STUDY OF EFFECT OF CIGARETTE SMOKING ON PLATELET COUNT AND PLATELET AGGREGABILITY IN YOUNG MALE SMOKERS

Mrunal R. Shenwai, N.V. Aundhakar

ABSTRACT
Background & Objective: Cigarette smoking has become a major avoidable cause of diseases like stroke, ischemic heart disease and occlusive vascular disease. Smoking increases mortality nearly five times between age group of 30-40 years who are likely to be free from other myocardial risk factors. Hyperthrombic state in smokers may be attributed to enhanced platelet activity which may be reflected in terms of platelet count and platelet aggregability. Previous studies have reported many conflicting results on these parameters. The present work was undertaken to study the effect of cigarette smoking on platelet count and platelet aggregability in young & well educated male smokers and compare the results with non-smokers.

Methods: Total sample size was 70 which included a mixed population of male doctors & engineers [smokers (n=35) & non-smokers (n=35)] between the age group 26-40 yrs. They were selected on the basis of smoking of filtered cigarettes minimum 5 per day for duration ≤ 10 years. Platelet count was done on Sysmex K-4500 Autoanalyser. Platelet aggregability was estimated by O’Brien’s method using ADP.

Observations: Our results showed a statistically significant increase in the platelet aggregability (P< 0.01 & Z >2). The change in the platelet count was statistically insignificant.

Conclusion: As per our study increased platelet adhesiveness is evidenced even in young smokers with duration of smoking less than 10 years. Such young population which is otherwise free from the predisposing factors like obesity, hypertension, diabetes etc. can be encouraged to adopt healthier lifestyles and quit smoking so that future health related consequences can be avoided.

Key words: young smokers, Platelet count, Platelet aggregability

INTRODUCTION
Cigarette smoking which was an uncommon behaviour earlier in the 19th century has increased tremendously over a period of years, despite of the fact that it is positively associated with many diseases. Growing incidence of smoking in young population of both sexes is still a matter of serious concern for health professionals. Majority of people who succumb to the habit are well educated. Coronary heart disease is one of the important causes of mortality in human beings and cigarette smoking is considered as an independent risk factor for it. Components of smoke like nicotine, carbon monoxide and tar exert direct as well as indirect effects on many systems of the body especially cardiovascular system. Heavy smoking is the commonest cause of ischemic heart disease and death in 30-40 yrs of age group who are likely to be free from other myocardial risk factors. Hyperthrombic state in smokers may be due to increase in the platelet aggregability and increased platelet activity which initiates clot formation leading to occlusive vascular disease. Many previous studies on this have reported conflicting results. According to some researchers, platelet aggregability is not affected by smoking; rather sometimes it may even decrease. Similarly effect of smoking on platelet count is a matter of debate. Hence the present work is undertaken to study the effect of cigarette smoking on platelet count & platelet aggregability. The focus of the study was on young well educated male smokers with an aim of creating awareness amongst them regarding their health & social responsibility.

MATERIAL & METHODS
Selection of subjects: The present study was conducted between two groups i.e. smokers and non-smokers. All were apparently healthy male subjects between the age group of 26-40 yrs. Total
sample size was 70, out of which study group included smokers (n=35) and control group included non-smokers (n=35). The study group included male smokers who have been smoking filtered cigarettes minimum 5 (maximum 10) per day for duration < 10 yrs. Out of 35, 18 subjects were engineers belonging to a Pune based Software Company & 17 were doctors from BJMC & Sassoon General Hospital. The control group included 18 subjects (non-smokers) from the same software company & 17 doctors (non-smokers) from BJMC & Sassoon General Hospital. Doctors & engineers were chosen as subjects because they are considered as role models of the society. The socio-economic status, age, height, weight, daily activity and levels of stress were comparable between study group and the control group. Subjects suffering from coagulation disorders, diabetes, hypertension or any infection and those who are on any medication like aspirin or non-steroidal anti-inflammatory drugs (NSAIDs) were excluded from the study. All subjects were free from other habits like tobacco chewing and alcohol intake.

Study Protocol: The study was conducted at B.J. Medical College, Pune. Study protocol was approved by the ethical committee of the B.J. Medical College, Pune.

For Platelet Aggregability, O'Brien’s method of ADP (Adenine DiPhosphate) induced platelet aggregation was used. PRINCIPLE: Addition of ADP to platelet rich plasma (PRP) causes change in the absorbance. The change in the absorbance reading is expressed as platelet aggregation time. “Absorbance of platelet rich plasma is directly proportional to the concentration of platelets in it”. More the decrease in the absorbance more is the platelet aggregability. Platelet count was estimated by Sysemex K-4500 Autoanalyser. It is an 18-parameter, 3 part differential analyzer which works on the principle of Aperture Impedance.

Written informed consent was taken from all the subjects before the procedure. By a clean venepuncture of anticubital vein, 7ml of blood was collected into a plastic syringe with all aseptic precautions out of which 2ml was immediately transferred to an EDTA (anticoagulant) bulb and automated counting was done using sysmex autoanalyser. The remaining 5ml was collected in a plastic centrifuge test tube containing 0.5ml of 3.8% trisodium citrate as an anticoagulant. The sample was centrifuged at 1300 rpm for 15 min to obtain platelet rich plasma (PRP). Colorimeter was adjusted for the operative wavelength of 550nm in such a way that the absorbance for dark was infinity and distilled water was zero. It was kept constant for each test. 2ml of PRP was taken in a colorimeter tube and absorbance reading (optical density) was noted. Then 0.1ml of ADP solution was added to the sample and mixed by stirring. ADP induces platelet aggregation and platelet clumps are formed which settle down at the bottom of the test tubes. Since the aggregates tend to disperse after 20 seconds absorbance readings were noted at the end of 20 seconds. Change in the optical density is taken as a measure of platelet aggregability. ADP solution used (200g/ml) was prepared by dissolving weighed amounts of ADP powder (Sigma Pharmaceuticals) (200 mg/L) in glass distilled water.

Sample collection time was kept constant between 9.30- 11.30A.M. to avoid the effect of diurnal variation on platelet aggregation.

STATISTICAL ANALYSIS OF THE DATA

The results are presented as mean ± S.D. All the results were statistically analysed by applying ’Z’ test, as the sample size was more than 30. ’Z’ value i.e. relative deviate and ’P’ value were found out. ’Z’value of > 2 and ’P’ value of < 0.001 has been taken as statistically significant.

RESULTS

Our results showed that platelet aggregability increases significantly in smokers as compared to non-smokers (P< 0.01 & Z >2). [Table I]. No significant difference was observed in the platelet count of smokers and non-smokers. [Table II]
Mrunai R. Shenwai et al, Effect of Cigarette Smoking

<table>
<thead>
<tr>
<th>Parameter</th>
<th>n=35</th>
<th>Mean ± SD</th>
<th>Z value</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smokers</td>
<td>0.066±0.021</td>
<td>7.32 *</td>
<td>P&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>Non smokers</td>
<td>0.035±0.012</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

TABLE I : EFFECT OF SMOKING ON PLATELET AGGREGABILITY

<table>
<thead>
<tr>
<th>Parameter</th>
<th>n= 35</th>
<th>Smokers Mean± S.D.</th>
<th>Non-Smokers Mean± S.D.</th>
<th>Z value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelet count (cells/cu.mm.)</td>
<td>225085.7 ± 46660.1</td>
<td>222657.1 ± 47589.9</td>
<td>0.27</td>
<td>N.S.</td>
<td></td>
</tr>
</tbody>
</table>

N.S. = statistically not significant

DISCUSSION

Increased platelet aggregability in smokers as observed in the present study goes in accordance with the findings reported by Davis, Rival J et al, Chiang V.L. The increase in platelet aggregability can be explained on the basis of raised levels of several platelet aggregating agents and injury to the endothelium or due to the direct effect of smoking on platelets. The most important platelet aggregating agent elevated in smokers is epinephrine and also nor-epinephrine. This is due to the nicotine induced stimulation of adrenal medulla & sympathetic ganglia. Epinephrine binds with specific receptors on platelet membrane, stimulates prostaglandin synthesis from the phospholipids leading to formation of thromboxane A₂, reduces cAMP levels and increases intracellular Ca²⁺ concentration. Increased intracellular Ca²⁺ stimulates the release of ADP and other substances from platelet granules leading to further activation & aggregation of platelets. Thus thromboxane A₂ & Ca²⁺ act as positive feedback mechanisms in the promotion of platelet aggregation. Smoking also stimulates thromboxane A₂ synthesis by the endothelial cells which also contributes to increased platelet aggregation in smokers. Epinephrine also induces exposure of fibrinogen receptors (Glycophorin IIb – IIIa) on the platelets and favours binding of fibrinogen with these receptors. This helps in the formation of interplatelet bridges causing 10 or reversible platelet aggregation. Increase in the levels of long chain fatty acids & cortisol in smokers also contributes to increased fibrinogen synthesis. Nicotine in tobacco smoke also causes endothelial cell injury which exposes subendothelial collagen and activates platelets. This leads to decreased nitric oxide production in smokers which is a known vasodilator thus leading to vasoconstriction and activation of platelets. Increased platelet aggregability may be responsible for the hyperthrombotic state and increased risk of cardiovascular disease in smokers. The result obtained in case of platelet count were statistically not significant. Some researchers have reported that an increase in the epinephrine levels due to nicotine causes contraction of spleen and release of platelets in circulation leading to increased platelet count. But many other workers found that there is no significant change in the platelet counts in smokers and it is only the increased reactivity and adhesiveness of platelets which is responsible for the hyperthrombotic state. It appears that the formation of platelet and fibrin thrombi in the coronary arteries during an acute coronary event is promoted by the adhesion of platelets to endothelial cells, platelet aggregation and increased procoagulant activity of platelets. Some of the recent studies have observed that Smoking apparently promotes the procoagulant activity of platelets and enhances the prothrombotic state. The sharp exacerbation of the prothrombotic state following smoking is also assumed to be related closely pathophysiologically to the onset of the acute coronary syndrome. Our study corroborates with all these findings. The younger age group and limited number of subjects are some of the limitations of our study. In future we would like to extend our study in smokers of higher age group with duration of smoking more than 15 years.

CONCLUSION

As per our study smoking causes significant increase in the platelet aggregability. These changes are evidenced even in younger age group who are smoking for a period between 6-10 years. Increased
platelet adhesiveness contributes to the hyperthrombic state in smokers. Thrombi in coronaries and cerebral vasculature can lead to myocardial infarction and stroke respectively. Hence, smoking is considered as one of the major avoidable risk factors for cardiovascular diseases and death. This is especially important for the younger age group which is otherwise free from the predisposing factors like obesity, hypertension, diabetes etc. We would like to conclude with the fact that encouraging results have been observed in smokers who show a rapid return of many hematological abnormalities towards normal on abstention from smoking. Also, the risk of adverse effects starts to decline quite rapidly after cessation of smoking. This is of immense importance for smokers of younger age as in case of our study group, as they have a bright future provided they exercise their will to stop smoking.

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