Evaluation of haemorheological, biochemical and microcirculatory parameters in young smokers

Ranjeet S. Ajmani*†, R. R. Puniyani and Varjeet S. Khanuja****

*National Institute of Health, Gerontology Research Centre, NIA, LCMB, 4-B-12, 4940 Eastern Avenue, Baltimore, MD 21224, USA
**School of Biomedical Engineering, Indian Institute of Technology, Powai, Bombay 400 076, India

We studied 44 young smokers and 40 controls, all male, for haemorheological, biochemical, physiological and microcirculatory parameters. This study was conducted between January 1994 and December 1994. All the smokers have been smoking 13–18 cigarettes per day for the last three years. None of them were taking any medicine nor did they have any major health problem which could affect the results of our study. The results of our study clearly indicate that smokers have disturbed haemodynamic profile as compared to normal controls. This could result in worsening of blood flow condition, leading to development of various disorders.

THE fact that cigarette smoking affects flow behaviour of blood has been well-documented[1]. The hazard of smoking greatly depends on the way of puffing and inhalation, and also on the number of cigarettes smoked per day[2]. It is composed of more than 4000 substances which have diversified adverse effects on the body. It contains nicotine, tar, polynuclear aromatic hydrocarbons, phenol, cresol and *N*-nitrosonornide, benzol in particulate phase, while the gaseous phase contains carbon monoxide, hydrocyanide, ammonia, acetaldehyde, various oxides of nitrogen and acrolein[3]. Out of these, nicotine is most characteristic of tobacco. It is an alkaloid whose inhalation is known to trigger the secretion of vasopressin, a potent vasoconstrictor hormone, with a possible action on coronary arteries. It also stimulates sympathoneurnal and sympathoadrenal activity and influences systolic blood pressure, heart rate and platelet aggregation. A study conducted by Arnow *et al.*[4] found that smoking high nicotine cigarettes causes rise in blood pressure, heart rate, left ventricular end diastolic pressure, coronary sinus, and in arterial and venous carbon monoxide levels, simultaneously causing a decrease in coronary sinus and in arterial and venous oxygen partial pressure levels with a partial recovery within 30 min. Another component is carbon monoxide, which has an affinity 245 times greater than oxygen and, therefore, reduces oxygen supply to the heart and increases the chances of coronary heart disease. This risk is 20 times if the carboxyhaemoglobin (COHb) level is 5% or more[5]. In patients with underlying coronary heart disease, smoking enhances greatly the incidence of acute myocardial infarction and sudden death. Premature coronary artery disease and mortality arising from it increases progressively with the number of cigarettes smoked and the fatal and nonfatal coronary artery disease (CAD) is 60–70% higher in smokers than in non-smokers.

Due to the more viscous blood, there is a stage of stagnation and deficient oxygen supply to the tissues, leading to hypoxia[6]. This reduced oxygen supply leads to: 1. lowered redox state, 2. rapid glycogen utilization, 3. increased osmolality and swelling of cells, and 4. metabolic acidosis.

Metabolic acidosis has a very strong negative effect on the structure and functions of red blood cells. The slower the blood flow in the capillaries, greater is the internal viscosity of the red blood cells and greater the chances of breakdown of haemodynamic mechanisms at macro-and microcirculatory levels. This decreases further the oxygen supply to the tissues, leading to hypoxia and further decrease in pH. Hypoxia stimulates the production of red blood cells, i.e. increase in haematocrit. Haematocrit is one of the strong determinants of blood viscosity. Gagnon *et al.*[7] found that increase in haematocrit coupled with blood viscosity has serious effects or organ perfusion. Thus, a vicious circle sets in which leads to further deterioration of blood flow, making smokers more susceptible to development of vascular disorders.

Indian Institute of Technology (IIT) Bombay is a residential campus where graduate and postgraduate students stay in different hostels. The total student population is about 2500. For conducting this study, students from different hostels were contacted and briefed about the nature of the study. About 152 students expressed their willingness to participate in this study. Only 44 smokers who did not have any major health problems and at the same time did not have any other addiction like alcohol/tobacco chewing, etc., were selected for this study. None of them were taking any treatment, particularly aspirin, at least two weeks before the laboratory tests were performed. All of them were smoking 13–18 filtered cigarettes/day for a variable period of 2–3 years. Out of 152 students, 40 were selected as control group, as they had not smoked cigarettes so far and also were clinically normal subjects. For getting their details and thorough medical, family and personal histories, a questionnaire was used. On the day of blood collection, their blood pressure, pulse rate and weight was taken. Arm blood pressure was taken under supine condition after 10 min of rest, using sphygmomanometer and peripheral

†For correspondence

Table 1. Details of the subjects

Group	Number	Age	Weight	Pulse	(SBP/DBP mm Hg)
NC	40	23 06 ± 1 86	65.23 ± 8 22	77 45 ± 9.01	130 6 ± 8 33 84 7 ± 3 9
SM	44	22 95 ± 2 14	67.93 ± 10 76	88 56 ± 10.19	145 2 ± 5 19 87 3 ± 5 83

NC Normal controls;
SM. Smokers
SBP/DBP: Systolic blood pressure/Diastolic blood pressure;
Subjects from both categories were males.

pulse was palpated. To minimize variability, one subject from each group was examined each morning. Details of these subjects are given in Table 1.

Blood collection: About 10 ml of venous blood was drawn from antecubital vein using a plastic syringe with 21-gauge stainless steel needle with minimal suction. Out of 10 ml, about 6 ml was anticoagulated with dipotassium salt of ethylene diamine tetraacetic acid (EDTA) 1.8 mg/ml and about 4 ml with citrate (3.8%). Citrated blood was used for platelet aggregation study. The blood sample of the subjects was collected in the morning between 9.30 and 10 a.m. (to avoid any diurnal variations), after 8 h of fasting, in the Laboratory of Haemorheology and Microcirculatory Sciences, School of Biomedical Engineering, IIT, Bombay.

The sample was analysed within 2 h of collection as per recommendation of International Committee for Standardization in Haematology[8–10]. The following parameters were investigated:

Hemorheological parameters: Whole-blood viscosity, plasma viscosity, red-cell rigidity, red-cell aggregation and platelet aggregation.

Biochemical parameters: Haematocrit, erythrocyte sedimentation rate, total proteins, fibrinogen, albumin, cholesterol and triglycerides.

Physiological parameters: Blood pressure, pulse and weight.

Microcirculatory parameters: Transcutaneous oxygen tension and skin blood perfusion.

Whole-blood viscosity, plasma viscosity and red cell rigidity were measured by using Contraves LS-30, as performed by Ajmani *et al.*[11]. For the measurement of red cell aggregation, haematocrit, platelet aggregation, erythrocyte sedimentation rate, cholesterol triglycerides and plasma protein the method of Puniyani *et al.*[12] was used. Biochemical parameters like plasma albumin, total proteins, cholesterol and triglycerides were estimated by using M-10 autoanalyser. Fibrinogen estimation was done using the method of Ratnoff and Manzie[13], where the fibrinogen present in the plasma is converted to fibrin by the action of thrombin and subsequently boiled with sodium hydroxide. Its tyrosine content is estimated using the Foil–Ciocalteu reagent.

For simultaneous flow dynamics and oxygenation studies, the subjects were called in the evening as they had to attend the regular course classes in the morning. Moreover, since the study of these parameters takes at least 2 h for each subject, evening was the most convenient time to carry out this study. These studies were carried out in nonfasting state.

The Periflus PF 3 laser Doppler perfusion monitor (LDPM) was used for continuous monitoring of local microvascular blood flow as per Schubert *et al.*[14].

The transparent transcutaneous triple sensor/monitor was used for measurement of oxygen tension ($TcPO_2$) and, simultaneously, flux pattern (flow/velocity) by laser Doppler equipment. The TTC-45 triple sensor is compatible with Periflux PF-3. Oxygen tension was measured by AVL triple sensor/monitor TTC-45 at 44°C on three anatomical sites (right ankle, right wrist and right elbow). AVL triple sensor TTC-45 takes 10–15 min to stabilize at each site and hence the readings are taken exactly after 15 min, when the slope of the curve becomes constant.

The $TcPO_2$ measurement is basically a polarographic determination, by means of a modified Clark-type electrode, of molecular oxygen diffusing into the skin surface. Local hyperaemia is necessary to measure PO_2 on the skin surface, which has a close relationship with change in arterial PO_2. The hyperaemic condition is achieved by heating the silver/silver chloride anode to 45°C. The PO_2 signal results from the reduction current of the platinum cathode, which is proportional to the amount of oxygen diffusing into the skin surface.

The results for smokers were compared with normal controls as nearly as possible for age and sex. Student's *t* test was used for the analysis of the data. Multivariate correlation analysis was carried out by standard statistical techniques.

The results of this study are presented in Table 2 and 3.

1. There was an increase in the whole-blood viscosity at both high and low shear rates. But changes were more significant at high shear rates ($p < 0.001$ to 0.004).
2. Increase in plasma viscosity was very significant ($p < 0.001$).
3. Red cell rigidity parameter was most significantly ($p < 0.0001$) affected as compared to other parameters.
4. Fibrinogen had the highest statistical significance among the biochemical parameters ($p < 0.001$).

Table 2. Means and standard error of haemorheological and biochemical parameters

Parameters	A-group (40)	B-group (44)
Whole blood viscosity (cP)at shear rate (s^{-1})		
94.5	4.32 ± 0.79	5 63 ± 1.03#
51.2	4.62 ± 1.03	6.44 ± 1.52#
20.4	5.44 ± 1.76	7.93 ± 2.01#
8.11	7.05 ± 1.23	10.53 ± 1.93**
4.39	8.76 ± 2.32	13.37 ± 3.56##
3.32	10.11 ± 2.15	14.94 ± 3.42**
1.28	15.74 ± 3.45	23.36 ± 4.36**
0.51	28.05 ± 4.34	37.52 ± 5.76**
Plasma viscosity (cP) at 51.2/s	1.29 ± 0.04	1.59 ± 0.19**
Red cell rigidity (cP) at 94.5/s	3.53 ± 0.61	4 76 ± 0.63***
Red cell aggregation	12.34 ± 3.76	17.22 ± 3.21**
Haematocrit (%)	43.29 ± 3.14	55.11 ± 5.48###
Fibrinogen (mg%)	311.33 ± 20.87	387.6 ± 21.74**
Erythocyte sedimentation rate (mm/h)	40.55 ± 8.22	90.34 ± 15.43###
Triglycerides (mg%)	88.43 ± 11.13	125.65 ± 10.78$
Cholosterol (mg%)	159.33 ± 15.21	190.28 ± 17.90$
Albumin (g%)	3.66 ± 0.11	4.22 ± 0.47
Total proteins (g%))	7.94 ± 0.78	8.36 ± 0 61

***$p < 0.0001$, **$p < 0.001$, *$p < 0.01$, ##$p < 0.004$. #$p < 0.003$, ###$p < 0.005$, $$p < 0.05$.

A-group: Normal controls.
B-group: Smokers.

Table 3. Microcirculatory parameters

Parameters	Non-smokers	Smokers	*P*
$TcPO_2$(1)	75.65 ± 11.01	62.90 ± 9.01	< 0.05
$TcPO_2$(2)	84.57 ± 15.20	68.75 ± 11.28	< 0.05
$TcPO_2$(3)	76.36 ± 7.98	65 46 ± 11.44	< 0.05
BL.PR. (1)	163.07 ± 38.77	124.79 ± 54.78	n s.
BL. PR. (2)	257.26 ± 77.38	201.04 ± 73 32	n.s.
BL. PR. (3)	203.09 ± 62.02	191.67 ± 62.72	n.s.

$TcPO_2$: Transcutaneous oxygen tension;
BL.PR.: Blood perfusion;
n.s : non-significant.
(1) At ankle; (2) At wrist, (3) At elbow.

5. Red cell aggregation had a significance of $p < 0.001$.
6. Erythrocyte sedimentation rate and platelet aggregation were also elevated ($p < 0.005$).
7. Haematocrit, triglycerides and cholesterol levels were also raised, with significance values of $p < 0.005$ and $p < 0.05$, respectively.
8. Total proteins and albumin fraction were unaltered.
9. Pulse rate was higher than normal controls. In a few cases the pulse was irregular and blood pressure readings, particularly diastolic, were on the higher side of the normal.
10. Skin blood perfusion and transcutaneous oxygen tension at all the three anatomical sites were found to be low. Although skin perfusion was reduced, it was not statistically significant.

Belch *et al.*[15] have shown that smokers have elevated levels of fibrinogen ($p < 0.04$), lower plasminogen ($p < 0.02$) and higher plasma viscosity ($p < 0.003$). Just after smoking three cigarettes there was a substantial increase in platelet aggregation ($p < 0.02$), alpha2 M ($p < 0.02$) and factor VIII RAG ($p < 0.05$). A prospective study conducted by Galea and Davidson[16] and Rampling *et al.*[17] on smokers reported significant improvement in overall haemorheological and haematological variables after 2 weeks of discontinuation of smoking. Billimoria *et al.*[18] divided smokers into two subgroups based on the number of cigarettes being smoked per day (> 20 cigarettes/day: heavy smokers, and 5–15 cigarettes/day: light smokers). Heavy male smokers showed higher fasting serum turbidity, cholesterol, phospholipid, triglycerides, esterified fatty acid index of beta and pre-beta lipoprotein and reduction in clotting time compared to normal controls. In the case of the women's group, though there was increase in triglycerides, pre-beta esterified fatty acid index, longer fibrinolysis time and reduction in clotting time, no change was observed in cholesterol, beta esterified fatty acid index and fasting serum turbidity. In the case of male heavy smokers, haematocrit, haemoglobin and mean corpuscular

volume were elevated. The white cell count was very significantly raised; in particular, there was a rise in neutrophils and lymphocytes. But these changes were not observed in the women's group. In the case of light smokers, changes did not achieve statistical significance but the trend was similar. We found increase in the blood viscosity at all the shear rates considered, although changes were more significant at high shear rates than at low shear rates. This indicates prime importance of red cell rigidity among smokers. Parallel to this, rise is haematocrit also contributes to rise in blood viscosity. There was significant positive correlation (0.846) between blood viscosity and haematocrit, indicating its role in haemoconcentration. Rise in haematocrit could be a response to consistent hypoxic state among smokers. Changes at low shear rates were mainly due to increase in red cell aggregation. Increase in the red cell aggregation was mainly due to rise in fibrinogen level and plasma viscosity. Increase in plasma viscosity is attributed to rise in whole-blood viscosity as there was positive correlation between them at all the shear rates. In our study we found rise in platelet aggregation over the range 48–76%. The rise in erythrocyte sedimentation rate among smokers could be due to the presence of many charged particles released in the blood while one is smoking[19].

Among the biochemical parameters, change in triglycerides and cholesterol was significant. The increase in cholesterol has an adverse effect on red cell rheology as well as plasma viscosity. These changes were coupled with changes in microcirculatory parameters, reduction in peripheral skin blood perfusion and transcutaneous oxygen tension. Although it is premature to comment on whether these microcirculatory changes precede or follow the haemorheological changes, if we consider this in totality, it is apparent that smokers have disturbed microcirculatory flow. Reduced skin blood supply along with low oxygen partial pressure will hamper perfusion processes, resulting in accumulation of metabolites, leading to haemorheological changes. Norton and Rand[20] have reported a reduction in the surface to volume ratio in red blood cells among smokers. It is indicative of increased red cell rigidity. However, Baker *et al.*[21] found no change in the red cell deformability among smokers. Hawkins[22] in his study found that platelet aggregation can be induced at lower concentrations in smokers than in nonsmokers. The coagulation time of whole blood in smokers were significantly shorter at 0.001% level. The thromboelastograph also reflects similar results. No difference in platelet counts is indicative of qualitative changes in the functioning of platelet cells in this context. In smokers the increase in maximum tensile strength of a clot was observed by 5% compared to nonsmokers. This indicates that there is a greater risk of formation of haemostatic plugs. This could offer a probable explanation of why smokers have higher rate of cardiovascular morbidity and mortality.

The microcirculatory changes mediated through vasopressin were studied by Waeber *et al.*[24]. In their study on 20 male volunteers using laser Doppler flowmetry, reduction in blood flow was observed by induction of concomitant stimulation of vasopressin secretion.

In conclusion, blood viscosity and microcirculatory parameters should be included in routine medical check-up for early detection of silent clinical conditions associated with smoking.

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