Pentraxin-3, Angiopoietin-Like Protein-3, Angiopoietin-Like Protein-4 and Angiopoietin-Like Protein-8 Levels in Morbid Obese Children

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ABSTRACT

A low-grade inflammatory state accompanies obesity and metabolic syndrome (MetS), which are risk factors for cardiovascular diseases (CVD) in children's future lives. The aim of this study is to investigate possible associations of pentraxin 3 (PTX3), a promising new marker for CVDs, with angiopoietin-like proteins (ANGPTL) in morbid obese (MO) children and their potential uses in the prediagnosis of MetS. Thirty normal weight (NW) and 50 MO prepubertal children without MetS symptoms, a total of 80, participated in the study. Using percentile tables for age and gender recommended by WHO, children whose percentiles were between 15-85 and above 99 were included in NW and MO groups, respectively. Anthropometric measurements, BMI and HOMA-IR values were recorded. Serum PTX3, ANGPTL3, ANGPTL4, ANGPTL8/betatrophin levels were determined by enzyme-linked immunosorbent assay (ELISA). Statistical analyses were performed by SPSS. $p < 0.05$ was the degree of statistical significance. There were no differences between PTX3, ANGPTL3 and ANGPTL4 levels of groups. ANGPTL8/betatrophin levels were significantly higher in MO children ($p \leq 0.001$). A significant correlation was observed between PTX3 and ANGPTL4 ($r = 0.297$, $p \leq 0.05$) in the same group. When adjusted for MetS parameters including HDL-Chol, correlations

between ANGPTL3-ANGPTL8/betatrophin (r = 0.327, $p \leq 0.05$) and PTX3-ANGPTL4 (r = 0.337, $p \leq 0.05$) were detected. When HDL-Chol was replaced with TRG these associations were disappeared. This study is the first to perform PTX3, ANGPTL3, ANGPTL4, ANGPTL8/betatrophin measurements in prepubertal NW and MO children. Upon evaluation of dyslipidemia in MetS, adjustment for MetS parameters pointed out HDL-Chol as the more valuable parameter than TRG, based upon the existence of ANGPTL3-ANGPTL8/betatrophin as well as PTX3-ANGPTL4 associations.

Keywords: Angiopoietin-like protein 3; Angiopoietin-like protein 4; Angiopoietin-like protein 8; Betatrophin; Childhood obesity; Pentraxin-3

1 INTRODUCTION

Obesity is a chronic clinical state associated with low-grade inflammation [1]. Many proinflammatory factors including C-reactive protein (CRP) contribute to the process. In the same family, PTX3, being detected mainly in atherosclerotic lesions, is suggested as a promising new marker for inflammatory cardiovascular diseases (CVD) such as atherosclerosis, acute coronary syndrome, chronic heart failure as well as MetS [2-9].

ANGPTL are structurally similar molecules to angiopoietins. Aside from their angiogenic effects, they also participate in several physiologic and pathologic processes. They have specific roles in lipid, carbohydrate, energy metabolisms, hematopoiesis, cancer and inflammation [10-12].

The potential roles of ANGPTL at the crossroads of lipoprotein, fatty acid and glucose metabolisms make these molecules important for the cardiovascular risk [13]. Particularly, ANGPTL3, ANGPTL4 and ANGPTL8/betatrophin are involved in the regulation of lipid and lipoprotein metabolisms and provide new hope for MetS [10-17].

ANGPTL4 represses the foam cell formation and may be protective against lipid accumulation [18]. ANGPTL3, by way of its effect on insulin, may lead to decreases in ANGPTL4 [10]. ANGPTL8/betatrophin contributes to the inhibition of lipoprotein lipase (LPL) by participating in the cleavage of ANGPTL3 and thus, activating it [16, 17]. ANGPTL8/betatrophin act together with ANGPTL3 to coordinate the trafficking of triacylglycerols (TRG) to tissues [19, 20].

Atherosclerosis is the most common cause of CVD, such as myocardial infarction and stroke. Inflammatory mechanisms are known to play a central role in the pathogenesis and progression of atherosclerosis. PTX3 is a modulator molecule of complement system, inflammatory response, angiogenesis, and vascular remodeling, which are critical determinants of CVD. Compared to most inflammatory markers PTX3 displays greater association with coronary artery disease. PTX3 may play an important role in maintaining plaque stability by mediating phagocytosis of late apoptotic macrophages. PTX3 affects lipid metabolism in human macrophages through increasing the uptake of oxidized low-density lipoprotein cholesterol and inhibiting cholesterol efflux pathways such as peroxisome proliferator-activated receptor-γ [21, 22].

ANGPTL-3, 4 and 8 act as important regulators of plasma lipoprotein levels by inhibiting LPL. ANGPTL4 plays a central role in lipid metabolism and pathophysiology of atherosclerosis. ANGPTL4 can destroy the endothelium and may lead to the initiation of atherosclerosis. The tagged single nucleotide polymorphisms and high serum ANGPTL4 levels are associated with large artery atherosclerotic stroke. MicroRNA-134 accelerates atherogenesis by promoting lipid accumulation and proinflammatory cytokine secretion via the ANGPTL4/LPL pathway. ANGPTL3 cooperates with ANGPTL8 to inhibit LPL. Inactivation of ANGPTL3 reduces plasma TRG and suppresses atherosclerosis. In human clinical trials, antisense oligonucleotide and monoclonal antibody-based inactivation of ANGPTL3 for the treatment of dyslipidaemia and atherosclerosis are being investigated. Oligonucleotides targeting ANGPTL3 retarded the atherosclerosis progression and reduced atherogenic lipoprotein levels [23, 27].

Obesity and MetS may be considered as risk factors for CVD. The aim of this study is to determine PTX3 and ANGPTL3, ANGPTL4, ANGPTL8/betatrophin concentrations in prepubertal NW and MO children for the investigation of their potential uses in the prediagnosis of MO children, who may develop MetS in the future. This study, for the first time, questions if MetS components observed in prepubertal MO children without MetS symptoms may be associated with the signs leading to CVD during their future lives.

2 MATERIALS AND METHODS

Thirty NW and fifty MO children without MetS symptoms participated in the study to compare the ANGPTL3, ANGPTL4, ANGPTL8/betatrophin and PTX3 levels in blood serum. All the children were in prepubertal stage (Tanner stage 1). Two groups were matched from the age and female/male ratio points of view. The study protocol was approved by Namik Kemal University Medical Faculty Ethics

Committee (NKU/TF/GOKAEK/2014/68/10/03-86). The study was supported by Namik Kemal University Scientific Research Fund Projects Coordination Unit Project No: NKUBAP.00.20.TU.14.01. All procedures were performed in accordance with the accepted international standards and the declaration of Helsinki. Written informed consent forms were signed by the parents of all participant children prior to their involvement in the study. Children were classified as NW and MO (15th-85th and ≥ 99th percentiles, respectively) based upon body mass index (BMI)-for-age and -gender tables recommended by World Health Organization and approved by Ministry of Health.

The basic anthropometric measurements (weight, height, waist circumference, hip circumference, head circumference and neck circumference) were taken and recorded. BMI values of children were calculated.

Blood samples were drawn after an overnight fasting. Venous blood was stored at -80°C following centrifugation until the analyses were performed. The complete blood counts, routine biochemical tests, HbA1c, lipid profile, glucose and insulin analyses were performed. Insulin resistance (IR) was calculated as Homeostatic Model Assessment for IR (HOMA-IR) index using the following formula: [insulin(μIU/ml) * fasting blood glucose (mg/dl)]/ [22.5/0.0555] .

Serum concentrations of the parameters were determined using human PTX3, human ANGPTL3, human ANGPTL4, human ANGPTL8/betatrophin (total) ELISA kits (AVISCERA BIOSCIENCE, Inc. Santa Clara, CA, USA). Inter- and intra-assay coefficient of variation were 8-12 % and 4-6 % for PTX3, 8-12 % and 4-8 % for ANGPTL3, 8-10 % and 4-6 % for ANGPTL4, 8-10 % and 4-6 % for ANGPTL8/betatrophin.

SPSS Version 20 was used for statistical analyses. Data were expressed in mean ± SD. Kolmogorov-Smirnov and Shapiro Wilk tests were used to test the normality for the distribution of the data. The differences between the groups were determined by Student's t-test or Mann-Whitney U test based upon the parametric or non-parametric distribution. The correlation analyses were performed using bivariate and partial correlation tests. p values < 0.05 were accepted as statistically significant.

3 RESULTS

This study was performed on eighty prepubertal children aged between 5.8-9.1 years. The study population was divided into two groups; NW healthy children (Group 1, n = 30) and MO children without MetS (Group 2, n = 50). No statistically significant differences were noted between ages as well as female/male ratios of Group 1 (F/M = 1.1, age = 7.5 ± 1.0 years) and Group 2 (F/M = 1.1, age = 7.6 ± 0.9 years) ($p \geq 0.05$).

Anthropometric measurements of children in groups were summarized in Table 1.

Table 1. Body mass index, waist circumference, hip circumference, head circumference and neck circumference values of children (mean ± SD)

Parameter	Group 1 (NW)	Group 2 (MO)	P-value
BMI (kg/m2)	15.2 ± 0.8	25.1 ± 3.1	≤ 0.001
Waist circumference (cm)	53.0 ± 3.8	79.7 ± 8.1	≤ 0.001
Hip circumference (cm)	59.5 ± 4.3	84.5 ±7.7	≤ 0.001
Head circumference (cm)	49.7 ± 1.6	52.0 ± 2.5	≤ 0.001
Neck circumference (cm)	25.4 ± 1.3	30.4 ± 2.9	≤ 0.001
Waist-to-Hip ratio	0.89 ± 0.04	0.95 ± 0.06	≤ 0.001
Head-to-Neck ratio	1.96 ± 0.09	1.72 ± 0.12	≤ 0.001

Statistically significant increases were found in MO children compared to NW healthy children in terms of their BMI, waist circumference, hip circumference, head circumference, neck circumference values and anthropometric ratios ($p \leq 0.001$).Blood pressure values and lipid profile of children were tabulated in Table 2.

Table 2. Systolic pressure (SP), diastolic pressure (DP), total cholesterol (TChol), triacylglycerol (TRG), low density lipoprotein cholesterol (LDL-Chol), high density lipoprotein cholesterol (HDL-Chol) values of children in groups (mean ± SD)

Parameter	Group 1 (NW)	Group 2 (MO)	P-value
SP (mm Hg)	102.6 ± 8.0	107.5 ± 9.2	≤ 0.05
DP (mm Hg)	66.7 ± 5.1	70.2 ± 9.2	NS
TChol (mg/dl)	163.2 ± 29.0	167.8 ± 27.5	NS
TRG (mg/dl)	60.1 ± 20.9	78.1 ± 35.3	≤ 0.05
LDL-Chol (mg/dl)	98.8 ± 26.5	101.8 ± 25.3	NS
HDL-Chol (mg/dl)	53.8 ± 11.8	50.5 ± 7.7	NS

In MO group, systolic blood pressure and TRG values were significantly higher than those in NW group ($p \leq 0.05$). Diastolic blood pressure, Total cholestereol (Tchol), LDL-Chol and HDL-Chol values did not differ between the groups.

Values for parameters related to IR were listed in Table 3.

Table 3. Blood glucose, insulin, HbA1c, HOMA-IR values (mean ± SD)

Parameter	Group 1 (NW)	Group 2 (MO)	P-value
Glucose (mg/dl)	86.1 ± 8.3	86.2 ± 5.6	NS
Insulin (μU/l)	3.3 ± 3.5	9.7 ± 7.6	≤0.001
HbA1c (%)	5.4 ± 0.8	5.7 ±1.7	NS
HOMA-IR	0.73 ± 0.80	2.11 ± 1.75	≤0.001

There were no statistically significant differences between groups in terms of fasting blood glucose and glycated hemoglobin levels. Significantly increased insulin and IR index, HOMA-IR values were observed in MO children ($p \leq 0.001$).

Findings related to PTX3 and ANGPTL concentrations of children were summarized in Table 4.

Table 4. Pentraxin 3 (PTX3), angiopoietin-like protein 3 (ANGPTL3), angiopoietin-like protein 4 (ANGPTL4), angiopoietin-like protein 8 (ANGPTL8) concentrations of children in groups (median)

Parameter	Group 1 (NW)	Group 2 (MO)	P
PTX3 (pg/ml) median	2601	2403	NS
ANGPTL3 (pg/ml) median	1813	1667	NS
ANGPTL4 (ng/ml) median	15.1	17.1	NS
ANGPTL8 (ng/ml) median	2.6	4.0	≤ 0.001

Decreasing PTX3 and ANGPTL 3 values as well as increasing ANGPTL4 values were recorded in MO group, however, differences were not statistically significant.

The only parameter, which exhibits significantly increased values in MO children was ANGPTL8/ betatrophin ($p \leq 0.001$).

In MO children, a correlation between PTX3 and ANGPTL4 was found (r = 0.297, $p \leq 0.05$). In the same group, a much stronger correlation was observed between ANGPTL3 and HOMA-IR (r = 0.396, $p \leq 0.01$) (Fig.1).

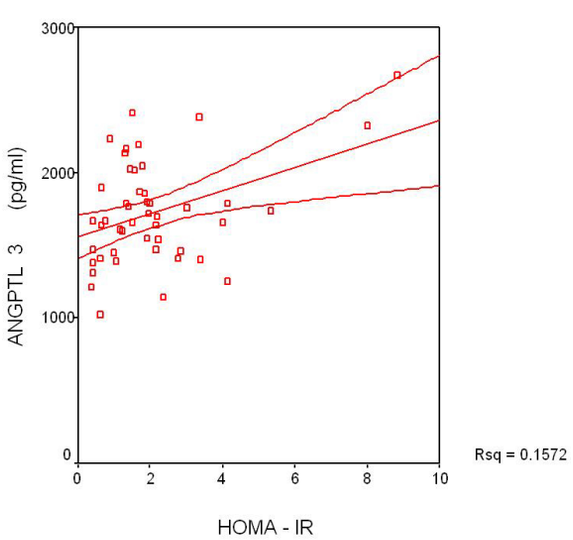

Fig. 1 Correlation between angiopoietin-like protein 3 and homeostatic model assessment of IR in MO group.

Controlling for waist circumference, systolic and diastolic blood pressures, fasting blood glucose, fasting blood insulin and HDL-Chol levels, positive correlations between ANGPTL3 and ANGPTL8/ betatrophin (r = 0.327, $p \leq 0.05$) as well as PTX3 and ANGPTL4 (r = 0.337, $p \leq 0.05$) were calculated in MO group (Fig. 2, Fig. 3).

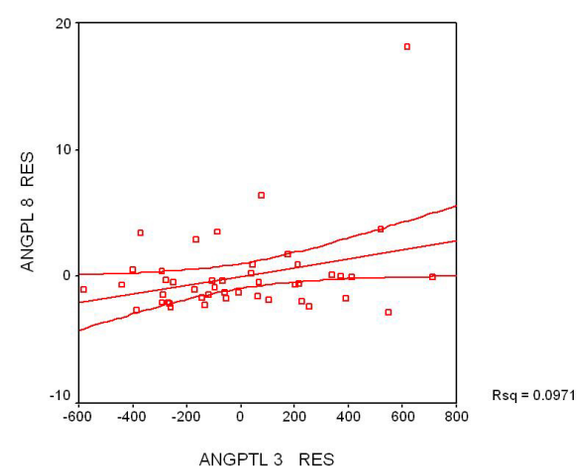

Fig. 2 Partial correlation between angiopoietin-like protein 8 and angiopoietin-like protein 3 in MO group.

None of these correlations were observed in NW group. In both NW and MO groups, when partial correlations were performed by replacing HDL-Chol with TRG, no correlations were detected.

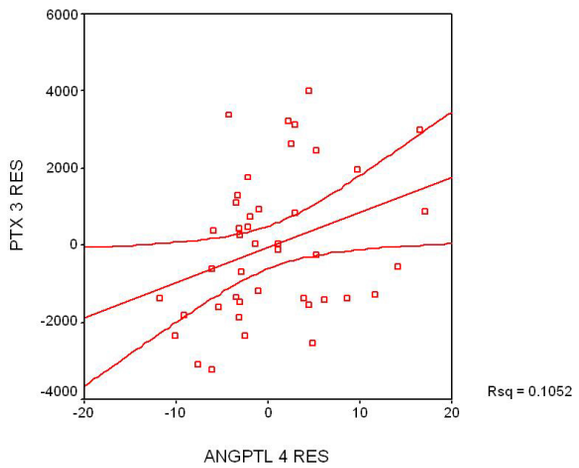

Fig. 3 Partial correlation between pentraxin-3 and angiopoietin-like protein 4 in MO group.

4 DISCUSSION

The association of obesity with critical chronic diseases including CVD is well-confirmed. PTX3 may act as a cardioprotective biomarker by decreasing inflammation in cardiovascular bed [2, 28]. However, it may also contribute to atherogenic process. These contradictory findings make PTX3 an important parameter to be investigated. Negative correlations between PTX3 and IR as well as TRG were reported in some studies [3-5, 29] however, such a relation couldn't be found in the others [4, 9] including our study.

There are reports explaining that PTX3 was reduced during obesity [30-32]. Our results agree with the results obtained in these studies. Plasma PTX3 showed a nonsignificant trend towards lower levels in MO group as in one of the above studies [30].

No correlations were found between this parameter and IR, most probably because our study population is composed of prepubertal children. PTX3 can not be described as a cardiovascular biomarker in this period.

While ANGPTL4 plays antilipotoxic role, it is also associated with inflammatory alterations and involved in human atherosclerosis. Increased ANGPTL4 levels were measured in patients with MetS and type 2 diabetes mellitus (T2DM) [18, 33-36]. In our study, ANGPTL4 levels did not differ between groups. It exhibits a pattern similar to that of PTX3. However, correlation analyses have shown that a hidden relation exists between ANGPTL4 and PTX3 in the very early stages of life.

Our study is the first childhood case-control study in this field. One of the remarkable findings was the detection of the correlation between PTX3 and ANGPTL4 in MO group ($r = 0.297$, $p \leq 0.05$). Controlling for waist circumference, systolic blood pressure, diastolic blood pressure, fasting blood glucose, insulin and HDL-Chol also resulted in even a more strengthened correlation ($r = 0.337$, $p \leq 0.05$). Considering studies reporting that ANGPTL4 represses foam cell formation and is protective against lipid accumulation to reduce atherosclerosis [18, 33], this consistent correlation between PTX3 and ANGPTL4 gives support to studies defending that PTX3 is also a cardioprotective parameter.

ANGPTL3 and ANGPTL4 are molecules with common features. ANGPTL3 is closely associated with arterial wall thickness, possibly it may have a potential role in atherosclerosis pathogenesis [37, 38]. In our study, concentrations of ANGPTL3 as well as ANGPTL4 did not differ between NW and MO groups as in the case of PTX3. These findings may be due to the characteristic features of the prepubertal study population.

In a recent study performed on a population, which is composed of both overweight and NW children, a significant association was noted between ANGPTL3 levels and HOMA-IR [39]. In our study, a positive strong correlation was noted between ANGPTL3 and HOMA-IR ($r = 0.396$; $p \leq 0.01$) in MO children. This correlation was not observed in children with normal BMI. This finding is important from this point of view. ANGPTL3 might act as a potential biomarker of IR in MO children. This data supports the reports emphasizing the role of ANGPTL3 in atherosclerosis.

Conflicting results reported for ANGPTL8/betatrophin concentrations have led to some controversy. In obese children and adolescents, ANGPTL8/betatrophin levels were reported as lower or did not differ compared to those observed in lean children [40-42].

In adults, ANGPTL8/betatrophin was introduced as an emerging potential player in dyslipidemia with strong association with HDL-Chol and a potential therapeutic tool for the treatment of dyslipidemia [43]. Significantly increased ANGPTL8/betatrophin levels were reported in T2DM with CVDs, T1DM, gestational DM, obesity and MetS [15, 44-49]. In an experimental study, ANGPTL8/betatrophin overexpression led to dramatically increased serum TRG levels [50]. It is also reported that overexpressed ANGPTL8/betatrophin increased serum TRG levels

in an ANGPTL3-dependent manner [20]. These results agree with our findings. In MO group, we have found significantly increased concentrations for this parameter ($p \leq 0.001$). This profile emphasized the fact that ANGPTL8/betatrophin is an important parameter because it exhibits an increase starting from the early periods of life in MO group.

Adjusting values for MetS parameters, partial correlation analysis emphasized a correlation between ANGPTL3 and ANGPTL8/betatrophin. This supports the notion that ANGPTL8/betatrophin and ANGPTL3 function in the same pathway, that is, ANGPTL8/betatrophin inhibits LPL in an ANGPTL3-dependent manner [51]. This finding may be the indicator of ANGPTL8/betatrophin's role in promoting cleavage of ANGPTL3. It may also point out that such correlation emphasizes the importance of ANGPTL8/betatrophin in MO children as a prediagnostic parameter for MetS. This suggests that ANGPTL8/betatrophin may be a therapeutic target for lowering serum TRG levels and may be useful in predicting newly-onset MetS and its progression in clinical settings in adults [52, 53].

Considering the limitations of this study, we have kept many parameters (e.g. age, female-to-male ratio, pubertal status, ethnic origin, living area, nutritional habits, socioeconomic status of the families, medications, and sleep durations) under control for the study groups, however, still some unidentified factors that may influence study parameters other than the above may exist.

It is well-known that these three ANGPTL work in a collaborative manner however, the exact mechanisms are not clearly defined yet [16]. In a study, ANGPTL8/betatrophin knock-out mice did not exhibit reduced levels of the ANGPTL3 N-terminal domain [54]. Another study reported that in mice with ANGPTL8/betatrophin overexpression, circulating ANGPTL3 levels were reduced [20]. The results of our study are consistent with the recently proposed ANGPTL3-4-8 model [51]. In this model, ANGPTL3 and ANGPTL8/betatrophin form a complex translocated into the capillaries to inhibit LPL, resulting in lowered levels of circulating ANGPTL3.

Common features of ANGPTL3 and ANGPTL4, their potential roles in atherosclerosis pathophysiology, cleavage of ANGPTL3 through ANGPTL8/betatrophin have shown that these proteins work in a coordinated manner in the regulation of lipid metabolism. Controlling for MetS parameters including HDL-Chol in MO group, correlations observed between ANGPTL3 and ANGPTL8/betatrophin as well as PTX3 and ANGPTL4 could not be detected when HDL-Chol was replaced by TRG. This showed that upon evaluation of dyslipidemia in children with MetS, HDL-Chol concentrations are more valuable than TRG concentrations. This study is the first from the evaluation of these four parameters together in MO children in comparison with NW children point of view. This study provides a basis for further future studies, which will be performed on the matter.

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CONFLICTS OF INTERET

The authors declare that they have no conflict of interest.

LIST OF ABBREVIATIONS

ANGPTL: Angiopoietin-like proteins

BMI: Body mass index

CRP: C-reactive protein

CVD: Cardiovascular diseases

DP: Diastolic pressure

ELISA: Enzyme-linked immunosorbent assay

HDL-Chol: High density lipoprotein cholesterol

HOMA-IR: Homeostatic model assessment for insulin resistance

IR: Insulin resistance

LDL-Chol: Low density lipoprotein cholesterol

LPL: Lipoprotein lipase

MetS: Metabolic syndrome

MO: Morbid obese

NW: Normal weight

PTX3: Pentraxin 3

SP: Systolic pressure

SPSS: Statistical package for social sciences

TChol: Total cholesterol

TRG: Triacylglycerol

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