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Research Article

Correlation between hormones, cytokines, MMP2 and α-fetoprotein among hyperlipidemic obese children

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ABSTRACT

Childhood obesity and its complications have become a very serious public health concern and threat. Obesity confers increased risk for cardiovascular diseases as a result of accumulation of visceral fat, which alters metabolism and insulin sensitivity. The objective of this study is to identify, correlate and validate certain biomarkers for detecting obesity in Egyptian children. Thirty patients and twenty healthy volunteers as control were enrolled in this study. All subjects were prepubertal (7-12 years) and of both sex. Anthropometric measurements; body mass index (BMI), waist and hip circumferences and abdominal skin fold thickness (ASFT) were taken into consideration. Leptin, matrix metalloproteinase -2 (MMP2), interlukin-10 (IL-10), alpha-fetoprotein (AFP) and tumor necrosis factor-α (TNF-α) were evaluated. Lipid profile; cholesterol, high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C) and triglycerides (TG) were also tested. The work was extended to measure liver function enzymes; aspartate and alanine aminotransferases (ASL&ALT). The results revealed significant elevation ($p<0.05$) of all the selected parameters in obese children as compared to healthy individuals, while HDL-C showed significant decrease. The pearson's correlation test (2-tailed) revealed significant ($p<0.01$) positive correlation between BMI and the selected parameters. In conclusion, these biomarkers succeeded for detecting children who may be at risk of being overweight or obese. Strong association between obesity and the selected biomarkers were observed.

Keywords: obesity; children; biomarkers, lipid profile, liver function

INTRODUCTION

Obesity is a chronic condition, prevalent in both developed and developing countries and affects both adults and children[1]. Globally, 22 million children under 5 years were overweight, with more than 75% of overweight and obese children living in low and middle income countries[2]. Childhood overweight and obesity is primarily a result of energy imbalance, whereby ingested calories exceed energy expended[3]. The biophysiological dynamics of this imbalance are very complicated and not yet fully understood[3]. In addition, the multifaceted causes of obesity make it difficult to fully determine the origin of an individual's obesity, particularly in children. Lifetime eating and physical activity habits are commonly developed in childhood. Recognizing this, it is important to acquire insight into adiposity and energy balance regulation during this period of development.

Obesity can affect the child's physical, emotional and social maturity and ultimately can lead to serious health concerns and early mortality, whether or not the child remains obese as an adult[4-6]. The rise in childhood obesity dictates that early intervention becomes a priority due to the significant acute and future chronic health consequences. Identification and validation of novel biomarkers for detecting children who may be at risk of being overweight or obese is of paramount importance.

One of these biomarkers is leptin. It is a hormone that is primarily synthesized and produced by adipocytes and it is identified as a key factor in maintaining energy balance and overall body weight composition[7]. Leptin may play a role in the complications of obesity. It was determined as the most sensitive adipokine marker for predicting the accumulation of cardiovascular risk factors and the presence of metabolic syndrome[8]. Elevated serum leptin may also be an indicator of fatty liver disease [9].

Tumor necrosis factor- α (TNF-α) is a proinflammatory cytokine, which is mainly produced by macrophages and lymphocytes, to a less extend by adipose tissue. The reason for increased circulating TNF- α level observed in obese people is not thought to be associated with over-production in the adipose tissue. It is hypothesized that systemic effects of leptin or other adipokines may induce TNF- α secretion from macrophages and lymphocytes[10]. The two clinically important effects of TNF- α in obese children and adults are insulin resistance and endothelial inflammatory changes. Increased plasma levels of the soluble fraction of tumor necrosis factor receptor 2 have been found to be associated with insulin resistance in healthy volunteers[11]. TNF-alpha activates the transcription factor nuclear factor-κB, which leads to a series of inflammatory changes in vascular tissue. These inflammatory changes of the vascular tissue have been

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Table 1: Anthropometric measurements of the selected groups.

Parameters	Control	Obese
Weight (kg)	35.70±11.25[b]	60.33±13.53[a]
Height (cm)	125.20±9.49[b]	135.58±8.93[a]
BMI (kg/m²)	22.88±4.51[b]	32.96±4.70[a]
Waist circumference (cm)	47.20±6.34[b]	80.56±5.76[a]
hip circumference (cm)	62.40±8.96[b]	89.45±6.78[a]
WHR	0.76±0.21[b]	0.91±0.16[a]
ASFT (cm)	6.78±1.22[b]	17.56±2.12[a]

- *All values are mean± SD of 20 individuals in control group and 30 subjects in obese group.*
- *BMI: Body Mass Index.*
- *WHR: Waist/hip ratio.*
- *ASFT: abdominal skin fold thickness.*
- *Superscript letters are significant difference between groups.*
- *Significance level is at P ≤0.0001.*

Table 2: Leptin, IL-10, MMP2, TNF-α and AFP in healthy and obese children

Parameters	Healthy children	Obese children	*P*<
Leptin (ng/ml)	15.83±2.88[b]	35.63±2.74[a]	0.00001
IL-10 (Pg/ml)	139.03±5.59[a]	172.40±4.88[b]	0.00001
MMP2 (ng/ml)	0.88±0.06[b]	1.43±0.38[a]	0.00001
TNF-α (Pg/ml)	111.53±7.05[b]	185.23±4.28[a]	0.00001
AFP (ng/ml)	2.65±1.78[b]	5.43±0.41[a]	0.00001

- *All values are mean± SD of 20 individuals in control group and 30 subjects in obese group.*
- *Superscript letters are significant difference between groups.*
- *Significance level is at P ≤0.05*

Table 3: Lipid profile in healthy and obese children.

Parameters	Healthy children	Obese children	*P*<
Cholesterol (mg/dL)	158.96±5.79[b]	326.20±16.14[a]	0.00001
TG (mg/dL)	117.26±4.95[b]	238.16±4.75[a]	0.00001
HDL-C (mg/dL)	61.60±3.84[b]	55.40±3.06[a]	0.0001
LDL-C (mg/dL)	84.37±5.19[b]	173.46±4.45[a]	0.00001

- *All values are mean± SD of 20 individuals in control group and 30 subjects in obese group.*
- *Superscript letters are significant difference between groups.*
- *Significance level is at P ≤0.05.*

shown to result in endothelial dysfunction and hypertension[12].

IL-10 is a cytokine secreted by activated macrophages and lymphocytes. Low production capacity of IL-10 has been demonstrated in obesity, metabolic syndrome, and type 2 diabetes13. IL-10 has insulin-sensitizing, anti-inflammatory and endothelial protective properties by antagonizing TNF- α and IL-6[12,13].

Several lines of evidence suggest a possible functional role of matrix metalloproteinase -2 (MMP2) in obesity. The main role of MMP2 is in the degradation of type IV collagen, the major structural component of basement membranes. However, the enzyme also has activity toward a spectrum of functional molecules including growth factor-binding proteins and growth factor

Table 4: Liver function enzymes in healthy and obese children.

Parameters	Healthy children	Obese children	*P*<
AST (IU/L)	19.70± 2.03[b]	24.73 ± 2.19[a]	0.00001
ALT (IU/L)	13.96± 2.02[b]	20.90± 1.58[a]	0.00001

- *All values are mean± SD of 20 individuals in control group and 30 subjects in obese gr oup.*
- *Superscript letters are significant difference between groups.*
- *Significance level is at P ≤0.05*

receptors, which are known to be involved in obesity. For example, MMP2 can cleave insulin-like growth factor-binding proteins and release insulin-like growth factors[14]. It has also been suggested that MMP2 plays an important role in adipose tissue development [15,16]. Furthermore, tissue degradation by MMP2 is pivotal to inflammation[17], and obesity is associated with low grade inflammation.

Alpha feto-protein (AFP) is a major plasma protein produced by the yolk sac and the liver during fetal development that is thought to be the fetal form of serum [18]. At birth, normal infants have AFP levels 4 or more orders of magnitude above the normal range that decreases to a normal range over the first year of life[19]. Alpha feto-protein is considered as one of tumor markers[20]. Higher risk of developing cancers is observed with being overweight or obese[21]. A recent case-control study observed a significant increase risk of hepatocellualr carcinoma among obese or diabetic patients without viral hepatitis[22].

The aim of the present study is to identify, correlate and validate certain biomarkers for detecting children who may be at risk of being overweight or obese.

METHODS

Subjects: This study included thirty patients and twenty healthy volunteers as control. All subjects were prepubertal (7-12 years) and of both sex (60% males and 40% females).The patients were randomly selected from Abu Elreash Children Hospital – Cairo University (2011-2012).

The subjects were divided into two groups. Group 1 comprised of twenty healthy prepubertal children of comparable age and sex and normal body mass index. Group 2 comprised of thirty obese prepubertal children with body mass index above +2SDS for age and diagnosed as exogenous obesity.

Exclusion criteria: Children with secondary obesity, obesity due to corticosteroid therapy or hypothyroidism and children with drug therapy interfere with lipid profile were excluded from the study.

Ethics and Examinations: Informed consents were taken from the parents of the selected groups according to guideline of Medical Ethical Committee of National Research Centre, Dokki, Giza. All the studied groups were subjected to full history report, clinical and general examination (weight, height, body mass index, waist and hip circumference and abdominal skin fold thickness) as well as systemic examination.

Samples collection: Three ml of venous blood were drawn from each subject after fasting for 12 hours. Sample was taken in a sterile tube; sera were obtained immediately after clotting of samples, collected in sterile tubes and stored at -80^{o}C until used.

Biochemical determinations: MMP-2 was estimated by the invitrogen MMP2 human kit (cat. no: KHC3081, Paisley, UK). It is a solid phase sandwich enzyme linked-immuno-sorbent assay (ELISA), where a highly purified antibody specific for MMP2 has been coated onto the wells of the microtiter strips provided. Standards of known MMP2 content, controls, and unknown samples are pipetted into the coated wells. A biotinylated secondary antibody, streptavidin-peroxidase (enzyme) and substrate solution were added. The intensity of the developed colour is directly proportional to the concentration of MMP-2 present in the original specimen at 450 nm.

TNF-α (cat. no: KHC3014, Paisley, UK) and IL-10 (cat. no: KHC0101, Paisley, UK) were carried out by using the invitrogen human TNF-α and IL-10 kits. It is a solid phase sandwich enzyme linked-immuno-sorbent assay (ELISA). The assay was carried out as the same method described above. The intensity of the developed colour is directly proportional to the concentrations of TNF-α or IL-10 present at 450 nm.

Alpha-fetoprotein (AFP) (cat. no: 0500, San Antonio, USA) and leptin (cat. no: 0010, San Antonio, USA) kits were estimated by the alpha diagnostic international human alpha-fetoprotein kit. It is a solid phase sandwich enzyme linked-immuno-sorbent assay (ELISA). The analytical procedures were carried out as the same method described above. The intensity of the developed colour is directly proportional to the concentration of AFP or leptin present at 450 nm.

Lipid profile was estimated through measuring cholesterol[23], high-density lipoprotein cholesterol (HDL-C)[24], low-density lipoprotein cholesterol (LDL-C)[25] and triglycerides[26].

Aspartate and alanine amintransferases were measured by the method of Gella et al.[27], where the transfer of amino group from aspartate or alanine formed oxalacetate or pyruvate, respectively and the developed colour was measured at 520 nm.

STATISTICAL ANALYSIS

Data was expressed as mean ± SD of 20 control individuals and 30 obese children. Analysis of data was carried out by one way analysis of variance (ANOVA), CoStat Software Computer Program (USA) accompanied with least significance difference between group at

Table 5. Pearson's correlations test between biomarkers in obese children.

Parameters	BMI	Leptin	IL-10	MMP-2	TNF-α	AFP	Cholesterol	TG	HDL-C	LDL-C	AST	ALT
BMI	1	0.835(**)	0.727(**)	0.667(**)	0.857(**)	0.638(**)	0.808(**)	0.903(**)	0.404(**)	0.837(**)	0.530(**)	0.769(**)
Leptin		1	0.844(**)	0.684(**)	0.945(**)	0.742(**)	0.906(**)	0.872(**)	0.487(**)	0.967(**)	0.582(**)	0.857(**)
IL-10			1	0.633(**)	-0.892(**)	0.689(**)	0.851(**)	0.798(**)	0.466(**)	0.887(**)	0.542(**)	0.795(**)
MMP-2				1	0.702(**)	0.563(**)	0.749(**)	0.647(**)	0.350(**)	0.718(**)	0.329(*)	0.623(**)
TNF-α					1	0.742(**)	0.929(**)	0.914(**)	0.468(**)	0.983(**)	0.584(**)	0.902(**)
AFP						1	0.685(**)	0.608(**)	0.431(**)	0.729(**)	0.415(**)	0.704(**)
Cholesterol							1	0.877(**)	0.399(**)	0.944(**)	0.456(**)	0.856(**)
TG								1	0.494(**)	0.911(**)	0.550(**)	0.824(**)
HDL-C									1	0.468(**)	0.284(*)	0.409(**)
LDL-C										1	0.582(**)	0.877(**)
AST											1	0.472(**)
ALT											.	1

- *Values are presented as coefficient factors.*
- *** Correlation is significant at the 0.01 level (2-tailed).*
- **Correlation is significant at the 0.05 level (2-tailed).*

$p<0.05$. The Pearson's correlation coefficient was used for measuring the linear correlation between variables (2-tailed).

RESULTS AND DISCUSSION

Concerning weight, height, body mass index (BMI), waist circumference, hip circumference, WHR and abdominal skin fold thickness, the results revealed significant increase in obese children as compared with healthy group. It was increased by 68.99, 8.29, 44.05, 70.67, 43.34, 19.73 and 158.99%, respectively (Table 1 and Fig.1a). This was in agreement with Nassef and Hamed[28] who found the same observations among obese and obese asthmatic children. Agirbasli et al.[29] added that BMI was a better predictor of waist- hip ratio and skin folds in obese children. However, Al-Attas et al.[30] postulated that waist circumference is more powerful in assessing metabolic disorders over other anthropometric values. LingHui et al.[31] stated that comparing with BMI, waist circumference contributed more to the development of dyslipidemia, fatty liver and non alcoholic fatty liver disease.

Leptin regulates body weight by signaling information to the brain regarding the availability of energy stored as fat; this negative feedback loop is disrupted in most obese individuals and results in a state known as leptin resistance[32]. Antunes et al.[33] and Watanabe et al.[34] added that greater BMI and total body fat in children were significantly associated with increased serum leptin levels. These observations are in accordance with our results through the observed increase in serum leptin level by 125.07% in obese children as compared with the control group (Table 2 and Fig.1b). Positive significant correlation was observed between BMI and leptin level is also recorded at $p<0.01$ (Table 5). Focusing on the liver, leptin is a potent profibrogenic cytokine and thus plays a key role in the progression of cirrhosis which is a precancerous condition of HCC[35]. Watanabe et al.[34] added that BMI, total body fat and leptin level were considered as significant risk factors for the recurrence of HCC. Elevated serum leptin levels were also observed in overweight children with or without fatty liver[36]. This finding indicates that increased serum leptin concentration, which might link obesity with liver carcinogenesis, is a preferable and useful biomarker for screening high-risk groups for the recurrence of HCC.

As obesity is consider being a risk factor for HCC[37], an elevation level of serum α-fetoprotein was recorded in patients with HCC that developed as a result of chronic viral hepatitis[38]. In patients with NAFLD higher level of α-fetoprotein was also observed[39]. In the present study α-fetoprotein recorded significant increase in obese children by 104.90% (Table 2 and Fig.1b). A significant positive correlation between BMI and α-fetoprotein was also noticed (Table 5). Therefore, we consider that α-fetoprotein may be used as a biomarker for predicting obesity contributing HCC or NAFLD. Conversely, Hashimoto et al.[40] recorded normal serum α-fetoprotein levels in patients with HCC and NAFLD and therefore they postulated that serum α-fetoprotein levels cannot be used to consistently rule out the presence of HCC in patients with obesity, or NAFLD.

IL-10 is a pleiotropic cytokine mainly derived from T-cells and macrophages in bone marrow, but increased visceral fat may be an alternative source for circulating IL-10 in obese subjects[41]. The mechanism of the link between abdominal obesity and serum IL-10 level is demonstrated to the presence of macrophages. The

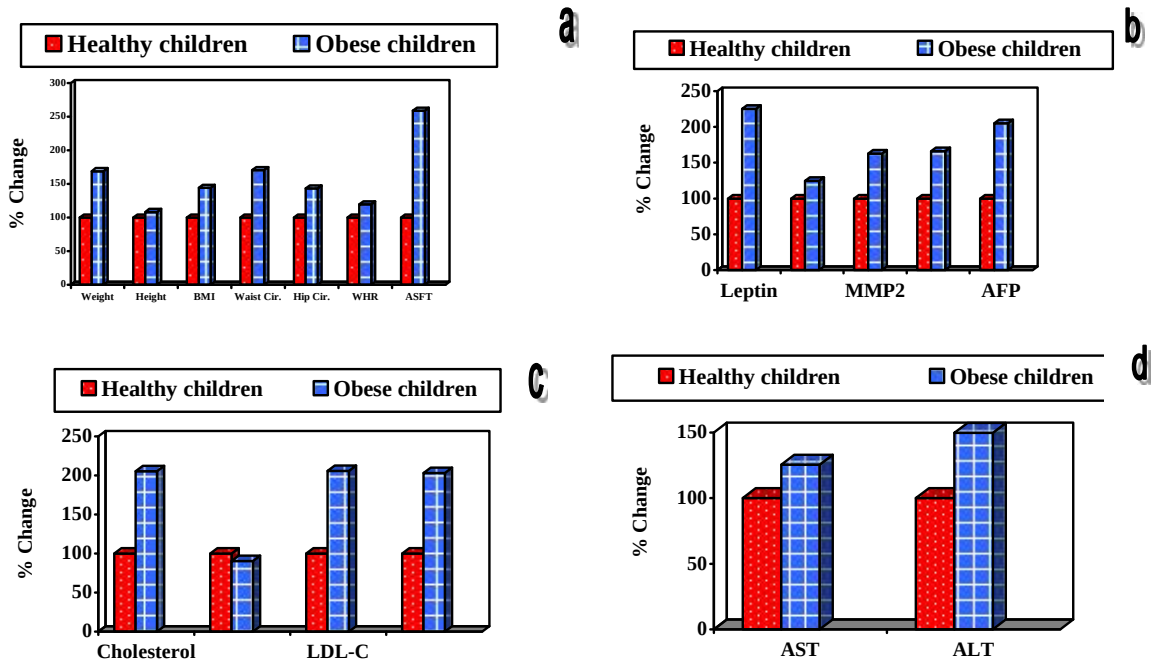

Fig.1Percentage changes of anthropometric parameters (a), selected biomarkers (b), lipid profile (c) and liver function enzymes (d) in normal and obese children.

finding of increased numbers of macrophages infiltrating the visceral fat tissue in obese individuals suggests that adipose tissue itself is a source and site of inflammation[42]. Therefore, the present results are consistent with prior reports of elevated inflammatory status in obese groups[43]. This elevation in inflammatory status was recoded by the significant increase of IL-10 by 24.53% and TNF-α by 66.08% in obese children (Table 2 and Fig. 1b). In a study including 47 obese children TNF-alpha and C-reactive protein levels were found to be significantly higher in obese children compared with the control group[44]. The elevated levels of IL-6 and TNF-alpha in children with atherosclerosis risk factors, particularly obesity, may confirm the presence of inflammatory process in early phases of atherosclerosis[13]. In line with our results, several authors reported a positive correlation between obesity and several inflammatory markers, including TNF-α and IL-10 in obese children[13,45].

MMP2 is an endopeptidase that degrades the basement membrane surrounding adipocytes and thus may facilitate hypertrophic development of adipocytes and formation of adipocyte clusters[46]. Dubois et al.[47] recorded an induction of MMP2 transcription in the adipose tissue of obese subjects. In line with the results of Han et al.[48], MMP2 is found to be associated significantly with overweight/obese subjects suggesting that MMP2 may be involved in the regulation of weight balance, where MMP2 recorded significant increase by 62.50% (Table 2 and Fig.1b) and positively correlate with the BMI (Table 5).

Regarding to the lipid profile and in parallel with the observations of Lai et al.[49], Miller et al.[50] and Nassef and Hamed[28], higher levels of cholesterol (105.20%), TG (103.10%) and LDL-C (105.59%) were recorded in obese children than control group. Contradictory, low level of HDL-C by 10.06% was noticed in obese children (Table3 and Fig.1c). LingHui et al.[31] also notice lower level of HDL-C among children contributing non alcoholic fatty liver disease associated with obesity. Positive correlation was also observed between BMI and lipid profile in obese individuals (Table 5).

Concerning liver functions enzymes, AST and ALT showed significant increase in obese children by 25.53 and 49.71% for AST and ALT, respectively (Table 4 and Fig.1d). Kelishadi et al.[51] showed that ALT and AST levels were significantly associated with age among children. In pediatric patients the increased of ALT level is related to excess weight in both gender [52,53] and the relative changes in BMI is related to the onset of fatty liver[54]. This association with BMI was significant for AST only in boys[51]. We found the same association between BMI and the levels of AST and ALT at $p<0.01$ (Table 5).

In conclusion, leptin, MMP2, IL-10, TNF-α and AFP served as potential biomarkers for detecting obesity in children. Strong association between body mass index and the selected biomarkers were observed. For clinical uses of theses biomarkers, further studies are needed due to the quite limited number of cases in this study; the small sample size makes it difficult to validate the utility of serum markers.

Contributorship: All authors were contributed equally in this work.

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