RESEARCH BRIEF

Adiponectin, Interleukin-6 and High-sensitivity C-reactive Protein Levels in Overweight/Obese Indian children

Running title: ADIPOCYTOKINES AND INFLAMMATION IN OBESE CHILDREN

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ABSTRACT

Objective: The aim of our study was to assess serum Adiponectin, Interleukin-6 (IL-6) and high-sensitivity C-reactive protein (hsCRP) levels and their correlation with conventional risk factors for cardiovascular disease and diabetes in overweight/obese Indian children.

Methods: Body mass index (BMI), waist circumference, blood pressure, fasting serum adiponectin, IL-6, hsCRP, blood glucose, triglycerides, and total and high density lipoprotein cholesterol were measured in children aged 7-15 years with BMI >85_{th} centile.

Results: Eighty-four overweight/obese children (48 boys) with mean (SD) age 10.2 (1.9) years were enrolled. Mean (SD) adiponectin, hsCRP and median (IQR) IL-6 levels were 6.0 (3.1) µg/mL, 3.4 (2.4) mg/L and 12.7 (5.0-90.0) pg/mL, respectively. Low adiponectin, high hsCRP and high IL-6 were noted in 16.5%, 49.4% and 54.4% participants, respectively. Adiponectin was inversely correlated with waist circumference, and IL-6 positively with BMI and blood glucose.

Conclusion: Inflammatory mediators, hsCRP and IL-6 were elevated in half of the overweight children. Adiponectin and IL-6 correlated well with traditional risk markers.

Keywords: *Cytokines, Inflammation, Metabolic syndrome*

INTRODUCTION

Over the last decade, several studies have indicated that inflammatory markers and adipocytokines mediate the evolution of the cardiovascular and metabolic complications of obesity. Low-grade chronic inflammation in association with obesity plays a major role in the pathogenesis of atherosclerosis and insulin resistance [1,2]. Adipose-tissue macrophages and adipocytes secrete interleukin-6 (IL-6), an inflammatory mediator that is postulated to affect lipid and glucose metabolism through several mechanisms, including adipose tissue-specific gene expression, triglyceride release, downregulation of lipoprotein lipase, and reduction of insulin sensitivity [2,3].

C-reactive protein (CRP) is another inflammatory biomarker, which is synthesized in the liver in response to stimulation by inflammatory cytokines [4]. It is considered to play an important role in vascular remodeling and plaque deposition (Buckley). High-sensitivity CRP (hsCRP) test measures low levels of CRP. Values above 3 mg/L are indicative of high risk for cardiovascular disease [5]. High levels

of hsCRP are also associated with impaired insulin sensitivity and the development of cardiometabolic syndrome [4].

Adiponectin is a collagen-like plasma protein secreted exclusively by adipocytes. This protein has anti-inflammatory, anti-atherogenic, and potent insulin-sensitizing effects, which may be partially mediated by suppression of TNF-α and IL-6 [4]. Low levels of adiponectin have been associated with metabolic syndrome, insulin resistance and type 2 diabetes [4,6].

Majority of the studies on these adipocytokines and inflammatory markers have been done in adults. The aim of our study was to assess serum IL-6, hsCRP and Adiponectin levels and their correlation with conventional risk factors for cardiovascular disease and diabetes in overweight/obese Indian children.

METHODS

This cross-sectional study was conducted at All India Institute of Medical Sciences, New Delhi after obtaining ethical approval from the Institute ethics committee. Children between 7-15 years, with BMI > 85_{th} centile according to Indian reference curves were enrolled from Pediatric outpatient department. Children with genetic, syndromic, endocrine or medication-related obesity were excluded. Informed consent was obtained from the parents, and assent from the children.

Weight, height, waist circumference and blood pressure (BP) were measured. BMI was calculated and pubertal staging was done. Fasting blood sample was collected for adiponectin, IL-6, hsCRP, glucose, total cholesterol, HDL cholesterol and triglycerides. Adiponectin, IL-6 and hsCRP were measured using quantitative colorimetric sandwich ELISA kits. Glucose was measured in the fresh sample by glucose oxidase method, total and HDL cholesterol and triglycerides were measured by Randox kits (Randox Ltd, Antrim, UK). LDL cholesterol was estimated using Friedewald's formula as total cholesterol – HDL cholesterol – (triglyceride/ 5) mg/dL.

The following cut-offs were considered abnormal: Adiponectin <3 µg/mL [7], hsCRP >3 mg/L [5], IL-6 >10 pg/mL [8], fasting blood glucose ≥100 mg/dL, Total cholesterol ≥200 mg/dL, LDL cholesterol ≥130 mg/dL, HDL cholesterol <40 mg/dL and Triglyceride>150 mg/dL [9]. Abdominal obesity was considered present if waist circumference was above 95_{th} percentile for age or >90 cm in boys/ >80 cm in girls [9]. Data was presented as mean (SD) or median (IQR), and as proportion with abnormal values. Spearman test was applied to check for correlation between adiponectin, IL-6, hsCRP, BMI, waist circumference and fasting blood glucose. Wilcoxon rank-sum (Mann-Whitney) test was

applied to test for difference in adiponectin, IL-6 and hsCRP in children with and without abdominal obesity and impaired fasting glucose.

RESULTS

Eighty-four children (48 boys) with a mean (SD) age of 10.2 (1.9) years were enrolled. Two-thirds of the children were prepubertal, and 87% were obese. The mean (SD) BMI Z-score was 2.7 (0.8). Mean (SD) waist circumference was 82.4 (10.3) cm, with abdominal obesity in 67.3%. Mean (SD) systolic and diastolic BP was 113 (10) and 74 (10) mm Hg, respectively with hypertension present in 15.5%.

The biochemical parameters are summarized in ***Table* I**. Values of lipid profile and fasting blood glucose could not be traced from the central laboratory for some patients. Elevation of inflammatory mediators constituted the commonest abnormality, with IL-6 being elevated in 54.4% and hsCRP in 49.4%. Low HDL was the commonest dyslipidemia, noted in 35.1%. Impaired fasting glucose was seen in 10.7% and low adiponectin levels in 16.5%.

Serum adiponectin was noted to have a significant inverse correlation with waist circumference (r= -0.28, P=0.047). Serum IL-6 had a positive correlation with BMI (r= 0.23, P= 0.09), and blood glucose (r= 0.24, P= 0.08), but was not statistically significant. Median (IQR) serum IL-6 in children with abdominal obesity was 45.2 (6.3,131.2) pg/mL as compared to 6.6 (4.5,22.5) pg/mL in those without abdominal obesity (P=0.047). Median (IQR) serum IL-6 was higher in the children with impaired fasting glucose as compared to those with normal levels (107.0 (22.5,197.5) *vs* 8.5 (5.0,116.0) pg/mL, P=0.06). There was no significant correlation between the serum levels of IL-6, hsCRP and adiponectin. No correlation was observed between hsCRP and BMI, waist circumference or blood glucose.

DISCUSSION

We noted a high prevalence of traditional risk markers of later cardiovascular disease and diabetes in our young study population. Abdominal obesity was present in nearly two-thirds, low HDL in one-third, hypertension in 15% and impaired fasting glucose in 10%. These values are comparable to those previously reported in Indian children. Half of our study group had elevated levels of inflammatory mediators IL-6 and hsCRP. To the best of our knowledge, no previous Indian study has reported IL-6 levels in obese children. However, higher levels of hsCRP and lower levels of adiponectin in obese post-pubertal adolescents as compared to lean have been reported by Vikram, *et al*. [10].

A limitation of our study is the lack of lean controls. Another limitation is that as a majority of our study participants were either pre-or early pubertal, and only 10% were in pubertal stage 3 or above, we could not ascertain the effect of puberty on the levels of IL-6, hsCRP and adiponectin.

In another study by our group (unpublished), serum adiponectin in lean children (*n*=29, mean (SD) age 11.5 (1.6) yrs, mean (SD) BMI z-score -0.4 (0.5)) was 8.7 (5.3) µg/mL, which is significantly higher as compared to the level in the overweight/obese children in the present study (6.0 (3.1) µg/mL). In a study in Austrian children with mean (SD) age of 12 (4) years, the mean (SD) hsCRP in the obese group was 4.1(4.8) mg/L [11]; and in another study in Caucasian pre-pubertal children with mean (SD) age of 8.0 (0.1) years, the median (IQR) hsCRP levels in the obese group were 2.5 (1.4–5.3) mg/L, [12], which were similar to the levels noted in our study. In these two studies, the levels in lean children were significantly lower at 0.9 (1.5) mg/L and 0.5 (0.2–1.7) mg/L, respectively [11, 12]. In a Spanish study, mean (SD) serum IL-6 in obese children aged 4-15 years was 7.5 (3.8) pg/mL, similar to our study [13].

We noted an inverse association of adiponectin with waist circumference, indicating that the association between abdominal obesity and insulin resistance may be mediated by lowered adiponectin. Similar observations of inverse association of adiponectin with obesity have been made in Taiwanese and Japanese children [14,15]. We also observed a positive correlation of IL-6 with BMI and fasting blood glucose, as has been reported previously in adults [16].

To conclude, our study showed that inflammatory mediators hsCRP and IL-6 were elevated in half of the obese/overweight children and adiponectin was low in 16.5%. Inverse correlation of adiponectin with waist circumference, and positive correlation of IL-6 with BMI and fasting blood glucose indicated the utility of these parameters as markers of metabolic risk in children.

*Contributors***:** VJ conceptualized and conducted the study and drafted the manuscript. AK helped in conducting the study, AA, NV and RK were co-investigators, AA helped in patient enrolment, NV and RK supervised the biochemical assays. All authors have given their inputs and approved the final manuscript.

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Competing interest: None stated.

WHAT THIS STUDY ADDS?

- Inflammatory mediators high-sensitivity C-reactive protein and Interleukin-6 are elevated and Adiponectin is low in a considerable proportion of obese/ overweight Indian children.
- Serum adiponectin has a significant inverse correlation with waist circumference, and serum IL-6 was significantly higher in children with abdominal obesity as compared to those without.

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TABLE I SUMMARY OF BIOCHEMICAL PARAMETERS IN OVERWEIGHT/OBESE INDIAN CHILDREN

Parameter	***N***	***Value***
Adiponectin* (µg/mL) <3	79	6.0 (3.1) 13 (16.5)
high-sensitivity C-reactive protein* (mg/L) >3	79	3.4 (2.4) 39 (49.4)
Interleukin-6# (pg/mL) >10	79	12.7 (5.0 – 90.0) 43 (54.4)
Total cholesterol* (mg/dL) ≥200	57	153.0 (33.0) 5 (8.8)
HDL cholesterol* (mg/dL) <40	57	43.9 (8.6) 20 (35.1)
Triglycerides* (mg/dL) >150	57	105.6 (39.4) 7 (12.3)
LDL cholesterol* (mg/dL) ≥130	57	88.0 (33.0) 4 (7.0)
Fasting blood glucose* (mg/dL) ≥100	56	85.0 (15.4) 6 (10.7)

*Value in No. (%), *mean (SD) or #median (IQR)*