ORIGINAL RESEARCH

Oxidative stress, Dyslipidemia and Inflammatory markers in patients with Rheumatoid Arthritis

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ABSTRACT

Background & Objectives: Rheumatoid arthritis is a chronic systemic inflammatory disorder with an unknown etiology that mainly involves a synovial joint. Cardiovascular disease is one of the more common complications in patients with rheumatoid arthritis (RA). Therefore, the present study was planned to evaluate RA patients in terms of clinical features, socio-demographic characteristics, behavioral traits, anthropometric measurements, traditional cardiovascular risk factors history, clinical examination, lipid profile, and inflammatory biochemical markers- (hs-CRP, Hcys, ESR) and MDA. **Methods**: In this observational case-control study, 150 confirmed cases of rheumatoid arthritis, and 150 age and sex-matched controls were included. Both rheumatoid arthritis subjects and healthy individuals were thoroughly investigated for traditional cardiovascular risk factors, oxidative stress, lipid levels, and inflammation. **Results**: The mean levels of smoking habit, physical activity, BMI, WHR, SBP, RA factor, RA duration, and ESR, lipid profile, hs-CRP, Hcys and MDA showed a statistically significant difference ($P<0.05$) between the two groups. RA subjects were associated with an increase in level of these parameters as compared to the control group. **Conclusion:** The levels of traditional cardiovascular risk factors, oxidative stress lipid levels, and inflammatory markers were statistically higher in rheumatoid arthritis compared to the control group. Therefore, patients with rheumatoid arthritis have to be screened for further complications in order to identify them earlier and to provide early treatment.

Keywords: Rheumatoid arthritis, BMI, ESR, Oxidative stress, Lipid profile, hs-CRP, Hcys, and MDA.

INTRODUCTION

Rheumatoid arthritis (RA) is the most common inflammatory autoimmune arthritis in adults [1].RA is most frequent in approximately one percent of the population around age 30-50 approximately and the disease is three times more common in women than in men, but the gender difference in older age groups decreases [2,3]. It includes primarily synovial joints, typically symmetrical in nature [4]. Major clinical manifestations of RA are divided into articular and extra-articular manifestations [5]. Fatigue, anorexia, general fatigue and ambiguous musculoskeletal symptoms are associated with the onset of RA disease in approximately 2/3 of patients, this initial phase may take several weeks or months [6]. Specific symptoms gradually appear along with symmetrical joints, in particular hand and foot joints [7]. During this disease and even before arthritis begins extra-articular manifestations of RA can occur [8]. Approximately 40 percent of patients acquire extra-articular manifestations during their lifetime. Cardiovascular disease is the most common cause of death in these patients, as an extra-articular manifestation of RA [9]. Cardiovascular Disease (CVD) is the leading cause of death among RA patients that takes place 10 years earlier than in the general population in RA patients on average [9].

Although traditional factors of risk, such as high blood pressure, tobacco, alcohol abuse, diabetes and dyslipidemia are taken into account, the risk of CVD is still high [10] . This increased risk of CVD in patients with RA is likely to be caused by both systemic inflammation in RA and by traditional CVD risk factors. Increased recognition of the role of

inflammation in pathogenesis of atherosclerosis has placed special emphasis on the relationship between RA and cardiovascular disease [11].Systemic inflammation markers confer a statistically important additional risk for cardiovascular death for RA patients even after control over the risk and comorbidity of traditional CV factors [16]. The concentration of high-sensitive C reactive proteins, homocysteine and malondialdehyde in baseline is an important predictor of cardiovascular death in patients, irrespective of other factors of disease severity [17].

Since subclinical manifestations occur many years before clinical occurrence, it is therefore essential for early recognition and to make the disease management better, in order to prevent these complications [18]. Therefore, the present study was planned to evaluate RA patients in terms of clinical features, socio-demographic characteristics, behavioral traits, anthropometric measurements and traditional cardiovascular risk factors history, clinical examination, lipid profile, and inflammatory biochemical markers - hs-CRP, Hcys and MDA.

MATERIALS AND METHODS

It was a case control study design based on the random sampling method which was conducted in the Central Laboratory of Adesh Institute of Medical Sciences and Research, Adesh University, Bathinda. In this study, 150 confirmed cases of rheumatoid arthritis and 150 age and sex matched were included. The study was conducted after taking institutional ethical clearance from the Ethics Committee of Adesh University, Bathinda and informed consent of the patients.

INCLUSION CRITERIA

Rheumatoid arthritis subjects more than 20 years of age who fulfilled the revised American College of Rheumatology/ European League Against Rheumatism Criteria (ACR/EULAR), both from outpatient and inpatient department of Adesh Institute of Medical Sciences and Research who fulfilled the revised criteria of American College of Rheumatology were included in this study.

EXCLUSION CRITERIA

Patients suffering from ischaemic heart disease, congenital heart disease, hypertension, pregnant females, patients with creatinine clearance < 30 ml/minute or serum creatinine > 3.0 mg/dl and patients with any immunosuppressive conditions or active infection were excluded from the study.

INVESTIGATIONS

Full history of subjects regarding age, duration of illness, socioeconomic status, drug history use and medical illness educational status, marital status, smoking and alcoholic habit, diet pattern, sleeping pattern, physical activity was gathered. All Samples were incubated at 37^0c for 10 minutes. After incubation samples were centrifuged at 3500 RPM for 10 minutes. Serum was then aliquoted in a separate tube and was used for various investigations like body mass index (BMI), rheumatoid arthritis (RA) factor, RA duration, erythrocyte sedimentation rate (ESR), total cholesterol (TC), high-density lipoprotein (HDL), lowdensity lipoprotein (LDL), very low-density lipoprotein (VLDL), triglyceride (TG), high sensitive C-reactive protein (hs-CRP), homocysteine (Hcys), malondialdehyde (MDA), were performed for all subjects.

STATISTICAL ANALYSIS

Data was expressed in terms ofnumber, percentage for categorical variables and mean ± standard deviation for continuous variables. The data was analysed with the help of excel software version 12. Independent sample t-test and chi-squared test were used to compare the continuous and categorical variables respectively between the study groups. A p value < 0.05 was considered statistically significant.

Table I. Comparison of clinical features, socio-demographic characteristics, behavioral traits, anthropometric measurements and traditional cardiovascular risk factors of the study participants.

Parameters	Categories	Cases		Controls		χ^2 p value
		n (150)	%	n (150)	%	
Age group	20-40	36	24.0	45	30.0	0.403
	41-60	87	58.0	76	50.7	
	61-80	27	18.0	29	19.3	
Gender	Male	59	39.3	67	44.7	0.105
	Female	91	60.7	83	55.3	
					43.3	
Smoking habit	Smoker	79	52.7	52	34..7	0.001
	Non- Smoker	71	47.3	98	65.3	
Alcoholichabit	Alcoholic	77	51.3	65	43.3	0.165
	Non- Alcoholic	73	48.7	85	56.7	
Physical activity	≤ 1hr	47	31.3	25	16.7	0.001
	> 1hr to ≤ 2hr	49	32.7	39	26.0	
	>2hr to <3hr	33	22.0	31	20.7	

	>3hr	21	14.0	55	36.7	
BMI >25 kg/m^2	<18.5 kg/m^2	05	3.3	07	4.7	0.001
	18.5-24.9 kg/m^2	54	36.0	128	85.3	
	25-29.5 kg/m^2	59	39.3	11	7.3	
	≥30 kg/m^2	32	21.3	04	2.7	
	90-99 mmHg	18	12.0	05	3.3	
	>100 mmHg	11	7.3	03	2.0	

Table II. Comparison of dyslipidemia of study participants.

Changes	**Cases**		**Controls**		χ^2 **p value**
	n (150)	**%**	**n (150)**	**%**	
Lipid profile changes					
TC > 200	80	53.3	12	8.0	0.001
HDL< 45	57	38.0	05	3.3	0.001
LDL > 150	24	16.0	07	4.7	0.001
VLDL > 40	63	42.0	08	5.3	0.001
TG > 180	71	47.3	10	6.7	0.001

TC: Total cholesterol; TG: Triglycerides; HDL: high-density lipoprotein cholesterol; LDL: low-density lipoprotein cholesterol; VLDL: Very low-density lipoprotein cholesterol.
Results are presented as percentages.
p value < 0.05, considered as statistical significant.
p value < 0.001, considered as highly significant.
p value > 0.05, considered as non-significant.

Table III. Comparison of different characteristics and biochemical parameters of study participants.

Parameter	**Mean ± SD**		**p value**
	Cases n (150)	**Controls n (150)**	
BMI (Kg/m^2)	25.89 ± 2.50	22.10 ± 1.42	0.001
RA factor (units/ml)	78.58 ± 39.75	10.64 ± 4.64	0.001
RA duration (yrs.)	5.07 ± 2.12	-	0.001
ESR (mm/h)	28.04 ± 11.78	9.74 ± 4.12	0.001
TC (mg/dl)	221.08 ± 37.59	168.32 ± 24.06	0.001
HDL (mg/dl)	42.42 ± 4.22	44.52 ± 3.13	0.001
LDL (mg/dl)	137.72 ± 42.66	73.1 ± 22.34	0.001
VLDL (mg/dl)	48.3 ± 28.02	29.58 ± 11.03	0.001
TG (mg/dl)	219.66 ± 103.68	146.34 ± 52.95	0.001
hs-CRP (mg/L)	3.90 ± 1.51	1.25 ± 0.69	0.001
Hcys (µmol/L)	13.52 ± 2.91	7.32 ± 1.21	0.001
MDA (nmol/mL)	8.23 ± 2.17	3.75 ± 1.34	0.001

p value < 0.05, considered as statistical significant.
p value < 0.001, considered as highly significant.
p value > 0.05, considered as non-significant.

RESULT

In the present study, the range of age was 20-80 years with the mean age of 46.75 ± 15.67 years in rheumatoid arthritis subjects and 45.01 ± 13.04 years in healthy controls. Majority of the cases were in the age group of 41-60 years. Out of 150 rheumatoid arthritis (RA) subjects, 91 were females constituting 60.7% of all. Males comprised of 59 (39.3%) of cases.In the study group, both rheumatoid arthritis subjects and control group didn't showed statistically significant difference (P < 0.05) for most of the socio-demographic characteristics like age group, gender etc. (Table I).

Both rheumatoid arthritis and control subjects were similar for various behavioral traits like alcoholic habits but were different for smoking habit and physical activity which showed statistically significant difference (P < 0.05) (Table I). The comparison between rheumatoid arthritis subjects and control group showed statistically significant difference (P < 0.05) of BMI >25 kg/m^2.

Dyslipidemia was present in 80 (53.3%) rheumatoid arthritis subjects and 12 (8.0%) control group. There was statistically significant difference (P < 0.05) of TC (53.3% versus 8.0%), HDL (38.0% versus 3.3%), LDL (16.0% versus 4.7%), VLDL (42.0% versus 5.3%), and TG (47.3% versus 6.7%) in rheumatoid arthritis subjects as compared to control group (Table II).

The mean RA factor, RA duration and ESR showed statistically significant difference (P<0.05) between the two groups (Table III). Significant (P<0.05) increase in TC, LDL, VLDL, TG concentrations and significant (P<0.05) decrease in HDL concentrations were observed in RA subjects as compared to control group respectively (Table III).

DISCUSSION

The present study was undertaken to study traditional risk factors, oxidative stress, lipid profile and inflammatory markers in the patients of rheumatoid arthritis who attended outdoor or were admitted in the Department of Medicine, Adesh Institute of Medical Sciences and Research, Adesh University, Bathinda. They were clinically assessed, along with relevant investigations.The mean age of RA subjects was 46.75 ± 15.67 years which was comparable to study conducted by Masooleh et al [19] i.e. 49.7±11.56. In the studies conducted by Vizzardi et al [20], Liang et al [21] and Raof et al [22], the mean age was 60.4±12.2, 60.5 and 53.31 ±2.14, respectively.The mean duration of disease was 5.07 ± 2.12 years. The mean duration of disease in the studies conducted by Vizzardi et al [20], Guedes et al [23] and Dawson et al [24] was found to be 10.6±7.1, 11±8.7, and 12.7±7.99 respectively.

The mean levels of hs-CRP, Hcys and MDA were statistically significantly higher in RA subjects as compared to healthy controls and have altered lipid profile that can lead to increased cardiovascular and other complications. It has been shown that hs-CRP, reflect the acute phase response more closely than erythrocyte sedimentation rate (ESR) in RA subjects because elevations in the ESR can be created by high titres of RF and immunoglobulins, which may not rise acutely [25]. High serum hs-CRP levels were documented extensively as an independent CVD predictor [26]. In the present study, dyslipidemia was present in 53.3% rheumatoid arthritis subjects which were comparable to the study by Hadda et al [27] and Nisar et al [28] in which it was 38.5% and 44.8% respectively. More studies are required in order to explore the importance of lipid profile monitoring and to determine the impact on CV outcomes of typical CVD risk factors and inflammation in patients with RA [29]. Dyslipidemia management should be considered in RA patients as part of cardiovascular risk management.

The present study showed that the values of Hcys were significantly higher among RA patients than among participants in the control group. This finding is in concord with previous studies ofFujimaki et al [30], Vasiljevic et al [31] which showed that with the increase of general inflammatory markers, plasma homocysteine levels were increased in RA subjects. . It is also proposed that the vascular mortality found in RA patients with thrombosis history may be affected by hyperhomocysteinemia[32,33]. In addition, the levels of plasma homocysteine and general inflammatory marker levels were increased. Therefore, the present study concluded that homocysteine can influence and can be a predictive factor for inflammation in patients with hyperhomocysteinaemia in patients with RA. The current study showed that the values of MDA were significantly higher among RA patients than among participants in the control group. These results are in concord with the studies by Gambhir et al [34], Jaswal et al [35], Cimen et al [36], Akyol et al [37] and Kalavacherla et al [38], in which higher levels of MDA were found in RA patients as compared to control, and concluded an increased oxidative stress in RA patients. These findings further support the idea of free oxygen radicals which play a major role in chronic inflammatory pathogenesis and treatments may have a beneficial effect by protecting from ROS damage [38]. One of the major risk factors for atherosclerosis is oxidative stress, which is a result of imbalance between pro-oxidants and antioxidants [38]. The current study showed that the values of hs-CRP were significantly higher among RA patients than among participants in the control group. CRP is a mediator involved in pathogenesis of atherosclerosis, as well as an indicator of generally inflammatory reactions. The CRP value was determined using high-sensitivity assays, appeared to be an independent and robust prognostic factor for cardiovascular events. Persistently high hs-CRP levels raise the risk of death from cardiovascular disease over the course of RA. These findings were in concord with the studies of Vasiljevic et al [31], Shrivastava et al [39].

CONCLUSION

The levels of traditional cardiovascular risk factors, oxidative stress, lipid levels and inflammatory markers were statistically higher in rheumatoid arthritis as compared to control group. In addition to controlling RA disease, it is essential to manage traditional CVD risk factors. Identification and management of dyslipidemia, inflammation and oxidative stress should be considered an integral part of RA therapeutic strategies to prevent Cardiovascular and other complications in RA patients. Further studies are required to determine the interaction between these risk factors and their impact on disease activity in RA patients.Therefore, patients with rheumatoid arthritis have to be screened for these abnormalities in order to identify earlier and to provide early treatment. For evaluation of cardiac involvement in RA patients, the present study recommends a prospective study with greater sample size and a long follow-up period.

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CONFLICTS OF INTEREST

None.

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