Original Article

Impact of chronic cigarette smoking on platelet aggregation and coagulation profile in apparently healthy male smokers

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Abstract

Background and Aim: Chronic cigarette smoking affects the normal hemostasis by influencing the coagulation pathways. However, the effect of smoking intensity on the degree of impairment of coagulation cascade still remains unclear. The present study was undertaken to assess the impact of smoking on the coagulation cascade and to study the association of smoking duration with coagulation defects.

Methods: A total of 120 apparently healthy subjects were enrolled for our study, were subsequently divided into 60 chronic smokers and 60 nonsmokers. The smokers group was further divided into group-1 (5-15 pack years) and group-2 (>15 pack years) depending on the duration of smoking. All the subjects were evaluated for complete hemogram, platelet count, mean platelet volume, prothrombin time, activated partial thromboplastin time (APTT), and platelet aggregation.

Results: The platelet count was significantly ($P < 0.05$) lower, APTT was significantly shorter ($P < 0.001$) and platelet aggregability was significantly higher ($P < 0.05$) in smokers. Smokers with >15 pack years of duration had significantly high platelet aggregation and shorter APTT compared to smokers with 5-15 pack years. Pearson correlation analysis suggested a strong negative correlation between platelet count and APTT with duration of smoking ($r = -0.557$ and $r = -0.342$, respectively).

Conclusion: Chronic smokers tend to have lower platelet count, shorter APTT, and higher platelet aggregability compared to non-smokers. Therefore, chronic smokers should be investigated for hemostatic dysfunctions.

Key words: Coagulation, duration of smoking, hemostatic dysfunctions, platelet aggregation

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INTRODUCTION

Coagulation is an important function of normal platelets. It is the process in which vessel damage is followed by sequential adherence of platelets to sites of vessel injury and start the coagulation cascade.[1] The platelets then activate internal signaling pathways, aggregate to form plugs and eventually localize the coagulation reactions, that lead to thrombin generation and fibrin strand formation.[1] The balance between fibrin formation and clot dissolution is crucial in maintaining the normal hemostasis and fluidity of blood. It involves a complex interaction of cellular components of endothelium interface with clotting factors and other plasma proteins of the coagulation system.[1,2] Any alterations of this complex balance may cause disruption of the normal hemostasis thus predisposing to thrombosis. Smoking has been shown to induce a state of hypercoagulability, thus influencing the normal platelet physiology and hemostasis mechanisms.[3] Evidence from the literature suggests that long-term effects of cigarette smoking are responsible for certain alterations in the coagulation parameters and thereby responsible for increased

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incidence of ischemic heart disease in smokers.[3-5] Smoking is known to increase the level of oxidative stress.[5] It has been estimated that 1016 radicals are present in one puff of cigarette smoke.[6] The biological effects of free radicals due to nicotine affect lipids, proteins, cellular and subcellular components, plasma constituents, circulating platelets and clotting factors.[6] The hyperthrombotic state in smokers may be due to the possible increased platelet activation and aggregation in response to the oxidative stress and endothelium injury induced by nicotine.[7] A study on the impact of smoking on platelets by Vyssouli *et al*., has suggested that, nicotine promotes endothelial cell damage, affects thrombopoiesis and activates thrombin, which inturn results in hemorrhage, thrombosis, and embolism in smokers.[8] Furthermore, Butkiewicz *et al*., has reported that chronic smoking affects the nitric oxide (NO) release from platelets by affecting synthetic functions of platelets, thus resulting in altered hemostasis.[9] Reports on effect of smoking on coagulation profile have been controversial. Earlier studies have observed an increase,[10,11] decrease,[12] or no change[13] in platelet aggregability and coagulation parameters following smoking. Even though, the previous studies has reported various coagulation defects in chronic smokers, the relationship between duration of smoking and degree of coagulation impairment in the coagulation pathways remains unclear. Thus, this study was undertaken to determine the impact of chronic cigarette smoking and smoking duration on hemostasis by studying the coagulation profile in smokers. Further the study intends to substantiate the predisposition of chronic smokers to coagulation disorders which may provide insight for treating them during hemostatic emergencies.

MATERIALS AND METHODS

A total of 120 apparently healthy male volunteers, aged between 30–60 years, attending the outpatients department of Gandhi Medical College and Hospital, Secunderabad, were recruited for the study after obtaining written informed consents from them. They were subsequently divided into smoker and nonsmoker groups based on their history of smoking, as per the World Health Organization's 10th revision of the international statistical classification of diseases and related health problems criteria of harmful use.[14] The smokers group was further categorized into two groups according to the duration of smoking (based on number of pack years of smoking). The group-1 comprised of smokers with 5-15 pack years history of smoking and group-2 consisted of smokers with duration >15 pack years (1 pack year = 20 cigarettes per day for 1-year).[15] Institutional Ethics Committee clearance was obtained. This study was conducted over a period of 2 years. Out of the 120 volunteers, 60 were smokers and 60 nonsmokers. Subjects with body mass index (BMI) >30 and those with hypertension, diabetes mellitus, rheumatic heart disease, thyroid disorders, hepatic and renal disorders, inflammatory disease and malignancy were excluded from the study. The subjects were not under any medication, which is known to affect platelet function, coagulation profile, fibrinolytic system or lipid metabolism, 2 weeks prior to the commencement of the study. Baseline parameters such as age, BMI, and blood pressure were documented in a well-designed proforma prior to collection of 10 ml of venous blood from each subject, for carrying out the following investigations.

Platelet aggregation

Venous blood was drawn by venous puncture into the vacutainer (containing 3·13% sodium citrate: Blood 1:9), was centrifuged at 800 g for 10 min at 22°C. The platelet-rich plasma (PRP) was separated from the upper two-third of the supernatant to avoid contamination with other cells, such as monocytes. Platelet aggregability was estimated colorimetrically by modified O'Brien's method,[16] based on the principle of change in the absorbance by the addition of adenosine diphosphate (ADP) to PRP. ADP solution of 0.1 ml was added to 5 ml of PRP sample. Once the platelet clumps are formed, the absorbance readings were noted after the dispersion of aggregates at the end of 20 s.

Activated partial thromboplastin time

This test was done by adding kaolin using the Hemoscann test kit (Quimica clinica applicada, S.A). After separating the PRP, the remaining blood was centrifuged again at 1500 g for 15 min to obtain platelet-poor plasma (PPP). Undiluted PPP was incubated at 37°C in aliquots with kaolin followed by addition of partial thromboplastin and $CaCl_2$. The time taken for clot formation, from the time of addition of $CaCl_2$ was recorded in seconds.

Prothrombin time

The prothrombin time (PT) was estimated by quick time method (one-stage) using the Hemoscann test kit (Quimica clinica applicada, S.A). The PPP was mixed with tissue thromboplastin at 37°C and an excess of calcium chloride (25 mm) was added to initiate coagulation. The time taken for the addition of calcium to the formation of the fibrin clot, known as the PT was recorded and expressed in seconds.

Complete blood picture

Whole blood (ethylenediaminetetraacetic acid as anticoagulant) was used for determining white blood cell (WBC) count, red blood cell (RBC) count, hematocrit (Ht%), hemoglobin (Hb) concentration, platelet count, and mean platelet volume (MPV) by an Auto-Hematology Analyser (Lab Life H3D, MINDRAY).

Blood pressure

The systolic blood pressure and diastolic blood pressure were recorded by sphygmomanometer in the morning, prior to collection of blood sample.

Statistical analysis of data

The data were expressed as mean ± standard deviation. Comparison of results between smokers and nonsmokers was done by Student's *t*-test. Association of smoking duration (pack years) with coagulation parameters was assessed by Pearson correlation analysis using SPSS software version 19 (SPSS, Chicago, IL). The $P < 0.001$ was taken as highly significant, a $P < 0.05$ as significant and a $P > 0.05$ as nonsignificant.

RESULTS

The complete blood picture of smokers and nonsmokers is described in Table 1, which suggested highly significant difference ($P < 0.001$) in total WBC count, RBC count, Ht and Hb concentration, in chronic smokers group in comparison to nonsmoker group. The comparison of parameters of coagulation revealed significantly lower ($P < 0.05$) platelet count and significantly shorter activated partial thromboplastin time (APTT) in smokers ($P < 0.001$) as depicted in Table 2. We did not find any significant difference in terms of MPV and PT. However, the platelet aggregation was significantly higher ($P < 0.05$) [Table 2] in smokers as compared to nonsmokers. The effect of duration of smoking on coagulation is depicted in Table 3, which revealed significantly lower platelet count, shorter APTT and higher platelet aggregability ($P < 0.001$) in smokers with >15 pack years of duration. There was no significant difference in MPV and PT while comparing smokers with mild to moderate (5–15 pack years) smoking to those with severe smoking (>15 pack years). The Pearson's correlation analysis is suggestive of significantly negative correlation with platelet count ($P < 0.001$), as well as APTT ($P = 0.007$) with smoking duration as depicted in Figures 1 and 2, respectively.

Table 1: Baseline characteristics and complete blood picture of the study population

Parameters	Nonsmokers (*n*=60)	Smokers (*n*=60)	*P*
Age (years)	51.9±1.2	52.3±1.5	0.109
Duration (pack years)	0	18±2.5	-
SBP (mm Hg)	128±12.59	130.81±20.1	0.360
DBP (mm Hg)	80.25±4.39	79.34±6.95	0.392
WBC count (10^9/L)	6.4±1.5	8.3±1.7	<0.001
RBC count (10^{12}/L)	5.24±0.6	5.61±0.43	0.002
Hematocrit (%)	45.14±0.93	45.82±1.07	0.003
Hemoglobin (%)	14.26±0.74	15.31±0.38	<0.001

Data expressed as mean±SD. Statistical analysis was done by Student's unpaired *t*-test. *P*<0.05 was considered statistically significant.
SBP: Systolic blood pressure, DBP: Diastolic blood pressure,
SD: Standard deviation, WBC: White blood cells, RBC: Red blood cells

Table 2: Comparison of coagulation profile between smokers and nonsmokers

Parameters	Nonsmokers (*n*=60)	Smokers (*n*=60)	*P*
Platelet count (cells/L)	4.8±0.54	4.55±0.53	0.011
MPV (fl)	8.4±0.23	8.56±0.61	0.06
APTT (s)	27.4±1.2	4.8±1.06	<0.001
PT (s)	14.64±1.5	25±1.15	0.112
Platelet aggregation (ODD)	0.061±0.026	0.069±0.013	0.035

Data expressed as mean±SD. Statistical analysis was done by Student's unpaired *t*-test. *P*<0.05 was considered statistically significant. MPV: Mean platelet volume, APTT: Activated partial thromboplastin time, PT: Prothrombin time, ODD: Optic density difference, fl: Femto liters, SD: Standard deviation

Table 3: Comparison of coagulation profile in relation to duration of smoking

Parameters	5-15 pack years (*n*=33)	>15 pack years (*n*=27)	*P*
Platelet count (cells/L)	4.76±0.45	4.28±0.49	0.0002
MPV (fl)	8.72±0.48	8.55±0.73	0.283
APTT (s)	25.28±0.89	24.27±1.02	0.0001
PT (s)	14.25±1.23	14.23±1.07	0.947
Platelet aggregation (ODD)	0.065±0.014	0.073±0.009	0.012

Data expressed as mean±SD. Statistical analysis was done by Student's unpaired *t*-test. *P*<0.05 was considered statistically significant. MPV: Mean platelet volume, APTT: Activated partial thromboplastin time, PT: Prothrombin time, ODD: Optic density difference, fl: Femto liters, SD: Standard deviation

DISCUSSION

In the present study, we observed significantly high WBC count in smokers, which is inagreement with previous studies.[17-19] The RBC count, Hb concentration, and Ht were found to be significantly higher ($P < 0.001$) in the smoker group. The high Hb concentration in smokers may be due to the fact that smoking causes excessive production of carbon monoxide (CO), which leads to the formation of carboxy Hb. Since Hb has 200 times more affinity for CO than O_2, there is the stark unavailability of Hb for O_2 carriage, shifting the Hb-O_2 dissociation curve to the left.[20] As a compensatory mechanism, the body tries to produce more Hb by increasing the rate of erythropoiesis. The high RBC count and Hb concentration is responsible for the higher Ht often observed in chronic smokers. Our study agrees with the results of Ugbebor *et al*., and Goel *et al*., who have reported similar findings.[21,22]

Changes in the platelet count, platelet morphology, and coagulation parameters enhance the risk of thrombosis. In the present study, we observed

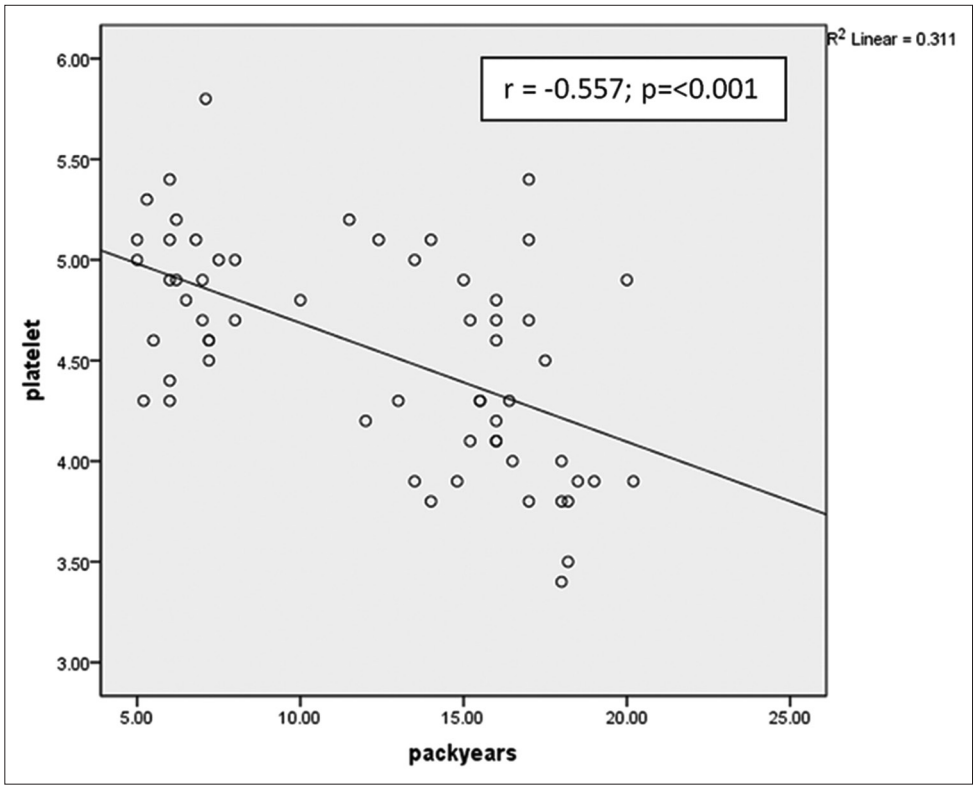

Figure 1: Effect of smoking duration (pack years) on platelet count. Statistical analysis was done by Pearson's correlation coefficient. $P < 0.05$ was considered statistically significant ($r = -0.557$; $P \leq 0.001$)

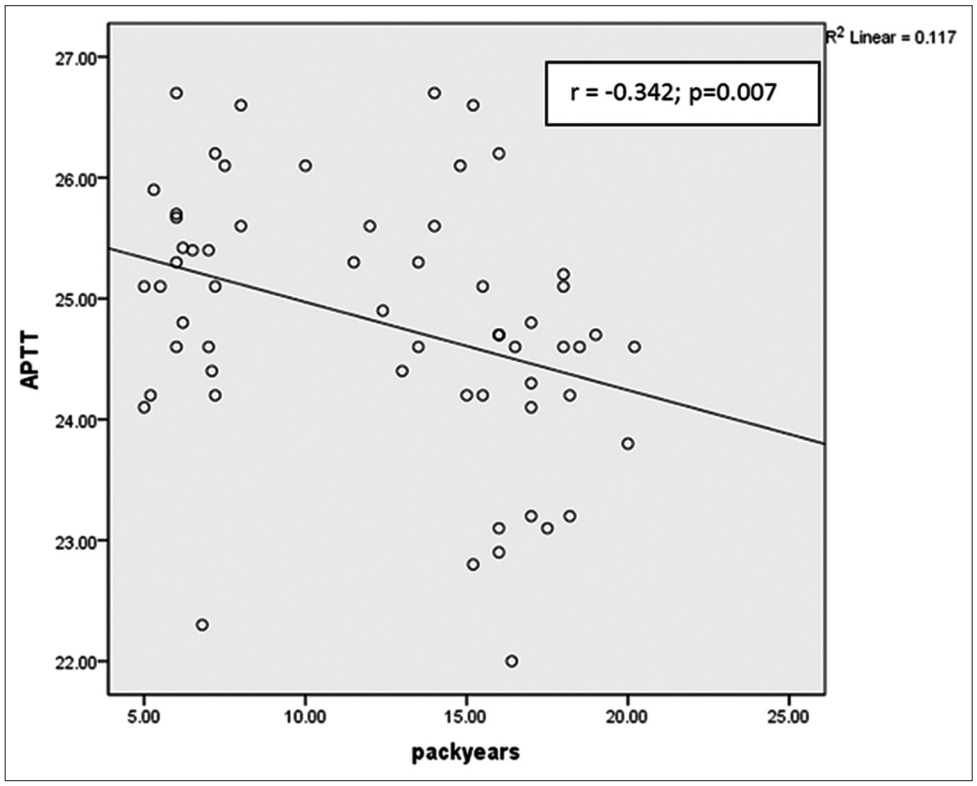

Figure 2: Effect of smoking duration (pack years) on activated partial thromboplastin time. Statistical analysis was done by Pearson's correlation coefficient. $P < 0.05$ was considered statistically significant ($r = -0.342$; $P = 0.007$)

significantly lower ($P < 0.05$) platelet count in chronic smokers [Table 2], however we did not observe any significant differences in the MPV. When the smokers were further compared based on the duration of smoking [Table 3], significant difference was observed in platelet count between mild and moderate smokers with that of severe smokers. Therefore, from the present study we observed that >15 years duration of smoking results in lower platelet count which could be due to the effect of nicotine-induced oxidative stress on thrombocytopoiesis. The Pearson's correlation analysis depicted strong negative correlation ($r = -0.557$, $P \leq 0.001$) between pack years of smoking and platelet count among smokers suggesting that, as the duration of smoking increases, there is a progressive decrease in the total platelet count [Table 4 and Figure 1]. Nair *et al*., has reported increased expression of thrombopoietic markers, such as P-selectin and sP-selectin and progressive decrease in platelet count. This decrease in platelet count could be due to nicotine-induced decreased thrombopoietic activity in chronic smokers.[23] Also Ridker *et al*., found a strong positive correlation of sP-selectin concentration with smoking which is an indicator of increased thrombopoietic activity followed by a decreased platelet count in smokers.[24] However, Butkiewicz *et al*., has reported marked decrease in platelet count only in female smokers suggesting platelets in women are more sensitive to smoking. However, there was no change in platelet count of males.[9] Conway *et al*., demonstrated a positive correlation between thrombopoietic activity and smoking, although the level of this marker of activation was lower in women than in men.[25]

The PT and APTT are the most commonly performed tests of hemostasis. The PT evaluates the extrinsic pathway of coagulation, whereas the APTT evaluates the intrinsic pathway of coagulation. While comparing the PT and APTT among nonsmokers and smokers, we observed that the APTT was significantly shorter in smokers, ($P < 0.001$) whereas there was no significant change in PT [Table 2]. However, while comparing the APTT and PT among the two groups of smokers based on their smoking duration, we observed significant change only in APTT among group-1 versus group-2. The study observed that smokers with longer duration (>15 pack years) of smoking had significantly shorter ($P < 0.001$) APTT in comparison to smokers with lesser (<15 pack years) duration [Table 3]. Also, the Pearson correlation analysis for association of pack years of smoking with that of PT and APTT in smokers suggested strong negative correlation between duration of smoking and APTT ($r = -0.342$, $P = 0.007$) [Table 4 and Figure 2]. Our findings suggest that nicotine-induced oxidative stress in platelets affects intrinsic pathways of coagulation thus producing a hypercoagulability state in smokers.[26] Our findings are in line with Takajo *et al*., who have reported shorter APTT in chronic smokers, making them more vulnerable to ischemic heart diseases.[6] The present study is concurrent with that of Ngozi and Ernest who found a negative correlation between smoking duration and APTT in male Nigerian smokers.[26] However, the present study does not agree with that of Al-Dahr who has reported significantly shorter PT, as well as APTT in chronic male smokers.[27] The differences in the above results may be due to the differences in smoking duration, intensity and other associated comorbid factors in the previous studies.

Table 4: Pearson's correlation analysis of duration of smoking with coagulation parameters

Parameters	Correlation coefficient (*r*)	*P*
MPV	−0.200	0.127
PT	0.126	0.336
Platelet aggregation	0.183	0.214

Statistical analysis was done by Pearson's correlation coefficient. *P*<0.05 was considered statistically significant. MPV: Mean platelet volume, PT: Prothrombin time

Platelet aggregation is one of the important markers for assessing coagulation profile.[1] In the present study, we observed significantly higher ($P < 0.05$) platelet aggregability in smokers in comparison with non-smokers, which is suggestive of hypercoagulability induced by nicotine. The smokers with >15 pack years of duration had significantly higher ($P < 0.05$) platelet aggregability in comparison to smokers with <15 years of duration [Table 3]. Deleterious effects of smoking are associated with the generation of free radicals and imbalance in platelet pro-antioxidants.[6] The impaired intra platelet redox state induced by nicotine is responsible for the impaired activity of platelet-derived NO (PDNO).[28] PDNO enhances the synthesis of thromboxane and has antithrombotic action by reducing the production of prostacyclin, thus leading to hemostatic disturbances in chronic smokers.[29] Takajo *et al*., has suggested that the increased risk of atherosclerosis and thrombotic disorders in chronic smokers is mediated primarily by repeated injury to the endothelium and decreased the production of PDNO.[6] According to Freedman *et al*., (1997), nicotine directly inhibits the production and bioactivity of PDNO.[30] Further, the sustained injury of endothelium in smokers expose the subendothelial collagen, which causes increased activation of platelets and enhances platelet aggregation.[4] Thus, in the present study it was observed that, chronic smokers (>15 pack years) tend to have lower platelet count, shorter APTT, and higher platelet aggregability, which are suggestive of disturbances in coagulation pathways induced by nicotine.

Limitations of the study

The sample size in the present study is relatively small and drawn from one limited geographical area, which

is inadequate for extrapolating the application of these findings to the general population. Therefore, future studies with larger sample size are warranted to further strengthen our results.

CONCLUSION

The findings from the present study along with evidences from literature enables us to suggest that along with conventional markers, individuals with >15 pack years history of smoking should be monitored for platelet count, APTT and platelet aggregability, which will be helpful in evaluating the altered coagulation status and hemostasis in chronic smokers. In light of these adverse effects, the cessation of smoking should be strongly encouraged.

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