To Study Endothelial Dysfunction in Rheumatoid Arthritis

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Abstract

Background: Rheumatoid arthritis (RA) is associated with an increased morbidity and mortality. This excess mortality attributable to cardiovascular events. Endothelial dysfunction represents the earliest stage of atherosclerosis. It can be measured noninvasively by peripheral tests of function, such as pulse wave analysis.

Objectives: To evaluate the influence of chronic inflammatory state on endothelial function in patients with RA free of cardiovascular disease or risk factors by measuring endothelial reactivity.

Method: A total number of 62 patients of RA and 18 normal healthy controls participated in the study. The pulse wave velocity (PWV), reflection index (RI) and augmentation or stiffness index (SI) were measured at baseline and vasodilatory response was measured. Waveform analyzer and Micromedical Pulse Trace Analyser were used.

Results: Heart brachial PWV and brachial ankle PWV were not statistically significant in healthy and RA patient groups. RI was higher in RA patients than controls. SI in RA patient group (7.94 ± 1.20) was statistically significant (p<0.0001) as compared to healthy controls (6.75 ± 0.65). RA patients with low SI had active disease indicated by DAS28 (5.03 ± 1.20) increased ESR and CRP levels as compared to the controls.

Conclusions: RA with high disease activity, free from cardiovascular risk factors and overt cardiovascular disease had premature aging (increased vascular stiffness). Inflammatory process associated with RA was responsible for findings. It is suggested that the increased arterial stiffness contributes to the observed increased cardiovascular mortality and morbidity in subjects with RA.

Introduction

Rheumatoid arthritis (RA) is a systemic immune and inflammatory disease associated with an increased morbidity and mortality.¹ This excess mortality is attributable to cardiovascular events. The standardized mortality ratio for RA patients is 2.0 and their median survival is 17 years less than that of the general population.¹

Atherosclerosis is a systemic disease and its effects on the vascular system may be measured noninvasively by peripheral tests of function, such as pulse wave analysis.² Arterial stiffness, assessed by the augmentation index and pulse wave velocity,³ is an independent risk factor for cardiovascular disease.

Pulse-wave velocity correlates well with arterial distensibility and stiffness and is a useful non-invasive index to assess arteriosclerosis. Arterial endothelial dysfunction is one of the key early events in atherogenesis, preceding structural atherosclerotic changes.

Augmentation index, a quantitative index of systemic arterial stiffness, refers to the difference between the first and second systolic peak of the central pressure waveform, expressed as a percentage of the pulse pressure.⁴ Patients with RA have increased augmentation index independent of cardiovascular risk factors. Augmentation index was associated with coronary artery calcification in patients with RA; this was attenuated after adjusting for cardiovascular risk factors.

Endothelial dysfunction represents the earliest stage of atherosclerosis. Damage to the arterial wall due to aging and atherosclerosis causes decreased arterial elasticity.⁵ A number of the

Editorial Viewpoint

• Increased cardiovascular (CV) morbidity and mortality is known in rheumatoid arthritis (RA).
• Arterial stiffness index was higher in RA patients compared to the controls.
• Increased arterial stiffness contributes to increased CV morbidity and mortality in RA patients.
major cardiovascular risk factors are reported to be associated with RA, such as smoking, dyslipidaemias, vasculitis associated with RA and treatment with corticosteroids. Endothelial function can now be assessed by flow mediated dilation of the brachial artery.

Inflammation is a potent vascular disease risk factor in the general population and is associated with endothelial dysfunction. The pathogenic events in atherosclerosis share many similarities with synovial inflammation in RA such as activation of inflammatory cells and increased expression of adhesion molecules and cytokines. Hence, inflammation may contribute to their increased vascular disease risk by promoting endothelial and vascular wall damage.

We carried out this study to evaluate the influence of chronic inflammatory state on endothelial function in patients with RA free of cardiovascular disease or risk factors by measuring endothelial reactivity.

Material and Methods

The study was conducted in the Department of Rheumatology and Department of Clinical Pharmacology and Therapeutics, Nizam’s Institute of Medical Sciences (NIMS), Hyderabad. Ethical approval was obtained from the Ethics Committee for the study and informed consent was obtained from each participant. The control population was from the general population and the patients were recruited from the outpatient Rheumatology Department from March 2005 to February 2006.

A total number of 62 patients and 18 normal healthy controls participated in the study. All subjects underwent screening by clinical history and physical examination and laboratory investigations. Sixty-two consecutive patients aged 50 years or younger, fulfilling the ACR Criteria for RA were included.

Patients with history of hypertension, diabetes, smoking or alcohol intake, pregnant or lactating, hepatic, renal, hemopoietic, and peripheral vascular disorders and those who were currently on drugs that influence endothelial function like statins, nitrates, ACE inhibitors and carvedilol were excluded from the study.

Age and sex matched volunteers were recruited as controls. The modified disease activity score (DAS28), was determined. Active disease was defined as DAS28 of >2.6.

Recording of Arterial Stiffness and Endothelial Function

The subject was examined in the supine position after a ten-minute rest on bed. Cuff was applied to the both brachium and ankle, and electrodes for echocardiography were attached to the both wrists. A microphone for phonocardiography was placed at the second intercostals space at the left margin of sternum.

The Hb PWV, heart rate (HR), systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP) and pulse pressure (PP), and electrocardiogram were recorded simultaneously by a waveform analyzer Colin Co Ltd, Komaki, Japan. First, an oscillometric pressure sensor measures blood pressure and then the plethysmographic sensor determines volume pulse forms.

Volume waveforms for the brachium were stored, for a sampling time of 10 seconds with automatic gain analysis and quality adjustment. The characteristic points of the waveform were determined automatically according to the phase velocity theory. The time interval between heart and brachium was defined as the time interval between the foot of the second heart sound and the dicrotic notch of the brachial waveform (Thb). The path length (Lb) from suprasternal notch to the brachium was obtained by the superficial measurements and was expressed using following equation Lb= 0.2195*height of patient (in cm)-2.0734.

Finally, the PWV was calculated as distance divided by time interval at that site (PWV in cm/s =Lb/Thb). This instrument automatically measures PWV twice and gives the mean of the velocity as the final pulse wave index.

The reflection index (RI) and augmentation or stiffness index (SI) were calculated using Micromedical Pulse Trace Analyser in 62 patients and patients with increased heart rates were excluded.

The first peak is formed mainly by the pressure transmitted along a direct path from left ventricle, where it generates a change in blood volume. The second peak is formed by reflected wave from lower part of the body along the aorta.

The RI is the height of the second wave of the arterial pressure waveform expressed as a percentage of the waveform peak. RI=b/a*100, where a and b represents heights of first and second waves respectively and the final reflection index is expressed as the mean of three reflection indices. The RI, SI and PWV were measured at baseline. Then the subject was asked to inhale 400 micrograms of salbutamol from a spacer and after 15 minutes; once again, RI and SI were measured. The percentage fall in RI at the end of the test was used to assess the endothelial dependent vasodilatory function. All recordings were performed while the patients were under the influence of their regular medications.

Hematological Analysis

Venous blood for full blood count, erythrocyte sedimentation rate (ESR), C reactive protein (CRP), plasma lipid concentrations (cholesterol, LDL, VLDL, HDL and...
triglycerides), and renal and liver function parameters was taken. IgM RF was measured by ELISA.

**Statistical Analysis**

The statistical analysis was carried out with SAS data analysis system. All the data was presented as mean, standard deviation. Unpaired “t” test was used for between group analysis. For statistical significance, the probability value of less than 0.05 was considered.

**Results**

**Characteristics of Patients and Controls**

Baseline characteristics of patients and controls are given in Tables 1 and 2. The patients and controls were similar with respect to age, height, heart rate, BP and a positive family history of cardiovascular disease.

Table 1 elicits the demographic characteristics of all the subjects. RA patients group had significantly higher ESR, CRP, Cholesterol, triglycerides and HDL compared to controls healthy controls.

The 62 patients had on average early RA. As indicated by the DAS28 values most had evidence of active disease, but mean CRP and ESR values were modestly raised and majority 77% was IgM RF positive.

A majority were receiving regular methotrexate, folic acid and low dose prednisolone.

Matching and haemodynamic parameters of patients with RA and matched healthy controls.

Table 3 shows the PWV, RI, and SI of healthy controls and RA patients. Heart brachial PWV and brachial ankle PWV are not statistically significant in healthy and RA patient groups. Heart rate is significantly high (p<0.0001) in both groups, RI is significantly low (p<0.001), and SI is significantly low (p<0.05), after salbutamol test in both groups.

Whereas, the SI in RA patient group (7.94 ± 1.20) is statistically significant (p<0.0001) as compared to healthy controls (6.75 ± 0.65).

The RA patients with low SI had active disease indicated by DAS28 (5.03 ± 1.20) increased ESR and CRP levels as compared to the controls.

**Discussion**

RA is associated with increased cardiovascular mortality1 which is not understood. There is evidence of increased prevalence of known cardiovascular risk factors, such as smoking and hyperlipidaemia, in patients with RA. The influence of the inflammatory process of RA itself on cardiovascular mortality has been suggested by epidemiological studies,1 but the mechanisms are not clear.

In this study we provide the preliminary evidence that RA is associated with an increase in arterial stiffness as assessed by non-invasive Micromedical Pulse Trace Analyser. Subjects with RA, selected for the absence of clinical cardiovascular disease and risk factors, had significantly increased stiffness index compared with that of well matched healthy controls.

Heart brachial PWV and brachial ankle PWV were not statistically significant in healthy and RA patient groups.

<table>
<thead>
<tr>
<th>Table 1: Demographic and other baseline characteristics</th>
<th>Table 2: Characteristics of patients with RA</th>
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<tbody>
<tr>
<td>Parameter</td>
<td>Healthy (n = 18)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>37.67 ± 7.2</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>153.78 ± 4.75</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>81.56 ± 10.82</td>
</tr>
<tr>
<td>Systolic BP (mm Hg)</td>
<td>119.00 ± 8.92</td>
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<tr>
<td>Diastolic BP (mm Hg)</td>
<td>75.56 ± 3.03</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>91.22 ± 3.72</td>
</tr>
<tr>
<td>PP (mm Hg)</td>
<td>43.56 ± 7.75</td>
</tr>
<tr>
<td>Hemoglobin (gm%)</td>
<td>11.93 ± 0.96</td>
</tr>
<tr>
<td>ESR (mm)</td>
<td>19.00 ± 9.56</td>
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</table>
| Cholesterol | 157.14 ± 14.37 | 174.10 ± 29.14 | **statistical significance, the probability value of less than 0.05 was considered.**

All the data is expressed as Mean ± SD. * = P<0.001, ** = P<0.0001 as compared to pre-salbutamol test in healthy controls; b = P<0.05, bb = P<0.001, bbb = P<0.0001 as compared to pre-salbutamol test in RA patients; ¶ = P<0.0001 as compared to healthy control.

**Table 3: PWV, RI and SI of healthy control and RA patients**

<table>
<thead>
<tr>
<th>Patients</th>
<th>Hb PWV</th>
<th>Ba PWV</th>
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<tr>
<td>Controls</td>
<td>523.80 ± 23.27</td>
<td>1317.94 ± 168.53</td>
</tr>
<tr>
<td>RA patients</td>
<td>536.48 ± 89.99</td>
<td>1369.86 ± 296.60</td>
</tr>
</tbody>
</table>

All the data is expressed as Mean ± SD; a = P<0.05, aa = P<0.001, aaa = P<0.0001 as compared to pre-salbutamol test in healthy controls; b = P<0.05, bb = P<0.001, bbb = P<0.0001 as compared to pre-salbutamol test in RA patients; ¶ = P<0.0001 as compared to healthy control.
which meant that endothelial was functioning properly in the subjects. These data indicate that non-invasively determined SI (stiffness or augmentation index) may be a sensitive marker of early cardiovascular dysfunction in patients with RA. The SI, as measured by pulse trace analysis, reflects predominantly the stiffness of large and medium-sized arteries. Abnormalities in these arteries may result from functional abnormalities, such as endothelial dysfunction, as well as structural changes, such as atherosclerosis. The support for this comes from studies in patients free of clinical vascular disease with conditions known to impair endothelial function, such as diabetes mellitus or hypercholesterolaemia, in which the stiffness index has been shown to be increased relative to control subjects matched closely for peripheral BP.

Arterial stiffness is not only a marker of cardiovascular dysfunction but also an independent risk factor for cardiovascular disease. Subjects with RA who have no cardiovascular symptoms with normal cardiac morphology on echocardiography may show impaired diastolic function indicating that diastolic dysfunction may be one of the first detectable cardiovascular abnormalities in this disease. As this study indicates, SI as measured by PWV, may also be an early marker of cardiovascular dysfunction and future studies should examine its relationship with diastolic dysfunction in rheumatoid disease. RA is characterised by chronic inflammation with an increase of acute phase response proteins, such as CRP and fibrinogen which have been shown to be predictors of future cardiovascular and cerebrovascular events in apparently healthy man and are strongly suspected to have a pathogenic role in cardiovascular disease. Acute systemic inflammation has been shown to lead to reversible endothelial dysfunction in man using forearm blood flow plethysmography.

Furthermore, chronic systemic vasculitis has been reported to impair endothelial function (as determined by brachial artery ultrasound) in vessels distant from the clinically inflamed sites, which improves after therapeutic suppression of the inflammatory process. There could be the possibility of a subclinical systemic vasculitis as a mechanism for increased arterial stiffness in rheumatoid patients. There is also new evidence of early atherosclerosis in rheumatoid subjects free of clinical cardiovascular disease and major risk factors. Two independent matched case-control studies found increased carotid intima-media thickness, an ultrasonographic parameter strongly associated with coronary atherosclerosis and cardiovascular risk, in rheumatoid subjects. Furthermore, carotid intima-media thickness correlated with disease duration.

In our patients, plasma concentration of CRP was increased. Several studies have suggested that CRP may contribute directly to the development of atherosclerosis, as it induces the expression of adhesion molecules on the endothelial surface and promotes the adherence of leucocytes. Thus CRP could be a direct link between autoimmune disease, characterized by systemic inflammation, and an increased risk for cardiovascular disease.

In this study we also found a significant correlation of augmentation index or stiffness index with disease activity parameters, such as CRP or DAS28. This suggests that the increased arterial stiffness in our patients reflected the degree of acute systemic inflammation. The SI represents structural and probably early atherosclerotic rather than functional arterial change, despite their relatively young age. Heart brachial PWV and brachial ankle PWV were not statistically significant in healthy and RA patient groups which meant that endothelial dysfunction was not significant in our subjects. A possible explanation may be that the effect of systemic inflammation on the functional (and potentially reversible) proportion reflected in the endothelial dysfunction may be masked or attenuated once structural atherosclerotic change is established. Recent epidemiological evidence suggests that cardiovascular mortality may already be increased during the earlier years of inflammatory polyarthritis. Indeed there is a need for future studies on RA patients who are not on DMARD’s and folic acid which can be considered as a prime limitation of our study as majority (82%) of our RA patients in the study were on methotrexate and folic acid, which would be responsible for the insignificant values of brachial ankle PWV and heart brachial PWV. Further, the current treatment with folic acid and DMARD’s would have led to the improvement of endothelial function.

Folic acid supplementation, taken by study subjects receiving methotrexate, may prevent hyperhomocysteinaemia during methotrexate treatment in RA and a recent prospective study suggests that methotrexate treatment reduces overall and cardiovascular mortality in rheumatoid patients. It is also shown that Folic Acid Improves Endothelial Function in Coronary Artery Disease via Mechanisms largely independent of Homocysteine lowering. Our study also lacks use of invasive techniques to assess arterial stiffness and endothelial function which is the gold standard.

In conclusion, this matched cohort pilot study demonstrates the premature aging (increased vascular stiffness) in patients with RA with high disease activity, free from cardiovascular risk
factors and overt cardiovascular disease. As our study subjects were free of cardiovascular disease and risk factors, we conclude that the inflammatory process associated with RA is responsible for our findings. It is suggested that the increased arterial stiffness contributes to the observed increased cardiovascular mortality and morbidity in subjects with RA.

Acknowledgements

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References