International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2013): 6.14 | Impact Factor (2013): 4.438

Effects of Cigarette Smoking on Blood Rheology and Biochemistry

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Abstract: ***Biomarkers of blood rheology and biochemistry were determined in cigarette smokers from Jordan. The study aimed at estimating 33 venous blood parameters in 606 subjects, comprising of 302 smokers and 304 control non-smoker subjects. The parameters were measured using the standard techniques and conditions applied in the clinical laboratories. Compared to non-smokers, significantly (P≤0.05) higher values exist in smokers for the following parameters: hemoglobin, hematocrit, MCV, MCH, MCHC, WBC count, absolute granulocyte count, fibrinogen, iron, triglyceride and erythrocyte malonydialdehyde (MDA), and lower values for absolute lymphocyte count and erythrocyte reduced glutathione (GSH). There was no difference between smokers and non-smokers in regard to glycoslylated Hb, RBC count, red cell distribution width (RDW), ESR, absolute Monocyte count, platelet count, platelet volume (PV), plateletcrit (PCT), platelet distribution width (PDW), total protein, total cholesterol, LDL cholesterol, HDL cholesterol, glucose, urea, creatinine, Na, K, Cl, AST, ALT and γGT. These results indicate higher blood and plasma viscosity, reduced erythrocyte deformability (rheology), presence of biomarkers of thrombosis, inflammation, oxidative stress, hyperlipidemia, reduced immunity and compromised blood flow in macro- and micro-circulation in smokers. These results, however, should alert public opinion towards the disastrous consequences of chronic smoking.***

Keywords: Cigarette Smoking, Hemorheology, Erythrocyte Deformability, Hyperviscosity, Biomarkers, Cardiovascular Disease

1. Introduction

The number of chemical compounds to which a smoker is exposed when smoking a cigarette has been estimated as 4800, including tars, nicotine, carbon monoxide, polycyclic aromatic hydrocarbons and others [Green CR & Rodgman A. 1996]. The rate and amount of exposure to these chemicals are complex functions of cigarette composition and design, rate of smoking, burn temperature and many other factors [Baker, RR. 1999]. Two phases of cigarette smoke exist: a tar or particulate phase and a gaseous phase, both of which contain extremely high concentrations of free radicals. In addition, cigarette smoke activates endogenous sources of free radicals as well [Pryor, WA & Stone, K, 1993].

Cigarette smoking is one of the major risk factors for cardiovascular disease [Ambrose, JA & Barua, RS. 2004, Bazzano, LA. et.al. 2003, Stavroula T. et.al.. 2003, Bottcher M & Falk E. 1999]. The mechanism responsible for this association is still unknown. Potential mechanisms include relative hypoxemia, inflammation, oxidative stress, endothelial dysfunction, lipid abnormalities, hemodynamic stress, coronary vasoconstriction, enhanced arrhythmogenesis, hyper-homocystinemia and insulin resistance, whereas thrombosis may play a greater role in the risk of acute cardiac events in smokers [Stricker H, et.al. 2006, Barua RS, etal. 2002, O'Callaghan P. et.al. 2002, Hioki, H. et.al. 2001, Miller, GJ, et.al. 1998, Cullen, P. et.al. 1998, Richards, GA. & van Antwerpen, VL. 1996, Van Antwerpen L. et.al. 1993]. Blood biomarkers have been used to assess the above mentioned potential mechanisms in smokers [Zedler, BK.et.al. 2006, Hatsukami, D.K., et.al. 2003, Szmitko, PEB, et.al. 2003, Eliasson B. et.al. 2001, Benowitz, NL. 1999]. Examples of such biomarkers for inflammation included fibrinogen levels, WBC count, and C-reactive protein [Kawada T. 2004, Szmitko PEB, et.al. 2003, Pradhan AD. et.al. 2002, Danesh J, et.al. 1998, Sunyer J. et al. 1996, de Maat MP, et.al. 1996], for thrombosis included enhanced platelet aggregation, decreased plasminogen activator release, increased levels of plasminogen activator inhibitor, and increased blood viscosity [Fusegawa Y, et.al. 1999], for oxidative stress included increased levels of oxidized LDL-cholesterol, oxidized proteins such as fibrinogen, F2 isoprostanes, and lipid-peroxidation products such as MDA and decreased levels of reduced glutathione and vitamine C [Charalabopoulos K. et.al. 2005, Richards, GA. & van Antwerpen, VL. 1996], and for endothelial cell function measurement of post-ischemic vasodilation, P-selectin, and ICAM. These potential mechanisms and other studies [Rampling, MW. 1999, Ernst E. 1995, Ernst E. et.al. 1988] suggest a role for blood rheology to play in the development of these vascular events, particularly in hypoxemia and thrombosis, which could be a consequence of reduced blood rheology. The present study therefore aimed to evaluate the effects of cigarette smoking on blood rheology and biochemistry of regular smokers from Jordan. Thirty three (33) laboratory tests as shown in table 1 were evaluated using venous blood taken from 302 smokers and 304 non-smokers.

2. Subjects and Methods

Six hundred and six (606) volunteers (342 males, 264 females) with a mean age of 32 years (range of 18 to 68 years) were studied, comprising of 302 smokers with duration of smoking from 5 to >20 years and number of cigarettes smoked per day ranging from 10 to >40 cigs, and 304 non-smokers, The volunteers were asked to fill out a questionnaire regarding their age, sex, weight, occupation, number of cigarettes smoked per day and duration of smoking after they signed an informed consent according to the Ethics Committee requirements of the university of Jordan. 10 ml of venous blood were withdrawn from each volunteer by 10 ml syringe, 5 ml of which was collected in a

plane tube in order to get serum for biochemical analysis, i.e. kidney and liver function tests, lipid profile tests, glucose and iron, 2.5 ml was collected in EDTA tubes to carry out the hematological tests, glycosylated hemoglobin and erythrocyte MDA and GSH, and 2.5 ml was collected in citrate tubes for estimation of plasma fibrinogen. Hematological tests were performed using fully automated hematology analyzer (the Roche diagnostic system, ABX hematology COBAS MICROSOT, open tube) that measures the following 15 parameters (RBC count, hemoglobin, hematocrit, MCV, MCH, MCHC, red cell distribution width (RDW), platelet count, mean platelet volume (PV), plateletcrit (PCT), platelet distribution width (PDW), WBC count, absolute granulocyte count, absolute lymphocyte count, absolute monocyte count). Glycosylated hemoglobin was measured by affinity chromatography using agarose column containing covalently bound aminophenylboronic acid (Trithdroxyaminophylborane). Erythrocyte MDA and GSH were measured as described before [Srour, MA et.al. 2000]. The other laboratory tests were performed according to the standard techniques used by the clinical laboratories.

2.1 Statistical Analysis

The statistical analyses were performed using the Statistical Package for Social Sciences (SPSS Inc., Chicago). For each parameter, the difference between the smoker and non-smoker was calculated. These differences were then compared using analysis of variance to test for statistically significant differences. These analyses were performed univariately and multivariately adjusting for age, sex and weight. In all cases, 2-tailed $P \leq 0.05$ was considered statistically significant.

3. Results and Discussion

Mean values of blood parameters of the studied smokers and non-smokers and the P values of the difference calculated after controlling for age, sex and weight are shown in table 1. Compared with non-smokers, smokers have significantly higher levels of hemoglobin, hematocrit, MCV, MCH, MCHC, WBC count, absolute granulocyte count, fibrinogen, iron, triglyceride and erythrocyte malonydialdehyde (MDA), and lower values for absolute lymphocyte count and erythrocyte reduced glutathione (GSH) (Table 1). Comparison of parameter values within the study group in terms of smoking and age showed significant differences with smoking and age in regard of MCV, MCH, absolute granulocyte count, fibrinogen and triglyceride. Comparison of parameter values within the study group in terms of smoking and sex showed significant differences with smoking and sex in regard of hemoglobin, hematocrit, MCV, MCH, MCHC, fibrinogen and iron. Comparison of parameter values within the study group in terms of smoking and weight showed significant differences with smoking and weight in regard of hemoglobin, hematocrit, WBC count, absolute granulocyte count and absolute lymphocyte count and triglyceride. However, there were no significant diffrences between smokers and non-smokers regarding the other tested blood parameters (Table 1).

Blood rheology is the study of flow properties of the cellular and plasma components of the blood. Blood rheology is influenced by several factors. These include: plasma viscosity, whole blood viscosity and the deformability of the erythrocytes [Bilto, YY. 1999]. Plasma viscosity is primarily a function of its protein concentration mainly fibrinogen and globulin fractions [International Committee for Standardization in Haematology 1986]. Because the presence of particles in a liquid raises its viscosity, the presence of cells in the blood raises its viscosity compared with that of the plasma. Therefore, the higher the hematocrit, RBC count or WBC count, the greater the whole blood viscosity [International Committee for Standardization in Haematology 1986]. Erythrocyte deformability (rheology) is the ability of the erythrocyte to change shape when passing through microcirculation or subjected to shear stress in macrocirculation. Normal erythrocyte deformability (rheology) is therefore important for blood flow in macro- and micro-circulation. The high deformability of the normal human erythrocyte is a consequence of its low cytoplasmic viscosity, its high ratio of membrane surface area to cell volume and its viscoelastic cell membrane [Bilto, YY. 1999]. Cytoplasmic viscosity depends mainly on the mean cell hemoglobin concentration (MCHC). The viscosity of hemoglobin solutions such as inside red cells increases exponentially above 32 gHb/dl, and hence erythrocyte deformability (rheology) decreases with increasing MCHC [Bilto Y.Y. et. al. 1987]. The unique geometry (biconcave disc shape) of normal erythrocytes and the normal excess ratio of surface area to volume (about 140%), allows the erythrocyte to undergo marked deformation. However, if the erythrocyte assumes a spherical shape as a result of reduction in surface area (due to membrane loss while maintaining its volume) or if there is an increase in cell volume (due to increase in MCV), then the cell deformability will be reduced. The membrane vicoelasticity is thought to depend on the lipid composition of the membrane, especially the cholesterol: phospholipid ratio [Bilto, YY. 1993]. Therefore abnormal blood lipid profile would have deleterious consequences on erythrocyte deformabilty (rheology). The importance of studying the effects of smoking on blood rheology comes from the fact that blood rheology plays an important role in the evolution and acceleration of occlusive arterial diseases. It is therefore reasonable to question whether the rheological properties of blood might be affected by cigarette smoking, as cigarette smoking is accepted as one of the major risk factors for ischemic heart disease. The present study screened the biomarkers of blood rheology and biochemistry that could be affected by smoking.

As shown in table 1, the present study showed that smokers have significantly higher levels of fibrinogen and triglyceride compared to non-smokers indicating higher plasma viscosity in smokers. However, age and sex were also other variables associated significantly with fibrinogen (Table 1), indicating that these two variables may modify the association between smoking and fibrinogen and that aging and being a man could magnify the adverse association with smoking. In fact, other studies [Bazzano, et al. 2003, Bermudez, EA et.al. 2002, Tuut M & Hense HW. 2001, Eliasson et al. 2001, Nascetti S. et.al. 2001, Meade et al. 1987] have found similar adverse association between smoking, sex, age and fibrinogen.

The present study also showed that smokers have significantly higher values for hemoglobin, hematocrit, WBC count and absolute granulocyte count (table 1), all of which contribute to whole blood viscosity, indicating higher blood viscosity in smokers, this is another marker for adverse blood rheology in smokers. However, sex and weight were also other variables associated significantly with these biomarkers (Table 1), indicating that the sex and weight variables could contribute to the adverse association between smoking and these biomarkers of blood rheology. Similar results were also obtained by others [Gregory A et.al. 2005, Nakanishi N et.al. 2003, Smith MR. et.al. 2003, Van Tiel E. et.al. 2002, Blann AD et.al. 1998, Opdenakker G et.al. 1998, Celada MM. et.al. 1997, Sunyer J. et.al. 1996, Freedman DS et.al. 1996, Schwartz J & Weiss ST. 1991, Schwartz J & Weiss, ST. 1994, Petitti DB & Kipp H. 1986, Hughes DA et.al. 1985, Maurel A et al 1997].

The present study also showed that smokers have significantly higher values for erythrocyte indices such as MCV, MCH and MCHC (Table 1). Higher erythrocyte indices are known to reduce erythrocyte deformabilty (rheology) by increasing cytoplasmic viscosity and decreasing the ratio of surface area to volume of the erythrocyte. An incease in MCV and MCHC puts also a burden on the erythrocyte in microcirculation, where erythrocytes with a diameter of 7-8 µm should pass through capillaries with a diameter of 2-3 µrn [Bilto Y.Y. et. al. 1987]. To our knowledge, there were no published reports measuring these indices in smokers, but reduced erythrocyte deformability in smokers or acute poisoning with CO was reported by others [Maurel A et al 1997, Ozturk B et al . 2014, Salbaş, K. 1994].

The increase in hemoglobin, hematocrit, MCV, MCH and MCHC could be due to the inhaled carbon monoxide gas (CO), which is one of the inhaled components of cigarette smoke. CO present in cigarette smoke in more than 600 times the concentration considered safe in industrial plants. A smoker's blood typically contains 4 to 15 times as much CO as that of a nonsmoker. CO combines reversibly with oxygen-carrying sites on the hemoglobin molecule with an affinity ranging from 210 to 240 times greater than that of oxygen, which results in decreased oxygen-carrying capacity of the blood, this decrease is compensated by an increase in hemoglobin and hematocrit (i.e. red cell mass). CO also alters the dissociation of oxygen from hemoglobin sites, which compromises the delivery of oxygen to the tissues [Varon et al. 1999]. However, chronic carbon monoxide exposure in smokers could lead to polycythemia (i.e. hyperviscosity syndrome), which also contributes to hypercoagulability and thrombosis as suggested by Varon et.al. (1999).

The present study showed an elevated levels of inflammatory markers such as the acute phase plasma protein fibrinogen, WBC count and absolute granulocyte count, which provide further evidence for smoking-induced inflammation and oxidative injury that leads to endothelial dysfunction, which accords with the postulated inflammatory and oxidative injury mechanisms that are blamed for the initiation and propagation of the atherosclerotic process in cardiovascular disease [Kawada T. 2004, Frohlich M. et.al. 2003, De Maat MP et.al. 1996]. Cigarette smoke is known as a potential source of oxidative stress, with each puff containing 1014 free radicals [Baker, RR. 1999]. More over smoking increases vascular production of free radicals, such as superoxide, which react with nitric oxide to decrease its availability, thereby impairing endothelium-dependent vasodilation and promoting other processes that accelerate atherosclerosis [Barua RS et.al. 2002, Powell JT. 1998, Pryor WA & Stone K. 1993]. Hence, the present study showed an increased erythrocyte MDA and decreased GSH in smokers compared to non-smokers (table 1), indicating an increased susceptibility of smokers to oxidative stress, which coincides with others [Charalabopoulos K et.al. 2005, Srour M.A. et. Al. 2000, Gokulakrishnan, A. et.al. 2010].

In consistence with other studies [Schuitemaker GE et.al. 2002, Eliasson et al. 2001, Brischetto CS et.al. 1983, Maurel A et al 1997], the present study also showed that smokers have significantly higher levels of triglyceride (Table 1), which is known as one of the traditional risk factors.

Unexpectedly, the present study showed that smokers have significantly ($p = 0.05$) elevated level of serum iron, this result is not reported by others and it can be explained by the reported increase in the release of tissue-damaging matrix metalloproteinases [Seagrave J et.al. 2004] that was found in smokers.

The present study did not find significant differences between smokers and non-smokers in regard to glycoslylated Hb, RBC count, red cell distribution width (RDW), ESR, absolute Monocyte count, platelet count, platelet volume (PV), plateletcrit (PCT), platelet distribution width (PDW), total protein, total cholesterol, LDL cholesterol, HDL cholesterol, glucose, urea, creatinine, Na, K, Cl, AST, ALT and γGT, similar results were reported by others for many of these parameters [Maurel A, et al. 1997].

4. Conclusions

1) the present study showed that smokers have higher levels of fibrinogen and triglyceride compared to non-smokers, indicating higher plasma viscosity, as a marker for adverse blood rheology and thrombosis in smokers.
2) The present study showed that smokers have higher values for hemoglobin, hematocrit, WBC count and absolute granulocyte count, all of which contribute to whole blood viscosity, indicating higher blood viscosity in smokers, this is another marker for adverse blood rheology and thrombosis in smokers.
3) The present study showed that smokers have higher values for erythrocyte indices such as MCV, MCH and MCHC, that are known to reduce erythrocyte deformabilty (rheology), which is important for macro- and micro-circulation.
4) The present study showed that smokers have elevated levels of inflammatory markers such as the acute phase plasma protein fibrinogen, WBC count and absolute granulocyte count, and oxidative stress markers such as increased erythrocyte MDA and decreased GSH, which provide evidence for smoking-induced inflammation and oxidative injury that leads to endothelial dysfunction,

5) The present study showed that smokers have lower values for absolute lymphocyte count, indicating reduced immunity.

5. Acknowledgement

This study was financed by the deanship of scientific research, The University of Jordan.

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Table 1: Mean values of blood parameters of the studied smokers and non-smokers.
P values of the difference calculated after controlling for age, sex and weight (n = 606). *P ≤ 0.05

Blood parameter & unit	Mean value			P value			
	Non smokers (n=304)	Smokers (n=302)	Difference	Smoking	Age	sex	Weight
Hemoglobin (Hb) (g/dL)	14.21	15.01*	+ 0.80	0.00	0.17	0.00	0.00
Glycosylated Hb (%)	4.52	4.62	+ 0.10	0.52	0.10	0.12	0.68
Hematocrit (%)	39.68	41.29*	+ 1.61	0.00	0.11	0.00	0.00
RBC count (x 10^{12}/L)	4.62	4.65	+ 0.03	0.13	0.04	0.00	0.00
MCV (fl)	85.93	88.87*	+ 2.94	0.00	0.00	0.00	0.74
MCH (pg)	30.76	32.24*	+ 1.48	0.00	0.00	0.00	0.53
MCHC (g/dL)	35.70	36.32*	+ 0.62	0.00	0.71	0.00	0.83
RDW (%)	13.39	13.34	- 0.05	0.58	0.23	0.00	0.17
ESR (mm/hr)	13.76	12.60	- 1.16	0.52	0.00	0.00	0.14
WBC count (x10^9/L)	7.19	7.73*	+ 0.54	0.00	0.19	0.51	0.00
Absolute granulocyte count (x10^9/L)	4.59	4.87*	+ 0.28	0.01	0.05	0.45	0.05
Absolute lymphocyte count (x10^9/L)	2.23	2.05*	- 0.18	0.00	0.57	0.14	0.00
Absolute monocyte count (x10^9/L)	0.54	0.58	+ 0.04	0.07	0.00	0.00*	0.02
Platelet count (x10^9/L)	245.74	242.74	- 3.00	0.78	0.06	0.00	0.14
Platelet volume (PV) (fl)	8.98	9.07	0.09	0.44	0.29	0.01	0.75
PCT (plateletcrit) (%)	0.22	0.22	0.00	0.77	0.13	0.00	0.10
PDW (%)	13.92	13.98	0.06	0.65	0.99	0.46	0.66
Fibrinogen (mg/dL)	279	296*	+ 17.0	0.00	0.00	0.00	0.46
Total Protien (g/L)	12.33	12.31	- 0.02	0.92	0.10	0.85	0.16
Iron (µg/dL)	160	174*	+ 14.0	0.05	0.89	0.00	0.06
Triglyceride (mmol/L)	1.48	1.73*	+ 0.25	0.00	0.00	0.54	0.00
Total Cholesterol (mmol/L)	4.85	4.74	- 0.11	0.44	0.00	0.07	0.00
HDL-Cholesterol (mmol/L)	1.15	1.17	+ 0.02	0.25	0.53	0.66	0.98
LDL-Cholesterol (mmol/L)	2.93	2.77	- 0.16	0.09	0.00	0.23	0.01
Glucose (mmol/L)	4.43	4.50	+ 0.07	0.24	0.00	0.88	0.54
Urea (mmol/L)	4.67	4.51	- 0.16	0.06	0.01	0.00	0.18
Creatinine (µmol/L)	90.63	86.47	- 4.16	0.32	0.42	0.41	0.92
Sodium (mmol/L)	142.88	143.67	+ 0.79	0.06	0.75	0.60	0.39
Potassium (mmol/L)	4.19	4.20	+ 0.01	0.95	0.16	0.71	0.03
Chloride (mmol/L)	101.69	101.49	- 0.20	0.44	0.87	0.97	0.85
ALT (U/L)	25.02	24.40	- 0.62	0.19	0.33	0.00	0.00
AST (U/L)	34.97	34.33	- 0.64	0.30	0.50	0.00	0.00
γ GT (U/L)	14.47	16.21	+ 1.74	0.55	0.02	0.00	0.23
Erythrocyte MDA (nmol/g Hb)	361.51 (n=25)	372.64* (n=25)	+ 11.13	0.05	-	-	-
Erythrocyte GSH (mg/dL RBCs)	80.93 (n=25)	72.42* (n=25)	- 12.51	0.01	-	-	-