RESEARCH PAPER

Risk Factors for Cardiovascular Disease in Obese Children

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Objective: To study the prevalence of cardiovascular risk factors in pediatric obesity. **Methods:** 50 obese children (age 5-17y) and 50 apparently healthy non-obese children (body mass index of over 95th percentile and between 5th to 95th percentiles, respectively) using Centre for Disease Control growth charts were included. Fasting blood sugar, lipid profile, insulin, homeostasis model assessment of insulin resistance, uric acid, fibrinogen, lipoprotein (*a*), homocysteine, malondialdehyde, ferric reducing ability of plasma and nitric oxide were measured. **Results:** Insulin, insulin resistance, triglycerides, uric acid, fibrinogen, malondialdehyde, ferric reducing ability of plasma and nitric oxide were significantly higher (P <0.001) in obese children. Body mass index showed significant positive correlation with insulin r=0.519, P<0.001; insulin resistance r =0.479, P<0.001; uric acid r= 0.289, P=0.005; fibrinogen r=0.461, P<0.001; and nitric oxide r=0.235, P=0.012. **Conclusion:** Pediatric obesity is associated with dyslipidemia, oxidative stress, insulin resistance and endothelial dysfunction, which are cardiovascular risk factors and components of metabolic syndrome. These children must be targeted for lifestyle and dietary modification.

Keywords: *Dyslipidemia, Endothelial dysfunction, Insulin resistance, Oxidative stress.*

Childhood obesity is a global phenomenon affecting all socio-economic groups, irrespective of age, sex or ethnicity [1]. Many of these children have risk factors for later cardiovascular disease (CVD), and early signs of atherosclerosis [2]. Childhood obesity tends to track to adulthood, and thus represents an early beginning of a potentially lethal pathologic process. The traditional cardiovascular risk factors, namely overweight/obesity, diabetes, hypertension and dyslipidemia may not account for all CVD-related deaths. Novel biochemical markers such as lipoprotein (*a*) (Lp(a)), uric acid, fibrinogen and homocysteineare increasingly being used to determine CVD related morbidity and mortality [3]. We planned this study to evaluate the prevalence of cardiovascular risk factors, oxidative stress markers as well as nitric oxide (NO) levels that influence endothelial function in pediatric obesity.

METHODS

Children (age 5-17y) attending the Pediatric endocrinology clinic at Sri Venkateshwara Institute of Medical Science, Tirupati (AP), India with body mass index (BMI) of over 95th percentile for their age and sex using Centre for Disease Control (CDC) growth charts 2000 [4] were included into the study as obese cases. We excluded those with hypothyroidism, Cushing's syndrome, type 1 diabetes mellitus, obesity syndromes, renal and liver diseases, and active infection. Non-obese children BMI between 5th to 95th percentile of the hospital faculty, staff and the neighborhood were taken as controls. The study was approved by Institutional Ethics Committee. Written informed consent was obtained from the guardians/parents of the participants after full explanation of the study protocol.

Anthropometric measurements were performed with the subject wearing minimal clothing, with no shoes and socks, with feet kept together, arms to the side, legs straight and shoulders relaxed, and looking straight ahead. A right-angled headboard and a measuring tape were used to measure the height. An electronic scale was used to measure the weight. BMI was calculated as weight in kilograms divided by the square of height in meters. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were recorded in the right arm of the relaxed, seated child. For Laboratory analysis, 6 mL of blood sample was collected from all the children 2 mL of the blood was transferred to ethylene diamino tetra acetic acid (EDTA) anticoagulant containing tube for fibrinogen estimation, 2 mL was transferred into sodium fluoride/potassium oxalate anticoagulant containing tube

for fasting blood sugar (FBS), estimation and 2 mL was transferred to plain vial for the estimation of lipid profile, uric acid, Lp (a), homocysteine and insulin. FBS, total cholesterol (TC), (Aspen Laboratories Pvt. Ltd., Delhi, India), triglycerides (TGL) (Futura system SRL), high density lipoprotein cholesterol (HDL-C) (Beckman system pack), uric acid (Crest Bio systems, Goa, India) and homocysteine (Dialab, Austria) were measured using enzymatic methods, and Lp(a) was measured by turbidimetric method (Randox Laboratories Limited, UK) by autoanalyzer (Beckman Synchron CX9 USA). Within run coefficients of variation (CV%) for glucose, cholesterol, triglycerides were 2%, 3.0% and 2.8%, respectively. Low density lipoprotein cholesterol (LDL-C) and very low density lipoprotein cholesterol (VLDL-C) were calculated using Friedewald formula [5]. Insulin was measured by radioimmuno assay (Immunotech, Prague, Czech Republic) on Wallac automated Gamma Counter-1480. Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated by using formula: fasting glucose (mmol/L) x fasting insulin (μIU/mL)/22.5 [6]. Fibrinogen was measured by turbidimetric method (Tulip Diagnostics (P) Ltd., Goa, India) on Chemwellautoanalyzer (Awareness Technology), with within run coefficient of variation (CV%) of 5.2%. Malondialdehyde (MDA) was measured as thiobarbituric acid reactive substances (TBARS) (CV% 4.6%), ferric reducing ability of plasma (FRAP) as a measure of antioxidant power (CV% 1.9%), and NO was measured by kinetic cadmium reduction method on spectrophotometer.

Statistical analysis: Distribution of data was studied by Kolmogrov Smirnov test. Parametric student's unpaired two–tailed t-test was applied for the data with normal distribution and non parametric Man Whitney U test was applied for the data that did not have normal distribution, to study the difference in the means between cases and controls. Correlation between variables was analyzed by Pearson's correlation for normally distributed data and Spearman's correlation for data which did not have normal distribution. '*P*' <0.05 was considered as statistically significant. Microsoft excels spread sheets and SPSS for V 11.5 were used for data analysis.

RESULTS

We enrolled 50 cases (2-10y=22, 11-17y=28; 29 boys) and 50 controls (2-10y=13, 11-17y=37; 27boys) Obese children had significantly higher insulin, HOMA-IR, TGL, VLDL, uric acid and fibrinogen levels (*P*<0.001) when compared to their lean peers (***Table* I**).

Oxidative stress marker, MDA, antioxidant status, FRAP and marker of endothelial dysfunction was found to be significantly higher (*P*<0.001) in cases as compared

TABLE I ANTHROPOMETRY AND BIOCHEMICAL PARAMETERS AMONG THE STUDY PARTICIPANTS

Parameter	*Controls (n=50) Mean (SD)*	*Cases (n=50) Mean (SD)*	*P value*
Age (years)	12.3 (2.81)	11.1 (3.02)	0.052
BMI (kg/m^2)	17.2 (3.18)	27.5 (6.27)	<0.001
SBP (mmHg)	95.8 (12.12)	99.7 (9.57)	0.216
DBP (mmHg)	65 (7.22)	67.1 (6.72)	0.131
FBS (mg/dL)	83.3 (11.75)	86.1 (7.32)	0.170
Insulin (μIU/mL)	7.0 (3.28)	12.9 (7.51)	<0.001
HOMA-IR	1.7 (1.33)	3.1 (2.62)	<0.001
Total cholesterol (mg/dL)	146.8 (22.27)	151.4 (32.55)	0.413
Triglycerides (mg/dL)	75.8 (30.82)	114.3 (68.65)	0.001
HDL (mg/dL)	35.5 (7.06)	35.5 (5.96)	0.126
LDL (mg/dL)	99.8 (20.94)	95.2 (27.07)	0.417
VLDL (mg/dL)	15.2 (6.16)	22.9 (13.73)	0.001
Uric acid (mg/dL)	3.8 (0.92)	4.3 (1.03)	0.036*
Fibrinogen (mg/dL)	224.1 (153.69)	474.3 (152.17)	<0.001*
Lipoprotein (a) (mg/dL)	16.0 (8.72)	18.1 (13.8)	0.895
Homocysteine (μmol/L)	19.3 (8.88)	20.3 (11.32)	0.888

**BMI – body mass index; SBP – systolic blood pressure; DBP – diastolic blood pressure; FBS – fasting blood sugar; HOMA-IR – homeostasis model assessment of insulin resistance; HDL-C – high density lipoprotein cholesterol; LDL-C – low density lipoprotein cholesterol; VLDL-C – very low density lipoprotein cholesterol.*

to controls (***Table* II**). A positive and significant correlation was observed for BMI with insulin (r=0.519, *P*<0.001), HOMA-IR (r=0.479, *P*<0.001), uric acid (r= 0.289, *P*=0.005), fibrinogen (r=0.461, *P*<0.001) and NO (r=0.235, *P*=0.012).

A significant elevation of homocysteine (*P*=0.039) and NO (*P*=0.017) was observed in female obese children when compared to male obese children (***Web Table* I**).

DISCUSSION

Obesity is associated with risk factors for CVD and accelerated atherosclerotic processes, including elevated blood pressure, atherogenic dyslipidemia, metabolic syndrome and type II diabetes mellitus [7]. In the present study, pediatric obesity was found to be associated with CVD risk factors, dyslipidemia, oxidative stress, insulin resistance and endothelial dysfunction.

The limitations of this study were: small sample size, convenience sampling, no strict age and sex matching, and hospital-based setting. Also we did not record data regarding the lifestyle and dietary habits of these children and the parental characteristics.

A significant elevation in fasting insulin levels with a significantly higher HOMA-IR (insulin resistance) in obese children has also been reported by Friedemann, *et al*. [8]. There is growing evidence for the association of insulin resistance with the development of type 2 diabetes mellitus in children [9]. Insulin resistance is the primary cause of hyperinsulinemia, which is associated with various risk factors including high triglyceride and uric acid levels, hypertension, type 2 diabetes, obesity and atherosclerosis [10]. Atabek, *et al*. [11] showed that serum lipid levels in obese children were significantly higher than those of healthy subjects. Defective insulin action in the liver and peripheral tissues gives rise to fasting hypertriglyceridemia. We did not find any significant difference in total cholesterol, HDL, LDL and Lp(a) levels in obese children when compared to controls, similar to the findings of DJ Stensel, *et al*. [13].

TABLE II OXIDANT, ANTIOXIDANT AND ENDOTHELIAL DYSFUNCTION MARKERS IN STUDY GROUPS

Parameter	*Control (n=50) Mean (SD)*	*Cases (n=50) Mean (SD)*	*P value*
MDA (µmol/L)	1.3 (0.19)	1.7 (0.53)	<0.001
FRAP (mmol/L)	1.2 (0.42)	1.3 (0.18)	0.014
NO (µmol/L)	67.1 (10.36)	76.0 (15.97)	0.001

MDA: malondialdehyde, FRAP: ferric reducing ability of plasma, NO : nitric oxide.

Hyperhomocysteinemia is a well-known independent risk factor for premature cardiovascular disease. However, we did not observe any significant difference in homocysteine levels between obese children and controls, similar to the study of Davis PH, *et al.* [13]. Many epidemiological studies have established an association between fibrinogen and CVD [14], as fibrinogen regulates cell adhesion, chemotaxis, proliferation, vasoconstriction at sites of vessel wall injury and stimulation of platelet aggregation.

Endothelial dysfunction is a predictor of CVD risk and is also the earliest indicator of atherosclerosis [17]. Our study has documented a significant elevation in NO levels in obese children when compared to controls, consistent with the findings of Codoner-Franch, *et al.* [16] who reported increased NO synthesis and nitrosative stress in obese children [16]. An increased generation of free radicals along with increased production of NO may lead to the increased conversion of NO to peroxynitrite, which diminishes the bioavailability of NO as a vasodilator, thereby leading to endothelial dysfunction [16]. Oxidative stress has been suggested to cause insulin resistance and may be a possible link with atherosclerosis [17]. We found significant elevation MDA and FRAP in obese children when compared to controls.

Given the importance of addressing CVD risk at a younger age, the findings of this study need to be validated in larger studies. Obesity in childhood is a major risk factor in the pathogenesis of type 2 diabetes and CVD. Therefore, these children must be targeted for life style and dietary modification, and if necessary therapeutic interventions must be initiated to arrest further progression of these risk factors.

Contributors: TC: study design, data collection, sample analysis; MMS: conception and study design, data analysis and interpretation of data, and reviewed and revised the manuscript. MP: data analysis and manuscript writing; PVLNSR: conception and study design; AS: conception and study design, and analysis of blood samples.

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WHAT THIS STUDY ADDS?

- Obesity in children was found to be associated with cardiovascular risk factors and endothelial dysfunction.

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WEB TABLE I ANTHROPOMETRY AND BIOCHEMICAL PARAMETERS BETWEEN FEMALE AND MALE OBESE CHILDREN

Parameter	*Females (n=21) Mean (SD)*	*Males (n= 29) Mean (SD)*	*P value*
BMI (kg/m^2)	26.4 (3.81)	28.3 (7.59)	0.671
SBP (mm of Hg)	98.5 (7.82)	100.4 (10.67)	0.608
DBP (mm of Hg)	65.7 (7.82)	68.1 (7.61)	0.174
FBS (mg/dL)	85.8 (8.50)	86.3 (6.48)	0.354
Insulin (µIU/mL)	12.5 (6.07)	13.1 (8.49)	0.802
HOMA-IR	3.4 (2.45)	2.8 (1.77)	0.465
Total Cholesterol (mg/dL)	149.0 (37.84)	153.1 (28.95)	0.745
Triglycerides (mg/dL)	111 (58.29)	116.7 (75.89)	0.984
HDL (mg/dL)	32.8 (5.65)	33.8 (6.23)	0.854
LDL (mg/dL)	94.0 (31.81)	95.9 (23.83)	0.839
VLDL (mg/dL)	22.12 (11.65)	23.3 (15.17)	0.984
Uric acid (mg/dL)	4.0 (1.04)	4.4 (1.01)	0.177
Fibrinogen (mg/dL)	465.4 (155.43)	481.4 (152.18)	0.507
Lipoprotein(a) (mg/dL)	11.0 (5.56)	23.0 (15.77)	0.186
Homocysteine(µmol/L)	24.2(11.37)	18.0 (10.82)	0.039
MDA (µmol/L)	1.7 (.39)	1.7 (.63)	0.665
FRAP (mmol/L)	1.3 (.18)	1.3 (.18)	0.867
NO (µmol/L)	82.4 (19.89)	71.0 (10.14)	0.017

**BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; FBS: fasting blood sugar; HOMA-IR: homeostasis model assessment of insulin resistance; HDL-C: high density lipoprotein cholesterol; LDL-C: low density lipoprotein cholesterol; VLDL-C: very low density lipoprotein cholesterol.*