] The acute phase reactants like SAP and CRP are indicators of chronic low-grade inflammation [45–48]. In the present study, we reported a marked increase in SAP levels of obese subjects (46.77 ± 6.43 mg/ml) as compared to the nonobese (17.89 ± 3.03 mg/ml) children. In 2008, Gómez-Ambrosi *et al*. reported increased levels of SAA in obese children and adolescents.[7] Here, we have introduced SAP as an acute phase protein associated with idiopathic childhood obesity. SAP levels were significantly corelated with body weight and hence adiposity. However, there was no corelation between SAP and endocrine markers. The difference in the concentration of SAP between the two groups may be attributed to the contribution of adipocyte-derived cytokines, which are secreted in direct proportion to the fat content. Moreover, a recent study also identified SAP gene present at the locus associated with increased plasma glucose levels and increased body weight.[8] Insulin and SAP were significantly corelated in obese patients indicating a possible role of insulin in amyloidosis. However, no apparent signs of skin amyloidosis were observed in these patients. Insulin resistance in the overweight and obese may also add to increased levels of SAP.[9]

Previous studies indicated that peripheral cortisol levels are higher in obese children as compared to normal subjects.[49,50] We did not find any significant difference in serum levels of cortisol between both groups, although mean concentration in obese was higher than the lean. As hyperphagia was reported in only 64% of the obese children included in this study, there is a strong suggestion that excessive weight in some of these subjects was put on due to an abnormal fat synthesis rather than due to increased food intake, and lack of satiety.

Ethnicity contributes to the prevalence of obesity in children due to differences in the gene pool, cultural backgrounds, and life styles. Various studies have been conducted in populations of different origin and numerous variations among them have been reported.[51,52] This study only focused on children of a

local population and hence ethnicity-based variations were not ruled out.

Conclusion

Conclusively, the present data indicated an acute risk of metabolic dysfunction, CVD and inflammation in the obese children, with progression of age. We propose SAP as a novel protein associated with obesity-related inflammation in children. It is expected that such findings in obese children will prompt further studies to probe into the causative factors, both genetic and physiological, leading to childhood obesity.

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Conflicts of interest

There are no conflicts of interest.

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