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

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ABSTRACT

Objectives: To assess the effects of cigarette smoking on thrombocytopenia 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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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 coagulatative factor VII.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{6} A study showed that acute smoking can change the platelet count and induce endothelial damage;\textsuperscript{7} other study did not lead to platelet aggregation stimulated by adenosine diphosphate (ADP), epinephrine, and collagen such effects.\textsuperscript{8} Although it was observed that inhalation of cigarette smoke can trigger platelet aggregation mediated by thrombin and ADP.\textsuperscript{9} Even, reduced platelet agreeability induced by aggregating processes was shown in smokers compared with non-smokers.\textsuperscript{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 means±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. 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).
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 and Pérez-Bautista et al. 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., 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. Roethig et al. 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. 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. It was also shown that smoking increased prostaglandin F (PGF) 2alpha formation, enhanced COX-mediated inflammation, and elevated levels of cytokines and isoprostanes. The mean 11-dehTxB2 as a triggering factor for platelet activation is statistically significantly higher in adult smokers than in nonsmokers. 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 et al. found no correlation between PLT and smoking in healthy volunteers. Also Dotevall et al. noted no changes in PLT in female smokers and non-smokers, and Suwansaksri et al. observed no alterations in platelets in male smokers and non-smokers. Mobarrez et al. showed that acute smoking can induce endothelial damage and increase the platelet and leukocyte count. Moreover, it was also shown that smoking increased reactive protein, fibrinogen, and Von Willebrand factor levels have been shown to be more increased in smokers than nonsmokers. The mean 11-dehTxB2 as a triggering factor for platelet activation is statistically significantly higher in adult smokers than in nonsmokers. 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. 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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. 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.

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