Regular blood donation improves endothelial function in adult males

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Abstract

Objective: Endothelial dysfunction, secondary to systemic inflammation and oxidative stress, is known to play a major role in the development and progression of atherosclerosis. It is hypothesized that the lower incidence of coronary artery disease in the premenopausal period in females when compared with males is associated with regular menstrual blood loss. We investigated whether regular blood donation (BD) is associated with improved endothelial function in healthy adult males.

Methods: Fifty young healthy male volunteers volunteers with a mean age of 30±6 years without overt cardiovascular disease were enrolled to participate in serial consecutive BDs. Serum iron levels as oxidative stress parameters, flow-mediated dilatation (FMD) for endothelial function, 24-h mean diastolic blood pressure for peripheral vascular resistance identification, and high-sensitivity C-reactive protein (hs-CRP) levels as systemic inflammatory markers were evaluated before and after BD. This study used a prospective observational cohort design. Patients with cardiovascular and inflammatory diseases were excluded.

Results: BD was found to improve FMD steadily and significantly when compared with the baseline (mean±SD: 9.9%±3.8%, 10.44%±3.9%, 10.65%±3.9%, and 10.75±3.9%, respectively, p=0.15, p=0.02, p=0.006 as compared with the baseline). A steady decrease was identified in hs-CRP levels after serial BDs, although this decrease was not statistically significant in the all phases (2.96±3.3 mg/L, 2.26±1.5 mg/L, and 2.12±1.5 mg/L, respectively, p=0.829, p=0.558). The 24-h mean diastolic blood pressures were significantly lower in the chronic phase (77±9 mm Hg, 75±7 mm Hg, and 72±8 mm Hg, respectively, p=0.50, p=0.003), whereas there was no significant change in iron levels in the acute and chronic phases (66±32 mg/dL, 72±43 mg/dL, and 68±33 mg/dL, respectively, p=1.000, p=1.000).

Conclusion: The results of the study indicate that regular BD improves endothelial function. *(Anatol J Cardiol 2016; 16: 154-8)*

Keywords: blood donation, endothelial function, atherosclerosis, flow-mediated vasodilatation, iron

Introduction

Vascular endothelium is the largest organ synthesizing and secreting various hormones that regulate the endothelium, vascular structure and functions, and vasomotor tonus (1). It is known that endothelial dysfunction secondary to systemic inflammation and oxidative stress plays the main role in the development and progression of atherosclerosis (2). Previous studies have shown that elevated C-reactive protein (CRP) levels are associated with inflammation, whereas decreased nitric oxide (NO) levels, which could potentially increase oxidized LDL levels, and increased iron levels are associated with oxidative stress (3-7).

There are several hypotheses to explain the lower incidence of coronary artery disease (CAD) before menopause in females when compared with males. According to one of these hypotheses, a decrease in the accumulation of iron (which is a pro-oxidant cofactor) due to menstruation leads to a decrease in oxidative stress (8, 9). Along with this hypothesis, a few studies shown that blood donation (BD) could be associated with better vascular function, confirming the link indirectly in case-control studies (10-12).

In the present study, we aimed to show a direct temporal link between BD and improved vascular function.

Methods

Study design

This study used a prospective observational cohort design.

Clinical data collection

Fifty consecutive healthy male participants without overt cardiovascular disease and who did not donate blood within the last 6 months were enrolled in the study between March 1, 2010 and March 10, 2011. Patients with hypertension, diabetes mellitus, chronic inflammatory diseases, recent infection, anemia

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(<13 gr/dL), or any other disease affecting any vascular territory as well as those taking antihypertensives, anti-diabetic medications, or any other drug were not included. All participants accepted to make three consecutive BDs (regular BDs) after providing informed consent.

In order to maintain literature-defined high-frequency BD (12), we defined regular BD as ≥3 consecutive BDs, with an interval of 2–3 months between BDs (12). Furthermore, we stratified BD into 3 phases, acute phase (period after the first BD), subacute phase (period after the second BD), and chronic phase (period after the third BD), and evaluated parameters along with these phases. All participants accepted to donate blood to the blood bank unit of our hospital at least three times (500 mL of blood was withdrawn into plastic bag, which was prefilled with 63 mL of citrate-phosphate-dextrose) with an interval of 2-3 months between BDs. The participants also underwent 24-h ambulatory blood pressure monitoring according to the protocol defined below (Del Mar Reynolds Medical CardioNavigator system).

All participants underwent flow-mediated vasodilatation (FMD) study and blood tests before and after BDs. A detailed description of the study plan is provided in Table 1. All FMD tests were performed on 35 of the 50 participants. For high-sensitivity C-reactive protein (hs-CRP) analysis, blood samples were collected 3 times from 17 participants. Twenty-nine participants were monitored 3 times for 24 h for ambulatory blood pressure monitoring. Finally, we were able to collect blood samples from only 12 participants for evaluation of iron levels.

Ultrasonographic examinations were performed via Vivid 4 system (GE Medical System) with 10-L transducer (4-10-Mhz probes). FMD was measured through the contralateral brachial artery before the initiation of regular BD and 1 month after each donation by an experienced author blinded to the study plan. Endothelial function was assessed after overnight fasting for at least 8 h. None of the participants smoked or consumed coffee for 1 h prior to the procedure. The technique, which conforms to the published guidelines (13), involved digital capture and storage of high-resolution end-diastolic longitudinal B-mode images of the brachial artery. The images were obtained at rest, during reactive hyperemia, induced by ischemia of the forearm for 5 min. Percent FMD was calculated as the brachial artery diameter after ischemia minus the brachial artery diameter before ischemia. All FMD measurements were recorded by labels without names and evaluated by a senior author at the end of the study in a random order.

This study was approved by the local ethics committee of Cumhuriyet University (registration number: 08-99, decision number: 2008-11/7) with the protocol mentioned above and was supported (and inspected with 6 month intervals) by a research grant from Cumhuriyet University Commission of Scientific Research Projects (registration number: CÜBAP T-394).

Table 1. Detailed description of the study plan

	Before the first BD	48 h after the first BD	1 month after the first BD	1 month after the second BD	1 month after the third BD
hs-CRP	+	+			+
Serum Iron	+		+		+
ABPM	+		+		+
FMD	+		+	+	+

APBM - ambulatory blood pressure monitoring; BD - blood donations; FMD - flow mediated vasodilation; hs-CRP - high-sensitivity C-reactive protein

Table 2. Baseline characteristics of the study participants (n=50)

Mean age, years	30±6
Male	50 (100%)
Hypertension	0 (0%)
Diabetes mellitus	0 (0%)
Smoking	49 (98%)
Hemoglobin, gr/dL	15.2±1.1
Hematocrit, gr/dL	45±5
Total cholesterol, mg/dL	168±44
Triglyceride, mg/dL	189±91
High-density lipoprotein cholesterol, mg/dL	34±9
Low-density lipoprotein cholesterol, mg/dL	107±35
FMD, %	10.25±3.9
hs-CRP, mg/L	2.66±2.5
24-h mean diastolic BP, mm Hg	79.2±8
Iron, mg/dL	77±29

BD - blood donation; BP - blood pressure; FMD - flow mediated vasodilation; hs-CRP - high sensitivity C-reactive protein; SD - standard deviation. Data are presented as mean±SD values

Statistical analysis

Continuous variables having normal distribution were described as mean±standard deviation (p>0.05 in the Kolmogorov-Smirnov test); continuous variables having abnormal distribution were described as median (minimum-maximum). One-sample Kolmogorov-Smirnov test showed that all variables were normally distributed. Given that the number of operations was higher than 2 and the variables were continuous, one-way repeated measures analysis of variance was used to evaluate whether the variables showed significant differences after regular BD. Power analysis was conducted by the Biostatistics Department of Cumhuriyet University Faculty of Medicine (as per the local ethics committee regulation) before the study. To yield a type I error of 0.05 and a type II error of 0.20 (80% statistical power), the minimum sample size was calculated as "50" up on 5 mm Hg decrease, in diastolic blood pressure in association with BD. All statistical procedures were performed using SPSS software version 14.0 (SPSS Inc., Chicago, IL, USA). A p value of ≤0.05 was considered statistically significant.

Results

The baseline characteristics of all study participants are summarized in Table 2. The mean age of the participants was

30±6 years. Of the 50 participants, 49 were current smokers and none had hypertension or diabetes mellitus. Furthermore, no participant reported previous BD within the last 6 months.

Participants who all completed or some of them exc. zthe FMD study as per the protocol and BD (500 mL of blood was withdrawn into plastic bag, which was prefilled with 63 mL of citrate-phosphate-dextrose) were considered for the analysis. A total of 35 participants underwent regulated FMD measurements. BD improved FMD steadily and significantly compared with the baseline (mean±SD: 9.9%±3.8%, 10.44%±3.9%, 10.65%±3.9%, and 10.75%±3.9%, respectively, p=0.15, p=0.02, p=0.006 compared with the baseline, Figure 1).

hs-CRP levels were evaluated before and after BDs as per the protocol, and a steady decrease in hs-CRP levels was identified after consecutive BDs. However, this decrease was not statistically significant in all phases (mean±SD: 2.96±3.3 mg/L, 2.26±1.5 mg/L, and 2.12±1.5 mg/L, respectively, p=0.829, p=0.558).

When the 24-h mean diastolic blood pressures of the participants were examined, the mean diastolic blood pressures were found to be significantly lower in the chronic phase (mean±SD: 77±9 mm Hg, 75±7 mm Hg, and 72±8 mm Hg, respectively, p=0.50, p=0.003). Of note, no significant fluctuation was noted with regard to systolic BP.

Finally, the changes in iron levels were evaluated as per the protocol; no significant difference could be found either in the acute or the chronic phase (mean±SD: 66±32 mg/dL, 72±43 mg/dL, and 68±33 mg/dL, respectively, p=1.000, p=1.000).

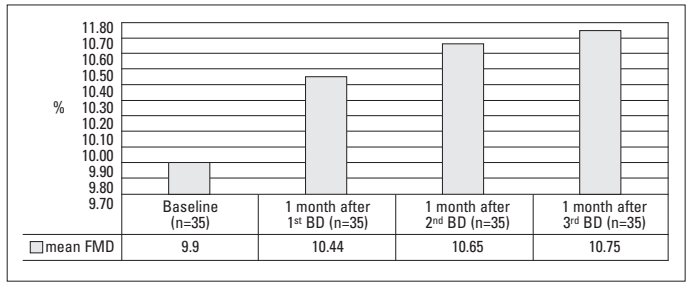

Figure 1. Temporal change in FMD
BD - blood donation; FMD - flow mediated vasodilatation

Discussion

Our results revealed a decrease in hs-CRP levels, improvement in endothelial function, and a decrease in diastolic blood pressure following regular BDs within 6 months, and the improvement rate was found to increase proportionally with the increase in the number of BDs.

Vascular endothelium releases various bioactive compounds, regulates vascular structures and functions, and maintains the equilibrium between vascular wall–thrombocyte interactions and fibrinolysis-thrombosis (1). In the event of endothelium dysfunction, there is an increase in oxidation, development of vasoconstriction, leukocyte adhesion, impaired coagulation, platelet activation, and thrombosis, leading to vascular inflammation (14). It is known that inflammation plays a major role in the onset and progression of atherosclerotic lesions as well as plaque disruption (15). CRP is the most important inflammation marker and plays a role in the pathogenesis of atherosclerotic lesions (16). It has been shown that hs-CRP levels in male and female patients with no cardiovascular disease increase in CAD (3). Furthermore, Ridker et al. (17) have shown that CRP levels are in fact a stronger predictor of atherosclerosis than LDL cholesterol. In our study, we found a steady decrease in hs-CRP levels, although this finding was statistically insignificant.

NO is an endothelium-derived relaxant and plays a key role in the protection of vascular tonus (18). During FMD measurement, the blood flow in arms is blocked for 5 min using a blood pressure meter under constant pressure. When the pressure is released, reactive hyperemia develops, resulting in shear stress-induced NO release and subsequent vasodilatation (FMD) (13). Approximately 30% of individuals in the general adult population are known to have hypertension, and it is known that the presence of CAD in patients is associated with morbidity and mortality (19). Recent studies have suggested that inflammation can lead to the development of HT and that oxidative stress and endothelial dysfunction are also involved in the inflammatory cascade (20). Similarly, in the present study, we found a significant improvement in FMD rates, which is a non-invasive method for evaluation of endothelial dysfunction.

Endothelial dysfunction has a key role in atherosclerosis and constitutes the first step of disease progression. Manganaro et al. (21) have found decreased FMD response in CAD. Another study has shown that FMD and CIMT are associated in patients with ACS. Fatma et al. (22) have demonstrated that ADMA and CIMT concentrations are associated with severe CAD. Feng et al. (23) have found that elevated ADMA levels are associated with disrupted FMD response in patients with vasospastic angina.

An increase in diastolic blood pressure is known to be associated with an increase in peripheral vascular resistance (24). Accordingly, an increase in the 24-h mean diastolic blood pressure could potentially be regarded as an indirect indicator of endothelial dysfunction (25). In our study, we found a significant decrease in diastolic blood pressure in the chronic phase, although such an effect was not observed in the early phase.

A correlation between increased levels of iron and atherosclerosis has been identified in a number of previous studies (7, 20, 26). In one study, investigating ischemia and reperfusion injury on rats, it was shown that diet-enriched higher levels of low-molecular-weight iron increased lipid peroxidation, which was partly prevented by antioxidant supplementation. Were given an iron-rich diet, following which higher levels of low-molecular-weight iron, increased lipid peroxidation, and decreased antioxidant concentration were observed (27, 28). The incidence of CAD in premenopausal women is significantly lower than that in postmenopausal women and males of the same age, and it is hypothesized that this correlation can be attributed to the decrease in pro-oxidant iron levels due to regu-

lar blood loss through menstruation (8). BD is another way of decreasing iron levels in the body, and the beneficial effects of donating blood have been demonstrated in various studies. These include decreased pro-oxidant iron levels, increased HDL cholesterol and apoA levels, decreased TG levels, decreased oxidant status of LDL particles, and increased antioxidant status of the body by increased NO_3 concentrations and decreased NO_2 concentrations (27). Hence, it is hypothesized that BDs improve endothelial function and may be useful in the prevention of atherosclerosis development (29). Contrary to the previous studies, we did not find a significant reduction in iron levels despite regular follow up of a small number of participants.

In this regard, one study has shown that improvements in endothelial functions in individuals who regularly donate blood are greater than those in individuals who donate blood less frequently (12). Improving endothelial function could potentially lead naturally to a decrease in the progression rate of CAD. Accordingly, a number of studies have shown that regular BDs could prevent CAD development (30, 31), although this correlation has not been noted in all studies (32).

Study limitations

There are several limitations of the current study that are worth mentioning. First, the study included only male subjects and hence does not represent females at all, although it is difficult to evaluate the discrete influence of BD in females. Second, some participants did not follow the entire study protocol either by not giving whole blood of 500 mL on each occasion or by violating the requirements for FMD testing after fasting; hence, they were not considered in the analysis. Of note, for the last FMD and BD, the participants were motivated carefully and complete protocol could be achieved. Third, the cohort does not represent the secondary prevention of endothelial dysfunction, although we cannot be completely sure that the participants were free of any vascular disease and can only give impression about the primary prevention of healthy endothelium. Fourth, all participants except one were smokers. None of the participants had cardiovascular diseases, and the participants did not smoke 1 h prior to the FMD measurement. Finally, some of the participants who were normotensive during 24-h blood pressure monitoring may have white coat hypertension, which may be associated with FMD outcome. However, because the current study also evaluated blood pressure change as an outcome, we preferred 24-h blood pressure monitoring over office readings, which may have confounded the results due to the white coat effect.

Conclusion

The present study investigated the effect of serial consecutive BDs on endothelial function-associated cardiovascular parameters. Our results revealed an improvement in clinical and laboratory findings in male volunteers who had no overt cardiovascular disease and who performed regular BDs. No decrease in blood iron levels after 1 month was identified in the participants; however, a decrease in inflammation-related hs-CRP levels and an increase in NO-related vasodilation were observed. From a clinical point of view, we determined a decrease in 24-h mean diastolic pressure in the participants and have shown that these improvements in the clinical and laboratory findings are higher with an increased frequency of BD. Finally, a decrease in inflammation could be the major factor of improvement in endothelial function after BD, and we believe that longer and more comprehensive studies in the future will validate this hypothesis.

Conflict of interest: None declared.

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