

Effects of cigarette smoking on coagulation screening tests and platelet counts in a Sudanese male adults population

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ABSTRACT

الأهداف: تقييم تأثير تدخين السجائر على تعداد الصفائح الدموية وزمن تخثر البروثرومبين وزمن الثرموبلاستين الجزئي ومعدل القياس العالمي للسيولة لدى المدخنين.

الطريقة: دراسة مقارنة أجريت في مدينتي كوستي والجبلين بالسودان خلال الفترة من أكتوبر 2016 إلى مايو 2017. شملت الدراسة 200 شخص من المتطوعين الذكور تتراوح أعمارهم من 20-67 سنة (100 من المدخنين و100 من غير المدخنين كمجموعة ضابطة) حيث تم جمع العينات في نوعين من مانعات تجلط الدم، وهي: ثلاثي سترات الصوديوم لتحليل ال (PT، PTT، & INR) وتم تحليل العينات باستخدام جهاز (Coagulometer) بينما تم جمع جزء اخر من العينات في مانع التجلط EDTA لتحليل تعداد الصفائح الدموية بواسطة جهاز Sysmex

النتائج: نتائج الدراسة أوضحت أن تعداد الصفائح الدموية كان قليلا لدى المدخنين مقارنة بغير المدخنين من الناحية الإحصائية (183×10^3 ميكرو لتر مقابل 244×10^3 ميكرو لتر ($p < 0.000$) وأن قيم زمن البروثرومبين كان اقل لدى المدخنين (12.9 ± 1.2 ثانية مقابل 13.7 ± 1.04 ثانية ($p < 0.000$) ومعدل القياس العالمي للسيولة لدى المدخنين اقل مقارنة بغير المدخنين (0.95 ± 0.09 مقابل 1.01 ± 0.08 ($p < 0.000$) أما زمن الثرموبلاستين الجزئي فلم يسجل فروق ذات دلالة إحصائية (3.8 ± 30 ثانياه مقابل 4.6 ± 9.37 ثانية ($p > 0.077$). طريقة بيرسول للتحليل أوضحت وجود ارتباط سلبى بين الصفائح الدموية ومدة التدخين ($r = -0.289$) ($P = 0.004$) وعمر المدخنين ($r = -0.238$) ($P = 0.017$).

الخلاصة: خلصت الدراسة الي قلة تعداد الصفائح الدموية، وقصر قيم زمن البروثرومبين ومعدل القياس العالمي للسيولة لدى المدخنين مقارنة مع غير المدخنين

Objectives: To study the effects of heavy cigarette smoking on coagulation (CGG) screening tests and platelet counts (PLTs) in a Sudanese male adults population.

Methods: A case control study was conducted at both Kosti and Gabalein towns, Sudan, during October

2016 to May 2017. A 100 adult cigarette smokers were selected and another 100 matched non-smokers were selected as healthy controls. Blood samples were collected in trisodium citrate anti-coagulant for prothrombin time (PT), partial thromboplastin time (PTT), and international normalized ratio (INR), analyzed using standard methods (co-agulometer machine) and Ethylenediaminetetraacetic acid for the platelet counts, using an automated haematology analyzer (Sysmex, Tokyo, Japan).

Results: The results showed that the mean platelet counts were significantly lower in the smokers ($183 \times 10^3/\text{cmm} \pm 64 \times 10^3/\text{cmm}$) versus ($244 \times 10^3/\text{cmm} \pm 38 \times 10^3/\text{cmm}$) in non-smokers, ($p < 0.000$). Pearson correlation analysis suggested a weak negative correlation between platelet counts with the duration of smoking ($r = -0.289$, $p < 0.004$) and the age of the smokers ($r = -0.238$, $p < 0.017$). The mean PT and INR were also significantly lower in smokers (12.9 ± 1.2 seconds) compared with the non-smokers (13.7 ± 1.04 seconds, $p < 0.000$), for PT and (0.95 ± 0.09 versus 1.01 ± 0.08 , $p < 0.000$) for INR. In contrast, PTT had no significant variation in smokers (30.5 ± 3.8 seconds) and the non-smokers (37.9 ± 4.6 seconds). A p -value > 0.05 was considered significant.

Conclusion: Cigarette smokers tend to have lower platelet counts, shorter PT, and INR values, compared to non-smokers. Therefore, smoking might be associated with bleeding disorders but further investigations are needed.

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Cigarette smoking could cause alterations in the main hemostatic systems, and coagulation factors which in turn could affect their functions.¹ Hypotheses have been forward to explain the effect of tobacco smoking such as alteration clot structure and oxidative stress or the generation of procoagulant micro vesicles.^{2,3} Cigarette smoking exposure (CSE) elevates the risk of arterial thrombosis, approximately 40% of cigarette smoking-related deaths associated with cardiovascular disease.⁴ Some research demonstrated higher risk of thrombosis related to cardiomyocyte mitochondria oxidative damage and increased in smooth muscle cell proliferation and platelet aggregation.⁵⁻⁷ Cigarette smoking exposure effects on hemostasis have been documented in decreased expression tissue factor pathway inhibitor and increased smokers plasma fibrinogen levels compared to non-smokers.^{8,9} Few studies examined the effects of acute CSE on clotting, but circulating tissue factor activity increased levels have been demonstrated after short-term of cigarette smoking.^{9,10} So, CSE shows increasing prothrombotic biomarkers and might directly promote thrombosis.

Blood haemostasis can be affected by a variety of factors one of them is cigarette smoking which may cause many health problems. Scant information on hemostatic studies of smokers were observed in the Sudanese population especially in the study area, thus, the current study aimed to study the effects of heavy smoking (more than 5 cigarettes per day) on the prothrombin time (PT), partial thromboplastin time (PTT), platelet counts (PLT) counts and international normalized ratio (INR) test values and compare these parameters with the results obtained from those non-smokers.

Methods. A case control study was carried out in 2 cities of Sudan, Kosti and Gabalein during October 2016 to May 2017. Two hundred male volunteers (100 cigarette smokers and 100 non-smokers as a control group) with ages between 20-67 years recruited to take place in this study. Smokers who had less than 3 years on smoking, history of alcoholism, presence of disease, which have a significant effect on coagulation, long-term medication, or current drugs, which have an effect on platelets and those who refused, were excluded from participation in the study. All the mentioned

criteria were applied for the non-smokers. Data was collected by using structural interviewing questionnaire. Venous blood specimens were collected in trisodium citrate anti-coagulant for PT, PTT, and INR, analyzed using standard methods (co-agulometer machine) and Ethylenediaminetetraacetic acid (EDTA) anticoagulant for the PLT counts, analyzed by (Sysmex, Tokyo, Japan) automated hematology analyzer.

Ethical considerations. According to Helsinki Declaration principles, prior to taking specimens, each participant completed the informed consent, which was approved by the Ethical and Research Committee of the University of El Imam El Mahdi, Faculty of Medical laboratory Sciences, Sudan and informed consent was taken.

Statistical analysis. Data analysis carried out by Statistical Package for the Social Sciences (SPSS) version21 (IBM Corp., Armonk, NY, USA). Frequency distribution, mean, standard deviation and percentage were calculated. Test of normality carried out using normal distribution test to choose the suitable statistical test, so t-test applied for the comparison means to assess the difference between the 2 study groups. The two-tailed tests, alpha error of 0.05 and p -value \leq 0.05 were considered significant. Pearson correlation analysis was applied for correlation calculations between the study parameters.

Results. All participants were males (aged between 20-67 years old), as in the area where the current study was carried out females did not smoke cigarettes. This is due to socio-cultural causes; tobacco is used mostly by male rather than female.¹¹ Table 1 shows smoked cigarettes per day and age comparison within the smokers group. Table 2 explains PT, PTT, PLTs, INR values of cigarette smokers, and non-smokers.

The mean PT is significantly shorter in smokers when compared to the non-smokers, (12.9 \pm 1.15s versus 13.7 \pm 1.04 s (p <0.000), whereas the mean APTT is not statistically significant between smokers and non-smokers (30.5 \pm 3.8s versus 37.9 \pm 4.6s), (p <0.077). Platelet count is statistically significantly lower in the smokers (183 \pm 64/ μ l versus 244 \pm 376/ μ l (p <0.000).

Table 1 - Smoked cigarettes per day and age comparison within the smokers group.

| Smoked cigarettes per day (%) | | Age (%) | |
|-------------------------------|------|------------|------|
| 5 to 9 | (39) | 3-5 years | (31) |
| 10 to 15 | (38) | 6-10 years | (27) |
| >15 | (23) | >10 years | (42) |

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Table 2 - The PT, PTT, PLTs count, INR values of cigarette smokers versus nonsmokers.

| Parameter | Cigarette Smokers n=100 | | | Non-cigarette Smokers n=100 | | | P-value |
|---------------|------------------------------|-----------|------------------------------|------------------------------|-----------|-------------------------------|---------|
| | Category (values) | Frequency | Mean±SD | Category (values) | Frequency | Mean±SD | |
| PT(s) | 10-10.9s | 3 | 12.9±1.15s* | 12.1-14s | 65 | 13.7±1.04* | 0.000* |
| | 11-13.5s | 70 | | 14.1-16s | 35 | | |
| | 13.6-16s | 25 | | | | | |
| | 16.1-17s | 2 | | | | | |
| | 19-23s | 3 | | | | | |
| 23.1-27s | 11 | | | | | | |
| APTT(s) | 27.1-29.9s | 31 | 30.5±3.8s‡ | 30-34s | 15 | 37.9±4.6s‡ | 0.077 |
| | 30-34.9s | 41 | | 34.1-39 s | 38 | | |
| | 35-40s | 14 | | | | | |
| | <150x10 ³ /cmm | 34 | | | | | |
| | 151-250x10 ³ /cmm | 51 | | 231-290x10 ³ /cmm | 49 | | |
| PLTs/ μ l | 251-350x10 ³ /cmm | 12 | 183±64x10 ³ /cmm* | 291-350x10 ³ /cmm | 13 | 244±376x10 ³ /cmm* | 0.000* |
| | 351-450x10 ³ /cmm | 3 | | | | | |
| INR | 0.7-0.8 | 34 | 0.95±0.09 | 0.80-0.95 | 36 | | |
| | 0.81-0.9 | 51 | | 0.96-1.05 | 30 | | |

PT - prothrombin time, APTT - activated partial thromboplastin time, PLTs - platelet counts, SD - standard deviation, INR - international normalized ratio, S - Seconds. * $p < 0.00$, † $p < 0.05$, ‡ $p > 0.05$.

Table 3 - Pearson correlation between age, duration, and number of cigarette per day with the coagulation parameters (PT, PTT, PLTs and INR) among cigarette smokers.

| Category | Pearson correlation | P-value |
|-----------------|---------------------|---------|
| <i>Age</i> | | |
| PLT | -0.238 | 0.017 |
| PT | -0.039 | 0.699 |
| PTT | 0.041 | 0.689 |
| INR | 0.013 | 0.900 |
| <i>Duration</i> | | |
| INR | 0.116 | 0.250 |
| PLT | -0.289 | 0.004 |
| PTT | 0.089 | 0.378 |
| PT | 0.089 | 0.806 |
| <i>Number</i> | | |
| PT | -0.044 | 0.663 |
| PTT | 0.112 | 0.268 |
| PLT | -0.132 | 0.192 |
| INR | -0.005 | 0.961 |

PT - prothrombin time, APTT - activated partial thromboplastin time, PLTs - platelet counts, SD - standard deviation, INR - international normalized ratio

Table 3 summarizes the correlation and significance between age, duration, and number per day with the coagulation parameters (PT, PTT, PLTs and INR) among cigarette smokers.

The findings of the current study observed that the highest number of cigarette smokers in this study was

in the 20-30 years age range, however the relationship between the 4 age groups of smokers (20-30 years), (31-40 years), (41-50 years) and (>50 years) and PT, PTT, and INR parameters showed no significant differences ($p > 0.05$); but there was a significance relationship between age and PLTs ($r = -0.238$, $p < 0.017$), this indicated that cigarette smoking was a significant public health problem among youths.

Discussion. Hypercoagulability state could be caused by cigarette smoking, potentially leading to thrombosis.¹² Cigarette smoking appears to play a significant role in coagulation factors malfunctions.^{13,14} The current study was conducted to know the effects of heavy cigarette smoking on PTT, PT, PLTs and INR among cigarette smokers in Kosti and Gabalein, Sudan. The present study findings state that, the mean PLT was statistically significantly lower with smokers ($183 \pm 64/\mu$ l versus $244 \pm 376/\mu$ l, $p < 0.000$). This result was in the line with the ROA Mustafa study,¹⁵ that, smoking significantly reduced platelet count (212.30 ± 55.71 vs. $238.23 \pm 36.89 \times 10^9/l$) and previous study carried out by Metta S et al,¹⁶ in 2015 reported that, platelet count was significantly lower in chronic smokers. The current results was not in agreement with the findings stated by Ghahremanfard et al,¹⁷ who concluded that, the mean platelet count was significantly higher in adult smokers than in adult non-smokers and Al-Dahr study,¹⁸ reported

that, no significant difference between adult smokers and non-smokers regarding platelet count. Concerning PT the current study revealed that, the mean PT was significantly shorter in smokers when compared to the non-smokers, ($12.9 \pm 1.15s$ versus $13.7 \pm 1.04s$, $p < 0.000$), the significantly reduced PT value was agreed with the results obtained by Akpotuzor et al,¹⁹ who reported that, mean prothrombin time values of smokers were significantly lower when compared with the non-smokers and disagree with the findings obtained from the study carried out by Metta S et al,¹⁶ who showed that no significant difference in PT, on the other hand the current study results regarding PT value inconsistent with the results of Al-Dahr study,¹⁸ who reported that PT was not changed significantly in the smoker groups. Regarding the results of the APTT there is no statistically significant difference between smokers and non-smokers ($30.5 \pm 3.8s$ versus $37.9 \pm 4.6s$, $p < 0.077$) in the current study, however the results recorded by Akpotuzor et al,¹⁹ showed that mean APTT values of smokers were significantly lower ($p < 0.01$) when compared with the non-smokers. According to the present study findings, the mean of INR was significantly shorter in smokers when compared to the non-smokers, (0.95 ± 0.09 versus 1.01 ± 0.08 , $p < 0.000$). This findings were agreed with a previous study reported by Nascetti et al,²⁰ who argued that, the decrease in INR value associated with cigarette smoking is proportional to the number of cigarette smoked per day. Pearson correlation analysis suggested a weak negative correlation between platelet count with the duration of smoking ($r = -0.289$, $p < 0.004$), these findings were not in agreement with the study carried out by Metta S et al,¹⁶ their Pearson correlation analysis suggested a strong negative correlation between platelet count with the duration of smoking. The decrease in platelet count could be due to nicotine-induced decreased thrombopoietic activity in smokers.²¹ The findings of the current study observed that the highest number of cigarette smokers in this study was in the 20-30 years age range, however the relationship between the 4 age groups of smokers (20-30 years), (31-40 years), (41-50 years) and (>50 years) and PT, PTT, and INR parameters showed no significant difference ($p > 0.05$); but there was a significant relationship between age and PLTs count ($r = -0.238$, $p < 0.017$), which indicated that cigarette smoking to be a concerned public health problem among youths. The participants are from only one state of the 18 states in Sudan.

In conclusion, cigarette smokers tend to have lower platelet counts, shorter PT, and INR values, compared to non-smokers so, the efforts need for governmental

and non-governmental organizations (NGOs), and clinicians to develop public awareness to mitigate the risk of cigarette smoking targeted at youths.

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