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IS THERE A REAL CORRELATION BETWEEN RED CELL DISTRIBUTION WIDTH AND PERIPHERAL ARTERIAL DISEASE?

DA LI POSTOJI STVARNA KORELACIJA IZMEĐU RASPODELE ERITROCITA I PERIFERNOG ARTERIJSKOG OBOLJENJA?

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Summary

Background: Few data is available concerning the association between peripheral arterial disease (PAD) and red cell distribution width (RDW). In this study, we analyzed the relationship between RDW and atherosclerosis of the vessels other than coronary arteries in patients who had undergone digital substraction angiography (DSA).
Methods: This study included 730 patients who had undergone DSA. Patients were divided into two groups according to their angiographic images. The association between RDW and atherosclerosis of peripheral arteries was analyzed. The relationship between atherosclerosis and smoking, hypertension (HT), diabetes mellitus (DM), hs-CRP, hemoglobin, white blood cell (WBC), triglyceride, total cholesterol, HDL and LDL cholesterol levels was assessed.
Results: Atherosclerosis was observed more common in male and patients with older age, HT, DM and smoking ($p<0.001$). hs-CRP and WBC levels were both in significantly positive association with atherosclerosis ($p<0.05$). However, there were no significant differences in the RDW levels, hemoglobin, triglyceride, total cholesterol, LDL and HDL cholesterol levels in the groups ($p>0.05$).
Conclusions: Our results seem to demonstrate that older age, male gender, HT, DM and smoking are powerful risk factors for PAD. In contrast to the previous reports, RDW levels are found not to be associated with atherosclerosis of peripheral arteries.

Keywords: red blood cell distribution width, peripheral arterial disease, digital substraction angiography, atherosclerosis

Kratak sadržaj

Uvod: Ima nekoliko podataka koji govore o vezi između perifernog arterijskog oboljenja i raspona raspodele eritrocita (RDW). U ovom izučavanju analiziran je odnos između RDW i ateroskleroze krvnih sudova osim koronarnih arterija u pacijenata koji su bili podvrgnuti digitalnoj angigrafiji (DSA).
Metode: Ovo izučavanje obuhvatilo je 730 pacijenata podvrgnutih DSA. Pacijenti su podeljeni u dve grupe prema angigrafskoj slici. Analizirana je veza između RDW i ateroskleroze perifernih arterija. Praćena je povezanost između ateroskleroze i pušenja, hipertenzije (HT), diabetes mellitus-a (DM), hs-CRP, hemoglobina, leukocita (WBC), triglicerida, ukupnog holesterola, i nivoa HDL i LDL holesterola.
Rezultati: Ateroskleroza je bila češća kod muškaraca i pacijenata starije dobi, sa HT, DM i pušača ($p<0,001$). Nivoi hs-CRP i WBC bili su u značajnioj pozitivnoj korelacijhi sa aterosklerozom ($p<0,05$). Međutim, nije bilo značajnih razlika u nivoima RDW, hemoglobina, triglicerida, ukupnog holesterola i nivoa LDL i HDL holesterola u grupama ($p>0,05$).
Zaključak: Rezultati ukazuju da su starije doba, muški pol, HT, DM i pušenje značajni faktori rizika za PAD. Nasuprot ranijim istraživanjima, nije nađeno da su nivoi RDW povezani sa aterosklerozom perifernih arterija.

Ključne reči: širina raspodele eritrocita, periferno arterijsko oboljenje, digitalna angiografija, ateroskleroza

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Introduction

Red blood cell distribution width (RDW) reflects the variation of red blood cell volume. It can easily be measured by routine analysis of whole blood count (1). Increased RDW results from heterogeneity of erythrocyte size and erythrocyte fragmentation in the circulation (2). Factors that cause increased erythrocyte size heterogeneity include iron, vitamin B_{12} and folate deficiency, decreased erythrocyte life span and impaired erythropoiesis. Increased RDW is also related to oxidative stress and release of cytokine in response to inflammation (3) RDW has been demonstrated to be associated also with cardiovascular and pulmonary diseases (4). Several studies have shown that high RDW is associated with coronary artery atherosclerosis in hypertensive patients and acute myocardial infarction (5, 6).

Peripheral arterial disease (PAD) is associated with increased mortality and morbidity (7). It is important to identify factors that might contribute to such high morbidity and mortality in PAD. Patients with PAD show high levels of inflammation and oxidative stress, which play roles in the progression of atherosclerotic diseases (8). Whether RDW is predictive of mortality in patients with PAD is unknown. A recent study demonstrated that increased RDW is associated with greater mortality in patients with PAD identified by noninvasive lower-extremity arterial testing (9). Several studies have been done to assess the relationship between RDW and coronary artery atherosclerosis. However, few data is available concerning the association between PAD and RDW. In this study, we analyzed the relationship between RDW and atherosclerosis of the vessels other than coronary arteries in patients who had undergone digital substraction angiography (DSA). Our study is the first to use an invasive method, DSA, for peripheral arterial evaluation.

Methods

A total of 730 patients, who underwent DSA (Artis Zee, Siemens Germany, and Integris V, Philips Netherland) in our interventional radiology unit between 2013 and 2014 were included in this study. All patients provided written informed consent and the local institutional ethics commitee approved the study protocol. Patients had undergone aortography (n:92), pelvic-iliac angiography (n:142), lower exremity angiography (n:202), renal angiography (n:122), carotid angiography (n:134) and upper extremity angiography (n:38). We divided patients into two groups according to their angiographic images. Patients who had extensive stenosis and atherosclerosis or occlusion were included in group 1. Patients with no stenosis and atherosclerosis or who had normal findings were included in group 2.

The patients' demographic characteristics including age, sex and smoking status were acquired through a standardized questionnaire. The mean age of the subjects in group 1 was 64.72±10.56 (427 males and 133 females), and the mean age of the subjects in group 2 was 54.86 ± 13.96 (105 males and 65 females). Smoking was defined as at least one cigarette daily for 1 year. Hypertension (HT) was defined as systolic blood pressure (SBP) 140 mmHg and (or) diastolic (DBP) 90 mmHg or previous diagnosis of hypertension with use of antihypertensive medication (10). Diabetes mellitus (DM) was defined as a fasting blood glucose level 6.99 mmol/L, a random glucose measurement >11.1 mmol/L or hemoglobin A_{1c}>0.065 proportion of total hemoglobin or a previous diagnosis with any use of oral antidiabetic agent and/or insulin (11). Exclusion criteria included previous cardiovascular disease, the presence of hemolytic, hepatic and renal diseases that could affect white blood cells (WBC), red blood cells (RBC) or hemoglobin. Patients who received erythrocyte suspension for any reason within the past 6 months were not considered for the study.

RDW, hemoglobin and WBC levels were measured using Abbott CELL-DYN 3700 automated CBC analyzer. The normal reference range for RDW in our laboratory is 11.6% to 15.5%. Serum total cholesterol, LDL and HDL cholesterol, triglyceride and hs-CRP levels were also determined.

Statistical analyses were performed with Statistical Package for the Social Sciences software (Version 9.0; SPSS, Inc., Chicago, IL). For continuous variables, Kolmogorov-Smirnov test was applied to test normal distribution. Mann-Whitney U test was applied to compare ages, LDL cholesterol, triglyceride, RDW, hemoglobin, WBC and hs-CRP levels between two groups as the data was not distributed normally. Independent samples t test was used to compare total cholesterol and HDL cholesterol levels because of normal distribution. The chi-square test was used for comparison of categorical variables. For continuous variables; numeric values are expressed as mean±SD. A p value of <0.05 was considered statistically.

Results

Aortography (thoracic or abdominal) was performed in 92 patients. Although 26 of these patients had no stenosis or occlusion, they had extensive atherosclerosis. 15 patients had abdominal aortic stenosis. 18 patients had abdominal aortic occlusion. 59 of these patients were included in group 1. 33 of these patients were included in group 2.

Pelvic and iliac angiographies were performed in 142 patients. 20 patients had no atherosclerosis. 66 patients had stenosis. 56 patients had total occlusion. We enrolled 122 of these patients in group 1 and 20 of these patients in group 2.

Lower extremity angiography was performed in 202 patients. 138 patients had undergone femoral angiography. Of the patients who had undergone femoral angiography, 16 patients had no atherosclerosis. 22 patients had stenosis. 100 patients had total occlusion. 64 patients had undergone knee angiography. Of the patients who had undergone knee angiography, 4 patients had no atherosclerosis. 8 patienst had stenosis. 52 patients had total occlusion. We enrolled 182 of these patients in group 1 and 20 of these patients in group 2.

Renal angiography was performed in 122 patients. 49 patients had normal findings. 68 patients had stenosis. 5 patients had total occlusion. So 73 of these patients were enrolled in group 1 and 49 of these patients in group 2.

Carotid angiography was performed in 134 patients. 27 patients had normal findings. 87 patients had stenosis. 20 patients had total occlusion. 107 of these patients were included in group 1 and 21 of these patients in group 2.

Upper extremity angiography was performed in 38 patients. 21 patients had normal findings. 7 patients had stenosis. 10 patients had total occlusion. 17 of these patients were enrolled in group 1 and 21 of these patients in group 2.

The baseline characteristics are shown in *Table 1*. 190 patients had DM. 165 of these diabetic patients were in group 1 and 25 of these diabetic patients were in group 2. A total of 558 patients had HT. 464 of these patients were in group 1. 94 of these hypertensive patients were in group 2. Number of the patients who had smoked was 456. And 396 of these patients were in group 1, the others in group 2.

As shown in in *Figure 1*, the analysis of RDW values revealed no significant differences in any of the groups (p=0.192). However, atherosclerosis was observed more common in male patients compared

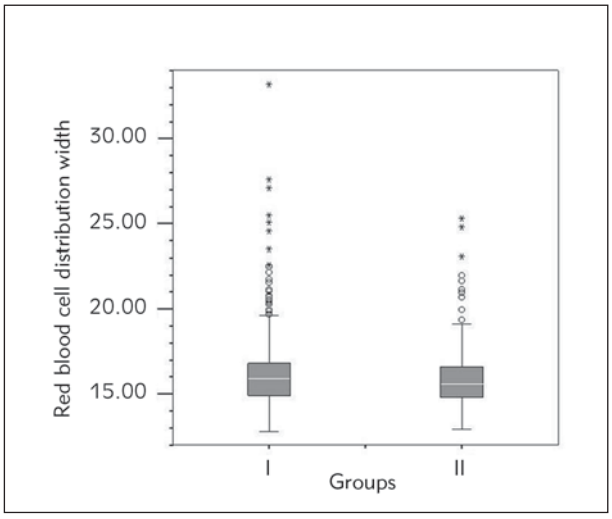

Figure 1 Red Blood Cell Distribution Width.

Table I Median and demographic levels of RDW and other parameters in each group.

Groups	Group I (n=560)	Group II (n=170)	P value
Male/female	427/133	105/65	Chi-square=15,552;p<0.001
Age (y)*	64.72 ± 10.56	54.86 ± 13.96	Z=-8,712; p<0.001
Total cholesterol (mmol/L)*	4.83± 1.17	4.79± 1.49	t=-0.363; p=0.717
HDL cholesterol (mmol/L)*	1.0± 0.24	1.024 ±0.26	t=0.428; p=0.669
LDL cholesterol (mmol/L)*	2.99 ± 0.86	2.92 ± 0.84	Z=-0.449; p=0.652
Triglyceride (mmol/L)*	1.75 ± 0.95	1.66 ± 0.76	Z=-0,451; p=0.653
RDW*	16.15 ± 2.03	16.04 ± 2	Z=-1,306; p=0.192
HT +/-	464/96	94/76	Chi-square=58,557; p<0.001
DM +/-	165/395	25/145	Chi-square=12,332; p<0.001
Smoking +/-	396/164	60/110	Chi-square=72,546; p<0.001
Hemoglobin (g/L)	130.26 ± 20.14	130.45 ± 20.23	Z=-1,167; p=0.243
WBC count (×109/L)	9.06 ± 3.55	8.27 ± 2.77	Z=-1,167; p=0.003
hs-CRP (nmol/L)	312.76 ± 490.96	212.95 ± 277.91	Z=-1,971; p=0.049

*Values are means ± standard deviation

*DM: Diabetes Mellitus; HDL: high-density lipoprotein; HT: hypertension; LDL: low-density lipoprotein; RDW: red blood cell distribution width; hs-CRP: high-sensitive C-reactive protein; WBC: white blood cell.

to female patients ($p<0.001$). Patients with older age had particularly extensive atherosclerosis in peripheral arteries ($p<0.001$). Patients who had DM, HT and smoking were more prone to atherosclerosis when compared to the control group ($p<0.001$). And also, hs-CRP and WBC levels were both in significantly positive association with atherosclerosis ($p<0.05$). But there were no significant differences in hemoglobin, serum total cholesterol, LDL and HDL cholesterol and triglyceride levels in these groups ($p>0.05$).

Discussion

The prevalence of PAD is increasing worldwide. It is well known that the morbidity and mortality of patients with PAD remain high despite current advances in revascularization techniques (12). Identification of risk factors for atherosclerotic PAD will provide insights into underlying pathophysiologic mechanisms and facilitate the development of diagnostic and therapeutic approaches.

PAD prevalence and incidence are age related. With aging of the population, it seems likely that PAD will increase in the future. And also, PAD seems to be higher among men than men. In our study, atherosclerosis was observed more common in male patients compared to female patients ($p<0.001$). Patients with older age had particularly extensive atherosclerosis in peripheral arteries ($p<0.001$). Our result is in agreement with Criqui and associates, who observed that PAD incidence rises >10% among patients in their 60s and 70s (13). Smoking, HT and DM are particularly strong risk factors for PAD. In our study, patients with HT, DM and smoking had increased atherosclerosis in peripheral arteries ($p<0.001$).

High levels of inflammation and oxidative stress play roles in the progression of atherosclerosis in PAD. hs-CRP is one of the most well-established biomarkers of chronic inflammation. hs-CRP produced by tissue macrophages and vascular smooth muscle cells could appear in the atherosclerotic plaques (14). Jia and associates showed that coronary artery atherosclerotic patients have higher hs-CRP levels than non coronary artery atherosclerotic patients (15). In our study, hs-CRP and WBC levels were both in significantly positive association with atherosclerosis in peripheral arteries ($p<0.05$). Although the underlying biological mechanisms remain unclear, RDW is also assessed as a marker of chronic inflammation and oxidative stress (3). Recent studies have demonstrated that high RDW is associated with poor clinical outcomes in patients with heart failure, coronary artery disease, pulmonary hypertension and PAD (9, 16). Inflammatory cytokine release in heart failure and acute myocardial infarction might affect bone marrow function and inhibit erythrocyte maturation induced by erythropoietin. Therefore , RDW becomes elevated (17). And also increased oxidative stress directly damages erythrocytes and shortens their survival, resulting in high RDW (18). According to Almer et al. (19) coronary artery disease is significantly related with inflammatory response of the vascular endothelial injury, while RDW is involved in the process of inflammation. Jia et al. (15) and associates demonstrated that higher RDW levels were associated with carotid artery stenosis and intimal medial thickness. They assessed atherosclerosis and intimal medial thickness via ultrasound. Also, Wen conducted a study to demonstrate the relationship between RDW and carotid artery atherosclerosis by using carotid ultrasonography (20).

Recently, Ye and al. (9) and associates reported a study which evaluated the utility of RDW to predict mortality in patients with PAD. They used ankle-brachial index which is an established noninvasive test for peripheral arterial disease. They demonstrated that higher RDW is associated with greater all-cause mortality in patients with PAD identified in the noninvasive vascular laboratory. And also, Zalawadiya et al. (21) demonstrated a strong and independent relationship between RDW and PAD. These findings are particularly noteworthy as RDW appears to be an emerging cost-effective biomarker. However, in our study, no statistically significant difference was found between two groups in terms of RDW levels. To date, RDW has been evaluated in various clinical settings both in PAD and other conditions before. However, this is the first study to use an invasive method, DSA, for peripheral arterial evaluation. DSA is stil considered the reference standard in vascular imaging (22).

However, there are some limitations of our study that have to be mentioned. Lack of data on serum folate, vitamin B_{12} and iron levels exists in our study. Therefore considering the data on the levels of serum iron, folate and vitamin B_{12} should also be assessed. And also, the patients are not examined for any morbidity or mortality event. In this context, a longer period of prospective observation may provide more prognostic information.

Conflict of interest statement

The authors stated that they have no conflicts of interest regarding the publication of this article.

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