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General Cardiology

Pentraxin 3 as a Marker for Cardiovascular Disease Risk in Overweight and Obese Children

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Background: Pentraxin 3 is an inflammatory mediator that may be associated with subclinical inflammation in atherosclerosis and cardiovascular diseases. This study investigated the predictive value of pentraxin 3 as an inflammatory biomarker in overweight and obese children.

Methods: Participants were categorized into three groups: overweight (n = 35), obese (n = 35), and healthy controls (n = 70). Cardiovascular parameters and pentraxin 3 were measured in all participants.

Results: The mean pentraxin 3 level was significantly higher in the overweight (10.23 ± 4.42 ng/ml) and obese (11.20 ± 4.12 ng/ml) groups compared to the control (7.93 ± 4.35 ng/ml) group. Pentraxin 3 was significantly correlated with carotid intima media thickness and epicardial adipose tissue thickness in the overweight group. In the linear regression analysis, body mass index and systolic blood pressure were significantly correlated with pentraxin 3 levels in the overweight group, whereas only heart rate was correlated with pentraxin 3 levels in the obese group. In receiver operating characteristic analysis, the optimal cut-off value for pentraxin 3 in the obese group was 9.321 ng/mL, with sensitivity and specificity of 77.1% and 74.3%, respectively [area under the curve (AUC) = 0.764, $p < 0.001$]. In the overweight group, the optimal cut-off value of pentraxin 3 was 9.263 ng/mL, with sensitivity and specificity of 62.9% and 72.9%, respectively (AUC = 0.687, $p = 0.002$).

Conclusion: Pentraxin 3 may be an early marker of cardiovascular risk in overweight children. Future longitudinal studies are needed to evaluate the predictive value of pentraxin 3 for cardiovascular disease.

Key Words: Atherosclerosis • Children • Obesity • Overweight • Pentraxin 3

INTRODUCTION

Obesity is one of the most common nutritional disorders in childhood. Coinciding with the increasing prevalence of childhood obesity is the increasing frequency and earlier onset of obesity-related complications. While obesity is an independent risk factor for cardiovascular disease, the presence of comorbidities including hypertension, dyslipidemia, impaired glucose metabolism and atherosclerosis further increases an individual's risk.[1,2] Atherosclerosis is the primary cause of cardiovascular disease and is the leading cause of death worldwide. A growing body of evidence suggests that the atherosclerotic process begins in early childhood, which marks an important opportunity for early diagnosis and treatment among high-risk children.[3] Although the underlying mechanism remains unclear, obesity is associated with systemic inflammation, resulting from the secretion of hormones, cytokines and chemokines from adipose tissue. Moreover, individuals with chronic inflammation have increased rates of cardiovascular morbidity.[4,5] These findings suggest that biomarkers of systemic inflammation may affect an individual's cardiovascular disease risk. Pentraxins are one of the mediators secreted during systemic inflammation.[6] They are multimeric proteins that are synthesized by several dif-

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ferent genes and exist in different protein sizes (i.e., short and long arms). C-reactive protein (CRP) is a well-known short pentraxin, and CRP levels increase during the acute phase response of inflammation. Pentraxin 3 (PTX3) was one of the first identified proteins in the long pentraxin family, and PTX3 levels have been shown to increase in response to various inflammatory stimuli including tumor necrosis factor, interleukin 1β and lipopolysaccharide.[7] Recent research has shown that PTX3 is predominantly secreted from macrophages and vascular endothelial cells, whereas CRP is secreted from hepatocytes. These findings suggest that PTX3 may be a more useful cardiovascular risk marker.[8,9] This study aimed to evaluate the relationship between PTX3 levels and cardiovascular disease risk in overweight and obese children.

MATERIALS AND METHOD

Study design and population

A case control study design was used. In total, 140 children were included in this study and divided into overweight (n = 35), obese (n = 35), and control (n = 70) groups. The study protocol was approved by Sakarya University Local Ethical Committee. All participants provided informed assent and written informed consent was obtained from all parents or caretakers of the children. Body mass index (BMI) values were calculated as weight (kg) divided by height squared (m^2). The BMI reference curves established by Bundak et al.[10] for Turkish children were used for the study group allocation. Given the age range of the study participants, BMI standard deviation scores (SDS) were used. Patients with BMI SDS of 1-2 were considered to be overweight and those with BMI SDS > 2 were considered to be obese.[11] Morbidly obese children, in whom morbid obesity may be accompanied by many adverse conditions such as hyperlipidemia, insulin resistance, diabetes mellitus, and metabolic syndrome, were not included in the study. In addition, participants with secondary and genetic causes of obesity, and those with a history of glucose intolerance, dyslipidemia, hypertension, chronic disease or early cardiovascular disease were excluded from the study. Each participant's blood pressure (BP) was measured three times on their right arm after ten minutes of rest. The right arm was preferred for repeated BP measurements due to consistency and the possibility of aortic coarctation, which may result in false (low) readings in the left arm in comparison with standard tables.[12-14] All measurements were performed using the same automated oscillometric device (53000, Welch Allyn, New York, USA). The average of the three BP measurements was used in the analysis. Heart rate was measured three times during blood pressure measurements, and the mean value was used.

Imaging techniques

All ultrasounds were performed using a Philips iE33 ultrasound machine with 5 MHz and 8 MHz phase transducers (Philips Ultrasound, Bothell, USA). Examinations were performed in the left lateral position using standard parasternal long-axis and apical four-chamber views. Two-dimensional targeted M-mode echocardiographic tracings were obtained in the parasternal long-axis view. All measurements were completed by the same specialist. Left ventricular (LV) mass was automatically calculated by the device using the current standardized formula, and height was used for allometric scaling, where LV mass was divided by height raised to the allometric exponent of 2.7 (LVMI = LV mass/height$^{2.7}$).[15,16] Epicardial adipose tissue thickness (EATT) was measured from the free wall of the right ventricle in the echo-free area of the pericardium.[17] The carotid intima-media thickness (CIMT) was measured from the posterior wall of the left common carotid artery, approximately 10 mm proximal to the bifurcation using a 7.5-mm linear ultrasound probe. Measurements from each site were recorded and the mean values were calculated.[18]

Detection of PTX3

Commercial enzyme-linked immunosorbent assay (ELISA) kits were used for the measurement of PTX3 (Uscn Life Science, Houston, TX, USA). The sandwich-ELISA principle was used as per the manufacturer's protocol. Micro ELISA plates were pre-coated with Human PTX3/TSG-14 specific antibodies, and standards and samples were added to the micro ELISA plate wells. Biotinylated detection antibody specific for Human PTX3/TSG-14 and Avidin-Horseradish Peroxidase (HRP) conjugate were added to each well and incu-

bated. Free components were washed away, and substrate solution was added to each well. Plate wells that contained Human PTX3/TSG-14, biotinylated detection antibody and Avidin-HRP conjugate appeared blue in color. The enzyme substrate reaction was terminated by the addition of stop solution, resulting in yellow coloration. The optical density (OD) was measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The OD values are proportional to the concentration of Human PTX3/TSG-14. Sample concentrations of Human PTX3/TSG-14 were calculated by comparing the OD values of the samples to the standard curve.

Statistical analysis

All statistical analyses were conducted using the Statistical Package for Social Sciences (SPSS) software package (version 21.0, SPSS® Inc., Chicago, Illinois, USA). Descriptive statistics were reported as frequencies, percentiles, means ± standard deviations, and minimum and maximum values. One-sample Kolmogorov-Smirnov tests were used to assess the normality assumptions of continuous variables. Pearson's correlation coefficient tests were used for variables with normal distribution and Spearman's rank correlation coefficient tests were used for non-normally distributed variables. Pearson's chi-squared tests were used to compare categorical variables. Variance analysis was performed using one-way ANOVA for continuous data showing normal distribution, and post-hoc binary comparisons were performed using Tukey's test for data with equal variance and Tamhane tests for data with unequal variance. Linear regression analysis was used to assess the effect of continuous variables on PTX3 levels. Receiver operating characteristic (ROC) curve analysis was used to assess the sensitivity and specificity of PTX3 levels in predicting cardiovascular risk in the obese and overweight participants. A p-value < 0.05 was considered to be statistically significant. Variance analyses of non-normally distributed variables were performed using the Kruskal-Wallis test. The three study groups were compared using Bonferroni correction and the Mann-Whitney U test for binary comparisons at the significance level of $p < 0.017$ (0.05/3).

RESULTS

Demographic and health characteristics of the three study groups are summarized in Table 1. The mean age was 11.96 ± 1.68 years in the overweight group, 11.11 ± 2.10 years in the obese group and 11.76 ± 1.59 years in the control group. There were no significant differences in age or gender among the three groups ($p > 0.05$). The mean BMI SDSs were significantly higher in the obese (2.23 ± 0.17) and overweight (1.72 ± 0.24) groups compared to the control group (0.15 ± 0.50) (both $p < 0.001$).

Table 1. Demographic characteristics, vital signs, BMI SDS, echocardiographic data and pentraxin 3 values for each study group

	Control group (n = 70)	Overweight group (n = 35)	Obese group (n = 35)	p
Age (years)	11.76 ± 1.59	11.96 ± 1.68	11.11 ± 2.10	0.103
Gender (F/M)	36/34	21/14	14/21	0.243
BMI SDS	0.15 ± 0.50$^{\#,\dagger}$	1.72 ± 0.24*,†	2.23 ± 0.17*$^{,\#}$	< 0.001
SBP (mm Hg)	116.57 ± 9.12$^{\#,\dagger}$	127.6 ± 6.81*	127.97 ± 7.32*	< 0.001
DBP (mm Hg)	74.94 ± 7.88$^{\#,\dagger}$	79.14 ± 6.06*	80.74 ± 8.80*	< 0.001
HR (beat/minute)	80.51 ± 9.86†	84.26 ± 9.31	85.03 ± 6.82*	0.027
CIMT (mm)	0.43 ± 0.10$^{\#,\dagger}$	0.63 ± 0.09*,†	0.70 ± 0.05*$^{,\#}$	< 0.001
EATT (mm)	4.46 ± 0.12$^{\#,\dagger}$	5.17 ± 0.30*,†	5.46 ± 0.17*$^{,\#}$	< 0.001
LVMI (g/m$^{2.7}$)	28.71 ± 6.40$^{\#,\dagger}$	36.15 ± 9.19*,†	45.14 ± 11.51*$^{,\#}$	< 0.001
Pentraxin 3 (ng/mL)	7.93 ± 4.35$^{\#,\dagger}$	10.23 ± 4.42*	11.20 ± 4.12*	< 0.001

* Statistically significant compared to control; $^{\#}$ Statistically significant compared to overweight; † Statistically significant compared to obese.

Genders are reported as total number of participants and all other parameters are expressed as means ± standard deviations. One-way ANOVA and chi-squared tests were performed and p-values < 0.05 were considered statistically significant.

BMI SDS, body mass index standard deviation score; CIMT, carotid intima media thickness; DBP, diastolic blood pressure; EATT, epicardial adipose tissue thickness; HR, heart rate; LVMI, left ventricular mass index; SBP, systolic blood pressure.

The obese and overweight groups had significantly increased systolic blood pressure (SBP) and diastolic blood pressure (DBP) compared to the control group. No significant difference was observed in blood pressure between the obese and overweight groups (Table 1). A statically significant difference was observed in heart rate (HR) between the obese and control groups (p = 0.027). EATT, CIMT and LVMI were significantly different between the three groups (p < 0.001). The mean PTX3 level was significantly higher in the overweight (10.23 ± 4.42 ng/ml) and obese (11.20 ± 4.12 ng/ml) groups compared to the control (7.93 ± 4.35 ng/ml) group. PTX3 was significantly correlated with BMI, HR, SBP, CIMT and EATT in the overweight group and with BMI, HR and LVMI in the obese group (Table 2). Linear regression analyses indicated that PTX3 was independently affected by BMI and SBP in the overweight group, and HR in the obese group (Table 3). In the ROC analysis, the optimal cut-off value for PTX3 in the obese group was 9.321 ng/mL, with sensitivity and specificity of 77.1% and 74.3%, respectively [area under the curve (AUC) = 0.764, p < 0.001] (Figure 1). In the overweight group, the optimal cut-off value for PTX3 was 9.263 ng/mL, yielding sensitivity and specificity of 62.9% and 72.9%, respectively (AUC = 0.687, p = 0.002) (Figure 2). Since AUC provides an estimate of the diagnostic value, PTX3 levels provided greater predictive value in the obese group compared to the overweight group (AUC of 0.764 versus 0.687, respectively).

Table 2. Results from the correlation analyses between pentraxin 3 levels and vital signs, BMI SDS, and echocardiographic data in the overweight and obese groups

	Overweight group pentraxin 3 level		Obese group pentraxin 3 level*	
	R	p	rho	p
BMI	0.482	0.003	0.331	0.052
HR*	0.348	0.041	0.374	0.027
SBP	0.469	0.004	0.059	0.737
DBP	-0.139	0.424	0.238	0.168
CIMT	0.403	0.016	-0.035	0.840
EATT	0.401	0.017	-0.032	0.853
LVMI	0.235	0.174	0.373	0.027

Pearson's and * Spearman's correlation tests were performed and p-values < 0.05 were considered statistically significant. BMI SDS, body mass index standard deviation score; CIMT, carotid intima media thickness; DBP, diastolic blood pressure; EATT, epicardial adipose tissue thickness; HR, heart rate; LVMI, left ventricular mass index; SBP, systolic blood pressure.

DISCUSSION

Obesity has become a serious health problem in recent years. Many studies have investigated the association between obesity and cardiovascular risk. Biomarkers such as inflammatory markers (high sensitive CRP, serum amyloid A, procalcitonin), cytokines [interleukin 6 (IL-6) ,tumor necrosis factor alpha (TNF-α), monocyte chemotactic protein-1], and adipokines (leptin, adiponectin, resistin, omentin, fibroblast growth factor 21) have been widely investigated. However, the clinical application of any of these biomarkers has yet to be clarified.[19,20]

To the best of our knowledge, this is the first study to assess PTX3 levels in children, and to show that PTX3 levels were significantly higher in overweight and obese children compared to healthy controls. The ROC analysis indicated that the optimal cut-off value for PTX3 levels in the obese group was 9.321 ng/mL, yielding 77.1% sensitivity and 74.3% specificity, and the optimal cut-off value for PTX3 levels in the overweight group was 9.263 ng/mL, with 62.9% sensitivity and 72.9% specificity. Several studies have investigated the relationship between PTX3 and obesity in adults and identified that PTX3 is produced by vascular cells in response to inflammatory signals,[21] and evaluated the role of PTX3 in the pathology of atherosclerotic lesions.[22] Although PTX3 is widely accepted as a biomarker in the inflammatory response,

Table 3. Multiple linear regression analysis results outlining the effect of independent variables on pentraxin 3 levels

	Coefficients β	p-value	95.0% confidence interval for β
Overweight group			
BMI SDS	6.498	0.032	0.609-12.388
SBP (mm Hg)	0.217	0.042	0.009-0.424
Obese group			
HR (beat/minute)	0.237	0.020	0.040-0.433

Body mass index standard deviation score (BMI SDS), heart rate (HR) and systolic blood pressure (SBP) values significantly affected pentraxin 3 levels in the linear regression analyses.

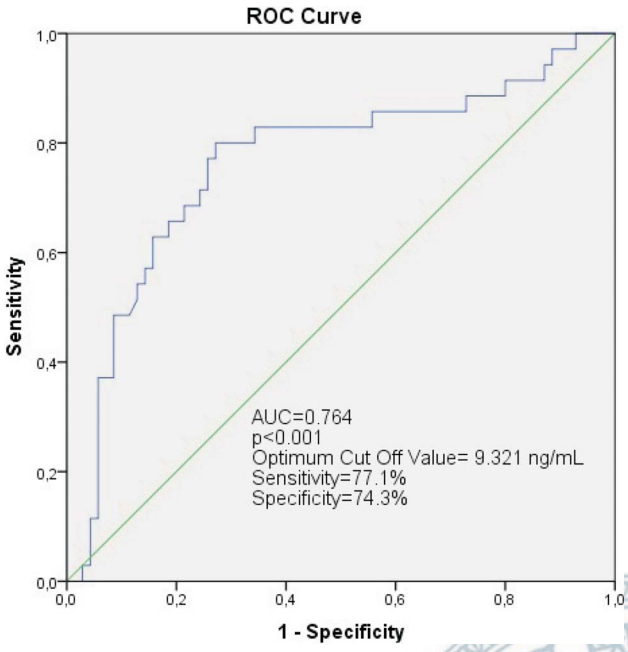

Figure 1. *Receiver operator characteristic curve analysis of the pentraxin 3 optimal cut-off value in the obese group. AUC, area under the curve.*

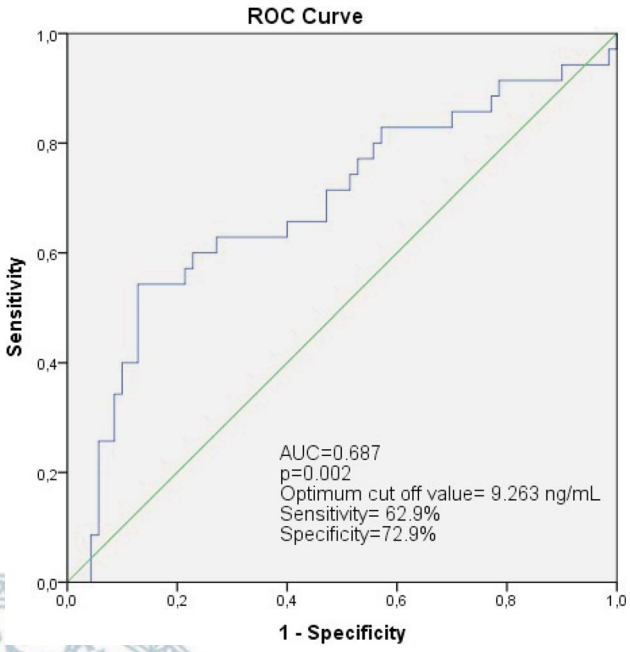

Figure 2. *Receiver operator characteristic curve analysis of the pentraxin 3 optimal cut-off value in the overweight group. AUC, area under the curve.*

whether PTX3 mediates the inflammatory response remains unclear. PTX3 deficiency was found to be associated with increased atherosclerosis and macrophage deposition in atherosclerotic lesions in apolipoprotein E-deficient mice, and the authors suggested that PTX3 had cardioprotective effects as an acute phase protein in atherosclerosis.[23] Another study investigating atherosclerosis and subclinical inflammation in obesity found that PTX3 levels were elevated in the obese men compared to controls;[24] however, the study was limited by a small number of participants. In a larger trial, PTX3 levels were increased in normal weight participants compared to overweight and obese participants, with PTX3 levels being negatively correlated with BMI. The authors concluded that low PTX3 levels contributed to chronic inflammation in overweight and obese individuals. Despite these findings, little is known about the underlying mechanisms of PTX3 in atherosclerosis.[25] In our study, PTX3 levels were higher in the overweight and obese groups than in the controls, but there was no significant difference between the overweight and obese groups. This may suggest that PTX3 starts to increase in the overweight period, which is the initial stage of obesity. These findings may be attributed to the subclinical inflammation associated with overweight and obesity and the secretion of PTX3 from vascular cells secondary to the inflammatory response.[26] However, in the transition from overweight to obesity, during which obesity was progressing, no significant difference was found between the two groups. This suggests that other factor may affect PTX3 levels during the obese period. In addition, this study found that EATT and CIMT were higher in the overweight and obese groups than in the control group, and PTX3 was positively correlated with CIMT and EATT in the overweight group, but not in the obese group. Previous studies have identified EATT and CIMT as important biomarkers for subclinical atherosclerosis.[27,28] In our study, the increases in EATT and CIMT in the overweight and obese groups compared to the control group support initiation of the atherosclerotic process in both groups. However, the fact that PTX3 correlated only with the overweight group suggests that there are metabolic differences between both periods. It is possible that subclinical inflammation started during the overweight period and became more severe than during the obese period, and then gradually became more stable in obesity. Further studies are needed in this regard. In this study, systolic and diastolic blood pressure values were significantly higher in the overweight group compared to the control group, with PTX3 being positively correlated with

SBP in the overweight group. The linear regression model found that SBP independently affected PTX3 levels in the overweight group. Several observational studies have assessed the relationships between blood pressure and cardiovascular mortality.[29] Although the relationship between obesity and hypertension in children and adults has been well-established in the literature,[30,31] the mechanism by which obesity affects blood pressure is unclear.[32,33] According to our findings, SBP and PTX3 values may be used as predictive markers for cardiovascular risk in overweight children. Moreover, PTX3 was positively correlated with HR in the obese group, with HR independently affecting PTX3 levels in the obese group in a linear regression model. Previous studies have shown that childhood obesity results in autonomic nervous system imbalance, characterized by reduced parasympathetic modulation.[34,35] Our study suggests that childhood obesity is characterized by reduced parasympathetic activity, resulting in sympathetic activity dominance. These results are consistent with other studies that showed childhood obesity was associated with cardiac autonomic dysregulation.[36]

CONCLUSION

In conclusion, overweight and obese children were at an increased risk for early atherosclerosis symptoms, despite having normal ranges of hemodynamic values. These findings suggest that serum PTX3 levels may be used to assess cardiovascular risk in overweight children, enabling early interventions and preventing cardiovascular diseases.

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P.D. designed the study and prepared the manuscript. B.E. performed the data collection and statistical analyses. All authors reviewed the manuscript.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

ETHICAL STANDARDS

The authors assert that all study procedures comply with the ethical standards of the relevant national guidelines on human experimentation in Turkey and adhere to the Helsinki Declaration of 1975, as revised in 2008.

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