Effects of cigarette smoking on morphological features of platelets in healthy men

Farahnaz Ghahremanfard, MD, Vahid Semnani, MD, Raheb Ghorbani, PhD, Farhad Malek, MD, Ali Behzadfar, MD, Mehrdad Zahmatkesh, RN.

ABSTRACT

الأهداف: تقييم تأثير تدخين السجائر على تكون الصفائح الدموية، وكذلك مدى تأثيره على المؤشرات التشكلية لهذه الصفائح لدى الذكور الأصحاء.

الطريقة: شملت هذه الدراسة المقطعية 542 من الأصحاء الذكور (يتراوح العمر ما بين 20 إلى 88 عاماً) الذين تم تحويلهم من أجل عمل الفحوصات الدورية إلى قسم المختبرات، مستشفى فاطمة، سيمنان، إيران وذلك خلال الفترة من نوفمبر 2011م إلى نوفمبر 2012م. ولقد قمنا بتقسيم المشاركين في الدراسة إلى مجموعتين وهما مجموعة المدخنين (العدد=258 والذين كان معدل تدخينهم ما بين 10 إلى أكثر من السجائر في اليوم الواحد وقد استغرق ذلك 12 شهراً من التدخين)، بالإضافة إلى مجموعة غير المدخنين (العدد=284). وقمنا بجمع عينات الدم من المشاركين من أجل تحليل قيم مؤشرات الصفائح الدموية وذلك باستخدام ABX 60 ميكروز للتعداد الخلوي.

النتائج: أشارت نتائج الدراسة بأنه عند مقارنة مؤشرات الصفائح الدموية بين مجموعتي المدخنين وغير المدخنين فقد كان معدل تعداد الصفائح الدموية لدى مجموعة المدخنين أعلى بصورة واضحة من الناحية الإحصائية منه لدى غير المدخنين (264.1 ± 81.2 ميكرولتر مقابل 247.7±83.9 ميكرولتر) (p=0.021). وبالمقابل فقد كان معدل قيم الصفائح الدموية الحرجة أقل لدى مجموعة المدخنين البالغين مقارنة بغير المدخنين (18.0 ± 12.0% مقابل 25.0 ± 10.0%) (p<0.001). فيما كانت مؤشرات الصفائح الدموية الأخرى غير مختلفة بين مجموعتي المدخنين وغير المدخنين.

الخاتمة: أظهرت الدراسة بأن تدخين السجائر لدى الأصحاء قد يؤثر تأثيراً واضحاً على المؤشرات التشكلية للصفائح الدموية. وذلك يظهر بتأثر التدخين على زيادة معدل تعداد الصفائح الدموية، ونقص قيم الصفائح الدموية الحرجة وذلك عند المدخنين مقارنة بغير المدخنين.

Objectives: To assess the effects of cigarette smoking on thrombocytopoiesis and some platelet morphological parameters in healthy male smokers.

Methods: In this cross-sectional study, 542 consecutive healthy men (aged 20 to 88 years), referred to the laboratory of Fatemieh Hospital, Semnan, Iran, between November 2011 and November 2012 for checking up were enrolled. The subjects were divided into 2 groups of smokers (n=258 with frequency of 10 or more cigarette per day with more than 12 months duration of smoking) and non-smokers (n=284). The blood samples were extracted to examine values of platelet indices using an ABX Micros 60 cell counter.

Results: Comparing platelet indices across smokers and non-smokers showed that the mean platelet count was statistically significantly higher in adult smokers than in nonsmokers (264.1 ± 81.2/µl versus 247.7 ± 83.9/µl, p=0.021), while the mean plateletcrit value was contrarily lower in the adult smokers (18.0 ± 12.0% versus 25.0 ± 10.0%, p<0.001). Other platelet indicators were not discrepant between the smokers and non-smokers.

Conclusion: Cigarette smoking in healthy individuals results in significant and considerable effects on platelet morphological indices. The mean platelet count is significantly increased, and plateletcrit values are reduced, compared with non-smoking status.

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From the Internal Medicine Department (Ghahremanfard, Malek, Behzadfar, Zahmatkesh), the Pathology Department (Semnani), the Research Center for Social Determinants of Health (Ghorbani), Semnan University of Medical Sciences, Semnan, Iran.

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Address correspondence and reprint request to: Dr. Vahid Semnani, Pathology Laboratory, Kowsar Hospital, Amin Ave. Basidj Boulevard, Semnan, Iran. Tel. +98 (23) 33437822. Fax. +98 (23) 33437838. E-mail: vahidsemnani1345@yahoo.com

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The International Agency for Research on Cancer (IARC) in its recent reports introduced cigarette smoking as one of the main carcinogenic agents because of thousands of chemical compounds that induce the generation of free radicals, reduce prostacyclin production leading to clot formation, as well as increase the production of fibrinogen and coagulative factor VII.[1,2] Smoking has been also identified as a principal underlying etiology for the occurrence and progression of cardiovascular diseases, inflammatory disorders, and oxidative stress stimulation.[3] Cigarette smoking's crucial role in disrupting platelet activation and aggregation, as well as other coagulation processing components leading to thrombotic formations has been recently suggested.[4,5] The pathophysiological effects of cigarette smoking on platelet activation have been recently investigated. Cigarette smoking can induce both acute and chronic potential effects on platelet function. Shortly after smoking, acute platelet potentiating can be occurred that may be resulted in chronic desensitization of the cell to activating agents over time.[6] A study showed that acute smoking can change the platelet count and induce endothelial damage,[7] other study did not lead to platelet aggregation stimulated by adenosine diphosphate (ADP), epinephrine, and collagen such effects.[8] Although it was observed that inhalation of cigarette smoke can trigger platelet aggregation mediated by thrombin and ADP.[9] Even, reduced platelet agreeability induced by aggregating processes was shown in smokers compared with non-smokers.[10] With due attention to this matter which was mentioned above, and because there are few reports on the effect of smoking on thrombocytopoiesis, we assessed the effect of cigarette smoking on thrombocytopoiesis and some platelet morphological parameters in healthy male smokers.

Methods. In a cross-sectional study, we enrolled 542 consecutive healthy men (aged 20 to 88 years) referred to the laboratory of Fatemieh Hospital in Semnan, Iran, between November 2011 and November 2012 for check up. Subjects who had been taking antiplatelet drugs for at least 10 days prior to blood collection, those with a history of hematological disorders, those suffering from heart and/or pulmonary system disorders, and those addicted to various types of substances, were excluded from the study. This cross-sectional study was approved by the ethical and research committee of Semnan University of Medical Sciences, and informed written consent was obtained from each subject. The subjects were divided into 2 groups of smokers (n=258 with a frequency of 10 or more cigarette per day, and more than 12 months duration of smoking) and non-smokers (n=284). Two milliliters of venous blood was withdrawn with minimum stasis into a clean disposable 2 ml syringe. The blood samples were stored in ?EDTA for examination of platelet indices using cell counter (ABX Micros 60, (Micros 60, Horiba ABX, Montpellier, France). The following platelet parameters were assessed: platelet count (PLT), mean platelet volume (MPV), platelet distribution width (PDW), and plateletcrit (PCT). We carried out a PubMed, and Google scholar search using the key words: cigarette smoking, platelet, and PCT to identify previous article and research. The study was carried out according to the principles of the Helsinki Declaration.

Statistical analysis. Data was reported as mean±standard deviation (SD) for the quantitative variables, and percentages for the categorical variables. We used Kolmogorov-Smirnov test to assess the normality of quantitative data in the 2 groups. The continuous variables were compared using the Student's t-test (or Mann-Whitney test). P-values of 0.05 or less were considered statistically significant. All the statistical analyses were performed using the Statistical Package for Social Sciences version 13.0 (SPSS Inc., Chicago, IL, USA) for Windows.

Results. The study enrolled 542 subjects, of which 258 adult smokers (mean age of 47.5 ± 16.4 years, range 20 to 85 years) and 284 nonsmokers (mean age of 48.5 ± 16.3 years, range 20 to 88 years) were evaluated. No meaningful difference was found in average age between smokers and non-smokers (p=0.443). Comparing platelet indices across the smokers and non-smokers (**Table 1**) showed that the mean PLT was statistically significantly higher in adult smokers than in nonsmokers (264.1 ± 81.2/µl versus 247.7 ± 83.9/µl, p=0.021), while the mean PCT value was contrarily lower in the adult smokers (18.0 ± 12.0% versus 25.0 ± 10.0%, p<0.001). Other platelet indicators were not discrepant between the smokers and non-smokers. The age of the participants was categorized in the 3 subgroups of ≤39 years, 40 to 59 years, and ≥60 years to examine any association between smoking and platelet indicators in different age groups. As shown in **Table 2**, smoking was only associated with PCT value in the group containing the youngest participants (p<0.001). In the smoker group, the average number of cigarettes smoked per day was 17.2 ± 8.4 (range from 10 to 60/per day). The assessment of the relationship between the number of cigarettes smoked and platelet indices showed that the increase in the number of cigarettes resulted in increasing MPV (p=0.019), and adversely reducing mean PCT value (p=0.017).

Table 1 - Values of platelet indicators in smokers and non-smokers included in a study in Iran.

Characteristics	Smokers (n=258)	Non-smokers (n=284)	*P*-value
	n (%)		
*PLT(/µl)**	264.1 ± 81.1	247.7 ± 83.8	0.021
<100	0 (0.0)	4 (1.4)	
100 - 199	44 (17.1)	82 (28.9)	
200 - 299	136 (52.7)	128 (45.1)	
300 - 399	64 (24.8)	60 (21.1)	
≥400	14 (5.4)	10 (3.5)	
*MPV (fl)**	9.9 ± 1.1	9.8 ± 1.2	0.615
8.0 - 9.9	54 (20.9)	52 (18.3)	
9.0 - 9.9	102 (39.5)	126 (44.4)	
10.0 - 10.9	52 (20.2)	50 (17.6)	
11.0 - 11.9	34 (13.2)	34 (12.0)	
12.0 - 12.9	14 (5.4)	18 (6.3)	
≥13.0	2 (0.8)	4 (1.4)	
*PDW (fl)**	15.1 ± 2.5	15.1 ± 2.2	0.999
<10.0	6 (2.3)	4 (1.4)	
10.0 - 14.9	90 (34.9)	80 (28.2)	
≥15.0	162 (62.8)	200 (70.4)	
*Plateletcrit**	18.0 ± 12.0	25.0 ± 10.0	<0.001
<0.10	84 (32.8)	0 (0.0)	
0.10 - 0.19	32 (12.4)	62 (21.8)	
0.20 - 0.29	108 (41.9)	168 (59.2)	
0.30 - 0.39	32 (12.4)	42 (14.8)	
≥0.40	2 (0.8)	12 (4.2)	

*mean ± standard deviation. PLT - platelet count, MPV - mean platelet volume, PDW - platelet distribution width

Table 2 - *P*-value of the platelet count and platelet indices between smokers and non-smokers of all ages.

Age subgroup	PLT count	MPV	PDW	PCT
≤39 years	0.70	0.71	0.16	<0.001
40 - 59 years	0.02	0.91	0.92	0.31
≥60 years	0.61	0.27	0.60	0.44

PLT - platelet count, MPV - mean platelet volume, PDW - platelet distribution width, PCT - plateletcrit

Discussion. According to our study findings, cigarette smoking in healthy individuals resulted in significant and considerable effects on platelet morphological indices so that mean PLT was significantly increased and the PCT value was reduced compared with a non-smoking status. Similar results were also reported in other studies. Pretorius[11] and Pérez-Bautista et al[12] with a focus on changes in platelet membrane fluidity found differences in the globular nature of the platelet membrane of smokers that was not visible in non-smoker ones. According to the results of the study by Biljak et al[13] patients with obstructive pulmonary disorders induced had a significantly increased PLT, along with a reduced MPV when compared with healthy controls that might be triggered by an increase in inflammatory biomarkers. Platelet activity measured by the platelet function analyzer was increased significantly after cigarette smoking, and can explain the increase in the platelet aggregation process leading to a progression of ischemic heart diseases.[14] Roethig et al[15] also showed that lower exposure to cigarette smoke in long-term adult smokers led to statistically significant decreases of up to 9% in platelets within only 3 days. However, cigarette smoking is an important risk factor for thrombogenesis and this effect results from enhancement of platelet function.[6] It seems that the deleterious effects of cigarette smoking on arterial thrombosis can be mediated by its influence on 3 groups of biomarkers including biomarkers for inflammation, biomarkers for oxidative stress, and biomarkers for platelet activation. In the first group (biomarkers for inflammation), high-sensitive-C reactive protein, fibrinogen, and Von Willebrand factor levels have been shown to be more increased in smokers than nonsmokers.[5,16-18] It was also shown that smoking increased prostaglandin F (PGF) 2alpha formation, enhanced COX-mediated inflammation, and elevated levels of cytokines and isoprostanes.[19] The mean 11-dehTxB2 as a triggering factor for platelet activation is statistically significantly higher in adult smokers than in nonsmokers.[20] On the other hand, cigarette smoking, directly by changes in morphological features of platelets as well as indirectly by inducing production of stressor and inflammatory biomarkers, facilitated an increase in the extension and severity of ischemic heart diseases leading to an increased risk of mortality and morbidity in smokers.

Our main purpose was to focus on the changes in thrombocytopoiesis in male smokers, and the results showed a significant increase in PLT, along with decreased PCT values. Literature reports on the effect of smoking on PLT seem to be controversial. Brummit[21] et al found no correlation between PLT and smoking in healthy volunteers. Also Dotevall et al[22] noted no changes in PLT in female smokers and non-smokers, and Suwansaksri et al[23] observed no alterations in platelets in male smokers and non-smokers. Mobarrez et al[7] showed that acute smoking can induce endothelial damage and increase the platelet and leukocyte count. Leone[24] indicated that cigarette smoking exposure resulted in hematologic changes including: increased white blood cells, platelet aggregation and adhesiveness and fibrinogen level. Chao et al[25] reported that in chronic smokers there was a significant increase in PLT, fibrinogen, and platelet factor-3 (PF-3) activity, and decrease in the lag period of collagen-induced platelet aggregation. It was reported that the hormonal pathways regulating platelet production may be potentially impaired following smoking inducing production

of platelets and increased platelets count. It has been demonstrated that chronic cigarette smokers had higher circulating thrombopoietin levels (a humoral growth factor that primes platelet activation and production) than nonsmoking controls.[26]

There is a scarcity of studies that evaluate all of the platelet parameters including MPV, PDW, and PCT. In our study cigarette smoking in healthy men was accompanied by significant and considerable effects on platelet indices with a significantly increased mean PLT, and decreased PCT value in comparison with non-smokers. Further studies are required to explain these morphological changes in platelets following smoking. Some limitations of our study include the relatively small sample size and lack of investigation of women due to their denial of smoking.

In conclusion, cigarette smoking in healthy men may be accompanied by significant effects on platelet indices, such as an increase in the mean PLT, and a decrease in PCT values in comparison with non-smokers. Future research should be carried out with larger sample sizes including females.

References

1. World Health Organization International Agency For Research on Cancer. Tobacco Smoke and Involuntary Smoking. International Agency for Research on Cancer (IARC) monographs on the evaluation of carcinogenic risks to humans. Lyon (France): WHO; 2014.
2. Padmavathi P, Reddy VD, Maturu P, Varadacharyulu N. Smoking-induced alterations in platelet membrane fluidity and Na(+)/K(+)-ATPase activity in chronic cigarette smokers. ***J Atheroscler Thromb*** 2010; 17: 619-627.
3. Yasue H, Hirai N, Mizuno Y, Harada E, Itoh T, Yoshimura M, et al. Low-grade inflammation, thrombogenicity, and atherogenic lipid profile in cigarette smokers. ***Circ J*** 2006; 70: 8-13.
4. Madsen C, Nafstad P, Eikvar L, Schwarze PE, Ronningen KS, Haaheim LL. Association between tobacco smoke exposure and levels of C-reactive protein in the Oslo II Study. ***Eur J Epidemiol*** 2007; 22: 311-317.
5. Liu J, Liang Q, Frost-Pineda K, Muhammad-Kah R, Rimmer L, Roethig H, et al. Relationship between biomarkers of cigarette smoke exposure and biomarkers of inflammation, oxidative stress, and platelet activation in adult cigarette smokers. ***Cancer Epidemiol Biomarkers Prev*** 2011; 20: 1760-1769.
6. Inoue T. Cigarette smoking as a risk factor of coronary artery disease and its effects on platelet function. ***Tob Induc Dis*** 2004; 2: 27-33.
7. Mobarrez F, Antoniewicz L, Bosson JA, Kuhl J, Pisetsky DS, Lundbäck M. The effects of smoking on levels of endothelial progenitor cells and microparticles in the blood of healthy volunteers. ***PLoS One*** 2014; 9: e90314.
8. Siess W, Lorenz R, Roth P, Weber PC. Plasma catecholamines, platelet aggregation and associated thromboxane formation after physical exercise, smoking or norepinephrine infusion. ***Circulation*** 1982; 66: 44-48.
9. Blache D, Bouthillier D, Davignon J. Acute influence of smoking on platelet behaviour, endothelium and plasma lipids and normalization by aspirin. ***Atherosclerosis*** 1992; 93: 179-188.
10. Schmidt KG, Rasmussen JW. Acute platelet activation induced by smoking -in vivo and ex-vivo studies in humans. ***Thromb Heamost*** 1984; 51: 279-282.
11. Pretorius E. Ultrastructural changes in platelet membranes due to cigarette smoking. ***Ultrastruct Pathol*** 2012; 36: 239-243.
12. Pérez-Bautista O, Ramírez-Venegas A, Escobar-Arriaga E, Sansores RH. [Differences in inflammatory markers in a non-smoking and smoking Mexican population]. ***Rev Invest Clin*** 2009; 61: 205-211. Spanish.
13. Biljak VR, Pancirov D, Cepelak I, Popović-Grle S, Stjepanović G, Grubišić TŽ. Platelet count, mean platelet volume and smoking status in stable chronic obstructive pulmonary disease. ***Platelets*** 2011; 22: 466-470.
14. Pamukcu B, Oflaz H, Onur I, Cimen A, Nisanci Y. Effect of cigarette smoking on platelet aggregation. ***Clin Appl Thromb Hemost*** 2011; 17: E175-180.
15. Roethig HJ, Koval T, Muhammad-Kah R, Jin Y, Mendes P, Unverdorben M. Short term effects of reduced exposure to cigarette smoke on white blood cells, platelets and red blood cells in adult cigarette smokers. ***Regul Toxicol Pharmacol*** 2010; 57: 333-337.
16. Madsen C, Nafstad P, Eikvar L, Schwarze PE, Ronningen KS, Haaheim LL. Association between tobacco smoke exposure and levels of C-reactive protein in the Oslo II study. ***Eur J Epidemiol*** 2007; 22: 311-317.
17. Smith GD, Harbord R, Milton J, Ebrahim S, Sterne JA. Does elevated plasma fibrinogen increase the risk of coronary heart disease? Evidence from a meta-analysis of genetic association studies. ***Arterioscler Thromb Vasc Biol*** 2005; 25: 2228-22233.
18. Kumari M, Marmot M, Brunner E. Social determinants of von Willebrand factor: the Whitehall II study. ***Arterioscler Thromb Vasc Biol*** 2000; 20: 1842-1847.
19. Helmersson J, Larsson A, Vessby B, Basu S. Active smoking and a history of smoking are associated with enhanced prostaglandin F (2alpha), interleukin-6 and F2-isoprostane formation in elderly men. ***Atherosclerosis*** 2005; 181: 201-207.
20. Nowak J, Murray JJ, Oates JA, FitzGerald GA. Biochemical evidence of a chronic abnormality in platelet and vascular function in healthy individuals who smoke cigarettes. ***Circulation*** 1987; 76: 6-14.
21. Brummit 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 Haematol*** 2000; 22: 103-107.
22. Dotevall A, Rongemarck C, Eriksson E, Kutti J, Wadenvik H, Wennmalm A. Cigarette smoking increases thromboxane A2 formation without affecting platelet survival in young healthy females. ***Thromb Haemost*** 1992; 68: 583-588.
23. Suwansaksri J, Wiwanitkit V, Soogarun S. Effect of smoking on platelet count and platelet parameters: an observation. ***Clin Appl Thromb Hemost*** 2004; 10: 287-288.
24. Leone A. Biochemical markers of cardiovascular damage from tobacco smoke. ***Curr Pharm Des*** 2005; 11: 2199-2208.
25. Chao FC, Tullis JL, Alper CA, Glynn RJ, Silbert JE. Alteration in plasma proteins and platelet functions with aging and cigarette smoking in healthy men. ***Thromb Haemost*** 1982; 47: 259-264.
26. Lupia E, Bosco O, Montrucchio G. Thrombopoietin contributes to enhanced platelet activation in cigarette smokers. ***Atherosclerosis*** 2010; 314-319.