Endothelial function, arterial wall mechanics and intima media thickness in juvenile idiopathic arthritis

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Abstract

Objective

Juvenile idiopathic arthritis (JIA) is a chronic inflammatory disease of children which might persist into adulthood. Systemic inflammation seen in adult RA patients has been shown to be associated with alteration in endothelial function, arterial wall mechanics and intima media thickness. Our study was planned to assess similar changes in JIA patients.

Methods

Thirty-one newly diagnosed JIA patients and a similar number of age- and sex-matched controls were enrolled in the study. Endothelial function was assessed by measuring flow mediated dilation and glyceryl trinitrate (GTN)-mediated dilation of the brachial artery. To assess arterial stiffness, various arterial wall mechanic parameters such as cross-sectional compliance, cross-sectional distensibility, shear stress and elastic modulus were derived. Intima media thickness of the common carotid artery was measured as a marker of subclinical atherosclerosis.

Results

The brachial artery diameter at rest was found to be slightly lower in the patients than controls (0.258±0.042 vs. 0.264±0.039; p=0.54). No significant difference was found in flow mediated dilation (17.71±9.26 vs. 16.31±8.23; p=0.53), GTN mediated dilation (25.25±10.02 vs. 23.66±9.79; p=0.53) or FMD: GTN mediated dilation ratio (0.730±0.432 vs. 0.717±0.280; p=0.89) between the cases and controls. There was also no significant difference in carotid artery intima media thickness (0.065±0.0068 vs. 0.068 ± 0.007; p=0.084) between cases and controls. Cases in different subsets of JIA were also analysed separately with regards to FMD, GTN mediated dilation and cIMT but no difference was found between cases in each subset and their controls.Cross-sectional compliance was significantly lower in cases than controls (0.0016±0.0005 vs. 0.002±0.001; p=0.034). Cross-sectional distensibility (0.009±0.003 vs. 0.011±0.006; p=0.14) was also found to be lower whereas diastolic wall shear stress (299.9±47.08 vs. 294.9±59.5; p=0.72) and elastic modulus (1138.5±1085.8 vs. 911±453; p=0.19) were found to be higher in cases as compared to controls. But these differences were not statistically significant. When the subsets were analysed separately for vessel wall indices, cross-sectional compliance was found to be significantly lower in systemic arthritis patients as compared to controls. A high level of intra- and inter-observer agreement was found for all the ultrasonographically evaluated parameters.

Conclusion

Arterial wall indices were found altered in JIA patients indicating increased arterial stiffness. Larger studies are required to assess endothelial dysfunction, intima media thickness and arterial stiffness in each subset of JIA patients.

Key words

endothelial function, arterial wall mechanics, intima media thickness, juvenile idiopathic arthritis

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Introduction

Juvenile idiopathic arthritis (JIA), the most common chronic rheumatic disease in children, encompasses all forms of arthritis beginning before 16 years of age, persisting for at least 6 weeks and is of unknown cause (1).

The International League of Associations for Rheumatology (ILAR) recognised seven subsets of JIA on the basis of features present in the first 6 months: systemic arthritis, oligoarthritis, rheumatoid factor (RF) positive polyarthritis, RF-negative polyarthritis, psoriatic arthritis, enthesitis-related arthritis (ERA) and undifferentiated arthritis (1, 2).

JIA is characterised by inflammatory synovitis of the joints and systemic inflammation in systemic arthritis and RF-positive polyarthritis.

Systemic inflammation has been reported to cause endothelial dysfunction, alter vessel wall mechanics and accelerate atherosclerosis. Acute phase reactants (*e.g.* CRP), inflammatory cells, cytokines (IL-1, IL-6, TNF-α, etc.), chemokines, reactive oxygen species (ROS), insulin resistance, dyslipidaemia, etc, associated with inflammation, have been reported to be involved (3-6). Endothelial dysfunction (ED), a systemic pathological state, is thought to be a key initial event in the pathogenesis of atherosclerosis. It leads to events that promote atherosclerosis, such as vasoconstriction, leukocyte adhesion, platelet aggregation, thrombosis, smooth muscle proliferation and migration and oxidative stress (3-11). Atherosclerosis in coronary vessels leads to cardiovascular disease. Thus, detection of endothelial dysfunction could be used as marker for early atherosclerosis and hence cardiovascular disease.

Endothelial function of macrocirculation could be assessed ultrasonographically by measuring flow mediated dilation (FMD) of the brachial artery in response to reactive hyperemia, generated by occlusion of the brachial artery by increasing cuff pressure and then releasing it. Low FMD indicates poor endothelial function. Evaluation of microcirculation could be done by an invasive procedure involving injection of acetylcholine into the coronary artery or a peripheral artery, followed by measuring the change in blood flow by colour doppler ultrasonography in the coronary, and by plethysmography in the peripheral artery (12, 13). A close correlation has been reported between peripheral and coronary endothelial function (14, 15).

Atherosclerotic change could be evaluated by measuring carotid artery intima media thickness (cIMT). Vessel wall indices such as cross-sectional compliance, cross-sectional distensibility, shear wall stress and elastic modulus have been used as indicators of arterial wall stiffness. Lower cross-sectional compliance and distensibility and higher wall shear stress and incremental elastic modulus indicated greater arterial stiffness (16).

Several studies carried out in adult rheumatoid arthritis (RA) patients revealed that RA is associated with endothelial dysfunction, increased carotid intima media thickness and altered vessel wall mechanics in the form of greater stiffness of the arteries (17-24). However, there is paucity of published data on endothelial dysfunction and arterial wall mechanics in JIA patients (25, 26). Hence, this study was made to evaluate endothelial function, cIMT and arterial wall mechanics in JIA patients.

Materials and methods

This study was carried out at the Department of Paediatrics and Radiology, PGIMER, Dr RML Hospital from November 2011 to March 2013.

Sample characteristics

Since at the time of start of our study, there was no published study in JIA patients in indexed English journals, the sample size was calculated with reference to studies in adult RA patients.

Based on the study by Mondal *et al.* (17) in which FMD was 4.03±1.9% in RA patients as compared to 8.7±1.7% in controls, sample size for comparing FMD was calculated to be 3.

Based on the study by Mahajan *et al.* (18) in which common carotid artery IMT was 0.519±0.18mm in RA patients as compared to 0.387±0.085mm in controls, sample size for comparing IMT was calculated to be 24.

Based on the study by Grover *et al.* (19)

in which cIMT was 0.558±0.137 mm in RA patients as compared to 0.416± 0.002 mm, sample size was calculated to be 10 while based on the study by Singh *et al*. in which cIMT in RA patients was 0.80±0.15 as compared to 0.59±0.11, sample size was calculated to be 8.

Therefore, sample size was taken to be 24 JIA patients.

Thirty-one consecutive JIA patients who were diagnosed for the first time at the paediatric rheumatology clinic at Dr Ram Manohar Lohia Hospital, who had not received any anti-rheumatic treatment and who were sub-grouped into a subset according to the ILAR classification were enrolled in the study. Thirty-one age- and sex- matched healthy children were enrolled as controls from the relatives of doctors, nurses and those attending the well baby clinic for immunisation. The study subjects were enrolled after excluding: arthritis due to other causes, diabetes mellitus, hypertension, body mass index (BMI) above the eighty-fifth centile of BMI charts, deranged lipid profile, preexisting coagulation disorder and preexisting cardiovascular disease.

Informed written consent was obtained from parents of both the JIA patients and healthy controls and assent wherever necessary.

This study was conducted as per the good clinical practice guidelines. The patients were enrolled after receiving ethical clearance from the Institutional Ethics Committee.

Study design

This was a cross-sectional observational study.

Methology

- Clinical assessment

The following data were recorded for all enrolled subjects: subset of JIA (as per the ILAR classification) (2), duration of disease, number of joints involved, Juvenile Arthritis Disease Activity Score (JADAS-27), blood pressure, pulse pressure (systolic--diastolic pressure), mean arterial pressure (2/3 diastolic pressure + 1/3 systolic pressure), weight, height, BMI [weight in kg /(height in m)^2] and treatment history.

JADAS-27 was calculated as a linear sum of 4 components: physician global assessment of disease activity, measured on a visual analogue scale of 0 to 10, parent/patient global assessment of general well being, measured on a visual analogue scale of 0 to 10, normalised erythrocyte sedimentation rate (0–10) and active joint count (out of 27 joints). JADAS ranged from 0 to 57 (27).

Pain the patients complained of was measured on a visual analogue scale (VAS) of pain from 0 to 10.

- Laboratory assessment

Laboratory assessment included determination of haemoglobin, total leukocyte count, differential leukocyte count, erythrocyte sedimentation rate (ESR) and lipid profile.

Haemoglobin and total leukocyte count were estimated by an electronic automated machine; Merk Madonic CA 620/580. The differential leukocyte count was calculated manually.

ESR was calculated by an automated machine; Alifax Spa Padova-Italy.

RF estimation was based on Latex agglutination test and was detected both qualitatively and semi-quantitatively. In the qualitative test, the result was read within 2 minutes for agglutination and semi-quantitative estimation was done by dilution using normal saline.

The concentrations of total cholesterol, HDL and triglycerides were estimated after twelve hours of fasting by end-point enzymatic method using fully automatic biochemistry analyzer (Olympus AU400) and values of LDL and VLDL cholesterol were calculated using Friedwalds formula (28).

- Ultrasonography

All enrolled JIA and healthy children were subjected to ultrasonographic evaluation using a PHILIPS HD11 ultrasound system to determine flow mediated dilation (FMD), GTN mediated dilation, intima media thickness (IMT), lumen diameters and vessel wall indices (14).

1. Assessment of endothelial function: It was done in the brachial artery, which was scanned in longitudinal section 2–15 cm above the elbow and its diameter was measured:
   - at rest
   - during reactive hyperaemia
   - after glyceryl trinitrate (GTN) administration

   The first scan was taken after 15 minutes of rest. Then reactive hyperemia was induced by inflating BP cuff to a suprasystolic pressure (40-50 mm above the systolic pressure) for 4 minutes followed by deflation of the cuff. The second scan was taken after 45–60 seconds of deflation. Thereafter the patient was allowed to rest for 10 minutes. After this the patient was given 400 mcg of GTN by aerosol. Then the third scan was done after 3 minutes.

   In all the scans brachial arterial diameter was measured from anterior to posterior 'm' line (interface between media and adventitia) at end diastolic incident with the R wave on ECG (Fig. 1). It was measured in 4 cardiac cycles and mean was derived.

Based on the brachial artery diameters obtained, the following were derived:

i Flow mediated dilation (endothelium dependent vasodilation): defined as percentage change in arterial diameter in response to reactive hyperaemia.

ii GTN mediated dilatation (endothelium independent vasodilation): defined as percentage change in arterial diameter in response to GTN.

2. Assessment of arterial wall mechanics: Common carotid artery was scanned 1–2cms proximal to the carotid bifurcation to determine:

a) Intima media thickness (IMT): By the sound beam, lumen–intima and media-adventitia interface was identified on the arterial wall at the point of greatest thickness and at two points 1cm upstream and 1cm downstream from the point of greatest thickness on both right and left common carotid arteries. The distance between the two interfaces was the IMT (Fig. 2). Three readings were taken from each side and the average of these six readings was calculated.

b) Internal lumen diameter: was measured along the same distance between the near and far wall lumen-intima interfaces.

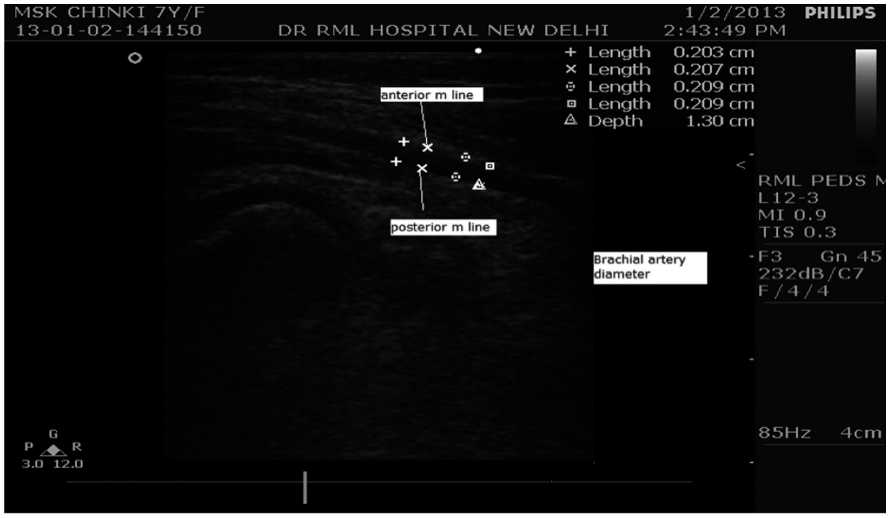

Fig. 1. Measurement of brachial artery diameter.

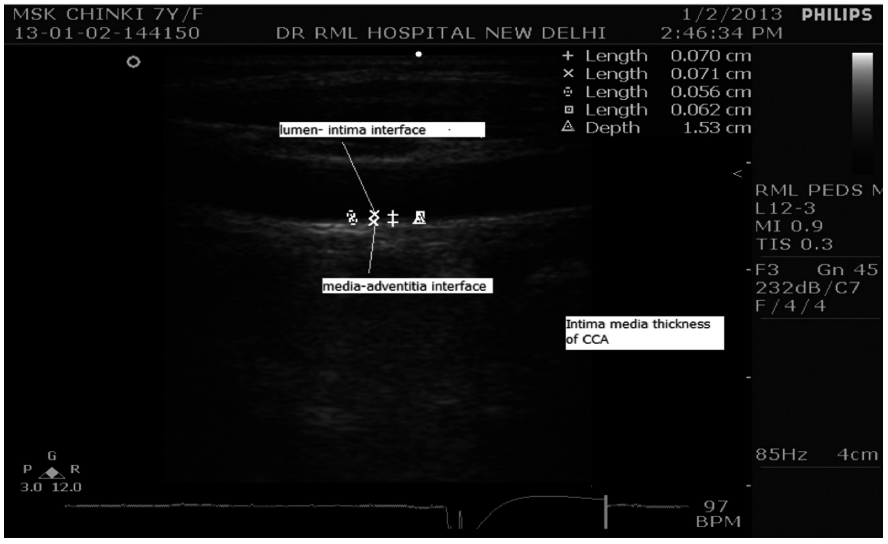

Fig. 2. Measurement of Intima media thickness.

Based on these observations the following were derived:

i Diastolic diameter (Dd) – mean of the minimum values of common carotid artery diameter for 5 consecutive cardiac cycles measured at R wave on ECG;

ii Systolic diameter (Sd) – mean of the maximum values of common carotid artery diameter for the same cardiac cycles at T wave on ECG.

Vessel wall indices

The following vessel wall indices were then derived using the formulae given (16):

i. Lumen cross-sectional area (LCA) = π $Dd^2 / 4$

ii. Wall cross-sectional area (WCA) = $\pi (Dd/2+IMT)^2 - \pi (Dd/2)^2$

iii. Cross-sectional compliance (CSC) ($mm^2.mm\ hg^{-1}$) = $\pi (Sd^2–Dd^2) / 4\ PP$

iv. Cross-sectional distensibility (CSD) ($mm\ Hg^{-1}\ 0.10^{-2}$) = $(Sd^2 – Dd^2) / Dd^2\ PP$

v. Diastolic wall shear stress (mm Hg 0.10^2) = $\frac{\text{Mean arterial pressure x Dd}}{2\ \text{IMT}}$

vi. Incremental elastic modulus (mm Hg 0.10^3) = $\frac{3\ (1+\ \text{lumen cross-sectional area / wall cross-sectional area})}{\text{cross-sectional distensibility}}$

Reliability study

The reliability study was made in ten children with JIA. In all these children, ultrasonographic evaluation of the brachial artery diameter at rest and after reactive hyperemia, carotid artery IMT and systolic and diastolic diameter was done by two researchers (NS and MS). Intra-observer reliability of ultrasonographic parameters was done by NS three days apart. Inter-observer reliability was evaluated by the two researchers (NS and MS) on each child two hours apart. Intra- and inter-observer reliability was not tested for GTN mediated dilation, since it was considered unethical to subject a child to three doses of GTN.

Statistical analysis

Statistical analysis was performed by the SPSS programme for Windows, version 17.0. Continuous variables are presented as mean ± SD, and categorical variables were presented as absolute numbers and percentage. Data were checked for normality before statistical analysis using Shaipro Wilk test. Normally distributed continuous variables were compared using student's *t*-test for two groups and ANOVA for three or more groups. If the F-value was significant in ANOVA and variance was homogeneous, Bonferroni multiple comparison test was used to assess the differences between the individual groups; otherwise, Tamhane's T2 test was used. The Kruskal Wallis test was used for those variables that were not normally distributed and further comparisons were done using Mann Whitney U-test. Categorical variables were analysed using the chi square test. Spearman's Correlation was also used among various variables. Intra- and inter-observer reliability was assessed by calculating the interclass correlation coefficient (ICC) for all ultrasonographic parameters. Since ours was a quantitative data, the correlation coefficient is given as fraction with 95% confidence interval. For all statistical tests, a *p*-value <0.05 was taken to indicate a significant difference.

Results

A total of 31 JIA patients of and their age- and sex-matched healthy controls were included in the study. None of the patients had received steroids or any disease-modifying anti-rheumatic drug. Some of the patients had received

Table I. Clinical and laboratory parameters in JIA patients and controls.

Parameter	Cases (n=31)		Controls (n=31)		*p*-value
	Mean ± SD	Range (min–max)	Mean ± SD	Range (min–max)	
Age (in years)	10.27 ± 3.99	3.5-16	10.27 ± 3.99	3.5-16	1.00
Sex (M:F)	1.38:1		1.38:1		1.00
Weight (in kgs)	26.36 ± 9.88	12-44	27.8 ± 10.28	13.5-45	0.58
Height (in metres)	1.29 ± 0.22	0.93-1.65	1.30 ± 0.23	0.87-1.64	0.89
BMI (kg/m^2)	15.18 ± 1.75	11.87 – 18.75	15.91 ± 2.06	12.08 – 19.53	0.138
Disease duration (in years)	3.097 ± 1.61	1 - 7			
Visual Analogue Scale (VAS) pain score	6.45 ± 1.12	4 - 9			
JADAS score	20.26 ± 5.74	10.2 – 43.5			
Systolic BP (mm Hg)	104.32 ± 12.77	80 – 126	104.68 ± 15.9	74 – 124	0.92
Diastolic BP (mm Hg)	68.74 ± 8.75	52 – 82	70.84 ± 9.53	54 – 84	0.37
Mean arterial pressure (mm Hg)	80.6 ± 9.67	64 – 96.67	82.12 ± 11.3	61.33 – 95.33	0.57
Pulse pressure (mm Hg)	35.58 ± 7.35	22 - 48	33.84 ± 8.78	18 – 44	0.40
Haemoglobin (gm/dl)	10.85 ± 1.42	8.2 - 13.5	12.00 ± 1.21	9.1 – 14.1	0.0012
Total leukocyte count(per mm^3)	8816 ± 3287	5400 – 19500	6851 ± 1464	4100 -10000	0.0035
ESR (mm in 1st hour)	35.55 ± 10.77	22 -62	10.58 ± 3.80	4 – 18	<0.001
S. Cholesterol (mg/dl)	120.93 ± 21.39	84 – 157	123.81 ± 22.03	78 – 164	0.60
S. Triglycerides (mg/dl)	99.71 ± 25.22	30 – 169	93.2 ± 21.52	58 – 145	0.28
VLDL (mg/dl)	22.35 ± 7.06	6 – 34	21.48 ± 7.42	9 - 40	0.64
LDL (mg/dl)	65.19 ± 14.73	36 - 106	64.26 ± 13.03	43 – 94	0.79
HDL (mg/dl)	34.61 ± 10.70	20 -63	37 ± 11.54	19 - 70	0.40

alternative medicines and anti-inflammatory drugs such as ibuprofen off and on for a short duration.

Of the 31 JIA cases enrolled in our study, 14 (45.16%) had systemic arthritis, 9 (29.04%) ERA, 4 (12.9%) RF-positive polyarthritis and 2 (6.45%) had RF-negative polyarthritis and oligoarthritis.

The majority (87.1%) of the patients and controls were in the age group of 5 to 15 years. Eighteen (58.06%) were male and thirteen (41.94%) were female.

The disease duration in cases varied between 1 to 7 years with a mean ± SD duration of 3.097±1.61 years. Of all the cases, five were of less than 1 year duration, twenty cases between 1 and 4 years, five of 5 years and one case of 7 years duration.

The majority of patients (74.19%) had moderate disease activity with juvenile arthritis disease activity score (JADAS) ranging from 15 to 25 and mean VAS pain score of 6.45±1.12.

The mean haemoglobin level was found to be significantly lower whereas total leukocyte count and ESR were found to be significantly higher in patients as compared to controls. The mean serum cholesterol and HDL levels were slightly lower whereas triglyceride, VLDL and LDL levels were slightly higher in cases as compared to controls but differences were not statistically significant (Table I).

In our study, the brachial artery diameter at rest was found to be slightly lower in the patients than controls. But no significant difference was found in Flow mediated dilation, GTN mediated dilation or FMD: GTN mediated dilation ratio between the cases and controls. There was also no significant difference in carotid artery intima media thickness between cases and controls (Table II). Cases in different subsets were also analysed separately with regards to FMD, GTN mediated dilation and cIMT but no difference was found between cases in each subset and their controls (Table III).

Cross-sectional compliance was significantly lower (by 20%) in cases than controls. Cross-sectional distensibility was also found to be lower (by 18%) whereas diastolic wall shear stress (by 2% approx.) and elastic modulus (by 20%) were found to be higher in cases as compared to controls. But these differences were not statistically significant. (Table II).

When the subsets were analysed separately for vessel wall indices, cross- sectional compliance was found to be significantly lower in systemic arthritis patients as compared to controls (0.0016±0.0006 *vs*. 0.0022±0.0008; *p*=0.018). In ERA and oligoarthritis subsets also, cross-sectional compliance and distensibility were lower and elastic modulus was higher in cases as compared to controls but the

Table II. Flow and GTN mediated dilation, intima media thickness and arterial wall mechanics in cases and controls.

Parameter	Cases (n=31)	Controls (n=31)	*p*-value
Diameter at rest (in mm)	0.258 ± 0.042	0.264 ± 0.039	0.54
FMD (% change)	17.71 ± 9.26	16.31 ± 8.23	0.53
GTN mediated dilation (% change)	25.25 ± 10.02	23.66 ± 9.79	0.53
FMD : GTN mediated dilation ratio	0.730 ± 0.432	0.717 ± 0.280	0.89
Intima media thickness	0.065 ± 0.0068	0.068 ± 0.007	0.084
Lumen cross-sectional area (LCA)	0.182 ± 0.028	0.192 ± 0.030	0.19
Wall cross-sectional area (WCA)	0.112 ± 0.016	0.126 ± 0.032	0.036
Cross-sectional compliance (CSC)	0.0016 ± 0.0005	0.002 ± 0.001	0.034
Cross-sectional distensibility (CSD)	0.009 ± 0.003	0.011 ± 0.006	0.14
Diastolic wall shear stress (SS)	299.9 ± 47.08	294.9 ± 59.5	0.72
Incremental elastic modulus (EM)	1138.5 ± 1085.8	911 ± 453	0.19

Table III. Flow and GTN mediated dilation, intima media thickness and arterial wall mechanics in various subsets.

Parameter	Cases Mean ± SD	Controls Mean ± SD	*p*-value
Systemic arthritis (n=14)			
FMD	17.30 ± 12.21	14.77 ± 9.91	0.552
GTN mediated dilation	25.15 ± 12.48	23.50 ± 12.52	0.730
IMT	0.064 ± 0.008	0.067 ± 0.007	0.347
Cross-sectional compliance	0.0016 ± 0.0006	0.0022 ± 0.0008	0.018
Cross-sectional distensibility	0.0089 ± 0.0004	0.012 ± 0.006	0.088
Diastolic wall shear stress	287.38 ± 49.40	294.52 ± 52.24	0.713
Incremental elastic modulus	1383.20 ± 1572.34	786.97 ± 375.39	0.179
Enthesitis related arthritis (n=9)			
FMD	18.23 ± 5.28	14.16 ± 4.53	0.098
GTN mediated dilation	23.50 ± 7.54	20.73 ± 5.73	0.393
IMT	0.065 ± 0.005	0.069 ± 0.005	0.156
Cross-sectional compliance	0.0015 ± 0.00019	0.0019 ± 0.0009	0.220
Cross-sectional distensibility	0.008 ± 0.001	0.0096 ± 0.0048	0.316
Diastolic wall shear stress	329.16 ± 39.06	318.85 ± 34.78	0.562
Incremental elastic modulus	1093.80 ± 304.71	964.22 ± 359.34	0.421
RF-positive polyarthritis (n=4)			
FMD	15.33 ± 8.39	23.98 ± 3.43	0.105
GTN mediated dilation	25.78 ± 7.76	27.00 ± 8.66	0.841
IMT	0.065 ± 0.005	0.073 ± 0.01	0.223
Cross-sectional compliance	0.0018 ± 0.0006	0.0017 ± 0.0014	0.943
Cross-sectional distensibility	0.010 ± 0.003	0.010 ± 0.009	0.955
Diastolic wall shear stress	313.64 ± 34.46	278.49 ± 27.80	0.163
Incremental elastic modulus	862.55 ± 321.73	1107.89 ± 725.26	0.559
RF-negative polyarthritis (n=2)			
FMD	20.61 ± 8.59	17.65 ± 12.39	0.807
GTN mediated dilation	32.27 ± 6.27	25.43 ± 4.86	0.347
IMT	0.069 ± 0.011	0.062 ± 0.005	0.534
Cross-sectional compliance	0.002 ± 0.0002	0.001 ± 0.0016	0.040
Cross-sectional distensibility	0.012 ± 0.0017	0.005 ± 0.0009	0.032
Diastolic wall shear stress	290.46 ± 4.29	354.66 ± 42.52	0.168
Incremental elastic modulus	568.27 ± 56.98	1554.93 ± 358.34	0.061
Oligoarthritis (n=2)			
FMD	20.10 ± 8.38	20.14 ± 7.75	0.997
GTN mediated dilation	25.75 ± 13.67	29.46 ± 11.35	0.796
IMT	0.064 ± 0.004	0.067 ± 0.009	0.725
Cross-sectional compliance	0.0015 ± 0.0002	0.0026 ± 0.0021	0.527
Cross-sectional distensibility	0.010 ± 0.003	0.014 ± 0.009	0.669
Diastolic wall shear stress	237.47 ± 19.12	163.50 ± 95.77	0.396
Incremental elastic modulus	748.58 ± 244.04	504.33 ± 175.60	0.369

Table IV. Comparison between systemic and non-systemic arthritis cases.

Parameter	Systemic arthritis (n=14)	Non-systemic arthritis (n=17)	*p*-value
Flow mediated dilation	17.30 ± 12.21	18.05 ± 6.275	0.83
GTN mediated dilation	25.15 ± 12.48	25.33 ± 7.86	0.96
Ratio of FMD:GTN mediated dilation	0.72 ± 0.61	0.73 ± 0.21	0.94
IMT	0.064 ± 0.008	0.065 ± 0.005	0.56
Cross-sectional compliance	0.0015 ± 0.0006	0.0016 ± 0.0004	0.81
Cross-sectional distensibility	0.009 ± 0.004	0.009 ± 0.003	0.85
Shear stress	287.38 ± 49.40	310.89 ± 43.78	0.17
Elastic modulus	1383 ± 1572	938 ± 326	0.26

difference did not reach significant levels. But in two patients of RF-negative polyarthritis we found increased cross-sectional compliance and cross-sectional distensibility.(Table III).

When all the JIA patients were divided into two groups – one, of systemic arthritis and the other, including all other subsets and evaluated, it was found that there was no difference in FMD, GTN mediated dilation, cIMT or vessel wall indices between systemic and non-systemic arthritis patients except that elastic modulus was higher in systemic as compared to non-systemic arthritis patients (Table IV).

Diameter of brachial artery at rest showed a negative correlation with number of active joints. IMT was found to have a significant positive correlation with ESR. A significant positive correlation was observed between FMD and number of active joints and JADAS (Table V).

Reliability studies revealed a high intra- and inter-observer agreement for all ultrasonographic parameters studied (brachial artery diameter at rest, FMD, carotid artery IMT, systolic and diastolic diameter) and derived arterial wall mechanics (Table VI).

Discussion

The majority of the patients enrolled in our study comprised two subsets *i.e.* systemic arthritis and ERA. This has been the usual pattern observed in various parts of India, as compared to western countries (1, 29, 30, 31).

In our study, we assessed endothelial function at macrovascular level using flow mediated dilation of brachial artery and also evaluated carotid artery intima media thickness and various derivatives of arterial wall mechanics in both cases and controls.

We did not find any endothelial dysfunction or increased cIMT in our cohort of JIA patients, which was contrary to our expectations. This could have been due to many reasons. First, we calculated our sample size on the basis of studies in adult RA patients which is a uniform disease unlike JIA which comprises different subsets. Second, the number of JIA patients in various subsets was probably too small to detect any real difference. Third, as mentioned earlier in the results, even though the disease duration was less than 4 years in most of the patients, our patients had moderate disease activity (as reflected by moderate VAS and low to moderate JADAS and mild to moderate increase in ESR) which might not have been sufficient to produce changes in endothelial function or cIMT in our patients. Even though,

this mild to moderate disease activity probably resulted in slight changes in lipid profile, a known risk factor for ED, but these changes were insufficient to produce ED in our patients. Fourth, the infrequent use of anti-inflammatory drugs and alternate system of medicine drugs in our patients also could have influenced our results.

Vessel wall indices were found to be altered in our cohort of JIA patients. When all the patients were analysed together, cross-sectional compliance was significantly reduced in cases as compared to controls meaning, therefore, that the arterial wall elasticity was reduced in the cases. Cross-sectional distensibility was also lower in the cases than in the controls but it was not found to be statistically significant. Diastolic wall shear stress and elastic modulus were higher in the cases than in the controls but difference was not statistically significant. These alterations in vessel wall indices in cases indicated greater stiffness of the arteries in cases as compared to controls. Subset analysis revealed similar results in ERA, systemic arthritis and oligoarthritis patients but divergent in two RF-negative patients. Since there were only two patients with oligoarthritis JIA and RF -negative JIA, their results may not be a true reflection of the change in arterial wall mechanics.

Our findings were in contrast with the study by Vlahos *et al*. (25) where they found FMD to be significantly lower in 30 JIA cases as compared to 33 matched controls, and increased IMT in systemic arthritis patients compared to either controls or patients with oligoarthritis or polyarthritis. They did not find any difference in arterial stiffness indices such as pulse wave velocity, large artery elasticity index and small artery elasticity index between cases and controls and also between the different subsets. Similarly, our findings were in contrast to what was reported by Pietrewicz *et al*. (26), who found significantly increased cIMT in their patients (only oligoarticular and polyarticular) and a positive correlation with disease duration.

Our study differed from these studies in many ways. In our study, the mean age of the cases was lower and also disease duration was lower as compared to their study. The majority of our patients had systemic arthritis and ERA whereas oligoarthritis and polyarthritis were the most common subsets in their study. Vlahos *et al*. used pulse wave analysis to assess arterial stiffness, whereas we used vessel wall indices such as cross-sectional compliance, cross-sectional distensibility, shear stress and elastic modulus for this purpose. Moreover, the majority of their patients were receiving treatment, whereas our patients were not on any anti-rheumatic drugs.

Table V. Correlation of FMD, GTN mediated dilation, cIMT and arterial wall mechanics with disease duration, JADAS score, number of active joints and ESR in cases.

		Disease duration	no. of active joints	jadas-27	ESR
Diameter of brachial artery at rest	r	-0.147	-0.412*	-0.255	0.132
	p-value	0.429	0.021	0.167	0.479
FMD (% change)	r	0.179	0.377	0.367	0.171
	p-value	0.336	0.037	0.042	0.357
GTN mediated dilation (% change)	r	-0.039	0.149	0.183	-0.154
	p-value	0.830	0.424	0.324	0.410
Intima media thickness	r	0.007	-0.105	0.069	0.432*
	p-value	0.971	0.572	0.711	0.015
Cross-sectional compliance	r	-0.140	0.192	0.182	0.193
	p-value	0.451	0.300	0.327	0.297
Cross-sectional distensibility	r	-0.128	0.084	0.047	0.167
	p-value	0.493	0.653	0.803	0.369
Shear stress	r	0.024	-0.014	0.042	-0.115
	p-value	0.898	0.942	0.823	0.537
Elastic modulus	r	-0.065	-0.137	-0.166	-0.169
	p-value	0.727	0.463	0.373	0.363

Table VI. Intra- and inter-observer reliability.

Parameter		Correlation coefficient	Confidence Interval Lower bound	Confidence Interval Upper bound	*p*-value
Diameter of brachial artery at rest	Intra-observer	0.971	0.890	0.993	0.000
	Inter-observer	0.964	0.864	0.991	0.000
FMD	Intra-observer	0.988	0.951	0.997	0.000
	Inter-observer	0.944	0.793	0.986	0.000
Mean cIMT	Intra-observer	0.860	0.535	0.963	0.001
	Inter-observer	0.952	0.821	0.988	0.000
Carotid artery systolic diameter	Intra-observer	0.987	0.950	0.997	0.000
	Inter-observer	0.992	0.970	0.998	0.000
Carotid artery diastolic diameter	Intra-observer	0.980	0.921	0.995	0.000
	Inter-observer	0.991	0.966	0.998	0.000
Lumen cross-sectional area	Intra-observer	0.974	0.901	0.994	0.000
	Inter-observer	0.991	0.963	0.998	0.000
Wall cross-sectional area	Intra-observer	0.938	0.773	0.984	0.000
	Inter-observer	0.979	0.918	0.995	0.000
Cross-sectional compliance	Intra-observer	0.986	0.942	0.996	0.000
	Inter-observer	0.837	0.475	0.957	0.000
Cross-sectional distensibility	Intra-observer	0.994	0.975	0.998	0.000
	Inter-observer	0.971	0.884	0.993	0.000
Shear stress	Intra-observer	0.964	0.862	0.991	0.000
	Inter-observer	0.984	0.937	0.996	0.000
Elastic modulus	Intra-observer	0.806	0.006	0.885	0.000
	Inter-observer	0.855	0.280	0.933	0.004

The difference in results could be due to difference in disease duration or the method used to evaluate arterial wall mechanics or the effect of drugs.

Our results were similar to those obtained by Doornum *et al*. (20), who found no significant difference in FMD in twenty five RA patients and their controls, but they found that large and small artery compliance measured by pulse wave analysis (PWA) was significantly impaired in cases as compared to controls.

We found high intra- and inter-observer agreement for ultrasonographically evaluated brachial artery diameter, FMD, carotid artery IMT, systolic and diastolic diameter and also for the derived values of arterial wall mechanics. This would mean that the ultrasonographic evaluation of macrovascular endothelial function is a valid and reliable method.

We found three significant correlations. The resting brachial artery diameter showed a negative correlation with number of active joints and IMT showed a significant positive correlation with ESR. This would suggest that inflammation does have some effect on arterial walls. However, we are unable to explain the positive correlation of FMD of the brachial artery with disease activity, which was contrary to our expectations.

Our study had some limitations. Firstly, we did not evaluate the levels of various cytokines, adhesion molecules and selectins which are thought to play a role in ED. The second limitation was the unequal distribution of cases in different subsets and a very small number of poly and oligo. Thirdly, we had calculated the sample size using adult studies. It is possible that if we had had access to a JIA study published earlier, we could have a different sample size and different results.

The results of our study suggest that arterial wall stiffness does occur to some extent in JIA patients. More studies with larger sample size in each subset would be required to exactly delineate any endothelial dysfunction, change in cIMT and arterial wall mechanics and their correlation with various inflammatory markers.

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