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**Name of Journal:** *World Journal of Clinical Pediatrics*

**Manuscript NO:** 66602

**Manuscript Type:** ORIGINAL ARTICLE

***Observational Study***

**Adipocytokine profile in children with Kawasaki disease at a mean follow-up period of 5.5 years - a study from North India**

Adipocytokine profile in children with Kawasaki disease

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## **Abstract**

### **BACKGROUND**

Kawasaki disease (KD) is an acute self-limited vasculitis with predilection for coronary arteries. Children with KD may have altered lipid metabolism and abnormal lipid profiles that may last for prolonged periods. However, there is paucity of literature on role of adipocytokines in KD.

### **AIM**

To estimate the levels of adipocytokines (viz. adiponectin, leptin and resistin) during convalescent phase of KD.

### **METHODS**

Twenty (20) children who had had KD at least three years earlier, were enrolled in this study. In addition, 20 healthy controls were also enrolled. Clinical and laboratory profiles of patients were obtained from hospital records. Serum adiponectin, leptin and resistin levels were estimated by enzyme-linked immunosorbent assay (ELISA).

### **RESULTS**

Mean age of patients in the study group was  $10.15 \pm 3$  years and male: female ratio was 1.5:1. Median serum resistin values in patients with KD (27.77 ng/mL; [IQR 18.66, 48.90]) was decreased compared to controls (21.20 ng/mL; [IQR 14.80, 27.00]) ( $P = 0.04$ ). Median serum leptin values in cases and controls were 1.83 ng/mL; (IQR 1.13, 3.80), and 1.10 ng/mL; (IQR 0.41, 2.88) respectively ( $P = 0.09$ ). Median serum adiponectin levels were similar in both cases (12.20  $\mu$ g/mL; [IQR 9.76, 17.97]) and controls (13.95  $\mu$ g/mL; [IQR 11.17, 22.58]); ( $P = 0.18$ ). There was no significant difference of all 3 adipocytokines between children with CAAs (4/20) and without CAAs (16/20).



## CONCLUSION

Serum resistin values were significantly elevated in patients with KD during convalescent phase compared to controls. Serum leptin levels appear to be higher in patients with KD, although the difference is not statistically significant. Adiponectin levels were similar in both cases and controls. Raised resistin and leptin levels may partially explain lipid perturbations seen during the convalescent phase of KD.

**Key Words:** Adipocytokines; adiponectin; resistin; leptin; lipid metabolism; Kawasaki disease; convalescent phase

Praharaj DL, Rawat A, Gupta A, Arora K, Pilia RK, Bhattad S, Singh S. Adipocytokine profile in children with Kawasaki disease at a mean follow-up period of 5.5 years – a study from North India. *World J Clin Pediatr* 2021; In press

**Core Tip:** Present study suggests that serum adipocytokine levels may impact lipid abnormalities seen during the convalescent phase of KD. Serum resistin values were significantly elevated in patients with KD during convalescent phase compared to controls. Serum leptin levels appear to be higher in patients with KD, although the difference is not statistically significant. Adiponectin levels were similar in both cases and controls.

## INTRODUCTION

### Introduction

<sup>3</sup> Kawasaki disease (KD) is a medium vessel vasculitis and the most common cause of acquired heart disease in children in most of the developed countries (1). There are data to support that incidence of KD is also rising in the developing world,





including India (1). Coronary artery abnormalities (CAAs) are noted in 15-25% of untreated children and treatment with intravenous immunoglobulin (IVIg) reduces this risk to 3-5% (2). Children with KD are known to have lipid abnormalities in the acute phase that may persist long after the initial episode of disease (3-8). It is known that serum lipid profiles may remain deranged for prolonged periods after the acute stage of the illness and this may probably contribute to the premature and accelerated atherosclerosis seen in patients with KD (9,10).

Adipocytokines play a significant role in lipid metabolism, inflammation and diseases associated with accelerated atherosclerosis (11-13). Moreover, their levels may impact lipid abnormalities (11-14). As some of the lipid abnormalities associated with KD persist during the convalescent phase, we hypothesized that the adipocytokine perturbations seen during acute phase of KD, may also persist during follow-up. There is a paucity of literature on this subject (15-19), and the results are difficult to interpret. We, and others, have previously shown that children with KD in India have a different clinical phenotype compared to those reported from developed world (20). We have also shown that lipid abnormalities are seen in up to 25.9% of children with KD at a mean follow-up of 5 years (6,7). We, therefore, embarked on this study to see whether adipocytokines are responsible for some of these lipid abnormalities.

## **MATERIALS AND METHODS**

**Patients and Methods:** The present study was a cross-sectional descriptive study conducted in the Paediatric Rheumatology Clinic (PRC), Advanced Paediatrics Centre, Postgraduate Institute of Medical Education and Research, Chandigarh. Our institute is a federally funded not-for-profit tertiary care centre catering to the population of North-West India. We follow the largest cohort of KD in India. Twenty (20) consecutive cases of KD with at least 3 years of follow-up, and 20 healthy controls were enrolled. Children with acute KD and convalescent cases with less than 3 years of follow-up were excluded. Diagnosis of KD was based on guidelines given by American Heart Association (21). During



the acute phase, children had received standard treatment i.e. IVIg 2 g/kg along with aspirin (initially in higher doses [30-50 mg/kg/day], followed by antiplatelet doses [3-5 mg/kg/day]). Written informed consent was taken from parents/guardians at time of enrolment in the study. Clinical records were reviewed. The study protocol was approved by the Institute Thesis Committee and Institute Ethics Committee. The manuscript has been approved by the Departmental Review Board.

**Evaluation of different adipocytokines:**

**Collection of blood sample:** Two ml of peripheral venous blood sample was collected from cases and controls in plain vials under aseptic precautions. Serum was extracted and collected in cryovials and stored at - 80°C without any delay. Hemolyzed and turbid samples were discarded.

**Estimation of serum resistin:** Serum resistin level was estimated using the AssayMax Human Resistin ELISA kit designed for determining human resistin in plasma, serum, urine, saliva and cell culture samples as per manufacturer's recommendations. Sensitivity of the assay was 0.2 ng/mL; intra-assay coefficient of variability (CV) was 4.5% and inter-assay CV was 7.0%. Absorbance was measured at 450 nm on a microplate ELISA reader (Infinite PRO 2000 TECAN Austria).

**Estimation of serum adiponectin:** Serum adiponectin level was estimated using the AssayMax Human Adiponectin ELISA kit designed for measuring human adiponectin in plasma, serum, urine, saliva and cell culture samples. Sensitivity of assay was 0.7 ng/mL, intra-assay CV was 4.3% and inter-assay CV was 7.2%

**Estimation of serum leptin:** Serum leptin level was similarly estimated using the DRG Human Leptin ELISA kit designed for determining human leptin in plasma and serum samples. Sensitivity of the assay was 1.0 ng/mL, intra-assay CV was 6-7% and inter-assay CV was 8.5-11.5%.



All 3 adipocytokines were measured in convalescent cases of KD and in healthy controls. Serum lipids were also estimated in 18 children in study group during follow-up. Reference values for lipids in healthy Indian children were obtained from a study by Marwaha *et al* (22).

**Statistical Analysis:** Data were collected on a pre-designed proforma and transferred to the Microsoft Office Excel sheet. Preliminary analysis was done by descriptive statistics, expressed as means (SD), medians (range) and proportions (centiles). Comparison of study and control group for levels of individual adipocytokines (i.e. leptin, resistin, and adiponectin) was done using Mann-Whitney test wherever data had skewed distribution and Student's t test was used for normal distribution. Analysis was done using the Statistical Package for Social Sciences (SPSS) Version 20.0 for Windows.

## **RESULTS**

**Observation and Results:** <sup>7</sup> Mean age of patients with KD and controls was 10.1 and 9.1 years respectively. Male:female ratio in patients with KD was 1.5:1. Mean duration of follow-up in the cases was 5.5 years. No case of IVIg resistance was documented in this cohort. Four children (20%) had CAAs at first admission that resolved on follow-up of 6-8 wk. Eighteen of 20 cases had lipid estimations during follow-up. Lipid abnormalities noted in these children have been tabulated (**Table 1**). No association was observed between the occurrence of CAAs and presence of lipid abnormalities.

Median serum resistin values in patients with KD (27.77 ng/mL; [IQR 18.66, 48.90]) was decreased compared to controls (21.20 ng/mL; [IQR 14.80, 27.00]) ( $P = 0.04$ ). Median serum leptin values in cases and controls <sup>6</sup> were 1.83 ng/mL; (IQR 1.13, 3.80), and 1.10 ng/mL; (IQR 0.41, 2.88) respectively ( $P = 0.09$ ). Median serum adiponectin levels were similar in both cases (12.20 µg/mL; [IQR 9.76, 17.97]) and controls (13.95 µg/mL; [IQR 11.17, 22.58]); ( $P = 0.18$ ) (**Table 2**). There was no significant difference of all 3 adipocytokines between children with CAAs (4/20) and without CAAs (16/20). We have performed correlation analysis of different lipid profile with adipocytokines (**Table 3**). No significant correlation was



observed between adipocytokines and lipid values. Body mass index has shown significant positive correlation with leptin values. There was no significant correlation of BMI with resistin and adiponectin.

## DISCUSSION

**Discussion:** KD is the most common cause of acquired heart disease in children in the developed world (1). KD is being increasingly reported in several developing countries, including India (23). Hospital based studies at our centre have shown that incidence of KD has risen significantly over the last 2 decades (23). Whether, this increase represents a true increase in incidence, or an increased ascertainment of disease as a result of heightened awareness, remains conjectural. We, and others, have previously shown that KD in India has a different phenotype inasmuch as a higher proportion of older children are seen in Indian cohorts (20,23,24). Further, periungual desquamation and thrombocytosis seem to appear earlier in children with KD in India (25).

Newburger and colleagues have previously reported that KD was associated with significant abnormalities of lipid metabolism and derangement in serum lipid profiles (3). In the first few days of illness, mean plasma concentration of total cholesterol and HDL-cholesterol was profoundly depressed, whereas mean triglyceride level was very high. Total cholesterol values rapidly returned to normal and remained stable more than three months after the onset of illness. HDL-cholesterol concentration recovered more slowly after illness onset. Mean HDL-cholesterol level was significantly reduced, even after three years of onset of illness. Lipid abnormalities in KD are in part attributable to concurrent reductions of lipoprotein lipase and hepatic lipase activities (4). Several other authors have also reported similar abnormalities in lipid profile in children with KD (4-6,26). We have shown that HDL-cholesterol was low in 6/18 and borderline in 11/18 patients with convalescent KD. Thus, 17/18 patients had abnormal HDL-cholesterol at follow-up. The persistence of low HDL-cholesterol for many years in our cohort suggests a long-lasting effect of KD on endothelial





function, perhaps attributable to the diminished activity of lipoprotein lipase. Normal values of lipids in general population have been studied in Indian children by Marwaha *et al* and this has been used as a historical reference standard for the present study (22).

Adipose tissue has long been considered as an inert organ and depot for energy storage. However, new advances have unraveled that it is also an important endocrine organ that produces numerous adipocytokines (11). Perturbations in adipocytokines are well known in obesity. These play a fundamental role in obesity-linked disorders like diabetes mellitus and metabolic syndrome (12). Now it is well recognized that adipocytokines play a pivotal role in immune response and inflammation (13). Studies have shown that adipokines may be an important biomarker for inflammation in chronic diseases (27,28). While some adipocytokines can induce pro-inflammatory effects (e.g. leptin, resistin, IL-6, TNF- $\alpha$ ), others have predominantly anti-inflammatory effects (e.g. adiponectin and IL-10) (14). Therefore, analysis of specific adiponectin isoforms may be necessary to prove these diverse effects. An imbalance between pro-inflammatory and anti-inflammatory adipocytokines leads to persistent inflammation and may contribute to accelerated atherosclerosis. Low adiponectin, high resistin and high leptin levels have been reported to produce this phenomenon.

As children with KD have lipid abnormalities (6,26), it is plausible that a disturbed adipocytokine milieu may contribute to early development of atherosclerosis. This may, in turn, predispose children with KD to acute coronary events at a young age. Adiponectin, resistin and leptin are the most explored adipocytokines in disorders of lipid metabolism and we, therefore, took up this study in the convalescent phase of KD. To the best of our knowledge, there is no published data on adipocytokine levels in children with KD from the Indian subcontinent.

Studies on adipocytokine profile in the follow-up of KD are sparse and have yielded conflicting results (5,9,19) (Table 3). Fukunaga *et al* (19) reported low, medium molecular weight (MMW) and LMW adiponectin levels in convalescent cases of KD compared to controls. In present study, serum resistin values were significantly elevated in patients with KD



during convalescent phase compared to controls. Serum leptin levels appear to be higher in patients with KD, although the difference is not statistically significant. Adiponectin levels were similar in both cases and controls. Cai *et al* performed meta-analysis to see the association of adiponectin and resistin with patients with KD (29). Authors have shown that that while **serum resistin levels in patients with KD were significantly higher compared with those in** controls, adiponectin levels were similar in patients with KD and controls. Our results have also in are consonance with these findings.

## **CONCLUSION**

Our results suggest that serum adipocytokine levels may impact lipid abnormalities seen during the convalescent phase of KD. The strength of our study is that it is a single centre study wherein all children were diagnosed and treated by the senior author in this manuscript (SS), thereby ensuring uniformity in sample recruitment. Further, the diagnosis of KD was based on standard criteria (AHA 2004). One of the obvious weaknesses is the small sample size, but this was unavoidable as the study had to be completed in a given time frame for the dissertation of the first author (DP). It is suggested that the leads provided by our work be taken up for a larger, and preferably multicentric, study.

## **ARTICLE HIGHLIGHTS**

### ***Research background***

Patients with Kawasaki disease (KD) may have abnormal lipid profiles that may last for prolonged periods. Reasons behind persistence of lipid abnormalities are not clear in patients with KD.

### ***Research motivation***



There is paucity of literature on role of adipocytokines and their effect on abnormal lipid metabolism in patients with Kawasaki disease (KD).

### ***Research objectives***

To estimate the levels of adipocytokines (viz. adiponectin, leptin and resistin) during convalescent phase of KD.

### ***Research methods***

Serum adiponectin, leptin and resistin levels were estimated by enzyme-linked immunosorbent assay (ELISA) in patients with KD and controls.

### ***Research results***

Mean age of patients in the study group was  $10.15 \pm 3$  years.

Median serum resistin values in patients with KD (27.77 ng/mL; [IQR 18.66, 48.90]) was decreased compared to controls (21.20 ng/mL; [IQR 14.80, 27.00]) ( $P = 0.04$ ).

Median serum leptin values and adiponectin values in cases and controls were similar.

There was no significant correlation of adipocytokine with lipid profile in patients with KD.

There was no significant difference of all 3 adipocytokines between children with CAAs and without CAAs.

### ***Research conclusions***

Our results suggest that serum adipocytokine levels may impact lipid abnormalities seen during the convalescent phase of KD.



### *Research perspectives*

The leads provided by our work be taken up for a larger, and preferably multicentric, study to confirm are results.





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