Research Article

Relationship Between Serum Level of Homocysteine, Leptin and Neopterin and Disease Activity in Rheumatoid Arthritis patients with or without Extra-articular Manifestations

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Abstract

Objectives: this study was aimed to determine serum homocysteine, leptin and neopterin levels in patients with RA and investigate the relationship between clinical and laboratory parameters of disease activity and presence or absence of extra- articular manifestations. **Patients and methods:** This study included 80 RA patients (16 males, 64 females; mean age 34.5±10.8 years; range 24.5 to 45.3 years) and age and sex-matched 80 healthy controls (16 males, 64 females, mean age 30.8 ± 10.4 range 20 to 65). RA patients were divided into two groups (A&B) depending on the presence or absence of Extra-articular manifestations. Of the patients, there was 40 patients with no Extra-articular while the other 40 with Extra-articular (9 patients with Cutaneous vasculitis, 7 with Nodules, 6 with Neuropathy, 5 with Reynaud's phenomenon ,7 with 2ndry. Sjogren, 2 with Fealty's syndrome, 2 with Interstitial nephritis, 2 with Interstitial lung disease). **Results:** In the RA group (A+B), mean serum Hcy, leptin and neopterin levels were (11.79 + 8.72 µmol/L), (22.43 ± 7.37 ng/ml) & (3.83±1.84 nmol/L) respectively with No statistically significant difference was found between RA and control groups regarding serum Leptin (p=0.674). While a significant difference was found between RA and control groups regarding serum Neopetrin (< 0.001) &Hcy. (< 0.001). Also, In RA groups (A, B) there was a statistically significant difference regarding serum Neopterin (p< 0.03) and DAS 28 ESR (p< 0.05). there was a Positive significant correlation between serum (neopterin - Hcy) and ESR, TNF-α, IL-6, and DAS-28 (p < 0.05) while no significant correlation was found between serum (neopterin- Hcy) and CRP (p > 0.05). **Conclusion:** Serum leptin cannot be considered of value as an inflammation marker in monitoring RA patients while Serum neopterin can be used as a sensitive marker for assaying background inflammation and disease activity score in RA patients while serum homocysteine can be used as a marker for probability of extra articular complication of RA.

Keywords: Homocysteine; Leptin; Neopterin; Rheumatoid arthritis.

Introduction

Rheumatoid arthritis (RA) is a chronic, systemic disorder with unknown etiology characterized by involvement of hand joints and deformation with the presence of many proinflammatory cytokines including tumor necrosis factor-alpha (TNF-a), interleukin-1, and interleukin-6 that have been involved in the pathogenesis.[1,2] with, increased comorbidity, and premature mortality, [3] due to increased atherosclerotic heart disease[4,5], These cytokines are playing a pivotal role in the development of RA also play a key role in atherosclerosis.

Hyper-homocysteinemia is an independent risk factor for coronary heart disease, peripheral vascular disorder, and stroke and that an independent predictor for cardiovascular mortality in atherosclerotic patients.[6,7] leptin is a component of cytokines that mediates immune response and plays an important role in the T-cell related inflammatory process and modulates T-helper cell activation in the cellular immune response.[8]

Neopterin is a pteridine derivative produced by monocytes and macrophages primarily as a response to interferon-gamma stimulation

induced by the activation of the cellular immune system.[9] thus, neopterin may be used as an indicator of the early stage of inflammation.[10]

In this study, we aimed to determine serum homocysteine, leptin and neopterin levels in patients with RA and investigate the relationship between clinical and laboratory parameters of disease activity and presence or absence of extra- articular manifestations.

Patients and Methods

Eighty consecutive patients (16 males, 64 females; mean age 34.5±10.8 years; range 24.5 to 45.3 years) with established RA, who were followed up in EL-Hussein University Hospital, Rheumatology Clinic of Department of Rheumatology and Rehabilitation between September 2018 and February 2019 as well as 80 age and sex-matched healthy controls (16 males, 64 females, mean age 30.8 ± 10.4 range 20 to 65). Mean disease duration was 9.57±7.29 years. Approval from Al-Azhar ethics committee was obtained and a written informed consent was obtained from each patient. The study was conducted in accordance with the principles of the Declaration of Helsinki.

Inclusion criteria were Fulfilling American College of Rheumatology/European League against Rheumatism (ACR/EULAR) Rheumatoid Arthritis classification criteria[11]

Exclusion criteria:

- Hypertension, hyperlipidaemia, cardiovascular disease, previous cerebrovascular disease, thromboembolic event,
- Smoking or alcohol consumption
- Acute or chronic infections, malignancies, known pulmonary, hepatic, or renal diseases, endocrinologic diseases, or pregnant females

Patients were categorized as the following 40 non-Extra Articular (non-ExRA) in **group A** & 40 with Extraarticular manifestations (ExRA) in **group B** while 80 healthy persons served as a control in **group C**.

Extra-articular manifestation in group B was assessed and were as following:

Table (1) Extra-articular manifestation in group B patients

- **Cutaneous vasculitis**	**9 patients**
- **Nodules**	**7 patients**
- **Neuropathy**	**6 patients**
- **Reynaud's phenomenon**	**5 patients**
- **2ry.sjogren**	**7 patients**
- **Felty's syndrome**	**2 patients**
- **Interstitial nephritis**	**2 patients**
- **Interstitial lung disease**	**2 patients**

Clinical assessments of the patient during routine clinical evaluations which included assessments of pain, and fatigue. Morning stiffness was assessed based on the duration of morning stiffness in minutes. Disease activity was evaluated using Disease Activity Scores in 28 joints (DAS28) criteria including the number of the tender or swollen joints, Erythrocyte Sedimentation Rate (ESR), and global health assessments. The patients had low (DAS 28 < 3.2 n=19; 23.7%), moderate (DAS28=3.2-5.1; n=32: 40.0%), and high (DAS28≥5.1; n=29: 36.21%) degrees of disease activity. [12]

Blood samples were drawn from all the participants who had fasted overnight. Results of routine laboratory tests (ESR, C-reactive protein, blood biochemistry, whole blood count, urinalysis, ESR and CRP are analysed on the same day.

CRP was measured using nephelometry. IL-6 & TNF-α was evaluated by enzyme-linked

immunosorbent assay (AVISCERA BIOSCIENCE TNF- α human ELIZA kit, cat No.: EK-072-28) & (Quantikie Human interleukin 6 (IL-6 immunoassay cat. No. D6050).

Blood samples to analyze Hcy, leptin, and neopterin levels were collected in citrated tubes and centrifuged at 2000 rpm for 15 minutes. The harvested serum was stored at - 20 °C. Serum Hcy (Organic diagnostika GmbH, Germany; Catalogue no: EIA-2395) Serum neopterin (Organic diagnostika GmbH, GmbH, Germany; Catalogue no: EIA-1476) and leptin (Organic diagnostika GmbH, Germany; Catalogue no: EIA-2395) levels were determined by enzyme-linked immunosorbent assay method using BioTek ELIZA reader (ELX50; USA sn 233754) and commercial kits in compliance with the manufacturer's directives and expressed in their units. Neopterin sensitivity was detected at 0.2 nmol/L /mL, while intra- and inter-assay coefficient of variations were <9.5% and <8.1%, respectively. Leptin sensitivity was detected at 1.0 ng/mL, while intra- and inter-assay coefficient of variation were <6.9% and <11.5%, respectively. Hcy sensitivity was detected at 0.3 μmol/L, while intra- and inter-assay coefficient of variations were <9.0% and <8.9%, respectively.

Statistical analysis

Data were analysed using the SPSS version 15.0 software (SPSS Inc., Chicago, IL, USA). Parametric tests (independent-samples t-test) were applied to data of normal distribution and non-parametric tests (Mann-Whiney U-test) were applied to data of questionably normal distribution. The distribution of categorical variables in both groups was compared using Pearson chi-square test. Continuous data were presented as mean ± standard deviation or median (minimum-maximum), as appropriate. All differences associated with a chance probability of $p<0.05$ were considered statistically significant.

Results

The age, sex, and body mass index (BMI) of the subjects were compared between RA and control groups (Table 2).

Duration of disease was 9.57±7.29 years in the RA group. The patients had low (DAS 28 < 3.2 n=13; 39.4%), moderate (DAS28= 3.2-5.1; n=12: 36.4%), and high (DAS28≥ 5.1; n=8: 24.2%) degrees of disease activity.

Significant differences were found between RA patients (group A + group B) and the control healthy group regarding the mean levels of Hgb, ESR, TNF-α and IL-6 ($p < 0.05$).

In the RA group (A+B), mean serum Hcy, leptin and neopterin levels were (11.79 + 8.72 μmol/L), (22.43 ± 7.37 ng/ml) & (3.83±1.84 nmol/L) respectively. In the control group C, mean serum Hcy, leptin and neopterin levels were (8.8 + 1.58 μmol/L), (20.43 ± 8.73 ng/ml) & (1.13±0.55 nmol/L), respectively. No statistically significant difference was found between RA and control groups regarding serum Leptin ($p=0.674$). While a significant difference was found between RA and control groups regarding serum Neopetrin (< 0.001) & Hcy. (< 0.001).

Table (2): Demographic, biochemical, and disease characteristics of all subjects.

	RA patients Group (A + B)	Control Group C	P
	Mean ± SD	Mean ± SD	
Age (years)	34.5 ± 10.8	30.8 ± 10.4	> 0.05
Sex (No. M/F)	16/64	16/64	
Body mass index (BMI)	31.1 ± 5.2	30.9±4.13	> 0.05
Hgb (g/dL)	8.8 ± 2.82	13.66 ± 3.66	< 0.001[a]
ESR (mm/h)	68.64 ± 13.43	13.82 ± 4.35	< 0.001[a]
CRP (mg/L)	8.42 ± 3.20		
TNF-α (pg/mL)	13.34 ± 8.44	1.9 ± 0.58	< 0.001[a]
IL-6 (pg/mL)	27.76 ± 14.28	3.26 ± 1.05	< 0.001
(Hcy)µmol/L	11.79 ± 8.72	8.8 ± 1.58	<0.001
Leptin ng/ml	22.43 ± 7.37)	20.43 ± 8.73	> 0.05
Neopterin (nmol/L)	3.83±1.84	1.13±0.55	< 0.001[a]

In RA groups (A, B) there was statistically significant difference regarding serum Neopterin (p< 0.03) and DAS 28 ESR (p< 0.05). Table (3)

Table (3): Demographic, biochemical, and disease characteristics of group A and group B patients.

	Non-EX RA group A	Ex RA group B	P
	Mean ± SD	Mean ± SD	
Age (years)	37. ± 5.8	38.84 ± 7.46	
Sex (No. M/F)	8/32	8/32	
Body mass index (BMI)	31.1 ±4.2	33.9±2.13	
Hgb (g/dL)	8.9 ± 2.48	7.21 ± 2.19	
ESR (mm/h)	51.64 ± 10.43	71.82 ± 9.9	
CRP (mg/L)	7.42 ± 2.20	11.2±3.52	
TNF-α (pg/mL)	9.34 ± 11.44	15.9 ± 5.11	
IL-6 (pg/mL)	29.81 ± 16.22	31.26 ± 5.05	
(Hcy)µmol/L	9.88±8.95	17.0±3.58	
Leptin ng/ml	19.43 ± 8.37)	21.43 ± 6.73	
Neopterin (nmol/L)	3.83±1.84	3.42±2.13	p < 0.03
DAS-28	3.56 ± 6.46	6.38 ± 8.36	P < 0.05

The correlations between serum (neopterin - Hcy), disease activity, and inflammatory mediators in patients are shown in table 4. Positive significant correlations were detected between serum (neopterin - Hcy) and ESR, TNF-α, IL-6, and DAS-28 (p < 0.05).

No significant correlation was found between serum (neopterin- Hcy) and CRP (p > 0.05).

Table (4) correlations between serum (neopterin - Hcy), inflammatory mediators and disease activity.

	Neopterin		Homocysteine	
	R	P	r	p
ESR	0.36	< 0.001[a]	0.36	< 0.001[a]
CRP	0.08	> 0.05	0.08	> 0.05
TNF-α	0.46	< 0.001[a]	0.46	< 0.001[a]
IL-6	0.24	< 0.05[b]	0.24	< 0.05[b]
DAS-28	0.33	< 0.001[a]	0.33	< 0.001[a]

Multiple linear regression analysis for the association of the different variables with serum neopterin & Hcy is shown in table 5 & 6 respectively. After adjustment for age, sex, and inflammatory markers, DAS-28 and ESR were the main predictors for the high neopterin levels seen in patients with RA ($p < 0.05$). The effects of TNF- α and IL-6 were attenuated by the adjustment of all the variables.

Table (5): Multiple linear regression analysis for Neopterin and different independent variables

Variables	Neopterin ($r^2 = 0.458$)		
	Standardized β coefficient	t	P
Age	0.065	0.710	> 0.05
Sex	-0.018	-0.203	> 0.05
ESR	0.517	4.934	< 0.001[a]
CRP	0.230	1.934	> 0.05
TNF-α	0.164	1.842	> 0.05
IL-6	0.315	1.872	> 0.05
DAS-28	0.534	4.862	< 0.001[a]

Table (6): Multiple linear regression analysis for Homocystein and different independent variables

Variables	Homocystein ($r^2 = 0.458$)		
	Standardized β coefficient	t	P
Age	0.065	0.710	> 0.05
Sex	-0.018	-0.203	> 0.05
ESR	0.517	4.934	< 0.001[a]
CRP	0.230	1.934	> 0.05
TNF-α	0.164	1.842	> 0.05
IL-6	0.315	1.872	> 0.05
DAS-28	0.534	4.862	< 0.001[a]

Discussion

RA is a chronic inflammatory disease that affects articular as well as extra-articular structures. It is characterized by inflammatory cell recruitment and in its later stage cartilage and bone destruction. Th1/Th2 balance plays an important role in RA, with Th1 and Th2 cytokines exerting proinflammatory and anti-inflammatory effects.[13] this study was designed to measure levels of homocysteine, leptin and neopterin in patients with RA either with or without extra-articular manifestations, and to correlate the findings with disease activity parameters.

In the present study, we founded a higher neopetrin &homocysteine level in male RA patients versus female patients and a significant correlation of plasma level of neopetrin, & homocysteine with age in both the RA & control. We also founded positive correlation between RA activity with ESR ($p = 0.001$), TNF- α ($p < 0.001$), CRP, RF & IL-6.

Otero et al.,[14] and Bokarewa et al.,[15] showed significantly higher serum leptin levels in RA patients when compared with healthy controls. Contrarily, Tokarczyk-Knapik et al.,[16] demonstrated lower leptin levels in their control group. Targonska-Stepniak et al.,[17] indicated that in patients with erosive RA, levels of leptin increased which demonstrated a positive correlation with disease duration and activation.

We founded correlation between serum neopterin level and severity of disease. Neopterin were significant predictor for disease activity in such patients. In our study, we also showed correlation between serum neopterin level and presence of Extra-articular manifestations in RA patients.

Schroecksnadel et al.,[18] and D'agostino et al.,[19] showed higher levels of neopterin levels in patients with RA when compared with those of the controls. Also, Shady et al.,[20] detected higher serum neopterin levels in patients with juvenile idiopathic arthritis compared to those of the control group.

Also, Fagerer et al.,[21], In agreement with the current study, they found high neopterin levels in RA patients and significantly elevated concentrations of neopterin in patients with RA plus cardiovascular disease (CVD) compared to RA without CVD.

In contrast, Ozkan et al.,[22] founded no difference between serum neopterin concentrations of patients with RA and healthy controls but detected a correlation between neopterin, ESR, and RF in patients with RA.

Also, a previous study in Japan detected significantly increased neopterin in SLE (Systemic Lupus Erythematosus) patients ($p < 0.001$) but not in RA patients. However, patients with RA had a greater concentration of neopterin in synovial fluid.[23]

In our RA patient, neopterin was significantly correlated with higher ESR ($p = 0.001$), TNF-α ($p < 0.001$), CRP, RF & IL-6 Rho et al.,[24] showed that neopterin concentrations were significantly higher in patients with SLE and RA than controls and were higher in SLE than RA (all $p < 0.001$). In SLE, neopterin was significantly correlated with higher ESR ($p = 0.001$), TNF-α, and homocysteine concentrations, but in RA only with ESR.

In our study, we founded that the serum Hcy levels in patients with RA were higher than the normal range and were significantly higher than the Hcy levels among participants in the control group. Also, we founded a correlation between plasma Hcy levels and disease activity in patients with RA. A statistically significant increase in the levels of plasma homocysteine was also observed in the patients with more severe disease when compared with those patients with low or moderate diseases activity. This result is agreement with Balkarli et al., study [25] that concluded Both mediators such as IL-6 and TNF-α and hyper-homocysteinemia are associated with atherosclerotic cardiovascular diseases.

We founded significantly elevated concentrations of homocysteine in patients with Ex RA compared to non ExRA ($p < 0.03$).

Other study has shown that myocardial ischaemia in patients with RA was associated with a high activity of the inflammatory process (high C-reactive protein levels, disease activity score, score on the HAQ disability index, and number of swollen and painful joints) and hyper - homocysteinaemia [(26)].

This finding might indicate that Hcy has a stronger role as a marker of atherosclerotic disease than as a risk factor for atherosclerotic disease. Timely identify-cation of patients with hyper-homocy-steinaemia enables adequate prevention of and treatment for CVD in rheumatoid arthritis patients.[(27)]

In our RA patient, plasma homocysteine was significantly correlated with higher ESR ($p = 0.001$), TNF-α ($p < 0.001$), CRP, RF& IL-6 which matched with results of Yang et al.,[(28)] who founded correlations between Hcy and CRP, anti-CCP antibody, rheumatoid factor, and disease activity (DAS28 score).

Balkarli et al.,[(25)] study suggested that Hcy can be used in the assessment of cardio-vascular risk in patients with RA. In Balkarli1etalstudy stated that serum Hcy levels were found to be increased in patients with RA compared to healthy controls. However, they failed to identify an assoc-iation between Hcy and IL-6 or TNF-α.

Conclusion

Serum leptin cannot be considered of value as an inflammation marker in monitoring RA patients. Serum neopterin can be used as a sensitive marker for assaying background inflammation and disease activity score in RA patients while serum homocysteine can be used as a marker for probability of extra articular complication of RA.

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