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Original Research Article

VEGF - a vasculitis marker in rheumatoid arthritis patients

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Abstract

Vasculitis is one of the most common complications in rheumatoid arthritis. Vascular endothelial growth factor, a potent growth factor for endothelial cells and inducer of angiogenesis, is important for endothelial integrity and thus for vascular function. Iron homeostasis differs in rheumatoid arthritis according to inflammation and disease activity status. We included 86 female patients with rheumatoid arthritis. They were separated into three groups according to RA activity – 29 with RA and ACD, 30 with RA and IDA, and 27 with RA no anemia (used as a control group). All included groups were analyzed for laboratory parameters: hepcidin, iron and TIBC, ferritin, CBC including CHr, CRP, liver enzymes, serum creatinine, blood glucose, selenium. Patients were diagnosed for RA in University hospital "Ivan Rilski", Department of Internal diseases. They were monitored for vasculitis in University hospital "Aleksandrovska", Department of Neurology. Patients from the control group showed hepcidin and VEGF concentrations into the reference ranges (hepcidin 14.6 ± 1.5 µg/L; VEGF 23.7 ± 1.5 pg/mL). The RA with ACD group shows elevated serum hepcidin and VEGF levels (hepcidin 53.9 ± 5.2 µg/L; VEGF 38.5 ± 2.4 pg/mL). The RA with IDA group has decreased iron regulatory hormone and slightly elevated vasculitis marker (hepcidin 1.8 ± 1.0 µg/L; VEGF 29.1 ± 1.6 pg/mL). We found a high positive correlation between serum hepcidin and VEGF levels in RA with ACD (r = 0.677, $p < 0.001$) and RA with IDA patients (r = 0.549, $p < 0.001$). Development of vasculitis in rheumatoid arthritis patients leads to elevated serum VEGF levels. Along with hepcidin changes in some cases it might cause oxidative stress and deterioration of patient's status.

Keywords: Anemia of chronic disease, Hepcidin, Iron deficiency anemia, Rheumatoid arthritis, Selenium, Vasculitis, VEGF

List of Abbreviations

ACD – anemia of chronic disease; **ADMA** – asymmetric dimethylarginine; **ALT** – alanin amino-transferase; **AST** – aspartat amino-transferase; **ATP** – adenosine triphosphate; **CBC** – complete blood count; **CHr** – hemoglobin concentration in reticulocytes; **CRP** – C-reactive protein; **DAS** – disease activity score; **ETAAS** – electrotermic atomic absorption spectrometry; **FAAS** – flame atomic absorption spectrometry; **IDA** – iron deficiency anemia; **RA** – rheumatoid arthritis; **Se** – selenium; **TIBC** – total iron-binding capacity; **VEGF** – vascular endothelial growth factor

INTRODUCTION

Vascular endothelial growth factor (VEGF) was originally described as an endothelial cell-specific mitogen (Ferrara N, et al., 1992). Different studies shows the VEGF role in various normal physiological functions such as bone formation (Gerber HP, et al., 1999), hematopoiesis (Ferrara N, et al., 1998), wound healing (Chintalgattu V, et al., 2003), and development (Reichardt LF, et al., 1991). Vascular endothelial growth factor is a potent growth factor for endothelial cells and inducer of angiogenesis. It is important for endothelial integrity and vascular function. VEGF may enhance the pathophysiologic mechanism of plaque formation and plaque destabilization (Holm PW, et al., 2009).

Hepcidin has been demonstrated to be a key peptide in the regulation of iron homeostasis (Ganz T, et al., 2012). Hepcidin promotes plaque destabilization partly by exaggerating inflammatory cytokine release, intracellular lipid accumulation, oxidative stress, and apoptosis in the macrophages with iron retention (Li JJ, et al., 2012).

The aim of our study was to evaluate serum VEGF levels, as a vasculitis marker and to compare its' results to iron metabolism parameters and selenium as an oxidative stress indicator.

MATERIALS AND METHODS

For a period of one year we measured 86 rheumatoid arthritis females, average age 49.5 ± 1.9. The patients were diagnosed for RA in University hospital "Ivan Rilski", Department of Internal diseases. All included cases were monitored for vasculitis as complication of the main disease in University hospital "Aleksandrovska", Department of Neurology. We evaluate iron homeostasis using serum hepcidin, hemoglobin in reticulocytes, iron, ferritin and TIBC levels. The presence of vasculitis process was evaluated by VEGF using ELISA method. Main parameters as liver enzymes, kidney function, carbohydrates metabolism and inflammation were evaluated. ESR was measured using citrate plasma; selenium was quantified in heparin plasma; all other parameters were evaluated in serum. The disease activity was evaluated by DAS 28. Serum hepcidin was measured by sandwich ELISA method. Iron and TIBC were quantified by FAAS (provided by Perkin Elmer). Selenium was evaluated by ETAAS (by Perkin Elmer). CRP and RF were measured by nephelometric method (by Siemens Healtcare). Other biochemical parameters were quantified on Dimension RXL MAX (provided by Siemens Healthcare). Advia 2120 (by Siemens Healthcare) was used for determination of CBC parameters, including hemoglobin in reticulocytes.

The patients with rheumatoid arthritis were separated into three groups according to RA activity – 29 with RA and ACD, 30 with RA and IDA, and 27 with RA no anemia (used as a control group).

Written informed consent was obtained from all patients (according to The Code of Ethics of the World Medical Association (Declaration of Helsinki, Directive 2001/20/EC). This study is part of Grants 2014 and 2015, sponsored by Medical University, Sofia, Bulgaria and was approved by its Ethics Committee.

Data were analyzed using SPSS 13.0 (IBM). All results are presented as mean value and standard deviation. For statistical evaluation of parameters we used paired Student's t-test and Pearson's correlation. As a significance we accept $p<0.05$.

RESULTS

All evaluated parameters of the included three groups are given in Table 1 as an average value and standard deviation.

We found statistically significant increased hepcidin levels in RA with ACD group, compared to RA no A (as a control) ($r = -0.628$, $p < -0.05$). Serum hepcidin showed decreased concentration in RA with IDA patients, compared to the control group (RA no anemia) ($r = 0.818$; $p < -0.05$) (Figure 1).

Serum VEGF concentrations were elevated in RA with ACD patients compared to the controls ($r = 0.698$, $p < -0.05$). Slightly increased VEGF levels were found and in RA with IDA group compared to RA no anemia cases ($r = 0.491$, $p < -0.05$) (Figure 2).

Figure 3 presents decreased plasma selenium levels in both RA with ACD and RA with IDA patients, compared to the controls (RA no anemia) ($r = -0.787$, $p <0.05$).

Patients from rheumatoid arthritis and ACD and IDA groups had elevated serum CRP levels, compared to RA no anemia cases (59.8 mg/L and 12.9 mg/L to 5.8 mg/L; $p<0.005$). Higher CRP levels in patients with ACD leads to increased ferritin concentration in this group (299.8 ng/mL to 112.5 ng/mL; $p<0.005$). Hemoglobin concentration in reticulocytes was increased in ACD patients compared to IDA group (35.9 pg to 24.4 pg; $r = 0.769$, $p<-0.05$).

RF and ESR showed no significant differences between three groups ($p>0.5$). Liver enzymes, glucose fasting and serum creatinine between included groups showed no differs ($p>0.5$).

Due to the differentiation between patients to ACD and IDA, serum iron was higher in RA with ACD group compared to ACD with IDA ($p<0.001$).

Table 1. Laboratory parameters of included patients

Parameter	RA no A		RA with ACD		RA with IDA	
	mean	SD	mean	SD	mean	SD
Hepcidin (µg/L)	14.6*;**	1.5	53.9*	5.2	1.8**	1.0
VEGF (pg/mL)	23.7*;**	1.5	38.5*	2.4	29.1**	1.6
RF (IU/mL)	25.5	2.9	28.7	3.1	27.7	2.7
ESR (mm/h)	25	3	29	4	30	3
Iron (µmol/L)	15.4*;**	1.9	19.5*	1.1	4.1**	0.8
Ferritin (ng/mL)	112.5*;**	5.9	299.8*	11.7	12.4**	1.4
CHr (pg)	31.1*;**	1.2	35.9*	1.1	24.4**	1.5
CRP (mg/L)	5.8*;**	0.1	59.8*	7.4	12.9**	2.1
ASAT (U/L)	10.4	0.8	12.9	1.0	11.8	1.0
ALAT (U/L)	9.9	0.7	10.8	0.9	10.4	0.8
Creatinine (µmol/L)	74.9	3.9	82.5	4.1	85.5	3.9
Glucose f. (mmol/L)	4.9	0.5	5.1	0.6	5.2	0.6
Se (µg/L)	101.4*;**	4.9	51.1*	3.8	49.7**	3.0

*-RA – rheumatoid arthritis, A – anemia, ACD – anemia of chronic disease, IDA – iron deficiency anemia, Glucose f. – glucose fasting; * - significance of differences among RA no A and RA with ACD is p<0.005; ** - significance of differences among RA no A and RA wit IDA is p<0.005.

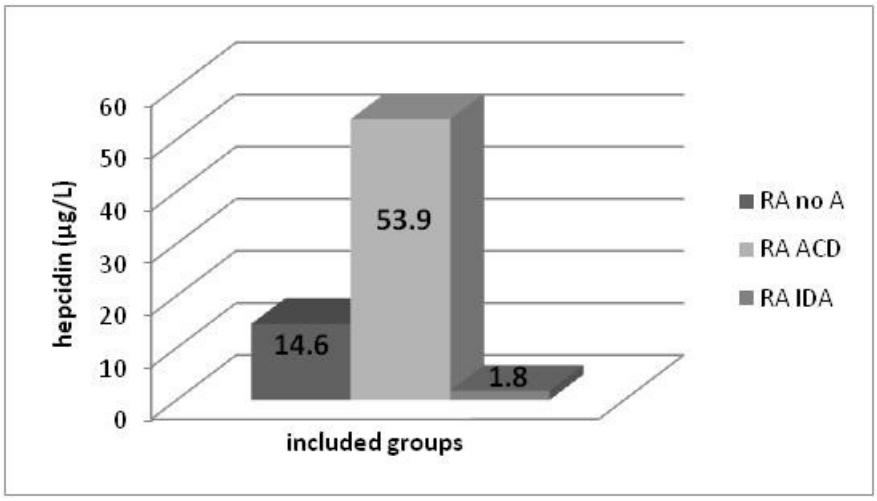

Figure 1. Serum hepcidin levels in included groups in µg/L (as mean value)

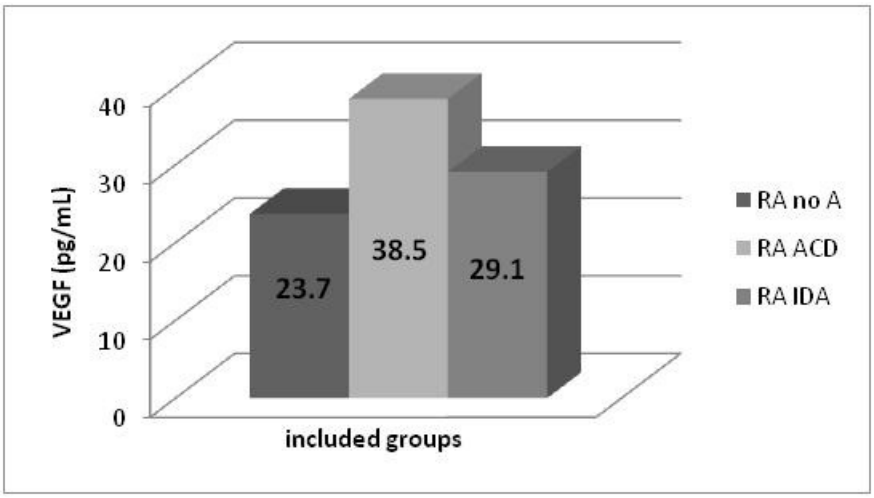

Figure 2. Serum VEGF levels in rheumatoid arthritis patients as mean value (in pg/mL)

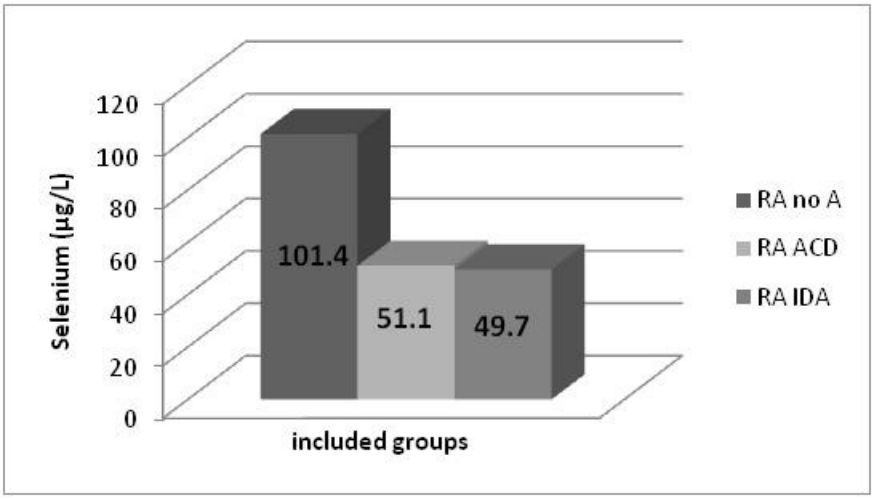

Figure 3. Selenium in plasma in µg/L (presented as mean value)

DISCUSSION

There were no significant differences in baseline characteristics of the patients (age, gender, $P > 0.5$). Included groups are homogeneous and with clear protocol. We found changes in VEGF levels, connected to oxidative stress parameter – selenium, and to iron regulatory peptide – hepcidin.

In two included groups we found a tight connection between VEGF and oxidative stress ($r = 0.677$, and $r = 0.549$, $P < 0.001$). Similar results were found in different studies (Furuki K, et al., 2008; Cooke JP, 2004; Bai Y, et al., 2013). This means that morphological changes in the vessel wall are connected to impaired endothelial function, despite of its level of change (Touboul PJ, et al., 2014).

Changes in CRP have their features. This might be the proof of active inflammatory process in RA disease, which may lead to vasculitis.

VEGF shows no relation to hepcidin in RA patients with no anemia ($r = -0.141$; $p < 0.001$). The ADMA/DDAH pathway regulates VEGF-induced angiogenesis (Fiedler LR, et al., 2009). The histological analysis shows that vulnerable plaques were correlated with higher CRP levels and VEGF levels (Fittipaldi S, et al., 2014). VEGF was higher in the blood of patients with anemia of chronic disease compared to RA and no anemia. VEGF expression which causes centripetal sprouting of adventitial vessels. This neovascularization is considered to be immature and highly susceptible to leakage (Michel JB, et al., 2014). The angiogenesis is initiated by tissue demands for oxygen and nutrients, resulting in a hypoxia/reoxygenation cycle, which, in turn promotes the formation of reactive oxygen species. The main mechanism of oxidative stress-induced angiogenesis involves hypoxia-inducible factor/VEGF signaling (Kim YW, et al., 2014). VEGF and VEGFR 1 could be key mediators (Russell DA, et al., 2008).

Development of vasculitis in rheumatoid arthritis patients leads to elevated serum VEGF levels. Along with hepcidin changes in some cases it might cause oxidative stress and deterioration of patient's status. Further investigations might be necessary to clear these mechanisms and probably establish a correlation to asymmetric dimethylarginine (ADMA), which acts an endogenous inhibitor of nitric oxide synthase and is involved in the pathogenesis of cardiovascular disease. High serum concentrations of VEGF were associated with carotid atherosclerotic lesions and may represent a new marker of vasculitis in elderly subjects.

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Conflict of Interest

Petrova Julia, Manolov Victor, Paskaleva-Peycheva Veneta, Hadjidekova Savina, Dimitrov Georgi, Tarnovska-Kadreva Rumiana, Yaneva-Sirakova Theodora, Velizarova Milena, Vasilev Vasil, Marinov Borislav, Emilova Radoslava, Bogov Ivo and Tzatchev Kamen declare that we have no-conflicts with any organization or institute during preparation of materials in short communication called "VEGF - a vasculitis marker in rheumatoid arthritis patients" that is given to *Merit Research Journals of Medicine and Medical Sciences*. All

patients included in the trial have signed Informed Consent according to respective requirements from The Code of Ethics of the World Medical Association (Declaration of Helsinki).

This article has been prepared after one year collection of samples from patients diagnosed with rheumatoid arthritis from the Department of Rheumatology at "St. Ivan Rilski" hospital. Vasculitis was diagnosed in Neurology Dept. at University “Aleksandrovska” hospital. During this period no pharmaceutical or other company was involved in the trial.

All authors disclose that have no any actual or potential conflict of interest including any financial, personal or other relationships with other people or organizations within three years of beginning the submitted work that could inappropriately influence, or be perceived to influence, their work.

There is no any potential Conflicts of Interest Related to Individual Authors' Commitments. All authors are responsible for disclosing all financial and personal relationships that might bias their work. All authors states that no potential conflicts exists.

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