

Original Research Article

Effects of cigarette smoking on coagulation profile among smokers

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Abstract

Introduction: Cigarette smoking is one of the major causes of cancer and cardiovascular diseases leading to millions of premature deaths each year all over the world. Scientists have identified about 4,000 different substances in tobacco all of which have certain degree of toxic effects. At least 43 of them are known carcinogens. Cigarette smoking is an important and independent risk factor for atherosclerosis, coronary artery disease and peripheral vascular disorders. Apart from active smokers, passive-smokers are also prone for the development of smoking related disorders. Smoking adversely affects the concentration of the coagulation profiles which causes abnormalities in circulation.

Aim and objectives: This study aimed to investigate the effects of smoking on coagulation profile in chronic smoking population.

Materials and methods: This present study was a case-control study conducted among 50 smokers (subjects) and 50 non-smokers (controls) aged 20-50. Coagulation profile markers such as Prothrombin time (PT), activated partial thromboplastin time (APTT) and fibrinogen levels were estimated with standard methods.

Results: The results showed that the mean values of PT and APTT of smokers were significantly prolonged compared to non-smokers ($P < 0.05$), while platelet and fibrinogen levels were significantly lowered in smokers compared to non-smokers ($P < 0.05$). PT and APTT were also significantly prolonged with increasing duration of smoking as well as number of cigarettes per day, while there was a relative decrease in platelet and fibrinogen levels with increasing duration and number per day.

Conclusion: It was concluded that cigarette smoking alters PT, APTT, platelet and fibrinogen values, while age, duration of smoking and number of cigarettes per day were determinant factors to the extent of coagulation dysfunction in smokers.

Key words

Activated partial thromboplastin time, Cigarette smoke, Fibrinogen, Platelet count, Prothrombin time.

Introduction

Smoking is the most important public health problem. Many studies conducted have proved its deleterious effects on many organ systems mainly respiratory, reticulo-endothelial systems and cardiovascular system [1]. According to data reported from the World Health Organization there is about 2.4 billion people worldwide that have consumed tobacco in the forms of smoking, chewing, snuffing or dipping. WHO also estimates that tobacco-related deaths will amount to 8.3 million in 2030 and one billion deaths during the 21st century. Cigarette smoke contains a variety of other compounds including oxidants and free radicals that are capable of initiating or promoting oxidative damage leading to various degenerative pulmonary and cardiovascular diseases as well as cancer. Although the effect of smoking on haematological parameters has been studied previously the literature is limited and controversial [2]. The balance between clotting and dissolution of clots is a function of the extremely complex interactions involving all of the cellular components in the blood arterial wall interface, especially the endothelial cells and platelets [3]. Haemostatic dysfunction, however, arises from any alteration of this complex system, leading to pathologic thrombosis or vascular occlusion by thrombus fragments. Smoking has been shown to induce hypercoagulability and a hyper thrombotic state, possibly by increased platelet aggregation and adhesiveness as a result of its nicotine content. About 10¹⁵-10¹⁷ free radicals are estimated to be contained in cigarette smoke per inhalation, and these are capable of oxidizing the fat components of the body [4]. Predictors such as age, duration and the average number of cigarettes smoked per day are established factors for assessing the absolute risk of developing smoke-related complications in long-term smokers [5]. The prothrombin time (PT) measures the extrinsic pathway of coagulation while activated partial thromboplastin time

(APTT) is a performance indicator of the efficacy of both the “intrinsic” (now referred to as the contact activation pathway) and the common coagulation pathways. Platelets, also called thrombocytes, are blood cells whose function (along with the coagulation factors) is to stop bleeding. They have no nucleus and are fragments of cytoplasm which are derived from the megakaryocytes. There have been only a few studies addressing the effect of smoking on platelets. In addition, many of the studies have not compared the data with those of non-smoking control groups [6].

Materials and methods

The study was carried out in Rajamuthaiah medical college and hospital in year of 2004 from February to August. The subjects included are 50 male smokers and 50 non-smokers. Cigarette consumption was classified according to the criteria of Rastogi, et al. [13]: mild, 1–10 cigarettes/day; moderate, 11–20 cigarettes/day; heavy consumption, 120 cigarettes/day.

Inclusion criteria

Control group were males, age range from 20-50 years and apparently healthy individuals. Inclusion criteria for study group were regular smokers (cigarette) for at least 7 years, males, age range from 20-50 years otherwise apparently healthy individuals.

Exclusion criteria

Any individual have any disease on examination/investigation in any of control or study group was excluded from the study. And any individual who smoke both cigar and shisha were excluded from study group.

5 ml of whole blood was collected. 2.5 ml was dispensed into an ethylenediaminetetraacetic acid container for platelet count while 2.5 ml was added to 0.3 ml of trisodium citrate giving a blood to anticoagulant ratio of 9:1. The blood

was mixed and spun within 2 h and the platelet poor plasma separated for use in PT, APTT, and fibrinogen assay. Prothrombin time was determined by 200 µl of calcium-rabbit brain thromboplastin mixture was placed in a clotting tube within a water bath at 37°C and incubated for 2 min at that temperature. 200 µl of Kaolin platelet substitute mixture was placed in a clotting tube in a 37°C water bath and incubated for 2 min to attain temperature. 100 µl of test plasma (or control) was added, and the tube gently tilted at intervals for exactly 2 min. 100 µl of 0.025 ml calcium chloride (pre-incubated at 37°C) was then added, and a stopwatch started. The tube was tilted at regular intervals and the time for clot formation was recorded as the APTT. 50 µl of plasma and 450 µl of trompene imidazole buffer were incubated at 37°C for 2 min to maintain temperature. 200 µl of the mixture was reacted with 100 µl of bovine thrombin reagent, and the stopwatch was started and time for clot formation was recorded. Filtered ammonium oxalate (0.38 ml) was measured and dispensed into a small test tube. Then, 20 µl (0.02 ml) of well mixed anticoagulant venous blood was added and mixed. The grid area of the Neubauer counting chamber was wiped clean; the cover slip was slid carefully over the grid area and pressed down on each side until rainbow colours (Newton's rings) were seen. The diluted blood was mixed properly and using a capillary tube the grid was filled with the sample. The chamber was left undisturbed for 20 min in a petridish on damped blotting paper covered with the lid to prevent drying of the

fluid. The underside of the chamber was wiped dry and placed on the microscope stage. 10X objective was used to focus the rulings of the grid, and × 40 objectives were then used for counting platelets. The data obtained was analysed using Statistical Package for Social Sciences (SPSS) version 20 Data were expressed as the mean ± standard deviation. The significance of the difference in mean values between groups was analysed using student t-test, while the significance of the difference among groups was evaluated using one-way ANOVA. P < 0.05 was considered statistically significant [7, 8].

Results

The mean values of PT and APTT were significantly prolonged in smokers compared to non-smokers (P < 0.05); while platelet count and fibrinogen levels were significantly lower in smokers when compared to non-smokers (P < 0.05). There was a significant prolongation in the PT and APTT of subjects who have smoked for >10 years, 6-10 years and 1-5 years when compared to subjects who have smoked for <1 year (P < 0.05). A significant decrease in the fibrinogen level (P < 0.05) was observed for smokers who had smoked for 1-5 years and 6-10 years when compared to subjects who have smoked for <1 year. A significant decrease in platelet count for smokers who had smoked for >10 years was evident when compared to subjects who have smoked for <1 year and 1-5 years respectively (P < 0.05) as per **Table – 1**.

Table - 1: Comparison of coagulation parameters of smokers and non-smokers.

Parameters	Smokers (n=50)	Non-smokers (n=50)	P value
PT (in seconds)	23.81±3.19	18.67±9.1	0.001
APTT(in seconds)	45.62± 6.0	39.53±4.3	0.001
Platelet count (10 ⁹ /L)	198.71±92	208.34±74	0.010
Plasma fibrinogen (mg/dl)	242.89 ±28.30	271.69.03±93.01	0.010

Discussion

The WHO has estimated that there are about 1300 million smokers globally and about 75% of them are in the developing countries. It is also

predicted that if the current pattern of smoking continues, by 2020, there will be 10 million tobacco-related deaths annually worldwide and seven million (70%) of these deaths will occur in

the developing countries. Tobacco use is the single most significant cause of preventable death worldwide. The World Health Organization (WHO) estimates that tobacco caused 5.4 million deaths in 2004 and 100 million deaths over the course of the 20th century. For decades, epidemiological data have demonstrated the association of smoking with the incidence of coronary heart disease, myocardial infarction and stroke [9]. In many of the acute clinical events, thrombotic occlusion of the vessel occurs, a process that is often associated with platelets. One study demonstrated smoking-stimulated platelet aggregate formation in habitual smokers. Another effect of smoking found in different groups is an increase in serum fibrinogen level [10]. Recent evidence implicates platelet activation and the consequent increased expression of platelet CD62 as direct mediators of vascular inflammation and atherosclerosis. Experimental data suggests that platelet CD62 is an important link between thrombosis and vascular injury. Platelet CD62 is rapidly expressed on the surface of activated platelets and mediates adhesion of platelets to neutrophils and monocyte [12]. Studies have revealed that oxidative stress from cigarette smoke impairs the functions of the liver cell which impairs the production of some coagulation factors. This could have led to the prolongation of PT and APTT. This is corroborated by our finding that the levels of fibrinogen (factor 1) which is also a coagulation factor produced in the liver was significantly reduced in smokers. Another inflammatory marker (fibrinogen) was also reported to be elevated in smokers compared to non-smokers in several published studies [14]. Although fibrinogen levels were not measured in our subjects, the short thrombin time value (the time required for thrombin to convert fibrinogen to an insoluble fibrin clot) reported in our study is a strong indicator of elevated levels of fibrinogen in smokers. This test has been reported to be directly affected by abnormal/high levels of fibrinogen. The higher levels of fibrinogen in male smokers may promote cardiovascular disease by affecting blood viscosity, platelet aggregation and general fibrin

formation. This finding was not seen in female smokers in our study Platelet counts in this study were reduced in cigarette smoking whereas it increased in shisha smokers compared to non-smokers. However there were mixed response from other authors some reported that platelet counts significantly more in smokers whereas some authors reported that platelet counts significantly lowered in smokers than non-smokers [15].

Conclusion

Cigarette smokers are also mediated in part by enhanced platelet reactivity and activation. Therefore, investigations of the influence of smoking on platelets remain an important field in research of the pathogenesis of acute thrombotic occlusions in the smoking population. From this present research, there was a significantly higher PT value in subjects who smokes more than 6 sticks per day compared to subjects who smoke 1 stick per day. The APTT value for subject that smoke more than 2 sticks per day was also significantly increased compare to individuals who smoke only 1 stick per day

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