Does smoking affect thrombocytopoiesis and platelet activation in women and men?

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Abstract

Purpose: Smoking is a significant risk factor of cardiac ischaemia. Changes in platelet count, morphology and platelet activation enhance the risk.

Material and methods: The objective of the study was to assess platelet parameters in smoking healthy subjects with reference to sex. In the group of women, 27% were smokers, in the group of men – 49%. All the subjects were tested for platelet count (PLT), mean platelet volume (MPV), percentage of large platelets (L_{PLT}), concentrations of β-thromboglobulin, sP-selectin (soluble) and thrombopoietin, percentage of reticulated platelets (RP) and absolute count of reticulated platelet.

Results: Lower platelet count (237.00±39.52 vs 258.34±40.81 x 10^9/l, p=0.0002), higher percentage of reticulated platelets (1.39±0.66 vs 1.04±0.35%, p=0.04) and higher concentration of sP-selectin (52.66±18.54 vs 43.94±17.14 ng/ml, p=0.03) were observed only in the group of smoking women, compared to non-smokers. In neither of the sexes smoking had an effect on the following parameters: mean platelet volume, percentage of large platelets, concentration of thrombopoietin, absolute count of reticulated platelet and concentration of β -thromboglobulin.

Conclusions: The results allow the hypothesis that women are more sensitive to smoking than men. Platelets in male smokers are less sensitive to smoking – the study showed no significant changes in the parameters.

Key words: platelet count, parameters of thrombocytopoiesis, platelet activation, smoking.

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Introduction

Tobacco smoking is one of the major factors accelerating atherosclerosis [1]. Deleterious effects of smoking are associated with generation of free radicals that break down NO, which on the one hand enhances thromboxan synthesis (the prothrombic action), but on the other reduces production of prostacyclin (the antithrombic action), thus leading to clotting disorders, additionally enhanced by increased production of fibrinogen and factor VII [2,3]. According to Tsiara et al., the increased risk of atherosclerosis and thrombotic disorders in chronic smokers is not only caused by the inhibition of NO release by platelets but also by enhanced production of PECAM-1, which stimulates migration of monocytes along the endothelium [4]. Moreover, smokers show increased potential for oxidation of lipids, particularly LDL cholesterol, which is then phagocytised by macrophages and finally constitutes an important component of atheroma. It has been also found that smoking leads to the inhibition of lecithin-cholesterol acyltransferases (present on the surface of HDL cholesterol molecules in combination with apolipoproteins), which contributes to a decline in the reversible transport of cholesterol, to an increase in concentration of lipoproteins rich in triglycerides (LDL) and to a decrease in HDL concentration [1]. Additionally elevated blood carboxyhaemoglobin level causes ischaemia and vascular endothelial damage. According to Bazzano et al., a strong positive correlation between smoking and elevated levels of reactive protein C, fibrinogen and homocystein indicates that inflammation and hyperhomocysteinaemia are an important mechanism through which smoking enhances atherosclerosis.

Undoubtedly, the unfavourable influence of both active and passive smoking is connected with the effect on platelets. Addicted smokers show increased potential for platelet aggregation, lower platelet survival rate and increased excretion of thromboxan metabolites [1,4]. Elevated platelet aggregation induced by passive smoking may cause an increase in the risk of cardiac ischaemic disease even by 34% [2].

Research data concerning the effect of smoking on platelet parameters, including activation, are equivocal and do not take sex into consideration. Besides, there are very few reports on the effect of smoking on thrombocytopoiesis. Given the above, we decided to assess the effect of smoking on thrombocytopoiesis, platelet activation and some morphological parameters in healthy male and female blood donors.

Material and methods

The study group consisted of 125 healthy blood donors (mean age 31.95 years), who reported to the Regional Centre of Blood Donation and Haemotherapy in Białystok. There were 60 women (mean age 30.85±10.32 years) and 65 men (mean age 33.06±9.01 years) in the group. Subjects who had been taking antiplatelet drugs for at least 10 days prior to blood collecting and those suffering from heart and/or circulatory system disorders and diabetes were excluded from the study. Referential blood morphology parameters were within the norm in all the study groups. The subjects were divided into 4 groups: F_1 – female smokers (16; mean age 32.94±11.05 years), F_2 – female non-smokers (44; mean age 29.48±10.21 years), M_1 – male smokers (32, mean age 33.94±9.15 years) and M_2 – male non-smokers (33, mean age 32.21±8.95 years).

The following platelet parameters were assessed: platelet count (PLT), mean platelet volume (MPV), percentage of large platelets (L_{PLT}), concentration of β-thromboglobulin (β-TG) and sP-selectin, concentration of thrombopoietin (TPO), percentage of reticulated platelets (RP) and absolute count of reticulated platelet and their correlation with smoking.

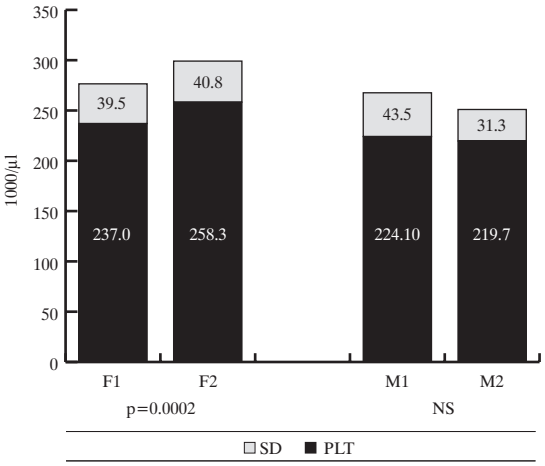
The material for analysis consisted of venous blood collected at 8-9 a.m., without stasis (to avoid platelet activation). Morphology and such platelet parameters as PLT, MPV, L_{PLT} were assessed in blood samples collected for EDTA-K_2, on haematological analyzer Advia 120 (Bayer). ELISA method was used to analyse the concentrations of β-thromboglobulin and sP-selectin (soluble) in venous blood collected to Vacutainer-type test-tubes containing anticoagulant CTAD. ELISA method was also used to determine thrombopoietin concentration in the blood collected for clot. The percentage of reticulated platelets was determined using a flow cytometer Epics XL, Coulter, with thiasole orange and CD 41Pe antibodies.

Study results were statistically analyzed based on the STATISTICA 8.0 PL. The hypothesis of normal distribution was verified with Kolomogorov test. Differences between the groups were evaluated with t-Student test for non-paired values. The level of $p<0.05$ was considered statistically significant. The correlation intensity for two variables was expressed by means of Pearson's correlation coefficient.

Results

The platelet count in the group F_2 (female non-smokers) was statistically significantly higher ($p=0.0002$) 258.34±40.81 x 10^9/l, compared to female smokers 237.00±39.52 x 10^9/l. However, no statistically significant difference was noted in platelet count between male smokers and non-smokers (*Fig. 1*). In neither of the sexes smoking had an effect on the following parameters: mean platelet volume, percentage of large platelets, thrombopoietin concentration, absolute count of reticulated platelet and β-thromboglobulin concentration (*Tab. 1* and *2*). The percentage of reticulated platelets was statistically significantly higher ($p=0.04$) in female smokers 1.39±0.66% in comparison to female non-smokers 1.04±0.35 %. In men, however, the percentage of reticulated platelets was similar in both male groups and did not show any statistically significant differences (*Fig. 2*). The concentration of sP-selectin in the group of women was statistically significantly higher ($p=0.03$) in smokers 52.66±18.54 ng/ml as compared to non-smokers 43.94±17.14 ng/ml. No statistically significant differences in this parameter were found in the group of men (59.58 ± 22.65 ng/ml in smokers and 53.62 ± 16.86 ng/ml in non-smokers) (*Fig. 3*).

Figure 1. **Platelet count in group of women (F1 – smoking, F2 – non smoking), and men (M1 – smoking, M2 – non smoking)**



Discussion

Literature reports on the effect of smoking on platelet count seem to be controversial. Brummit et al. found no correlation between platelet count and smoking in healthy volunteers [5]. Also Dotevall et al. noted no changes in platelet count in female smokers and non-smokers [6], and Suwansaksri et al. observed no alterations in PLT in male smokers and non-smokers [7]. According to Blann et al., smoking two cigarettes a day by chronic smokers of both sexes does not affect the platelet count [8]. No correlation has been also found between platelet count in pregnant women and the urinary level of nicotine metabolites [9]. However, Chao et al. have revealed that chronic male smokers have elevated PLT compared to male non-smokers [10].

In the current study, female smokers accounted for 27% of all the women involved, while in the group of men 49% smoked. We found lower platelet count, higher percentage of reticulated platelets and higher level of sP-selectin in the group of female smokers as compared to non-smokers. In the group of men,

Table 1. **Thrombopoietin concentration and absolute count of reticulated platelet in group of women (F1 – smoking, F2 – non smoking), and men (M1 – smoking, M2 – non smoking)**

	F_1 n=6	F_2 n=44	M_1 n=32	M_2 n=33
TPO (pg/ml)	145.32±36.61	161.47±64.08	177.23±63.74	182.53±58.50
absolute count of RP (x 10^9/l)	3.26±1.45	2.87±1.03	2.54±1.13	2.84±1.16

Table 2. **Mean platelet volume, large platelet count, β-thrombpoietin concentration in group of women (F1 – smoking, F2 – non smoking), and men (M1 – smoking, M2 – non smoking)**

	F_1 n=16	F_2 n=44	M_1 n=32	M_2 n=33
MPV (fl)	8.58±0.83	8.94±1.01	8.98±1.16	8.90±0.84
L_{PLT} (%)	6.42±4.12	6.88±3.95	6.84±4.32	6.93±3.58
β-TG (IU/ml)	216.68±54.34	223.59±36.82	230.98±27.49	222.59±37.28

Figure 2. **Reticulated platelet count in group of women (F1 – smoking, F2 – non smoking), and men (M1 – smoking, M2 – non smoking)**

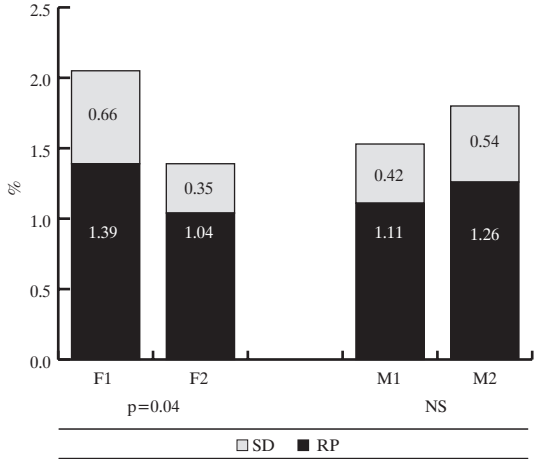
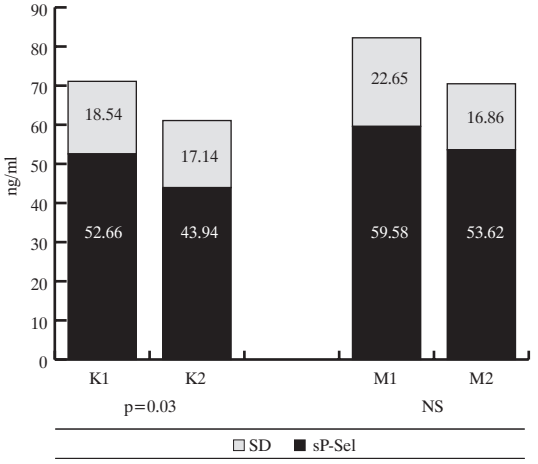


Figure 3. **Soluble P-selectin concentration in group of women (F1 – smoking, F2 – non smoking), and men (M1 – smoking, M2 – non smoking)**



platelet count was slightly higher (statistically insignificantly) and sP-selectin level higher in smokers than in non-smokers. Additionally, male smokers had minimally lower percentage of reticulated platelets, absolute count of reticulated platelet and thrombopoietin level, as compared to non-smokers. The lower platelet count in female smokers as compared to non-smokers in the current study is difficult to explain. Lack of changes in TPO concentration suggests that the observed phenomenon does not reflect thrombocytopoietic disturbances in female smokers in comparison to non-smokers. It is only the higher percentage of reticulated platelets that may indicate accelerated platelet restoration. Higher platelet turnover means the appearance of younger, more metabolically active platelets in the circulation. Compared to mature platelets, they have greater density and show higher expression of surface receptors [11]. Therefore, lower platelet count and higher percentage of young reticulated platelets in female smokers seem to be an unfavourable event, increasing the likelihood of platelet activation.

Our assumptions have been confirmed by higher sP-selectin concentrations in female smokers compared to non-smokers obtained in the current study. Similarly, Nair et al. demonstrated increased expression of P-selectin and sP-selectin concentration in smokers, which proved platelet activation [12]. Also Ridker et al. found a strong positive correlation of sP-selectin concentration with smoking [13]. However, Barbaux et al., studying a group of patients suffering from coronary diseases, noted significantly higher values of sP-selectin in smokers (135.9 ng/ml) as compared to non-smokers (123.4 ng/ml) [14]. According to this author, this is consistent with the effect smoking exerts on inflammatory markers, the adhesive molecules in particular. However, in a study by Ponthieux et al., men had higher levels of sP-selectin than women, and no effect of smoking on this marker

was found [15]. Conwey et al. demonstrated a positive correlation between sP-selectin concentration and smoking, although the level of this marker of activation was lower in women than in men [16]. Blann et al. found no changes in sP-selectin level in addictive smokers of both sexes, as compared to non-smokers [8]. Undoubtedly, smoking exerts a harmful effect on platelets, the effect being especially visible after even short-term abstinence from smoking. In healthy subjects, already after 6 weeks following smoking cessation, plasma sP-selectin concentration decreased by 29% [17]. Platelet aggregation potential improves in long-term smokers already after 2 weeks following smoking cessation [18].

We found no alterations in β-TG in the plasma of smoking women as compared to non-smokers. Similar results were obtained by Dotevall et al. [6]. At the same time, these authors observed increased urinary excretion of TXB_2 metabolites, which may indicate that these parameters are more sensitive markers of platelet activation than β-TG [6]. Smoking was also found to cause an increase in β-TG concentration in the urine and epinephrine in the blood of men with arterial hypertension as compared to the control group of subjects with normal blood pressure [19]. An increase in α granules: PF-4 and β-TG in the blood was observed in healthy smokers [4]. Chao et al. demonstrated that chronic male smokers had higher concentration of platelet factor 3 (PF-3) released from blood platelets [10]. However, Doteval et al., in a study involving a group of young healthy smoking women, found no changes in PF-4 concentration in the blood and β-TG in the blood and urine, in the mean platelet survival rate and in platelet production, as compared to non-smokers [6]. We found no effect of smoking on MPV and L_{PLT}. However, Calori et al. demonstrated that in monozygotic twins of both sexes smoking leads to increased MPV [20]. The changes observed in blood platelets are in the author's opinion due to the effect of smoking on vascular endothelium.

The data presented here allow the hypothesis that women show greater sensitivity to smoking than men. Responsible for this mechanism is probably the antiestrogenic effect of smoking on blood platelets. Smoking induces conversion in estradiol transformations, which leads, through the effect on P_{450} isoenzyme, to the production of metabolically inactive compounds [4]. According to Tsiara et al., especially in young and middle aged women, smoking enhances the risk of cardiovascular diseases, as compared to men [4]. Ultimately, irrespective of sex, the incidence of heart infarct and mortality rates due to ischaemic disease are higher in smokers than in non-smokers, especially under 65 [1].

Based on the current study and literature data, it cannot be definitely stated that nicotine and other cigarette components cause platelet activation. In smokers, platelet activation can be associated with structural or biochemical alterations in the circulation (changes in endothelium, increase in free fatty acids) rather than with direct effect of tobacco on platelets [21].

Conclusions

Women demonstrate higher sensitivity to smoking than men – differences in platelet count, parameters of thrombocytopoiesis and platelet activation were found only in the group of female smokers compared to female non-smokers.

References

1. Świątecka G. Choroby serca u kobiet. Via Medica wydanie I, Gdańsk 2000.
2. Stasiołek D, Kwaśniewska M, Drygas W. Palenie jako czynnik ryzyka chorób układu sercowo-naczyniowego. Czynniki Ryzyka, 2000; 4: 62-8.
3. Tatoń J, Czech A, Bernas M. Kardiodiabetologia. Via Medica, wydanie I, Gdańsk 2002.
4. Tsiara S, Elisaf M, Mikhailidis DP. Influence of smoking on predictors of vascular disease. Angiology, 2003; 54: 507-31.
5. Brumit DR, Barker HF. The determination of a reference range for new platelet parameters produced by the Bayer ADVIA TM 120 full blood count analyser. Clin Lab Haem, 2000; 22: 103-7.
6. Dotevall A, Rongemarck C, Eriksson E, Kutti J. Wadenvik H, Wennmalm A. Cigarette smoking increases tromboxane A2 formation without platelet survival in young healthy females. Thromb Haemost, 1992; 68: 583-8.
7. Suwansaksri J, Wiwanitkit V, Soogarun S. Effect of smoking on platelet count and platelet parameters: an observation. Clin Appl Thromb Hemost, 2004; 10: 287-8.
8. Blann AD, Kirkpatrick U, Devine C, Naser S, McCollum CN. The influence of acute smoking on leukocytes, platelets and the endothelium. Atherosclerosis, 1998; 141: 133-9.
9. Cope GF, Nayyar P, Holder R. Measurement of nicotine intake in pregnant women – association to changes in blood cell count. Nicotine & Tobacco Research, 2001; 3: 119-23.
10. Chao FC, Tullis JL, Alper CA, Glynn RJ, Silbert JE. Alternation in plasma proteins and platelet functions with aging and cigarette smoking in healthy men. Thromb Haemost, 1982; 47: 259-64.
11. Saving KL, Mankin PE, Gorman MJ. Differences in adhesion receptor expression between immature and older platelets and red blood cells of neonates and adults. J Pediatr Hematol Oncol, 2002; 24: 120-4.
12. Nair S, Kulkarni S, Camoens HM. Changes in platelet glycoprotein receptors after smoking: A flow cytometry study. Platelets, 2001; 12: 20-6.
13. Ridker PM, Buring JE, Rifai N. Clinical investigation and reports soluble P-selectin and the risk of future cardiovascular events. Circulation, 2001; 103: 491.
14. Barbaux SC, Blankenberg S, Rupprecht HJ, Francomme C. Association between P-selectin gene polymorphism's and soluble P-selectin levels and their relation to coronary artery disease. Arteroscler Thromb Vasc Biol, 2001; 21: 1668-74.
15. Ponthieux A, Herbeth B, Droesch S, Haddy N, Lambert D, Visvikis S. Biological determinants of serum ICAM-1, E-selectin, P-selectin and L-selectin levels in healthy subjects: the Stanislas study. Atherosclerosis; 2004; 172: 299-308.
16. Conway DS, Pearce LA, Chin BS, Hart RG, Lip GY. Plasma von Willebrand factor and soluble p-selectin as indices of endothelial damage and platelet activation in 1321 patients with nonvalvular atrial fibrillation: relationship to stroke risk factors. Circulation, 2002; 106: 1962.
17. Lee KW, Lip GYH. Effects of lifestyle on hemostasis, fibrinolysis, and platelet reactivity. Arch Internal Med, 2003; 163: 2368.
18. Morita H, Ikeda H, Haramaki N, Eguchi H, Imaizumi T. Only two-week smoking cessation improves platelet aggregability and intraplatelet redox imbalance of long-term smokers. J Am Coll Cardiol, 2005; 45: 589-94.
19. Mundal MM, Hjemdahl P, Gjesdal K. Acute effects of cigarette smoking on platelet function and plasma catecholamines in hypertensive and normotensive men. Am J Hypertens, 1998; 11: 677-81.
20. Calori G, D'angelo A, Della Valle P, Ruotolo G, Ferini-Strambi L, Giusti C, Errera A, Gallus G. The effect of cigarette-smoking on cardiovascular risk factors: a study of monozygotic twins discordant for smoking. Thromb Haemost, 1996; 75: 14-8.
21. Mikhalidis DP, Barradas MA, Jeremy JY et al. Heparin-induced platelet aggregation in anorexia nervosa and in peripheral vascular disease. Eur J Clin Invest, 1985; 15: 313-9.