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Incidence and effect of insulin resistance on progression of atherosclerosis in rheumatoid arthritis patients of long disease duration



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ABSTRACT

Background: The continued atherosclerotic risk in rheumatoid arthritis (RA) has been inadequately explained by conventional factors. Chronic inflammation and endothelial activation seems responsible for developing insulin resistance (IR). The study was aimed to assess the role of inflammation and endothelial activation causing IR in long term RA patients leading to increased atherosclerotic risk.

Methods: Fifty (25 long-duration and 25 short-duration) RA patients and twenty-three healthy controls were recruited excluding potential confounding co-morbidities. Fasting insulin, proinflammatory cytokines, endothelial stress markers and adipokines were quantified by ELISA. Homeostasis Model Assessment (HOMA)-IR calculated using glucose and insulin values. Atherosclerotic indices were measured using ultrasound.

Results: Lipid profile was comparable among groups. Mean carotid intima media thickness (cIMT) was significantly higher in both RA groups ($p = 0.0062$) compared to controls. HOMA-IR was significantly higher in long-duration RA ($p = 0.005$); it showed significant associations with DAS 28 ($p = 0.01$) and hsCRP ($p = 0.03$) in this subset. Mean cIMT for short-duration RA ($p = 0.02$) and long-duration RA ($p = 0.0006$) respectively was also significantly associated with HOMA-IR. Pro-inflammatory markers like TNF- α , resistin and leptin were highest in long-duration RA, higher in short-duration RA when compared to control group respectively. HOMA-IR was significantly dependent on TNF- α ($p = 0.008$), resistin ($p = 0.031$), leptin ($p = 0.0054$). Mean cIMT showed association with all parameters mainly with TNF- α ($p = 0.001$), iNOS ($p = 0.001$), resistin ($p = 0.008$) and leptin ($p = 0.04$).

Conclusions: Persistent inflammation leads to altered adipokine secretion promoting IR in RA patients with long disease duration. Treatment with conventional disease modifying anti-rheumatic drugs (DMARDs) is incomplete to control chronic inflammation and limit progression of atherosclerosis.

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At a glance of commentary

Scientific background on the subject

Atherosclerosis is probably the major cause of cardiovascular risk in rheumatoid arthritis patients. Persistent systemic inflammation and a state of endothelial disorder causes a condition of insulin resistance in long-duration rheumatoid arthritis patients pushing them towards an enhanced risk of atherosclerosis.

What this study adds to the field

Insulin resistance prevalence is higher in rheumatoid patients with chronic low-grade inflammation mostly facilitated through cytokines and adipokines. Even in the absence of obesity, primarily tumor necrosis factor (TNF)- α mediated inflammation emerges as the key factor. Hence, controlling TNF in the very early stages could be beneficial in preventing co-morbid conditions in rheumatoid arthritis patients.

It is increasingly recognized that premature atherosclerosis accounts for the increased cardiovascular disease (CVD) and mortality associated with rheumatoid arthritis (RA) [1]. Both classical and non-classical risk factors contribute to atherosclerosis progression in RA which increases with disease progression. High prevalence of premature atherosclerosis has been documented both in early RA patients with disease duration less than one year [2,3] and in established RA with long duration [4–6]. Moreover, various studies have observed that merely presence of classical risk factors do not adequately explain the increased risk of atherosclerosis in these patients [7].

Inflammation is considered a very important non-conventional risk factor in this regard [2]. Inflammatory cytokines play a major role by regulating the immunomodulatory and tissue-destructive events underlying the etiopathogenesis and progression of RA [8]. In RA, increased secretion of proinflammatory cytokines, altered secretion of adipokines like adiponectin, leptin, resistin, visfatin etc from adipose tissue and increased circulating levels of free fatty acids can alter cell signaling in vascular endothelium causing insulin resistance (IR) [9]. Increased tumour necrosis factor (TNF)- α secretion leads to activation of inflammatory cascade which in turn interferes with the insulin receptor substrate (IRS)-1 and phosphatidylinositol 3-kinase (PI3K)-dependent signaling pathway thus leading to IR [10]. In general population, IR is an established risk factor for CVD and Type II diabetes mellitus [11]. Obesity induces alterations in skeletal muscle, adipose tissue and the liver resulting in localized inflammation and IR through endocrine signaling [12]. During the last decade, literature started identifying metabolic syndrome, especially IR in RA patients [13,14]. Recently some studies have shown an increased presence of IR in patients with RA [15,16]. RA patients also experience a prevalence of impaired glucose tolerance (IGT) of 10–20% [17,18].

The increased atherosclerotic risk in RA have been explained by various factors including lipid profiles [19] and prolong steroid therapy [17,18] which have been elucidated as the reasons for continued risk of CVD in these RA patients with disease progression. Till date, only a few studies have reported the concurrent presence of IR and increased risk of CVD in RA patients [20–22]. Primarily, chronic inflammation and stress induced endothelial activation seems to be responsible for developing IR which leads to premature atherosclerosis and can further enhance the risk of CVD in RA. In this background, a study was conducted to elucidate the relation of inflammation and endothelial stress with IR in long disease duration RA patients in turn leading to cardiovascular (CV) risk.

Materials and methods

Patients and controls

Fifty consecutive patients with RA fulfilling the inclusion criteria were recruited from the patients attending the outpatient clinic of the Rheumatology Department of the hospital.

Inclusion Criteria: All patients were within age 18–50 years and were diagnosed as per ACR 1987 [23] guidelines.

Long duration RA patients (RA > 5 years) or the study group:

- Disease duration/onset of symptoms \geq 5 years at inclusion
- Not currently under any form of steroids (6 months) and hydroxychloroquine (3 months).

Short duration RA patients (RA < 1 year) or the comparator group:

- Disease duration/onset of symptoms < 1 year at inclusion
- Not taken any DMARDs (Disease modifying anti-rheumatic drugs) or glucocorticoids.

Healthy volunteers or the control group (n = 23) of age within 18–50 years willing to participate in the study and not having any systemic disease and/or not on any chronic medication were recruited as controls.

Exclusion criteria used for both patients and controls were as follows:

A. Co-morbid diseases/conditions like:

Metabolic syndrome and its related diseases, diabetes (if incident before onset of RA), family history of diabetes, Obesity (BMI \geq 30), familial dyslipidemia, hypertension, coronary artery disease, cerebrovascular disease, peripheral vascular disease, hypothyroidism, renal disease (serum creatinine \geq 3.0 mg/dL or creatinine clearance \leq 30 mL/min), liver disease, polycystic ovarian syndrome, current or recent (within the past 3 months) pregnancy.

B. Concurrent treatment with

Anti-diabetic drugs, lipid lowering drugs, biological DMARDs (esp. Anti-TNF), beta blockers, oral contraceptives, estrogens, progestin, thyroxin, Vitamin E.

C. Current or past Smoking.

Written informed consent was taken from all study participants. The study protocol has been approved by the institutional ethics committee and have been performed in accordance with the ethical standards as laid down in ICMR Ethical Guidelines 2006.

Study design

Assessment of patients included duration of disease, Tender Joint Count (TJC), Swollen Joint Count (SJC), Health Assessment Questionnaire–Disability Index (HAQ-DI) score [24] and Visual Analogue Scale (VAS) (0–100 scale). A composite disease activity index Disease Activity Score (DAS28) [25] was calculated using 4 variables: SJC (28), TJC (28), VAS (0–100 scale), and Westergren erythrocyte sedimentation rate (ESR). The low disease activity is defined by $DAS28 \leq 3.2$, moderate disease activity as $3.2 < DAS28 \leq 5.1$ and severe disease activity as $DAS28 > 5.1$ [26]. All patients were receiving methotrexate (MTX) of 10–20 mg/week monotherapy or in combination with other standard drugs.

Blood sampling

Overnight fasting (12 h) blood samples were obtained from both patients and the control group. The blood samples were immediately centrifuged; sera were separated and stored at $-40\text{ }^{\circ}\text{C}$ until analyzed. All sera analyses were performed within 3 months of blood collection.

Laboratory analysis

Serum levels of total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL) and fasting glucose were determined using a semi-auto-analyzer (Randox Laboratory India Private Ltd, Mumbai, India) with commercially available enzymatic kits. High sensitivity C reactive protein (hs-CRP) was measured by nephelometry (BN Prospec, Siemens Healthcare Diagnostic Ltd, IL, USA) using closed system commercially available kits. Fasting insulin levels were measured by ELISA using standard Calbiotech Inc. insulin assay kits.

Homeostasis Model Assessment for Insulin resistance (HOMA-IR) was calculated using fasting glucose and fasting insulin levels.

$$\text{HOMA} - \text{IR} = [\text{Insulin}(\mu\text{m}/\text{ml}) \times \text{Glucose}(\text{mg}/\text{dl})]/405$$

The quantification of the biomarkers was done from the serum obtained from both RA patients' group and healthy controls by ELISA

Pro-inflammatory cytokines like $\text{TNF}\alpha$ and IL (Interleukin)-1 β ; Adhesion molecule: sICAM (soluble intracellular adhesion molecule); Adipokines or mediators released from adipose tissues: Adiponectin, Leptin, and Resistin were all estimated using RayBio® Human ELISA Kits. Oxidative stress was estimated by evaluating the levels of anti-oxidative stress markers like Superoxide dismutase (SOD), inducible nitric

oxide synthetase (iNOS) and glutathione reductase (GSH). SOD and iNOS were quantified using YH Biosearch Laboratory Human ELISA Kits whereas GSH was measured from serum using a biochemical method all reagents being provided by Cayman Chemicals Company.

Radiological assessment by ultrasound

Atherosclerotic burden was assessed by two very simple non-invasive ultrasound techniques performed by a single blinded radiologist.

A. Flow-Mediated Vasodilatation (FMD) of Brachial Artery

The subject was made to lie in a supine position for 10 min. The right brachial artery was scanned in longitudinal section 2–15 cm above the antecubital fossa with B-mode ultrasonography images using 7–12 MHz broadband linear array transducer in an Acuson Antares ultrasound system premium edition (Siemens Healthcare Diagnostic Ltd, Deerfield, IL). The centre of the artery was identified where the clearest picture of anterior and posterior intimal layers was available. In this suitable transducer position, which was kept constant throughout the procedure, a resting scan was obtained. The luminal diameter of the brachial artery was measured by pulsed Doppler. A sphygmomanometer cuff placed around forearm distal to the scanned region was inflated to 200 mm Hg for 4.5 min and then released, which induced increased flow. A second scan was taken at this stage and again luminal diameter of the artery was measured 60 s after cuff deflation. Endothelial dysfunction (ED) is considered to be present when FMD is below 4.5% [27,28].

B. Carotid Ultrasonography

The procedure was performed on the same ultrasound machine as used above. Both carotid arteries were scanned, with the subject in supine position with slight hyperextension of the neck, to identify atherosclerosis (plaque protruding into arterial lumen $\geq 50\%$). Intima-media thickness was measured from end-diastolic M-mode images (minimum distension) of the far wall of the distal common carotid artery in a location not containing plaques. The greatest distance between lumen-intima and media-adventitia interface was measured. Mean value of right and left sides was evaluated as the mean carotid intima-media thickness (cIMT) of the subject. The mean value of the control group was taken as the cut-off for abnormal cIMT for the patients group [29].

Statistical analysis

All data were tested for normality by applying Shapiro-Wilk Test and has been presented as mean values with Standard Deviations (SDs) for parity but analyzed depending on its distribution. Between-group differences were estimated using one way ANOVA with post hoc tests by Bonferroni correction or Dunns multiple comparison test for normally distributed and skewed data, respectively. Comparisons between two groups were done by independent-sample t test and Mann-Whitney U test. Chi-square test or Fischer Exact test was

Table 1 Comparison of different parameters among the RA patients and healthy controls.

Variables	Healthy Controls (n = 23)	RA < 1 yr (n = 25)	RA > 5 yrs (n = 25)	p-value
Demographic features				
Age (yrs) [#]	36.58 ± 4.6	38.8 ± 11.4	41.2 ± 6.8	0.23
BMI [#]	23.7 ± 2.9	21.8 ± 3.2	21.5 ± 3.6	0.08
Gender (F:M) [#]	15:8	18:7	16:9	0.81
Lipid profiles				
Cholesterol (mg/dl) [#]	203.8 ± 59.8	189.7 ± 82.4	180.0 ± 47.1	0.43
Triglycerides (mg/dl) [#]	172.6 ± 63.9	157.0 ± 70.2	163.7 ± 49.7	0.73
HDL (mg/dl) *	55.6 ± 10.1	56.1 ± 24.8	72.1 ± 19.2	0.01
LDL (mg/dl) [#]	103.7 ± 22.6	86.7 ± 27.4	102.8 ± 29.4	0.08
Atherogenic index [#]	0.47 ± 0.20	0.44 ± 0.29	0.35 ± 0.17	0.25
Insulin resistance parameters				
Glucose (mg/dl)*	70.1 ± 9.7	86.96 ± 26.6	106.5 ± 31.9	0.002
Insulin (µm/ml) [#]	2.63 (0.5–8.4)	2.98 (0.04–11.8)	2.94 (0.7–13.6)	0.7
HOMA-IR **	0.42 (0.07–1.5)	0.59 (0.007–1.8)	1.04 (0.3–7.04)	0.005
Disease variables				
Disease duration (yrs)	–	0.46 ± 0.36	9.6 ± 3.3	<0.0001
DAS 28	–	6.7 (5.7–7.9)	4.3 (3.6–5.3)	<0.0001
Atherosclerotic indices				
Mean cIMT (mm) [#]	0.42 ± 0.07	0.52 ± 0.11	0.51 ± 0.09	0.0005
ED-FMD (%) [*]	12.23 ± 4.6	6.71 ± 4.32	11.68 ± 7.57	0.01
Inflammatory markers				
hsCRP (mg/l) *	3.50 ± 1.15	32.30 ± 43.53	14.16 ± 20.14	0.0002
ESR (mm/Hg) **	16.82 ± 7.1	66.34 ± 30.12	37.81 ± 19.5	<0.0001
TNF-α (ng/ml) ***	1.22 ± 1.2	2.41 ± 0.5	6.64 ± 4.2	0.0002
IL-1β (pg/ml) [#]	6.04 ± 1.8	7.82 ± 3.3	8.67 ± 3.1	0.08
Endothelial markers				
sICAM (pg/ml) [#]	107.8 ± 14.7	176.2 ± 52.5	179.5 ± 34.5	0.0016
SOD (U/ml) ***	1138 ± 769.2	235.7 ± 244.4	619.3 ± 251.2	<0.0001
GSH (µM) **	35.31 ± 12.9	3.28 ± 2.3	7.25 ± 3.5	<0.0001
iNOS (U/ml) *	53.0 ± 10.9	55.34 ± 27.5	38.49 ± 12.7	0.03
Adipokines				
Resistin (pg/ml) **	70.64 ± 42.24	381.8 ± 299.6	1084 ± 805.5	<0.0001
Leptin (ng/ml) **	0.41 ± 0.2	1.63 ± 1.2	2.69 ± 1.0	<0.0001
Adiponectin (µg/ml) [#]	100.6 ± 40.2	420.3 ± 220.5	297.5 ± 205.2	0.0001

Data are expressed as mean value ± standard deviation (SD), median (IQR) or number of individuals. Abbreviations: BMI: body mass index; ESR: erythrocyte sedimentation rate; HDL: high-density lipoprotein; LDL: low-density lipoprotein; hsCRP: high sensitivity C-reactive protein; cIMT: carotid intima-media thickness; ED-FMD: endothelial dependent flow mediated vasodilatation; TNFα: tumor necrosis factor α; IL-1β: Interleukin-1β; sICAM: soluble intracellular adhesion molecule; iNOS: inducible nitric oxide synthase; SOD: super oxide dismutase; GSH: Glutathione reductase; IQR = Inter Quartile range; yr(s): year(s). *p* < 0.05 is considered significant.

p* < 0.05; *p* < 0.01; ****p* < 0.001; #*p* = not significant; these are *p* values of t-test between RA < 1yr and RA > 5yrs.

used for comparing categorical variables. Risk of abnormal HOMA-IR in patients was quantified as Odds Ratio (OR) with *p* value. Correlation between variables was examined using the Pearson's correlation coefficient or Spearman's correlation coefficient and linear regression method as appropriate. All *p* values < 0.05 were considered statistically significant. All statistical analyses were performed using GraphPad Prism software ver 5.0.

Results

The comparison of demographic, clinical features and the serum biomarker levels of the long duration RA, short duration RA and the control group have been summarized in [Table 1]. The three groups were comparable among themselves in variables like age and gender. The mean body mass index (BMI) of healthy controls was more than that of the RA patients although values were not statistically significant. TC and

TG levels were comparable among the groups. HDL and to some extent LDL exhibited significant differences between the groups. Inflammatory markers like hsCRP and ESR were significantly higher in the patient groups than in the control.

DAS 28, hsCRP and ESR were significantly lower in the long duration RA group with severe disease activity as compared to the short duration RA [Table 1]. Median insulin levels were similar in all three study groups but the average glucose levels differed significantly between them. HOMA-IR was significantly higher in the long duration RA group when compared to short duration RA and controls [Table 1]. Taking the median of the control group (0.42) as the cut off for the normal HOMA-IR, 11 short duration RA patients (44%), 19 long duration RA patients (76%) and 5 controls (21%) had abnormal median HOMA-IR which showed high significance in chi-square test [*p* = 0.002]. Long duration RA patients had higher incidence of increased HOMA-IR values when compared to controls (OR = 10.86; *p* = 0.001) and to short duration RA (OR = 0.23; *p* = 0.05).

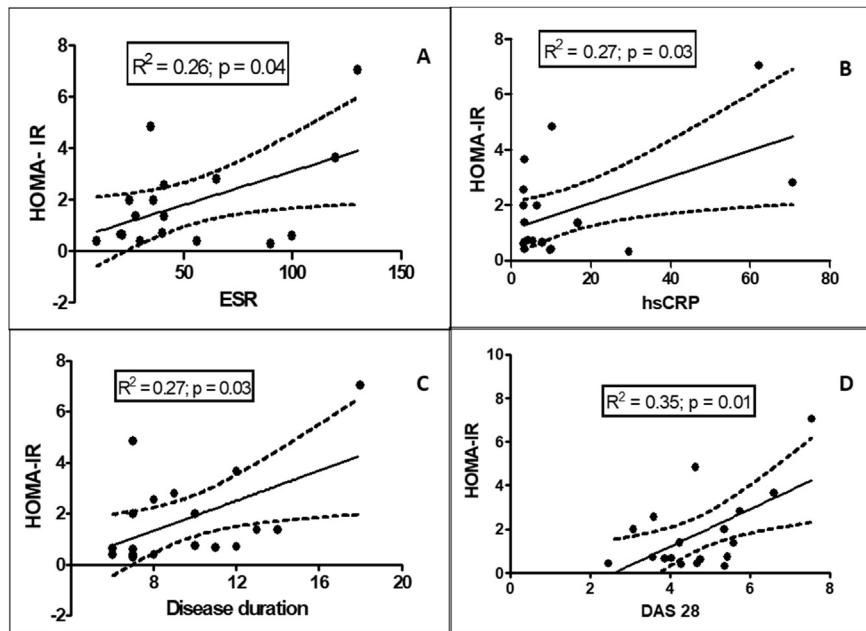


Fig. 1 Simple linear regression analysis representing the association of HOMA-IR with different variables with ESR (A) hsCRP (B) disease duration (C) and DAS 28 (D) of the long duration RA (RA>5 yrs) patients; R^2 = simple linear regression coefficient of determination; $p < 0.05$ is considered significant.

Carotid IMT was significantly higher in both the RA patients group than in the healthy controls [Table 1]. In short duration RA patients, median FMD% was significantly lower as compared to long duration RA patients and control.

When the patient groups were compared, pro-inflammatory markers like $TNF\alpha$, resistin and leptin were highest in long duration RA, higher in short duration RA when compared to control group respectively. IL- 1β , though not significant, showed a trend in change amongst the three study groups. Stress markers like SOD, GSH and iNOS were lower in the patient groups as compared to control group. Adiponectin showed a trend in significance and sICAM showed no difference between the two patients group [Table 1].

From [Fig. 1], the significant association of HOMA-IR with age ($r^2 = 0.21$; $p = 0.05$), ESR (A), CRP (B), disease duration (C)

and DAS28 (D) for long duration RA patients was evident. [Fig. 2] demonstrated significant correlation of HOMA-IR with the respective mean cIMT values of the short (A) and long duration (B) patients group. There was also trends of association of HOMA-IR with FMD% values ($r^2 = 0.20$; $p = 0.07$) of the long disease duration RA group.

Since long disease duration RA group is the study group, the association studies have been performed with parameters of this group.

Correlation analyses (as shown in [Table 2]) of the adipokines levels of long duration RA patients with their respective inflammatory and stress markers exhibited that leptin was positively correlated with BMI, ESR, $TNF\alpha$, hsCRP, DAS28 and showed trends in negative correlation with the anti-stress markers like SOD and GSH. Adiponectin showed significant

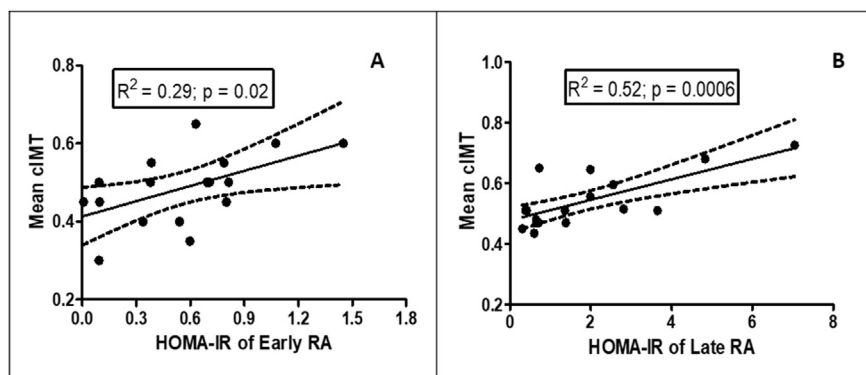


Fig. 2 Simple linear regression analysis representing the association of cIMT with HOMA-IR of (A) short duration RA (RA<1yr) and that of (B) long duration RA (RA>5yrs) respectively; R^2 = simple linear regression coefficient of determination; $p < 0.05$ is considered significant.

Table 2 Association of the adipokines levels of RA patients >5 years with their respective inflammatory and stress markers.

Variables	Resistin		Leptin		Adiponectin	
	Correlation Coefficient	p value	Correlation Coefficient	p value	Correlation Coefficient	p value
BMI	0.49	0.04	0.49	0.04	−0.22	0.38
ESR	0.34	0.18	0.49	0.04	−0.19	0.45
hsCRP	0.13	0.61	0.48	0.046	0.13	0.59
DAS 28	0.39	0.10	0.69	0.001	−0.079	0.75
TNF α	0.42	0.07	0.49	0.04	−0.35	0.14
SOD	−0.46	0.06	−0.45	0.06	0.53	0.02
GSH	−0.18	0.58	−0.03	0.92	0.37	0.19
iNOS	−0.17	0.55	0.47	0.05	0.39	0.14

Abbreviations: BMI: body mass index; ESR: erythrocyte sedimentation rate; hsCRP: high sensitivity C-reactive protein; DAS 28: Disease Activity Score for 28 joints; TNF α : tumor necrosis factor α ; iNOS: inducible nitric oxide synthase; SOD: super oxide dismutase; GSH: Glutathione reductase. $p < 0.05$ is considered significant.

positive correlation with only the anti-stress marker SOD and trends of negative correlation with BMI, ESR, DAS 28 and TNF α . However, resistin only showed significant positive correlation with BMI and some trends in correlation with TNF α and SOD.

Linear regression analysis was performed to see whether these markers of inflammation and stress together with the adipokines had any association with HOMA-IR, cIMT and FMD% values of the long duration RA patients [Table 3].

HOMA-IR was significantly associated with TNF α , resistin, leptin and showed some trends with adiponectin. Mean cIMT showed dependency on all the parameters producing significant results with TNF α , iNOS, resistin, leptin and showing tendency of association with SOD and adiponectin. However, FMD % of the long duration RA patients significantly associated with only iNOS ($r^2 = 0.32$; $p = 0.01$). BMI showed trends in association with both HOMA-IR and mean cIMT ($r^2 = 0.17$; $p = 0.08$ for each respectively).

Discussion

Inflammation and endothelial dysfunction (ED) has always been closely associated with RA. Both pathological conditions play a pivotal role in RA associated complications and mortality.

ED, being the principal underlying mechanism in atherosclerosis, was significantly pronounced in short duration RA patients as compared to controls suggesting endothelial activation; however, the FMD% values showed improvement in the long disease duration group which could be because of folic acid supplements that these patients have been receiving as a part of the regular treatment dose with methotrexate (MTX). Folic acid is known to have beneficial effect on uncoupling of nitric oxide and reduction of superoxide radicals [30]. This improvement in ED and the oxidative stress levels of the patients with long term treatment is also demonstrated by the levels of anti-stress markers like SOD and GSH which was higher in the long duration RA and healthy controls as compared to short duration RA. Similarly, iNOS is significantly higher in the short duration group when compared to long duration RA patients confirming the

improved ED state. Several studies have shown that hypoxia and endothelial stress precedes the initiation of both RA and IR pathogenesis [31] as like the present study; but here the endothelial stress later improves with decrease in disease activity and severity.

The mean cIMT, the major atherosclerotic index, was significantly higher in both RA patients' group than that in healthy controls. The atherogenic lipid profile of patients and controls were similar in our study. Several studies attempting to compare lipid profiles in RA patients to those of controls have reported that active RA leading to a fall in both LDL-C and HDL-C levels is generally accepted. This 'lipid paradox' phenomenon—reduction in serum lipids associated with increased CVD risk—is also seen in other autoimmune inflammatory diseases and sepsis [32]. However, in this study, long duration RA patients showed a marginal alteration in both HDL and LDL levels, unlike the aforesaid phenomena, inferring that the atherogenic lipid profile cannot completely explain the higher cIMT levels in early RA patients when compared to healthy controls.

We found that disease activity marker DAS 28 along with the inflammatory variables like hsCRP and ESR showed significant improvement in the long duration RA patients, suggesting that the effect of cDMARDs on the overall inflammatory state of the patients is quite effective thereby reducing the disease activity and severity. However, none of these variables were significantly correlated with the

Table 3 Linear regression of the clinical parameters of RA patients >5 years with their respective serum biomarkers.

Variables	HOMA-IR		Mean cIMT		FMD%	
	R ²	p value	R ²	p value	R ²	p value
TNF α	0.36	0.008	0.49	0.001	0.05	0.38
SOD	0.004	0.8	0.16	0.09	0.01	0.7
iNOS	0.08	0.25	0.35	0.001	0.36	0.01
Resistin	0.33	0.031	0.51	0.0008	0.02	0.59
Leptin	0.39	0.0054	0.23	0.04	0.03	0.51
Adiponectin	0.19	0.06	0.17	0.08	0.009	0.71

R² = Multiple coefficient of determination. $p < 0.05$ considered significant. For other definitions, see [Table 1].

increased cIMT for the RA patients, as supported by few previous studies [7], implying that the cDMARDs can control the articular damage but the extra-articular features and the pathophysiological damage is not being targeted or targeted only partially.

This study also demonstrated that HOMA-IR was significantly higher in the long duration RA group when compared to short duration RA and control group. Moreover, likelihood of higher HOMA-IR in long duration RA patients was reconfirmed by estimation of odds ratio. Several studies in literature have concluded that RA patients have a higher HOMA index than controls showing that the latter was associated with markers for inflammation and disease activity such as hsCRP, ESR and DAS28 [33] and with age disease duration and steroid use [34]. Unlike them, this study had strictly excluded patients on steroid use as glucocorticoid therapy strongly contributed to development of obesity and IR [17]. Meanwhile, as supported by literature [20], the strong associations of cIMT with HOMA-IR for both the groups respectively could suggest a common underlying pathophysiological process.

The proinflammatory cytokines, TNF α and IL-1 β , were highest in long duration RA patients, higher in short duration RA as compared to control group. IL-1 β did not show significant results unlike TNF α , possibly due to protective effect of MTX against IL-1 β [35]. Although the long duration RA patients were on cDMARDs, the TNF- α mediated inflammation in RA physiology does not seem to have completely receded at the cellular level thereby leading to a state of persistent low grade chronic inflammation which could be the factor inducing the increased incidence of IR by altering adipokine secretion and activation of proinflammatory signaling pathways as shown in few recent studies [36,37]. Results also indicate leptin and resistin levels were higher whereas adiponectin, known to have anti-inflammatory properties, was lower in long duration RA patients group clearly suggesting that with disease progression, the increased inflammatory state has stimulated the adipocytes to release resistin and leptin which have been associated with IR states in RA patients [21,22].

Interestingly, obesity induced inflammation could not be the reason for this increased risk of IR and atherosclerosis. The RA patients had lower BMI than the controls, which could be related to TNF mediated rheumatoid cachexia [38] or leptin mediated hunger control [39], in turn leading to break down of stored fat into free fat mass in the body thus causing IR. Though, BMI did not show significant difference among the groups, this lower BMI still appears as a contributor to the increased risk and could be projected as a major factor with a larger study group.

From correlation analysis, leptin emerged as the major contributing adipokine exhibiting strong correlation with BMI, inflammatory and disease activity variables and with stress markers like iNOS and SOD suggesting its dependency on inflammatory state [40] and stress levels of the patients [41] respectively. Resistin also showed significant correlation with BMI and trends in correlation with TNF α and SOD, suggesting similar function as leptin; both these adipokines increases with increase in free fat mass [42]. Moreover, adiponectin showed strong positive correlation with SOD indicating its improvement with the reduction of stress. Literature reports the relation of inflammatory and stress

markers with adipocytokines [43,44] but the information on the strong association amongst them in RA patients are scanty [45].

In the last few years there have been some studies establishing the relation between IR, RA and cardiovascular risk [15,46] but only few studies have emphasized on the relation of biomarkers with the clinical predictors of atherosclerosis [21]. HOMA-IR had significant association with TNF α , leptin and resistin and showed a trend in association with adiponectin thus re-establishing the fact that IR in RA patients is primarily dependent on the inflammatory cytokines and adipokines which on the other hand increases the risk of atherosclerosis in these patients. This study also demonstrated significant association of cIMT with leptin, resistin, TNF α and iNOS and trends of association with SOD and adiponectin implying that inflammation and insulin resistance are the major contributor to increased CVD risk in general [47] and more so in RA population [20,21].

The likelihood of long duration RA patients having higher HOMA-IR is quite high in this study although the sample size was small; the stringent exclusion of possible confounders might be of importance. Prospective follow up of short duration (early) RA patients would possibly throw more light on the issue.

Conclusions

Patients with RA have a higher probability of having IR than general population; more so in long duration RA compared to their short disease duration counterparts. In RA, even in absence of obesity, increased levels of proinflammatory cytokines and oxidative stress together with altered secretion of adipokines play a complex role in the atherosclerosis progression. Moreover, it could also be suggested that treatment with combination DMARDs in RA patients though effectively seem to target the clinical outcome of the patients, is not enough to control low grade chronic inflammation in the molecular level. The underlying molecular mechanisms of inflammation, primarily mediated by TNF- α , continue their interplay in various signaling pathways thus prolonging the risk of atherosclerosis and diabetes in RA patients, even after regular treatment with cDMARDs. Hence, controlling other pathways of inflammation, especially TNF- α , from the very early stages of the disease is important not only to control the disease but also to limit the comorbidities and imminent cardiovascular events of RA patients.

Conflicts of interest

The authors would like to state that there is no conflict of interest regarding any financial or personal issues with any individual or group.

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REFERENCES

- [1] Bacon PA, Stevens RJ, Carruthers DM, Young SP, Kitas GD. Accelerated atherogenesis in autoimmune rheumatic diseases. *Autoimmun Rev* 2002;6:338–47.
- [2] Hannawi S, Haluska B, Marwick TH, Thomas R. Atherosclerotic disease is increased in recent-onset rheumatoid arthritis: a critical role for inflammation. *Arthritis Res Ther* 2007;9:R116.
- [3] Adhikari MC, Guin A, Chakraborty S, Sinhamahapatra P, Ghosh A. Subclinical atherosclerosis and endothelial dysfunction in patients with early rheumatoid arthritis as evidenced by measurement of carotid intima-media thickness and flow-mediated vasodilatation: an observational study. *Semin Arthritis Rheum* 2012;41:669–75.
- [4] Roman MJ, Moeller E, Davis A, Paget SA, Crow MK, Lockshin MD, et al. Preclinical carotid atherosclerosis in patients with rheumatoid arthritis. *Ann Intern Med* 2006;144:249–56.
- [5] Gonzalez-Juanatey C, Llorca J, Martin J, Gonzalez-Gay MA. Carotid intima-media thickness predicts the development of cardiovascular events in patients with rheumatoid arthritis. *Semin Arthritis Rheum* 2009;38:366–71.
- [6] Chung CP, Oeser A, Raggi P, Gebretsadik T, Shintani AK, Sokka T, et al. Increased coronary-artery atherosclerosis in rheumatoid arthritis: relationship to disease duration and cardiovascular risk factors. *Arthritis Rheum* 2005;52:3045–53.
- [7] del Rincón ID, Williams K, Stern MP, Freeman GL, Escalante A. High incidence of cardiovascular events in a rheumatoid arthritis cohort not explained by traditional cardiac risk factors. *Arthritis Rheum* 2001;44:2737–45.
- [8] McInnes IB, Schett G. Cytokines in the pathogenesis of rheumatoid arthritis. *Nat Rev Immunol* 2007;7:429–42.
- [9] Dessein PH, Joffe BI, Singh S. Biomarkers of endothelial dysfunction, cardiovascular risk factors and atherosclerosis in rheumatoid arthritis. *Arthritis Res Ther* 2005;7:R634–43.
- [10] Draznin B. Molecular mechanisms of insulin resistance: serine phosphorylation of insulin receptor substrate-1 and increased expression of p85 α -the two sides of a coin. *Diabetes* 2006;55:2392–7.
- [11] Grundy SM. What is the contribution of obesity to the metabolic syndrome? *Endocrinol Metab Clin N Am* 2004;33:267–82.
- [12] de Luca C, Olefsky JM. Inflammation and insulin resistance. *FEBS Lett* 2008;582:97–105.
- [13] Wasko MC, Kay J, Hsia EC, Rahman MU. Diabetes mellitus and insulin resistance in patients with rheumatoid arthritis: risk reduction in a chronic inflammatory disease. *Arthritis Care Res* 2011;63:512–21.
- [14] Kaplan MJ. Cardiovascular complications of Rheumatoid Arthritis -Assessment, prevention and treatment. *Rheum Dis Clin N Am* 2010;36:405–26.
- [15] La Montagna G, Cacciapuoti F, Buono R, Manzella D, Mennillo GA, Arciello A, et al. Insulin resistance is an independent risk factor for atherosclerosis in rheumatoid arthritis. *Diabetes Vasc Dis Res* 2007;4:130–5.
- [16] Shahin D, Eltoraby E, Mesbah A, Houssem M. Insulin resistance in early untreated rheumatoid arthritis patients. *Clin Biochem* 2010;43:661–5.
- [17] Dessein PH, Joffe BI, Stanwix AE, Christian BF, Veller M. Glucocorticoids and insulin sensitivity in rheumatoid arthritis. *J Rheumatol* 2004;31:867–74.
- [18] Wolfe F, Michaud K. Corticosteroids increase the risk of diabetes mellitus in rheumatoid arthritis and contribute to the risk of myocardial infarction and heart failure [abstract]. *Ann Rheum Dis* 2004;63:495A.
- [19] Nurmohamed MT. Atherogenic lipid profiles and its management in patients with rheumatoid arthritis. *Vasc Health Risk Manag* 2007;3:845–52.
- [20] Pamuk ON, Unlü E, Cakir N. Role of insulin resistance in increased frequency of atherosclerosis detected by carotid ultrasonography in rheumatoid arthritis. *J Rheumatol* 2006;33:2447–52.
- [21] Kang Y, Park HJ, Kang MI, Lee HS, Lee SW, Lee SK, et al. Adipokines, inflammation, insulin resistance, and carotid atherosclerosis in patients with rheumatoid arthritis. *Arthritis Res Ther* 2013;15:R194.
- [22] Rho YH, Chung CP, Solus JF, Raggi P, Oeser A, Gebretsadik T, et al. Adipocytokines, insulin resistance and coronary atherosclerosis in rheumatoid arthritis. *Arthritis Rheum* 2010;62:1259–64.
- [23] Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 1988;31:315–24.
- [24] Bruce B, Fries J. The Stanford health assessment questionnaire (HAQ): a review of its history, issues, progress, and documentation. *J Rheumatol* 2003;30:167–78.
- [25] Prevoo ML, van't Hof MA, Kuper HH, van Leeuwen MA, van de Putte LB, van Riel PL. Modified disease activity scores that include twenty-eight-joint counts - development and validation in a prospective longitudinal study of patients with rheumatoid arthritis. *Arthritis Rheum* 1995;38:44–8.
- [26] Fransen J, van Riel PL. The disease activity score and the EULAR response criteria. *Clin Exp Rheumatol* 2005;23(5 Suppl 39):S93–9.
- [27] Corretti MC, Anderson TJ, Benjamin EJ, Celermajer D, Charbonneau F, Creager MA, et al. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. *J Am Coll Cardiol* 2002;39:257–65.
- [28] Bots ML, Westerink J, Rabelink TJ, de Koning EJP. Assessment of flow-mediated vasodilatation (FMD) of the brachial artery: effects of technical aspects of the FMD measurement on the FMD response. *Eur Heart J* 2005;26:363–8.
- [29] Persson J, Stavenow L, Wikstrand J, Israelsson B, Formgren J, Berglund G. Noninvasive quantification of atherosclerotic lesions. Reproducibility of ultrasonographic measurement of arterial wall thickness and plaque size. *Arterioscler Thromb* 1992;12:261–6.
- [30] Stroes ES, van Faassen EE, Yo M, Martasek P, Boer P, Govers R, et al. Folic acid reverts dysfunction of endothelial nitric oxide synthase. *Circ Res* 2000;86:1129–34.

- [31] Bornfeldt KE, Tabas I. Insulin resistance, hyperglycemia, and atherosclerosis. *Cell Metabol* 2011;14:575–85.
- [32] Robertson J, Peters MJ, McInnes IB, Sattar N. Changes in lipid levels with inflammation and therapy in RA: a maturing paradigm. *Nat Rev Rheumatol* 2013;9:513–23.
- [33] Dessein PH, Joffe BI. Insulin resistance and impaired beta cell function in rheumatoid arthritis. *Arthritis Rheum* 2006;54:2765–75.
- [34] Chung CP, Oeser A, Solus JF, Gebretsadik T, Shintani A, Avalos I, et al. Inflammation-associated insulin resistance: differential effects in rheumatoid arthritis and systemic lupus erythematosus define potential mechanisms. *Arthritis Rheum* 2008;58:2105–12.
- [35] Chang DM, Weinblatt ME, Schur PH. The effects of methotrexate on interleukin 1 in patients with rheumatoid arthritis. *J Rheumatol* 1992;19:1678–82.
- [36] Berg AH, Scherer PE. Adipose tissue, inflammation, and cardiovascular disease. *Circ Res* 2005;96:939–49.
- [37] Tilg H, Moschen AR. Adipocytokines: mediators linking adipose tissue, inflammation and immunity. *Nat Rev Immunol* 2006;6:772–83.
- [38] Elkan AC, Håkansson N, Frostegård J, Cederholm T, Hafström I. Rheumatoid cachexia is associated with dyslipidemia and low levels of atheroprotective natural antibodies against phosphorylcholine but not with dietary fat in patients with rheumatoid arthritis: a cross-sectional study. *Arthritis Res Ther* 2009;11:R37.
- [39] Elmquist JK, Elias CF, Saper CB. From lesions to leptin: hypothalamic control of food intake and body weight. *Neuron* 1999;22:221–32.
- [40] Peng DZ, Liu XW, Huang L, Zhu XF, Zheng YQ, Wang LX. Relationship between leptin and chronic inflammatory state in uremic patients. *Eur Rev Med Pharmacol Sci* 2014;18:2882–5.
- [41] Rajendran K, Devarajan N, Ganesan M, Ragunathan M. Obesity, inflammation and acute myocardial infarction - expression of leptin, IL-6 and high sensitivity-CRP in Chennai based population. *Thromb J* 2012;10:13.
- [42] Yannakoulia M, Yiannakouris N, Bluher S, Matalas AL, Klimis-Zacas D, Mantzoros CS. Body fat mass and macronutrient intake in relation to circulating soluble leptin receptor, free leptin index, adiponectin, and resistin concentrations in healthy humans. *J Clin Endocrinol Metab* 2003;88:1730–6.
- [43] Markaki A, Gkouskou K, Stylianou K, Dermitzaki E, Perakis K, Margioris A, et al. Relationship between adiposity, adipokines, inflammatory markers and lipid profile in hemodialysis patients. *Eur Rev Med Pharmacol Sci* 2014;18:1496–8.
- [44] Rajkovic N, Zamaklar M, Lalic K, Jotic A, Lukic L, Milicic T, et al. Relationship between obesity, adipocytokines and inflammatory markers in type 2 diabetes: relevance for cardiovascular risk prevention. *Int J Environ Res Publ Health* 2014;11:4049–65.
- [45] Hayashi H, Sato K, Sato-Mito N, Kaburagi T, Yoshino H, Higaki M, et al. Nutritional status in relation to adipokines and oxidative stress is associated with disease activity in patients with rheumatoid arthritis. *Nutrition* 2012;28:1109–14.
- [46] Deo SS, Chogle AR, Mistry KJ, Shetty RR, Nadkar UL. Increased prevalence of subclinical atherosclerosis in rheumatoid arthritis patients of Indian descent. *Exp Clin Cardiol* 2012;17:20–5.
- [47] Abu-Farha M, Behbehani K, Elkum N. Comprehensive analysis of circulating adipokines and hsCRP association with cardiovascular disease risk factors and metabolic syndrome in Arabs. *Cardiovasc Diabetol* 2014;13:76.