DOI: https://doi.org/10.54393/pjhs.v7i9.3714

PAKISTAN JOURNAL OF HEALTH SCIENCES
(LAHORE)
https://thejas.com.pk/index.php/pjhs
ISSN (E): 2790-9352, (P): 2790-9344
Volume 7, Issue 09 (September 2026)



OPEN ACCESS
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Original Article

Comparison of Whole Blood Impedance Platelet Aggregation Using Adenosine Diphosphate and Arachidonic Acid Between Smokers and Non-Smokers

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ARTICLE INFO

Keywords:
Whole Blood Aggregometry, Impedance Aggregometry, Platelet aggregation, Arachidonic Acid, Adenosine Diphosphate, Cigarette Smoking

How to Cite:
Sultana, R., Naz, F., Afzal, M., Rashid, Y., & Saqlain, N. (2026). Comparison of Whole Blood Impedance Platelet Aggregation Using Adenosine Diphosphate and Arachidonic Acid Between Smokers and Non-Smokers: Whole Blood Impedance Platelet Aggregation Between Smokers and Non-Smokers. Pakistan Journal of Health Sciences, 7(9), 27-33. https://doi.org/10.54393/pjhs.v7i9.3714

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Received Date: 19th December, 2025
1st Revision Received: 27th February, 2026
2nd Revision Received: 14th April, 2026
Acceptance Date: 21st April, 2026
Published Date: 30th September, 2026

ABSTRACT

Smoking enhances platelet aggregability and related ischemic and vascular events. However, data on whole blood impedance platelet aggregation in relation to both smoking duration and daily cigarette consumption separately in apparently healthy Pakistani adults are limited. **Objective:** To assess the association of smoking duration and number of cigarettes smoked daily with increased platelet aggregation and find out factors independently predicts increased aggregation. **Methods:** A comparative cross-sectional study was done at the Department of Pathology, Lahore General Hospital. Sixty-five smokers and non-smokers each (a total of 130) were enrolled using non-probability convenience sampling. History and venous samples were taken, and whole blood platelet aggregometry was performed using Arachidonic Acid and Adenosine diphosphate. Chi-Square and Logistic Regression tests were applied, p-value<0.05 was considered significant. **Results:** 84.6% of non-smokers had normal aggregation with both agonists. The percentage of individuals with enhanced aggregation increased as the duration of smoking and the number of daily cigarettes increased. Analysis showed a statistically significant association between abnormal aggregation and increased duration of smoking and number of daily cigarettes. Logistic Regression showed duration of smoking as an independent predictor of increased aggregation with ADP only (p-value=0.006, Exp(B)=15). The number of cigarettes smoked daily was not an independent predictor after adjusting for confounders. Results for age and hematocrit were not significant. **Conclusions:** Cigarette smoking is associated with increased platelet aggregation. Longer duration of smoking independently predicts high ADP-induced platelet aggregation after adjusting for age and hematocrit. The number of cigarettes smoked daily is not an independent predictor of increased aggregation.

INTRODUCTION

Tobacco is called the only "legal drug" which used as per the instructions of the manufacturer, killing its users. The World Health Organization estimates that around one in five adults' smokes tobacco. Globally, in 2024, the adult smoking prevalence was 19.5% [1, 2]. Cigarette smoking is among the primary causes of cancer. Cigarette smoking increases the risk of pulmonary and cardiovascular diseases, such as atherosclerosis and coronary artery disease [3]. It also increases the risk of myocardial infarction [4]. Several pathogenic mechanisms link platelet hyperaggregability to tobacco smoking. Among over 7000 hazardous compounds present in tobacco, nicotine and carbon monoxide cause most of the pharmacological effects [5, 6]. Deleterious free radicals are present in 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]. It induces clot formation in the coronary arteries, weakens the vascular activity, and increases endothelial dysfunction,

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DOI: https://doi.org/10.54393/pjhs.v7i9.3714

escalating several cardiovascular diseases [7, 8]. Nicotine creates oxidative stress and causes endothelial injury, causing platelet activation [9]. Smoking induced serotonin/5-HT related downstream signaling and a change in platelet trafficking dynamics that remodels platelet surface protein and glycans, predisposing to hyperactivation [8]. Platelets are central to the pathogenesis of atherosclerosis and arterial thrombosis [10]. Dysregulated platelet endothelial interactions are an important pathogenic mechanism in the development of atherosclerosis [11]. The impedance of whole blood aggregometry (WBA) allows assessment of platelet function using anticoagulated whole blood without any sample processing. It is a diagnostic test for platelet function defects and measures an increase in electrical impedance in relation to agonist-induced platelet aggregation [12]. Since the contribution of other blood elements may affect platelet function, by using Whole Blood (WB) aggregometry, platelet function is assessed under more physiological conditions. Currently, in Pakistan, around 17% of men and 1 - 2% for women use tobacco, and adult smoking prevalence is 9.3%. According to the data from 2023, 10.73% of the deaths in Pakistan are caused by tobacco [13]. Prevalence of smoking is on the rise, particularly in developing countries, including Pakistan, and at the same time, knowledge about the hazards of smoking is lacking among the public. The purpose of this study is to assess ADP and arachidonic acid-induced platelet aggregation defects in smokers and compare them with aggregation in non-smokers. It will help in understanding the nature of hemostatic derangements in apparently healthy smokers and in reducing the morbidity and mortality in smokers associated with thromboembolic complications by increasing awareness about the negative health consequences of tobacco. It will increase physicians' understanding that smokers are at higher risk of thromboembolic episodes and educate them better.

Most of the data available in the literature assesses smoking habits in the form of pack years. No data are available separately on whether the number of years the person has been smoking impacts more than the total number of cigarettes smoked daily. Therefore, this study aimed to understand whether new smokers show similar aggregation abnormalities as compared to smokers with a long history of smoking or not. Also, this study adjusts for confounders like age and hematocrit and aims to establish duration of smoking or total number of cigarettes smoked daily as an independent predictor.

METHODS

A comparative cross-sectional study was carried out between February 2025 and August 2025 in the Department of Pathology, Lahore General Hospital. Ethical approval was taken from Lahore General Hospital (Reference No. 00-53-A-2025; dated 15th January 2025). Using the standard formula for comparing two independent proportions, $n = (Z_{1-\sigma/2} + Z_{1-\beta})^2 [p_1(1-p_1) + p_2(1-p_2)] / (p_1-p_2)^2$, with $Z_{1-\sigma/2} = 1.96$ (95% confidence), $Z_{1-\beta} = 1.28$ (90% power), $p_1 = 0.15$ (enhanced aggregation in non-smokers), and p2 = 0.40 (enhanced aggregation in smokers), the numerator equals $(3.24)^2 \times [0.1275 + 0.24] = 10.4976 \times 0.3679$, and the denominator equals (0.15 – 0.40)2 = 0.0625. This gives n = 3.8579 / 0.0625 ≈ 61.7, which rounds to 62 participants per group. To account for potential dropouts or data exclusions, we increased this to 65 participants per group, yielding a final total sample size of 130 subjects (65 smokers and 65 non-smokers) [13]. Due to cultural norms, we could not find a large number of female smokers. Therefore, only adult males were included in the research. A total of 130 subjects were enrolled for this study using non-probability convenience sampling. Adult healthy males between 18 and 50 years of age who had smoked at least 100 cigarettes in their lifetime and were Grade 0 on the mMRC Dyspnea scale were included in Group 1 (smokers). Those who had never smoked in their life or less than 100 cigarettes and met other inclusion criteria were included in Group 2 (non-smokers). Children, subjects with diabetes, hypertension, or other comorbidities, and who had used medication during the past two weeks were excluded from the study. Smokers consuming tobacco in forms other than cigarettes, ex-smokers (adults who had smoked at least 100 cigarettes in their lifetime but quit at the time of the interview), and 'someday smokers' (adults who have smoked at least 100 cigarettes in their lifetime and smoke now but not every day) were also excluded. After informed consent, the subjects were interviewed about smoking habits (number of cigarettes smoked per day, duration of smoking, current smoking status) using a predesigned proforma. 5ml venous blood through a single venipuncture following strict aseptic measures was taken in EDTA and citrated vacutainers. Complete blood count was run on an EDTA sample on Sysmex XN-1000. The citrated samples of subjects having platelet counts between 200 x 109/l and 400 x 109/l were analyzed on Chrono-Log Model 591/592 Whole Blood Aggregometer using impedance aggregometry method. Agonists used were Adenosine diphosphate (ADP) (10 uM) and Arachidonic acid (0.5 mM).

Data were analyzed using SPSS version 27.0. Quantitative variables were represented as mean ± SD and qualitative variables as frequency and percentages. The association between smoking and platelet aggregability was calculated using the Chi-square test, and a p-value of less than 0.05 was considered significant. For regression analysis, to ensure stable estimates and avoid sparse-data bias, we

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collapsed categories with very small frequencies. Duration of smoking was re-grouped into three meaningful categories: 1–5 years, 6–10 years, and ≥11 years. Similarly, daily cigarette consumption was re-grouped as 5–10, 11–15, and ≥16 cigarettes. Multinomial logistic regression was then applied, adjusting for age and hematocrit, to identify independent predictors of enhanced platelet aggregation.

RESULTS

The study population had 130 subjects: 65 smokers and non-smokers, male patients. The mean age for the whole study group was 31.45 ± 7.87 years; the minimum was 18 years, and the maximum was 49 years. Median age was 31 years; inter-quartile range (IQR) 25.75 years – 37.25 years. In the smokers' group, the mean age was 29.89 ± 7.85 years; the minimum was 18 years, and the maximum was 48 years. Median age was 29 years; IQR 22 years – 37 years. In the non-smokers' group, the mean age was 33 ± 7.64 years; the minimum was 19 years, and the maximum was 49 years. Median age was 32 years; IQR 28 years – 38 years. The mean hematocrit for smokers and non-smoker groups was 44.15% ± 2.9% and 41.67% ± 2.3%, respectively. Duration of smoking was divided into four categories: 1-5 years, 6-10 years, 11-15 years, 16-20 years, and 21-25 years. The number of cigarettes smoked per day was categorized into four groups: 5-10 cigarettes, 11-15 cigarettes, 16-20 cigarettes, and 21-25 cigarettes. The largest percentage of the population (30%) smoked for 6 – 10 years, followed by 11–15 years (17%). The largest percentage fell in the category of 5–10 daily cigarettes (33%), followed by 11–15 daily cigarettes (17%). After category merging, the logistic regression model yielded more stable estimates. Duration of smoking ≥11 years remained a significant independent predictor of enhanced ADP-induced aggregation (OR = 15.24; 95% CI: 2.20–105.48; p = 0.006). For arachidonic acid, the odds ratio for duration ≥11 years was 22.60; however, the confidence interval remained wide (95% CI: 0.20–2515.25; p = 0.190), indicating insufficient precision, likely due to the limited number of events in this category even after merging. The number of cigarettes smoked daily was not an independent predictor for either agonist (p > 0.05). The frequencies and percentages are summarized in table 1.

Table 1: Frequency Distribution of Smoking Habits among the Smoker Group (n=65)

Duration of Smoking (Years)	n (%)	Number of Daily Cigarettes	n (%)
1-5	15 (23.1%)	5-10	33 (50.8%)
6-10	30 (46.1%)	11-15	17 (26.1%)
11-15	17 (26.2%)	16-20	10 (15.4%)
16-20	2 (3.1%)	21-25	5 (7.7%)
21-25	1 (1.5%)	–	–

With Arachidonic Acid, 12.3% of non-smokers had reduced, 84.6% normal, and 3.1% increased aggregation. In the 1-5 years sub-group, reduced, normal, and increased aggregation were seen in 1/15 (6.7%), 13/15 (86.6%), and 1/15 (6.7%) individuals, respectively. In the 6-10 years sub-group, reduced, normal, and increased aggregation was seen in none, 24/30 (80%), and 6/30 (20%) individuals, respectively. In the 11-15-year sub-group, reduced, normal, and increased aggregation was seen in none, 5/17 (29.4%), and 12/17 (70.6%) individuals, respectively. In the 16-20 years sub-group, reduced, normal, and increased aggregation was seen in none, 3/10 (30%), and 7/10 (70%) individuals, respectively. In the 21-25 years sub-group, reduced, normal, and increased aggregation was seen in none, 3/5 (60%), and 2/5 (40%) individuals, respectively. The results of platelet aggregation with Arachidonic Acid as affected by duration of smoking are shown in figure 1.

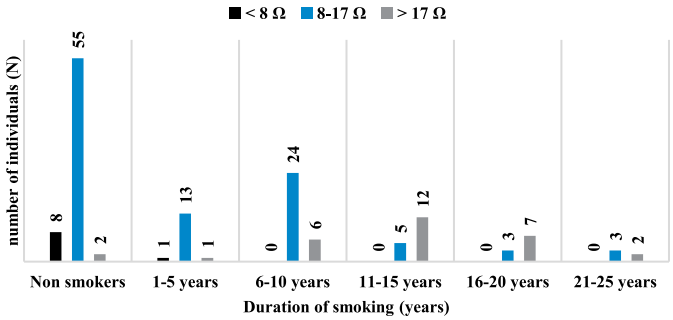


Figure 1: Effect of Duration of Smoking in Years on Platelet Aggregation with Arachidonic Acid

Analysis of platelet aggregation with Arachidonic Acid was done. In the daily 5-10 cigarettes group, reduced, normal, and increased aggregation were seen in 1/33 (3%), 28/33 (84.8%), and 4/33 (12.2%) individuals, respectively. In the daily 11-15 cigarettes group, normal and high aggregation were seen in 8/17 (47.1%) and 9/17 (52.9%) individuals, respectively. In the 16-20 daily cigarettes group, normal and increased aggregation were seen in 3/10 (30%) and 7/10 (70%) individuals, respectively. In the daily 21-25 cigarettes group, normal and increased aggregation were seen in 3/5 (60%) and 2/5 (40%) individuals, respectively. The results of platelet aggregation with Arachidonic Acid as affected by the number of cigarettes daily are shown in figure 2.

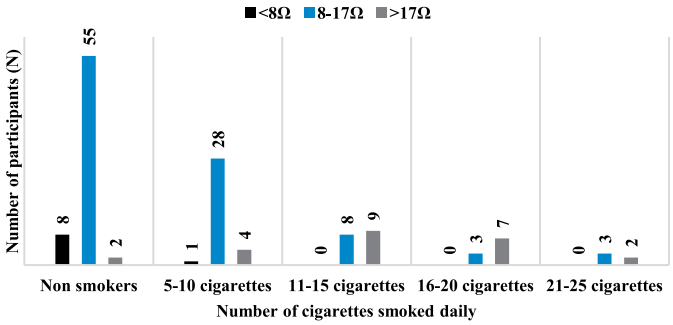


Figure 2: Effect of Number of Cigarettes Smoked Daily on Platelet Aggregation with Arachidonic Acid

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The distribution of population for aggregation with ADP was analyzed. 12.3% of non-smokers had aggregation between less than 9 ohms, 87.6% had between 9-14 ohms, and none had aggregation of more than 14 ohms. Among those who had a smoking duration of 1-5 years, less than 9 ohms, 9-14 ohms, and more than 14 ohms of aggregation were seen in 1/15 (6.7%), 13/15 (86.6%), and 1/15 (6.7%) individuals, respectively. Among those who had a smoking duration of 6-10 years, less than 9 ohms, 9-14 ohms, and more than 14 ohms aggregation was seen in 3/30 (10%), 16/30 (53.3%), and 11/30 (36.7%) individuals, respectively. Among those who had a smoking duration of 11-15 years, less than 9 ohms, 9-14 ohms, and more than 14 ohms aggregation was seen in none, 5/17 (29.4%), and 12/17 (70.6%) individuals, respectively. Among those who had a smoking duration of 16-20 years, no individuals showed less than 9 ohms, and 9-14 ohms of aggregation. More than 14 ohms of aggregation were seen in two (2/2) patients (100%). In the group with a duration of 21-25 years, there was only one individual. He showed an aggregation of more than 14 ohms. The results of platelet aggregation with ADP as affected by duration of smoking are shown in figure 3.

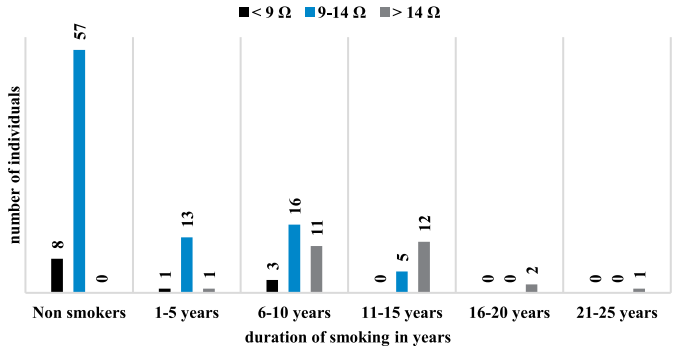


Figure 3: Effect of Duration of Smoking on Platelet Aggregation with ADP

The results for the number of cigarettes smoked daily and their effect on platelet aggregation were analyzed. In the 5-10 cigarettes daily sub-group, reduced, normal, and increased aggregation were seen in 2/33 (6.1%), 26/33 (78.8%), and 5/33 (15.1%) individuals, respectively. In the 11-15 cigarettes daily sub-group, reduced, normal, and increased aggregation was seen in 1/17 (5.9%), 3/17 (17.6%), and 13/17 (76.5%) individuals, respectively. In the 16-20 cigarettes daily sub-group, reduced, normal, and increased aggregation was seen in 1/10 (10%), 3/10 (30%), and 6/10 (60%) individuals, respectively. In the 21-25 cigarettes daily sub-group, reduced, normal, and increased aggregation was seen in none, 2/5 (40%), and 3/5 (60%) individuals, respectively. The results of platelet aggregation with ADP as affected by the number of cigarettes smoked daily are shown in figure 4.

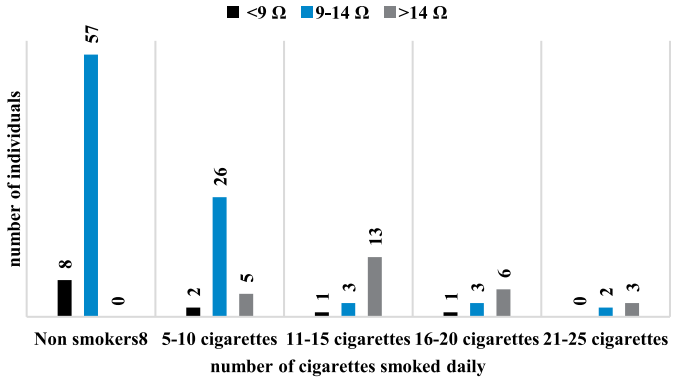


Figure 4: Effect of Number of Cigarettes Smoked Daily on Platelet Aggregation with ADP

For the ease of comparison and analysis, the study participants were divided into age groups. There was a total of 29 (22.3%), 52 (40%), 40 (30.8%), and 9 (6.9%) participants in the age groups of 18-24 years, 25-34 years, 35-44 years, and 45-54 years, respectively. Association of platelet aggregation with age, hematocrit, duration of smoking in years, and number of cigarettes smoked daily was sought through the Chi-square test. Duration of smoking in years (p-value for ADP <0.001, Arachidonic acid <0.001) and number of cigarettes smoked daily (p-value for ADP < 0.001, Arachidonic acid <0.001) were found to be associated with abnormal platelet aggregation. p-values are given in table 2.

Table 2: Statistical Significance of Variables (Chi-Square Test)

Variables	p-value	
	ADP	Arachidonic Acid
Age	0.320	0.270
Hematocrit	0.060	0.060
Duration of Smoking (Years)	<0.001	<0.001
Number of Cigarettes Smoked Daily	<0.001	<0.001

Next, Multinomial Regression Analysis was done to see which duration of smoking and number of cigarettes smoked daily independently predicted increased aggregation. Age and hematocrit were not found to be independent predictors. Duration of smoking was found to be an independent predictor of increased aggregation with ADP (p-value=0.006). It was not found to be an independent predictor of increased aggregation with Arachidonic Acid (p-value=0.190). The number of cigarettes was not found to be an independent predictor. Results of Multinomial Logistic Regression are shown in table 3.

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Table 3: Multinomial Logistic Regression Results Showing Comparison of Duration of Smoking and Number of Cigarettes Daily Using Arachidonic Acid and ADP

Variable	Aggregation in Ohms	p-value	Exp (B) (Odds Ratio)	Confidence Interval	
				Lower Bound	Upper Bound
Arachidonic Acid - Reference Category: < 8 ohms					
Duration of Smoking (years)	8 - 17	0.43	6.67	0.064	694.89
	>17	0.19	22.6	0.204	2515.25
Number of Cigarettes Daily	8 - 17	0.87	0.70	0.009	55.48
	>17	0.85	0.66	0.008	53.63
ADP - Reference Category: < 9 ohms					
Duration of Smoking (years)	9 - 14	0.410	1.92	0.406	9.088
	>14	0.006*	15.24	2.203	105.478
Number of Cigarettes Daily	9 - 14	0.300	0.47	0.111	1.983
	>14	0.540	0.63	0.146	2.735

DISCUSSION

The effect of smoking on platelet aggregation has been evaluated by many researchers. Increased incidence of ischemic heart disease due to hypercoagulability has been reported [14]. Ramotowski *et al.* demonstrated increased platelet aggregation in young smokers but no effect in older age [15]. The current results show a positive association between smoking (both duration and number of cigarettes smoked daily) and increased aggregation with Arachidonic Acid and ADP, as shown by the Chi-Square test. No association with age and hematocrit was found. The prothrombotic effect of smoking on platelet function has been cited in a recent systematic review as well [16]. Some studies also report temporary hyper-reactivity after cessation for 4 to eight weeks, which should be kept in mind. But this effect was seen with collagen [15, 17]. Positive correlation was found in another study, and early smoking cessation was recommended for reversal [18]. Duration of smoking was found to independently predict increased platelet aggregation with ADP after adjusting for confounders, as shown by Regression Analysis. A 15-fold increased risk was observed with increased years of smoking, and the results were statistically significant. (p-value=0.006). However, the confidence intervals were wide, and a higher sample size in future research in the abnormal aggregation group would be required for better reliability. The current study observed an increased risk of enhanced platelet aggregation with increased years of smoking, a 22-fold increased risk of high aggregation with Arachidonic Acid, but the result was not statistically significant (p-value=0.190), and the confidence intervals were wide. Therefore, duration of smoking was not found to independently predict increased platelet aggregation with Arachidonic Acid. The number of cigarettes smoked daily was not found to be an independent predictor of increased aggregation with ADP or Arachidonic Acid. The current study found that the percentage of individuals with increased platelet aggregation with ADP progressively increased across the groups with the increase in duration of smoking. In groups 16–20 years and 21–25 years, the current study had very few participants, but all of them showed increased aggregation. The current study did observe a rise in the percentage of individuals with increased platelet aggregation with Arachidonic Acid across the groups. Long duration of smoking was found to be associated with increased platelet aggregation with Arachidonic Acid, but was not found to be an independent predictor. Enhanced aggregation with ADP in smokers has also been shown by another research [19]. Some of the current results are also supported by some of the studies that found no association of smoking with platelet aggregation [20]. Summarizing the findings of the current research, both smoking duration and number of cigarettes smoked daily were associated with increased aggregation with Arachidonic Acid and ADP, as shown by the results of the Chi-Square test. When adjusted for age and hematocrit with Logistic Regression analysis, only smoking duration was found to independently predict increased platelet aggregation with ADP.

There are a few limitations of this study. The exclusion of female smokers due to cultural norms limits generalizability, and the use of convenience sampling introduces selection bias, which may affect the external validity of our findings. The confidence intervals were wide. The current study recommends studies on larger cohorts, with special attention to the groups who have smoked for more than 16 years, and smoke more than 20 cigarettes daily, for a better understanding of the topic, and preferential inclusion of female smokers in studies to demonstrate gender related effects. Also, this study was done in ex vivo, and its application in vivo should be done with caution.

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CONCLUSIONS

Cigarette smoking is associated with increased platelet aggregation, and longer duration of smoking is independently associated with high ADP-induced aggregation in whole blood impedance assays. This enhanced aggregability in vivo may contribute to the elevated risk of thrombotic ischemic events in chronic smokers. However, in vivo implications of these findings should be made with caution.

Authors' Contribution

Conceptualization: RS
Methodology: RS, FN, MA, YR
Formal analysis: MA, YR, NS
Writing and Drafting: RS, FN, MA
Review and Editing: RS, FN, MA, YR, NS

All authors approved the final manuscript and take responsibility for the integrity of the work

Conflicts of Interest

All the authors declare no conflict of interest.

Source of Funding

The authors received no financial support for the research, authorship, and/or publication of this article.

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