

ENDOCAN AS AN EARLY PREDICTOR OF ENDOTHELIAL DYSFUNCTION AND ATHEROSCLEROSIS IN EGYPTIAN PATIENTS WITH RHEUMATOID ARTHRITIS

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Abstract. Background: Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease. Inflammation leads to endothelial cells activation. Activated endothelial cells with increased leukocytes adhesion molecules expression can induce endothelial dysfunction (ED) and atherosclerosis. **Aim:** to measure the serum level of Human endothelial cell-specific molecule-1 (endocan) in patients with rheumatoid arthritis and to evaluate its relation to endothelial dysfunction and atherosclerosis. **Materials and methods:** The study included 30 consecutive RA patients, and 30 healthy control subjects. RA disease activity was measured by the DAS-28 score. Serum endocan was measured by enzyme-linked immunosorbent assay (ELISA). The collected data were statistically analyzed using SPSS program version 16. **Results:** There was a significant elevation in Endocan level in RA patients (1.81 ± 0.66 ng/ml) compared to control group (1.41 ± 0.59 ng/ml) ($P = 0.012$). Serum endocan levels showed significant positive correlation with disease activity score-28(DAS-28), ESR, CRP, LDL and carotid intima media thickness (cIMT) ($P = 0.015, 0.004, < 0.001, 0.008, 0.005$ respectively). Linear regression analysis revealed that the serum endocan level, age and disease activity were independent predictors for cIMT, and atherosclerosis development in RA patients. **Conclusion:** Endocan is a useful marker to predict endothelial dysfunction and atherosclerosis in patients with rheumatoid arthritis.

Key words: rheumatoid arthritis, atherosclerosis, endocan, carotid intima media thickness

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic systemic inflammatory, autoimmune disease with unknown etiology. Symmetrical joint involvement, erosion and joints deformities due to synovial inflammation are almost the main manifestations of the disease [1, 2].

Atherosclerosis (AS) and cardiovascular diseases (CVD) are among the major causes of morbidity and mortality in RA. A role for endothelial dysfunction (ED) in RA associated with CVD has been postulated although endothelial dysfunction in RA is still poorly understood [3, 4].

Endothelial dysfunction is a failure of endothelial repair mechanisms. Assessment of endothelial function may be a useful tool to identify and monitor RA patients [5]. The ED in RA may be due to the altered function of endothelial progenitor cells, which are involved in vascularization and vascular repair [6].

Human endothelial specific molecule 1 (endocan) is a proteoglycan expressed by the inflammatory vascular endothelial cells [7]. Endocan inhibits

the interaction between adhesion molecule and the integrin on lymphocytes and monocytes. So, it affects leukocytic migration and adhesion to the endothelium [8].

Carotid intima media thickness ultrasound scan (cIMT) is used to detect subclinical atherosclerosis and the progress of associated cardiovascular diseases in patients with rheumatoid arthritis [9, 10].

Our aim is to measure the serum level of endocan in patients with rheumatoid arthritis and to evaluate its relation to endothelial dysfunction and atherosclerosis.

MATERIALS AND METHODS

This study was a cross-sectional study conducted on thirty consecutive RA patients, diagnosed according to the 2010 American College of Rheumatology/European League against Rheumatism Classification Criteria for Rheumatoid Arthritis [11], and 30 age and sex matched healthy subjects as the control group.

The study subjects were collected from the outpatient clinics of Physical Medicine, Rheuma-

tology & Rehabilitation department, Tanta University Hospitals.

The study had been performed in accordance with the Declaration of Helsinki ethical standards. The study was approved by the Local Research Ethics Committee of Faculty of Medicine, Tanta University (code no. 34423). Written informed consent was obtained from all the participants.

All RA patients had a minimum diagnosis of one year and must be under treatment with disease-modifying anti-rheumatic drugs (DMARDs).

Patients with known history of malignancy, smoking, or acute infection were excluded from this study. Patients who had diseases such as diabetes mellitus, hypothyroidism, liver or kidney disease, Cushing's syndrome, obesity, history of familial dyslipidemia, myocardial infarction during the last six months, and patients receiving medications affecting lipid metabolism such as lipid-lowering drugs, beta-blockers, oral contraceptives, or vitamin E were also excluded.

The participants were subjected to the following assessment:

- Full history taking
- Thorough clinical examinations.
- Assessment of disease activity:

The disease activity score of the patients was calculated with the DAS-28 index [12]. $\text{DAS-28} = (0.56 \times \sqrt{\text{number of tender joints}}) + (0.28 \times \sqrt{\text{number of swollen joints}}) + (0.014 \times \text{visual pain scale}) + (0.7 \times \text{erythrocyte sedimentation rate})$.

- Laboratory investigations:

CBC, RF, ESR, CRP, and lipid profile for both subject groups were measured.

- Carotid intima-media thickness measurement (cIMT)

Noninvasive measurements of the arterial wall thickness. Imaging was conducted using B-mode ultrasound with a 12-MHz transducer [13].

- Serum endocan measurement:

Blood samples were collected from both the patient and the control group after 12-h fasting. After appropriate centrifugation, the samples were stored at -80°C until testing. The level of endocan was assessed by the enzyme-linked immunosorbent assay (ELISA). The ELISA process was performed according to the manufacturer's instructions. Absorbance was evaluated by the ELISA reader [14].

Statistical Analysis SPSS version 16 was performed to analyze collected data [15]. Quantitative data were expressed as mean $\pm$ standard deviation; categorical variables were expressed as number and percentage. Paired t test and chi-square tests were used in comparing groups for significant differences, and the Pearson correlation was used in measuring the correlation coefficients. Linear regression model was performed to determine the predictors of cIMT levels. Statistical significance was evaluated at the $P\text{-value} < 0.05$.

RESULTS

The study included thirty RA patients (24 were females, and six were male); their ages ranged from 35 to 55 years with a mean of 45.08 ± 9.73 years. Thirty apparently healthy controls (21 were females, and nine were males) were included; their ages ranged from 32 to 54 years with a mean of 41.97 ± 10.01 years. There were no significant differences between the two studied groups according to age, sex, Hemoglobin, Triglycerides and LDL cholesterol. In RA patients, laboratory parameters including ESR, CRP, RF, and lipid profiles (TC, and HDL) were significantly higher than controls. Also, there was a significant increase in cIMT in rheumatoid arthritis patients compared to control groups with statistically significant difference (Table 1).

Serum endocan level showed statistically significant difference between both studied groups ($P = 0.012$). It was 1.81 ± 0.66 ng/ml in RA patients and 1.41 ± 0.59 ng/ml in the healthy controls (Table 2).

Serum endocan levels showed significant positive correlation with disease activity score-28 (DAS-28), ESR, CRP, LDL and carotid intima-media thickness (cIMT) ($P = 0.015, 0.004, < 0.001, 0.008, 0.005$ respectively). At the same time, no correlation was found with age, disease duration, Hemoglobin, RF, Triglycerides, Total cholesterol, and HDL cholesterol (Table 3) (Fig. 1).

According to a linear regression model, serum endocan level, age, and disease activity were independent predictors for cIMT, and atherosclerosis development in RA patients ($P = 0.015, 0.029, 0.045$ respectively) (Table 4).

Table 1. The clinical and laboratory data of both studied groups

Parameters		RA patients (n = 30)	Control (n = 30)	P value
Age		45.08 ± 9.73	41.97 ± 10.01	0.308
Sex (F/M)		24 (80%) / 6 (20%)	21 (70%) / 9 (30%)	0.410
DAS 28	remission	5 (16.67%)		
	Low activity	7 (23.33%)		
	Mod activity	9 (30%)		
	High activity	9 (30%)		
Hemoglobin(g/dl)		12.42 ± 1.47	13.54 ± 1.57	0.111
RF (IU/dl)		76.16 ± 112.57	7.12 ± 2.2	0.018*
ESR (1STh) mm/h		32.81 ± 10.76	17.75 ± 2.57	0.004*
CRP (mg/dl)		13.74 ± 7.39	5.58 ± 0.62	0.031*
Triglycerides (mg/dl)		132.79 ± 4.68	132.53 ± 3.41	0.408
Total cholesterol(mg/dl)		207 ± 11.88	188.5 ± 8.99	0.031*
LDL-cholesterol (mg/dl)		114.53 ± 6.93	112.28 ± 9.22	0.397
HDL-cholesterol (mg/dl)		40.41 ± 5.21	45.57 ± 6.04	< 0.001*
cIMT (mm)		0.67 ± 0.11	0.46 ± 0.8	.001*

RA: rheumatoid arthritis, DAS-28: disease activity score-28, RF: rheumatoid factor, ESR: erythrocyte sedimentation rate, CRP: C-reactive protein, LDL: Low density cholesterol, HDL: high density cholesterol, cIMT: carotid intima-media thickness.

Table 2. Serum endocan levels in rheumatoid arthritis patients and controls

Endocan (ng/ml)		RA patients (n = 30)	Control (n = 30)
Range		1.03-3.89	1.04-2.79
Mean ± SD		1.81 ± 0.66	1.41 ± 0.59
T-Test	t	0.395	
	P-value	0.012*	

*Significant < 0.05

Table 3. Correlation between serum endocan level and RA patients' data

	Endocan	
	r	P-value
Age	0.187	0.455
Disease duration	0.082	0.659
DAS 28	0.515	0.015*
Hemoglobin	0.18	0.414
RF	0.178	0.350
ESR	0.486	0.004*
CRP	0.609	< .001*
Triglycerides	0.161	0.328
Total cholesterol	0.150	0.369
LDL-cholesterol	0.393	0.008*
HDL-cholesterol	0.366	0.472
cIMT	0.444	0.005*

RA: rheumatoid arthritis, DAS-28: disease activity score-28, RF: rheumatoid factor, ESR: erythrocyte sedimentation rate, CRP: C-reactive protein, LDL: Low density cholesterol, HDL: high density cholesterol, cIMT: carotid intima-media thickness.

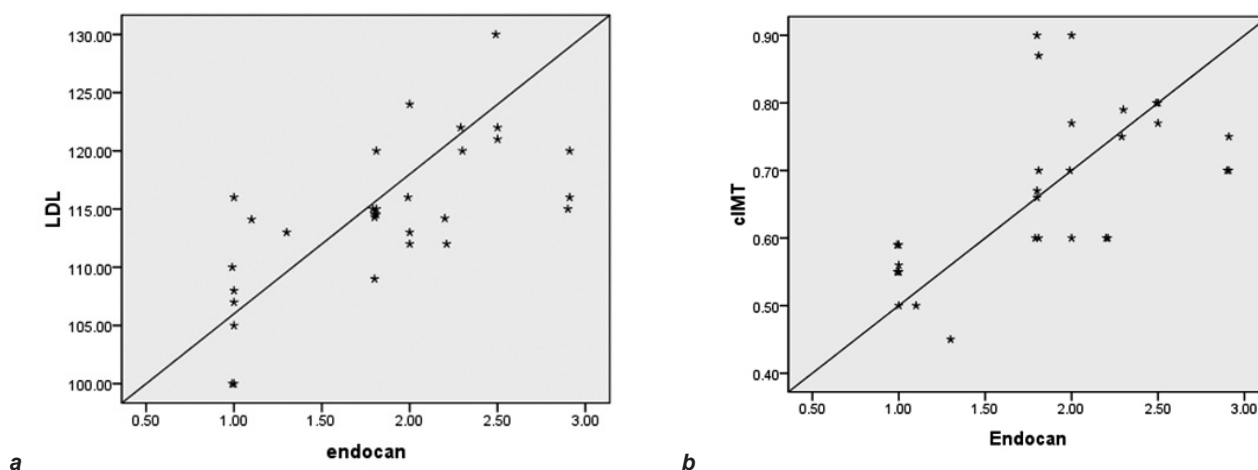


Fig. 1. Correlation between serum endocan level and; a. carotid intima-media thickness, b. LDL cholesterol

Table 4. Linear regression analysis for cIMT in the patients with RA.

	cIMT	
	Beta coefficient	P value
Age	0.318	0.045*
Disease duration	0.047	0.729
DAS-28	0.322	0.029*
Hdl-cholesterol	0.123	0.265
LDL-cholesterol	0.059	0.640
Endocan	0.597	0.015*

RA: rheumatoid arthritis, DAS-28: disease activity score-28, LDL: Low density cholesterol, HDL: high density cholesterol, cIMT: carotid intima-media thickness

DISCUSSION

Rheumatoid arthritis (RA) is characterized by severe inflammation and angiogenesis [16]. The production of Human endothelial cell-specific molecule-1 (endocan) is controlled by pro-inflammatory cytokines as tumor necrosis factor alpha and by pro-angiogenic agents, like vascular endothelial growth factor [17, 18].

Serum endocan levels are suggested to be increased in inflammatory disorders as in rheumatoid patients. Endocan can induce angiogenesis, and was detected in inflamed joints correlating with the severity of inflammation [19].

Endocan is a specific endothelial cell molecule and may be a useful marker for endothelial dysfunction. Endothelial dysfunction is the initial process in atherosclerosis and cardiovascular diseases development. [20, 21].

Carotid intima-media thickness measured by B-mode ultrasound is an easy, cheap, and reliable

method for atherosclerosis evaluation. Atherosclerosis is a systemic disease responsible for most cardiovascular events [22].

In our study, we tried to explore the possible relation between endocan levels and cardiac involvement in RA patients. Serum endocan levels were significantly higher in RA patients compared to control group. Also, RA patients had a significantly higher cIMT compared with control subjects (Table 1).

In agreement with our results Abdelsalam et al. [23] found that endocan levels were higher in patients with RA than healthy controls and significantly higher in patients with severe disease activity, Tuzcu et al. [24] also reported that endocan serum values of the RA patient group were significantly higher than those of the control group ($P = 0.009$), Balta et al. [25] found that serum endocan levels in Behçet's patients are higher than those in controls and higher in patients with increased disease activity. Icli et al. [21] and Ozalper et al. [26], showed that endocan levels in patients with systemic lupus erythematosus and familial Mediterranean fever respectively were higher compared with their controls.

In our patients, there was a significant positive correlation between serum endocan levels and the disease activity (DAS-28), severity of inflammation (ESR & CRP), and cardiovascular risk (LDL & cIMT). While no correlation was found with age, disease duration, hemoglobin, RF, triglycerides, total cholesterol, and HDL cholesterol (Table 2).

Our results coincided with those of Abdelsalam et al. [23] who found serum endocan levels positively correlated with disease activity (DAS-28), HAQ-DI, ESR, CRP modified Larsen score, and RF levels. Another study reported the same results in JIA but failed to report correlation with the severity of inflammation

[27]. Others reported that ESM-1 was elevated and correlated with ESR and CRP in Behcet's disease [25], systemic lupus erythematosus [21], systemic sclerosis [28] & inflammatory bowel disease [29].

Tuzcu et al. [24] showed that endocan values showed a significant positive correlation with age, DAS-28, and cIMT. There was no correlation with duration of disease, BMI, rheumatoid factor, anticyclic citrullinated peptide, triglycerides, total cholesterol, HDL or LDL-cholesterol in the RA patients.

Icli et al. [21] showed that endocan levels in patients with systemic lupus erythematosus were associated with cIMT.

In our study, the linear regression analysis revealed that the serum endocan level, age, and disease activity were independent predictors for cIMT, and atherosclerosis development in RA patients (Table 3).

Tuzcu et al. [24] revealed that there was an independent relationship between endocan and cIMT in RA patients ($P = 0.029$). Age and serum creatinine were also independent predictors for cIMT.

In multivariate regression analysis Kundi et al. [30] found that the endocan levels and hsCRP were independently associated with the presence of Slow Coronary Flow phenomenon.

In agreement with our results; Bicer et al. [31] revealed by multiple linear regression analysis that circulating endocan level is an independent predictor for cIMT in women with polycystic ovary syndrome ($\beta = 0.128$, $P = 0.011$).

In another study the multivariable regression analysis showed that serum endocan, ischemia-modified albumin (IMA) and high-sensitivity C-reactive protein (hsCRP) were independent risk factors for carotid intima-media thickness and endothelial dysfunction in type 2 diabetes mellitus ($P = 0.004$, 0.008 & 0.003) [32].

CONCLUSION

Elevated concentration of endocan in RA patients and its correlation with cIMT and DAS-28 scores suggest that serum endocan may be a novel biomarker that predicts cardiovascular risk, disease activity and severity in RA patients. So, endocan is an interesting target for future research. Anti-endocan therapies may be used in the future to possibly manage RA cardiovascular morbidity and disease activity.

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Declaration of interest

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Author contributions

HE had performed the conception and idea, SG made design, SE formulation of overarching research goals, HE had analyzed and interpreted the data; AE performed the draft of paper and revised it for intellectual content; and RG conducted the research and investigation process, specifically the laboratory measurements; ES conducted the research and investigation process, specifically the imaging, and all authors shared the final approval of the version to be published and agree to be accountable for all aspects of the work.

Ethics approval and consent to participate

The study was approved by the local Ethics Committee of Faculty of Medicine, Tanta University. Approval Code no. 34423. The written informed consent from all the patients was obtained and the trial was conducted according to the Declaration of Helsinki principles.

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