Lipid profile and fat patterning in children at a mean of 8.8 years after Kawasaki disease: a study from Northern India

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ABSTRACT

Objective. *Kawasaki disease (KD) is an acute vasculitis that can result in coronary artery abnormalities (CAA). Higher risk of atherosclerosis has also been documented in those who do not develop CAA. We report herein the lipid profile and fat patterning in children with KD in a cohort from Northern India at a mean follow-up of 8.8 years after the acute stage.*
There is a paucity of literature on this aspect of KD.

Methods. *Twenty children, who had developed KD at least 5 years previously were enrolled along with age- and sex-matched controls.*
Cases and controls underwent anthropometric assessment using standardised techniques and instruments. Lipids were assayed only in the cases.

Results. *There was no significant difference in weight, height, mid-upper arm circumference, waist circumference, hip circumference and waist-to-hip ratio between cases and controls. Skinfold thickness (ST) at triceps, subscapular, midaxillary and suprailiac regions was similar in cases and controls. Biceps and medial calf ST was, however, significantly higher among girls with KD in 10–14.9 years age group. On comparison with cut-offs enumerated by the National Cholesterol Education Program (NCEP), 2 children with KD had borderline while 1 had undesirable levels of total cholesterol. Undesirable triglyceride levels were seen in 12 children. Ten children had HDL levels <35 mg/dl while 1 had borderline LDL levels.*

Conclusion. *Lipid abnormalities at a mean of 8.8 years after KD suggest that these patients may be prone to premature atherosclerosis. There were no significant differences in the anthropometric parameters and most of the ST.*

Introduction

Coronary artery abnormalities (CAA) are the most significant long-term complications of Kawasaki disease (KD) (1, 2). It is difficult to predict which subset of children with KD would develop CAA (3). These CAAs can cause myocardial ischemia due to thrombotic occlusion or progressive stenosis (1, 2). However, use of intravenous immunoglobulins (IVIg) has led to significant reduction in development of CAA from 25% to 3–5% (2). Higher risk of atherosclerosis has also been documented in those who do not develop CAA during acute phase (4). Endothelial dysfunction (5), abnormal lipid profiles (6-10), increased carotid intima-media thickness (11) and systemic arterial stiffening (11) have been implicated in premature atherosclerosis. Abnormal lipid profiles have been reported in many studies**.** Whereas some studies have documented proatherogenic lipid profiles (6-9), others have documented normal lipid profiles in children with KD (10).

There is paucity of literature on fat patterning during follow-up of children with KD. We hypothesise that patients with KD who develop abnormal fat patterning may be at more risk of coronary atherosclerosis in future. This may be useful in detecting high risk patients and initiating risk modification strategies. This study was aimed to study the lipid profile and fat patterning in children 5 years after the diagnosis of KD in a cohort of children from Northern India.

Materials and methods

This study was carried out in the Paediatric Rheumatology Clinic of Advanced Paediatrics Centre at the Post Graduate Institute of Medical Education and Research, Chandigarh, India. Our institute serves as a tertiary level referral centre for north-west India. All cases with the diagnosis of KD made at least 5 years

before recruitment were eligible for enrolment into the study. The diagnosis of KD was based on the standard criteria given by American Heart Association (12). Children with KD were subjected to echocardiography at the time of admission and then 6–8 weeks later. All patients were treated with 2 gm/kg Intravenous immunoglobulin (IVIg) along with aspirin (initially 70–80 mg/kg/day followed by 3–5 mg/kg/day). Aspirin was discontinued if the follow-up echocardiography at 6–8 weeks was normal. In patients with CAA, however, it was continued for longer periods of time.

The data regarding initial diagnosis, investigations and treatment were recorded in a predesigned proforma from the follow-up clinic file. The study protocol was approved by the Institute Thesis Committee and the Institute Ethics Committee. Parents were explained about the study and its procedure in detail and after obtaining an informed consent, all children underwent a detailed anthropometric assessment using standardised techniques and instruments in the Growth Laboratory of Advanced Paediatrics Centre. The anthropometric assessment included a measurement of weight, height, mid upper arm circumference (MUAC), waist circumference, hip circumference and skinfold thickness at biceps, triceps, suprailiac, subscapular, midaxillary and medial calf regions. Body weight was measured by an electronic scale (Avery Ltd, India) with a least count of 0.05 kg. Height was measured by Stadiometer (Make: Holtain Ltd, UK) with a least count of 1 mm. Harpenden skinfold caliper (Holtain Ltd, UK) was used to measure skinfold thickness with a least count of 0.2 mm. Circumferential measurements were taken with the help of fibreglass measuring tape with a least count of 1 mm. Each parameter was measured twice by the investigator and an average of two values was recorded. All anthropometric measurements were carried out under the supervision of a senior physical anthropologist (AKB).

Four ml fasting venous blood sample was collected in a plain vial after sterile venepuncture from all cases. The sample was allowed to clot at room temperature for 2 hours and the serum separated. Serum was divided equally in two vials and sent to the Lipid Research Laboratory of the Department of Experimental Medicine and Biotechnology – one with the subject's name and the other with a random number so as to ensure blinding of the laboratory personnel. These samples were stored at -20˚C till analysis. Tests were carried out in duplicate in a blinded manner to assess interassay variability.

Table I. Comparison of lipid profiles in unblinded and blinded samples among the study population.

Variable (mg/dl)	Unblinded sample Mean ± SD (range)	Blinded sample Mean ± SD (range)	*p*-value
Total cholesterol	134.20 ± 29.89 (100 – 208)	134.7 ± 30.47 (100-210)	0.53
Triglycerides	133.25 ± 29.93 (50.23 – 174)	131.42 ± 31 (50-173)	0.44
HDL cholesterol	37.90 ± 11.34 (21 – 56)	38.64 ± 11.84 (21-58)	0.20
VLDL	26.65 ± 5.99 (10.05 – 34.80)	26.28 ± 6.20 (10-35.36)	0.44
LDL cholesterol	69.65 ± 26.37 (33.95 – 124.20)	69.78 ± 26.47 (36-126.20)	0.91

Biochemical methods

The kit manufactured by ACCUREX Biochemical Pvt. Ltd. (Mumbai India) was used for measurement of lipid profile. This kit has been previously used extensively and standardised in the department of Experimental Medicine and Biotechnology, PGIMER, Chandigarh.

Total cholesterol was determined after enzymatic hydrolysis and oxidation by CHOD PAP method in our reference laboratory using standard kit. HDL cholesterol was measured by precipitation of chylomicrons and VLDL by addition of phototungstic acid and magnesium chloride. After centrifugation the supernatant fluid contains the HDL fraction which is assayed for HDL cholesterol with the standard cholesterol liquicolor test kit. The LDL cholesterol concentration was calculated from total cholesterol (TC) concentration, the HDL cholesterol concentration and the Triglycerides (TG) concentration (LDL = TC – {HDL + (TG/5) [mg/dl]. VLDL (TG/5 mg/dl) was derived indirectly for the purpose of this study. TG was determined after enzymatic hydrolysis with lipases by GPO-PAP method using standard kit. Indicator was quinoneiminie formed from hydrogen peroxide, 4-aminoantipyrine and 4-chlorophenol under the catalytic influence of peroxides.

For the purpose of analysis, cut-offs given by the National Cholesterol Education Program (NCEP) were taken. Serum cholesterol levels <170 mg/dL were considered desirable and ≥200mg/dL were considered undesirable (13). Cholesterol levels in between these limits were considered borderline. Similarly, LDL-C levels <110 mg/dL were considered desirable and ≥130 mg/dL were considered undesirable (13). LDL-C levels in between these limits were considered borderline. HDL-C levels >35 mg/dL were considered desirable and <35 mg/dL were considered undesirable (14). For triglycerides, levels <125 mg/dL were considered desirable (14).

Statistical methods

Conventional statistical methods were used to calculate mean, standard deviation and coefficient of variance. Unpaired *t*-test was used to compare means of study population and those of published controls, after ensuring a normal distribution. WHO Anthroplus software was used to calculate 'z' scores for BMI for all the children.

Results

Twenty children with a diagnosis of KD at least 5 years before enrolment were included in the study. There were 17 boys and 3 girls; mean age of the study group was 13.2 years (5-19 years).

Table II. Anthropometric profile of cases and controls according to age and sex.

Age (in years)	5.0–9.9			10.0–14.9						15.0–19.9				
	Boys (n=2)			Boys (n=10)			Girls (n=2)			Boys (n=5)			Girls (n=1)	
	Cases	Controls	*p* value	Cases	Controls	*p* value	Cases	Controls	*p* value	Cases	Controls	*p* value	Cases	Controls
Weight (mean+SD, in kg)	22.43+ 8.10	21.43+ 5.69	0.89	41.66+ 13.62	38.62+ 10.37	0.58	49.23+ 4.63	34.40+ 8.70	0.58	57.42+ 25.49	3.09+ 57.47	0.73	53	43.75
Height (mean+SD, in cm)	119.40+ 19.09	124.05+ 18.59	0.83	151.24+ 11.54	146.42+ 9.71	0.33	154.10+ 3.11	144.15+ 10.68	0.35	166.96+ 11.69	164.62+ 6.97	0.71	161.40	159.50
MUAC* (mean+SD, in cm)	17.65+ 2.62	16.00+ 0.28	0.47	19.94+ 3.79	21.08+ 3.46	0.49	23.60+ 0.85	18.85+ 2.62	0.14	24.64+ 6.64	23.70+ 2.64	0.78	24.50	22.70
Waist circumference (mean+SD, in cm)	56.70+ 3.54	50.65+ 0.92	0.14	64.82+ 11.92	62.57+ 10.63	0.66	68.55+ 6.43	56.25+ 5.30	0.17	68.50+ 20.82	71.92+ 9.14	0.75	66.50	57.40
Hip circumference (mean+SD, in cm)	61.70+ 8.34	58.35+ 4.03	0.66	76.85+ 11.91	76.85+ 9.05	1.00	89.75+ 4.59	75.25+ 10.96	0.23	88.92+ 17.28	83.80+ 4.38	0.54	89.00	85.40
Waist to hip ratio	0.92+ 0.07	0.87+ 0.04	0.45	0.85+ 0.09	0.81+ 0.06	0.32	0.77+ 0.11	0.75+ 0.04	0.86	0.76+ 0.11	0.86+ 0.08	0.13	0.75	0.67

*Mid-upper arm circumference.

Table III. Skinfold thicknesses of cases and controls according to age and sex.

Age (in years)	5.0–9.9			10.0–14.9						15.0–19.9				
	Boys (n=2)			Boys (n=10)			Girls (n=2)			Boys (n=5)			Girls (n=1)	
	Cases	Controls	*p*-value	Cases	Controls	*p*-value	Cases	Controls	*p*-value	Cases	Controls	*p*-value	Cases	Control
Biceps (mean+SD, in cm)	5.40+ 3.11	4.90+ 1.56	0.85	6.90+ 3.32	6.42+ 5.78	0.82	8.30+ 0.14	4.80+ 0.57	0.01	7.44+ 4.40	8.20+ 4.55	0.80	6.00	5.20
Triceps (mean+SD, in cm)	10.70+ 6.36	7.50+ 2.40	0.57	11.00+ 6.27	11.44+ 9.77	0.91	18.00+ 2.83	10.40+ 1.98	0.09	13.12+ 8.78	13.60+ 9.44	0.94	15.60	10.00
Subscapular (mean+SD, in cm)	6.40+ 3.11	6.00+ 3.11	0.90	10.88+ 9.07	8.92+ 8.20	0.62	20.00+ 10.75	7.00+ 1.98	0.24	14.84+ 12.23	11.40+ 5.82	0.59	14.00	8.40
Midaxillary (mean+SD, in cm)	5.50+ 2.69	4.40+ 0.85	0.64	7.58+ 7.64	8.18+ 8.14	0.87	11.50+ 5.23	6.40+ 1.69	0.32	13.08+ 11.40	10.32+ 5.29	0.64	11.00	7.20
Medial calf (mean+SD, in cm)	10.70+ 5.23	8.20+ 1.69	0.59	13.68+ 8.57	11.68+ 9.15	0.62	17.90+ 2.40	10.80+ 0.28	0.05	14.28+ 5.88	14.44+ 8.11	0.97	13.20	13.20
Suprailiac (mean+SD, in cm)	8.70+ 7.49	6.65+ 2.05	0.90	15.00+ 12.08	12.56+ 12.69	0.67	28.80+ 19.23	12.20+ 5.94	0.36	18.60+ 12.50	20.28+ 11.05	0.83	10.80	16.20

Mean interval between initial diagnosis and enrolment was 8.8 years with a range of 5.3–13.6 years. One child had a 6 mm aneurysm of left anterior descending artery which resolved on follow-up. Two children had perfusion defects on thallium (Th^{201}) scintigraphy.

Lipid profiles

When blinded and unblinded samples were compared, there was no significant interassay variability in different parameters of the lipidogram (Table I). On comparison with NCEP cut-offs, 2 children had borderline while 1 had undesirable levels of total cholesterol. Undesirable triglyceride levels were seen in 12 children. Ten children had HDL levels less than 35 mg/dL. One child had borderline LDL levels.

Anthropometry

Means±SD obtained for various anthropometric parameters like weight, height, MUAC, hip circumference, waist circumference and waist to hip ratio amongst cases and controls according to age and sex are shown in Table II. A regular increase in the mean attainment of all anthropometric parameters was seen between 5 to 19.9 years with advancement of age. Girls in the age group 10–14.9 years possessed higher weight, height, MUAC, hip circumference and waist circumference as compared to boys of same age group. There were no significant differences in any of the anthropometric parameters between cases and controls.

Skinfold thicknesses (ST)

Means±SD obtained for skinfold thickness obtained at biceps, triceps, subscapular, midaxillary, medial calf, and suprailiac regions amongst cases and controls according to age and sex are depicted in Table III. A regular increase in these anthropometric parameters was seen with advancement of age. Girls in the age group 10–14.9 years showed higher skinfold thickness compared to

boys of same age group. The only statistically significant difference between cases and controls was for biceps and medial calf ST in girls 10–14.9 years of age.

Discussion

We have previously shown that the phenotype of KD at Chandigarh is different from that in Japan and the Western countries (15-17). Whether these differences are due to a different genetic background is not clearly understood. KD appears to affect a relatively older age-group of children in north-west India as compared to the West. Furthermore, thrombocytosis and periungual desquamation appears earlier in our cohort of children with KD. It is possible, therefore, that there could be differences on follow-up of these children as well. There is virtually no international literature on follow-up of KD from India or from other developing countries. Furthermore, there is hardly any literature available on fat patterning in children with KD. We have recently reported on the fat patterning of children with KD at a mean follow-up of 3.7 years (18). The only other report on this subject has been from Canada (19). We found that a proportion of our patients had abnormal lipid profiles even after 5 years of KD. HDL levels lower than the accepted level, were seen in 50% children. Triglyceride levels higher than accepted level were seen in 60% children. Two children had borderline while 1 had an undesirable level of total cholesterol. One child had borderline LDL levels. Cheung YF *et al*. showed elevated cholesterol and LDL levels in children with KD after 6–7 years of diagnosis, irrespective of whether they had CAA or not (6). Mitra et al also showed significant elevated cholesterol and LDL levels and lower HDL levels in children with KD (7). Gupta-Malhotra *et al*. have shown higher cholesterol and triglyceride levels in children with KD (9). Noto N *et al*., on the other hand, have not shown any significant difference in lipid profiles of patients with KD versus controls (10). The mean time period from onset of KD to enrolment has varied from 2.6 to 18.6 years (6-10). Number of subjects in all the previously published studies, as also in our study has been small (6-10). This is understandable considering the fact that these are essentially single-centre studies. Lower HDL and higher triglyceride levels in boys in our study may predispose them to atherosclerosis. No previously published studies have documented lower LDL levels compared to published controls. Lower LDL levels in boys in our study are likely to be beneficial in atherosclerosis risk. However, it has also been documented that Indian population tends to have lower HDL levels compared to Western populations (20-22). It has also been reported that coronary artery disease occurs at much lower levels of total cholesterol and LDL in Asian-Indians (20, 21).

We have taken care to ensure good quality control for lipid estimation in our study. Assays were carried out in the Lipid Research Laboratory, Department of Experimental Medicine and Biotechnology under the supervision of a senior biochemist. The laboratory personnel were blinded to the samples. All laboratory tests were run in duplicate. The absence of significant inter-assay variability of laboratory parameters showed a good correlation.

We also do not know whether these abnormalities would abate, persist or worsen with age. Hence more long-term longitudinal multicentric studies would be needed to clarify this issue; as such abnormalities may accelerate atherosclerosis and make these young adults more prone to coronary events later in life.

We attempted to study anthropometry and fat patterning in our cohort. As expected, a regular increase in various anthropometric parameters was observed in cases with advancement of age in both cases and controls. At a mean of 8.8 years after KD, there was no significant difference in any of the anthropometric parameters between cases and controls. Amongst the skinfold thicknesses, the only statistically significant difference seen was for biceps and medial calf skinfold thickness in girls with KD 10-14.9 years of age. However, as the numbers are small it would be imprudent to extrapolate and make generalisations from these data.

In conclusion, therefore, our preliminary study suggests that at a mean of 8.8 years after the acute episode of KD, children in our cohort appear to have significant abnormalities in their lipid profiles. These may put these patients at high risk for coronary events later in life. However, these are results on a relatively small sample size from a single centre. Longer follow-up and multicentric studies would help clarify these issues.

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