ORIGINAL ARTICLE

Cigarette Smoking, a Risk Factor for Chronic Subclinical Inflammation and a Predictor of Metabolic Syndrome in Adult Healthy Population of Bangladesh

Rehnuma B[1], Eva SN[2], Ibrahim M[3], Nasir TA[4], Ali L[5]

Abstract

Background and Aims: Smoking is a classical and major risk factor for cardiovascular disease. Inflammatory activators and metabolic disorders are the mediators of smoking-induced atherosclerotic progression. The aim of the present study was to investigate whether smoking alter inflammatory or metabolic status and affect subclinical atherosclerosis in apparently healthy persons. **Methods:** A total number of 149 adults, age 30-60 yrs, were recruited in the study. Participants were divided into sub-groups of smokers (76) and non-smokers (73). All participants were interviewed and underwent physical examinations and blood collection. High-sensitivity C reactive protein (Hs-CRP) was measured to assess degree of underlying inflammation. Fasting plasma glucose and lipid profile were measured to assess metabolic condition. Data were analyzed using statistical Package for Social Program (SPSS) for Windows version 17. **Results:** Hs-CRP (p=0.017), Fasting glucose (p=0.003), Triglyceride (p=0.005) was significantly high in smokers in comparison with nonsmokers. BMI (p=0.012) and BFM (%) (p= <0.001) showed significantly lower in comparison with the counterpart. HDL-c (p=.030) was also significantly lower in smoker group than non-smoker group. In Spearman's correlation analyses Triglyceride (p=0.037) and smoking (p= 0.042) showed positive correlation with Hs-CRP. HDL-c is less in smoker subjects but not statistically up to the significant level. **Conclusion:** The rising Hs-CRP concentration reflects presence of chronic subclinical inflammation in middle aged Bangladeshi smokers and thus may have a risk for future cardiovascular disease.

Key words

High sensitive C-reactive protein (Hs-CRP), smoking, subclinical inflammation

Introduction

The link between smoking and increased morbidity and mortality have been long established, and current trends indicate that there are one billion smokers worldwide and 500 million will die prematurely from smoking-related diseases.[1] Smoking has been shown to have harmful effects on numerous organs of the body and the list of diseases where smoking has been recognized as contributory factor is extensive.[2] It has long been accepted that cigarette smoking is a classical and major risk factor in the development of cardiovascular disease (CVD) and atherosclerosis.[3,4] More recently, it has been recognized that CVD contains a component of inflammation and has even been referred to as an inflammatory disease.[5,6] In addition, a link has been

1. Registrar, Clinical Biochemistry, Lab Medicine Dept. Apollo Hospitals Dhaka, 2. Specialist, Clinical Biochemistry, Pathology Laboratory, United Hospital Ltd, Dhaka, 3. Consultant, Clinical Biochemistry, Lab Medicine Dept. Apollo Hospitals Dhaka, 4. Sr. Consultant and Coordinator, Dept. of Lab Medicine, Apollo Hospitals Dhaka, 5. Professor and Coordinator, Dept. of Biochemistry and Cell Biology, BIRDEM, Bangladesh.

established between several other chronic inflammatory diseases and smoking, including chronic obstructive pulmonary disease (COPD)[7], rheumatoid arthritis, systemic lupus erythematosus[8] and Crohn's disease.[9] Although the mechanisms linking smoking to these diseases are not well understood, interest in finding relationship between inflammatory markers and smoking has been gathering pace to provide explanations for smoking-mediated morbidity and mortality. One such inflammatory marker, is C-reactive protein (CRP), which may be easily and sensitively measured in a variety of clinical situations to monitor disease progression.[10]

C-reactive protein (CRP) is an inflammatory marker whose expression is markedly up regulated during inflammation.[11] It is the acute phase protein synthesized in the liver and regulated to a large extent by pro inflammatory cytokine interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α).[12,13] Furthermore, new, highly sensitive assays for CRP (Hs-CRP assays) measure levels within the "normal" range i.e., low grade inflammation or subclinical inflammation thus enabling careful evaluation of underlying systemic inflammation in apparently healthy people as well as those with established cardiac and metabolic diseases. Moreover, Hs-CRP assay is the inflammatory marker with improved precision, standardization and other characteristics. Recent prospective studies have demonstrated that subjects with low grade inflammation have a higher risk of cardiovascular diseases.[14,15,16] Evidence suggests that elevation of CRP reflect not only local inflammation at atherosclerotic lesions but also systemic abnormalities related to insulin resistance, such as increase in fasting insulin, body mass index (BMI), systolic blood pressure and triglyceride (TG) as well as decrease in high density lipoprotein cholesterol (HDL-c).[17,18,19] Unhealthy lifestyle might increase the risk of CVDs.[20,21,22] These habits include physical inactivity, calorie dense diets, alcohol drinking, smoking and psychosocial stress. Previous studies have demonstrated the adverse effect of smoking on CVDs, e.g. tobacco use increases triglycerides (TG) and decreases HDL-c levels. It has also been shown that total cholesterol (TC), low density lipoprotein cholesterol (LDL-c), and Total Cholesterol/HDL-c ratio are strongly related to smoking.[23] There are significant improvements in LDL-c, HDL-c and the HDL-c to LDL-c ratio 8 weeks after cutting down on smoking.[23]

Globally, tobacco use accounts for about 10% of all CVD related mortality, with the highest occurrences being in low to mid-income countries.[24] Yet awareness about the cardiovascular implications of cigarette smoking is still unacceptably low considering that smoking is a completely preventable cause of CVD. Previous studies have demonstrated the adverse effect of smoking on serum lipid profile.[20,21,22,23] We set out to examine lifestyle factors such as smoking, and levels of CRP, Focusing on the use of CRP measurement to predict long-term outcome in smokers.

Subjects and Methods

An invitation was made to the volunteers of the study through personal contact to report in the department of Biochemistry and Physiology, BIRDEM in fasting condition.

Subjects reported were examined for their wellbeing. Purpose and nature of the study were

explained to them. Consented respondents were given appointment for blood sampling. Detailed medical and personal history was recorded on the day of blood sampling in a pre-designed case record form.

Anthropometric measurements and blood pressure recording

Volunteer's height (in meter) and weight (kg), waist and hip (cm) circumference were taken following standard procedure. Cut-off values for BMI (normal 22.9 Kg/m2; over weight 23-27.5 and obese 27.5) and WHR (male-0.90 and female-0.80) were used as per WHO, 2004 guidelines for Asian population.

Blood pressure (average of two independent measurements) was recorded using barometric Sphygmomanometer.

Five milliliter of venous blood was drawn from each subject by vein puncture at fasting and drawn blood was allowed to clot. After 20 minutes samples were centrifuged at 3000 rpm for 10 minutes. Separated serum was aliquoted in micro centrifuge tubes, labeled and preserved at (-30°C) for biochemical analyses.

Biochemical methods

Glucose was measured by (glucose-oxidase) and Total Cholesterol, Triglyceride and HDL-c were measured (by enzymatic colorimetric) method using in the Biochemistry Auto-analyzer 'Hitachi 704' reagents of RANDOX Laboratories Ltd., UK. LDL- c was calculated using Fried wald formula. The method was not applied when triglyceride level exceeded 400 mg/dL. Serum Hs-CRP was estimated by enzyme linked immunosorbant assay (ELISA) method.

Statistical methods

Data were expressed as mean ± SD and number (percent). Statistical analyses were performed using Statistical Package for Social Science (SPSS) for Windows Version 17. Two tailed P value <0.05 was taken as significant level.

Recruitment criteria

A total number of 149 volunteers aged 30-60 yrs were finally recruited in this study. Subjects suffered from acute illness in the last three months, subjects with secondary obesity, pregnancy, known primary hyperlipidemias, hereditary or systemic inflammatory diseases, on any regular medications or significant physical training program were excluded.

Ethical Consideration

The Helsinki Declaration on medical ethics was respected in the surveys. The protocol was approved by the Ethical Committee of Diabetic Association of Bangladesh.

Results

Of the total subjects 73 (48.9%) were nonsmoker and 76 (51%) were smokers.

Table I showed Hs-CRP was significantly high (2.89±2.2) in smokers in comparison with nonsmokers (2.3±1.8; p=0.017). Fasting glucose (p=0.003) and Triglyceride (p=0.005) was also significantly high in smokers (5.25±0.45, 187.8±90.04) compared with nonsmokers (5.02±0.46, 134.0±69.0).

BMI (p=0.012) and BFM (%) (p= <0.001) and showed significantly lower (22.4±2.9 and 20.9±4.2 respectively) in comparison with the counterpart (24.1±3.3 and 26.8±6.9 respectively). HDL-c (p=.030) also showed significantly lower levels in smoker group (34.8±7.0) than nonsmoker group (39.9±8.5).

Table I: Clinical and biochemical variables between smokers and non-smokers study subjects, n =92

Variables	Non-Smoker (63)	Smoker (29)	t/p values
Age	39.72±7.5	43.0±9.6	ns
BMI ($kg/m^{2)}$	24.1±3.3	22.4±2.9	0.012
Body fat mass%	26.8±6.9	20.9±4.2	<0.001
WHR	0.87±.05	0.88±0.06	ns
SBP(mmHg)	114.1±9.9	114.3±7.4	ns
DBP(mmHg)	72.8±8.55	73.8±7.3	ns
Fasting glucose (mmol/l)	5.02±0.46	5.25±0.45	-2.992/0.003
Triglyceride (mg/dl)	134.0±69.0	187.8±90.04	0.005
Total cholesterol (mg/dl)	182.6±36.7	189.9±28.8	ns
HDLc (mg/dl)	39.9±8.5	34.8±7.0	2.228/0.030
LDLc (mg/dl)	117.8±31.8	116.5±26.6	ns
hsCRP (mg/L)	2.3±1.8	2.89±2.2	0.017

Results were expressed as mean±SD. Unpaired Student's t test was performed to compare between groups.

Note: BMI (Body mass index), hsCRP (High sensitive C-reactive protein), WHR (waist hip ratio), SBP (systolic blood pressure), DBP (diastolic blood pressure), HDL-c (High density lipoprotein cholesterol), LDL-c (Low density lipoprotein cholesterol).

Correlation analyses

Bivariate correlation analyses

Spearman's correlation analyses were performed for variable Age, BMI, WHR, fasting glucose, TG, Total Cholesterol, HDL-c and LDL-c and smoking habit.

Age (r = - 0.028 p = 0.733), BMI (r = 0.026 p = 0.757), WHR (r = 0.132 p = 0.110) did not show any significant correlation with Hs - CRP. Triglyceride (r = 0.171, p = 0.037) and smoking (r = 0.168, p = 0.042) showed positive correlation with Hs - CRP and Fasting glucose (r = 0.030 p = 0.716), Total cholesterol (r = 0.016 p=0.842), and LDL -c (r = 0.150 p = 0.067) did not show any significant correlation with Hs-CRP. HDL - c (r = -0.157 p = 0.056) showed negative correlation but did not reach up to the significant level (Table II).

Table II: Spearman's correlation analysis for Hs-CRP with independent variables (n=149)

Variables	r	p
Age	-0.028	0.733
BMI	0.026	0.757
WHR	0.132	0.110
Fasting glucose	0.030	0.716
Triglyceride	0.171	0.037
Total Cholesterol	0.016	0.842
HDL-c	-0.157	0.056
LDL-c	0.150	0.067
Smoking	0.168	0.042

Results were expressed as Spearman's correlation and statistical significance p. BMI (Body mass index); WHR (waist hip ratio); HDL-c (High density lipoprotein cholesterol); LDL- c (Low density lipoprotein cholesterol).

Discussion

Low grade inflammation may be part of the 'common soil' underlying the metabolic syndrome, Type 2 Diabetes Mellitus and cardiovascular diseases. CRP is a nonspecific marker widely used to monitor treatment of cardiovascular diseases (high serum CRP levels indicate poor outcome of heart disease). A healthy lifestyle decreases serum CRP levels, while obesity, physical inactivity, and smoking increase them.[20,21,22]

Our findings support the hypothesis that lifestyle factors such as smoking adversely affect inflammatory and metabolic processes. Our results showed an association between smoking and elevated Fasting Serum Glucose, Triglyceride, Hs-CRP as well as low HDL-c. The Hs-CRP concentration found to be elevated by smoking[17,25] which can be explained by three possible mechanisms: (i) smokers have chronic airway inflammation because cigarettes contain potent airway irritants. [26] Indeed, smokers have a higher prevalence of chronic bronchitis, bronchial asthma and pulmonary emphysema than nonsmokers.[27] (ii) Smokers are likely to have a tendency to have dyslipidemia, coronary vasomotor reactivity, platelet aggregation, and a prothrombotic state.[28,29] Accumulation of these risk factors promotes the initiation and progression of atherosclerosis. This hypothesis is supported by reports that smokers have elevated concentrations of soluble intercellular adhesion molecule type 1, E-selectin, interleukin-5, and P-selectin[30]; and (iii) smoking deteriorates insulin resistance, resulting in an increase in Hs-CRP.[31] Insulin resistance in smokers could be caused by an increase in counter regulatory hormones,

such as, Growth hormone, Cortisol, Glucagon and Catecholamines, all of which raise blood glucose level.[32] A case control study based on this showed that smokers had higher levels of Hs-CRP, FPG and TG but lower levels of HDL-c than nonsmokers.[17] Smoking had a dyslipidaemic effect and can increase Total Cholesterol, LDL-c and Trigliceride; furthermore, it can decrease serum HDL-c level.[17] In agreement with Oh et al,[33] we find that chronic smoking is associated with higher Triglycerides and lower HDL-c.

In our study, BMI, body fat and WHR in smokers were lower than in non-smokers. Some other studies have demonstrated lower BMI in current smokers. Numerous cross-sectional studies indicate that body weight, or body mass index (BMI; in kg/m2), is lower in cigarette smokers than in nonsmokers.[34-37] In the World Health Organization Monitoring Cardiac Disease (WHO MONICA) surveys, BMI was lower in smokers than in smokers, and there was no population in which smokers had a higher BMI than did nonsmokers.[38] In the second National Health and Nutrition Examination Survey (NHANES II) study (1976 –1980), smokers weighed less than nonsmokers, and body leanness increased with the duration (but not with the intensity) of smoking.[39] The effect of smoking on body weight could lead to weight loss by increasing the metabolic rate, decreasing metabolic efficiency, or decreasing caloric absorption (reduction in appetite), all of which are associated with tobacco use. The metabolic effect of smoking could explain the lower body weight found in smokers. Smoking a single cigarette has been shown to induce a 3% rise in Energy Expenditure within 30 min.[40] And could reduce appetite, which likely explains why smokers tend to have lower body weight than do nonsmokers.

Conclusion

Elevated Hs-CRP has been closely linked with the development of atherosclerosis and is associated with the development of and mortality from CVD. Though not proven here, our study suggests that aggravation of systemic inflammation by cigarette smoking may account for the increased risk of CVD in smokers. Finally, we can conclude that subclinical atherosclerosis is independently accelerated via continuous smoking and that the smoking-induced promotion of atherosclerotic change is closely associated with inflammatory reactions.

References

1. World Health Organization. WHO Report on the Global Tobacco Epidemic, 2008: The MPOWER Package. Geneva, World Health Organisation; 2008.
2.Centers for Disease Control and Prevention. 2004 Surgeon General's Report – The Health Consequences of Smoking. Atlanta, GA:Centers for Disease Control and Prevention, National Center for Chronic Disease Prevention and Health Promotion, Office on Smoking and Health; 2004.
3.Ockene IS, Miller NH. Cigarette smoking, cardiovascular disease, and stroke: a statement for healthcare professionals from the American Heart Association. American Heart Association Task Force on Risk Reduction. Circulation. 1997;96:3243–7.
4.Smith SC Jr, Milani RV, Arnett DK. Atherosclerotic Vascular Disease Conference: Writing Group II: risk factors. Circulation. 2004;109:2613-16.
5. Libby P, Ridker PM, Maseri A. Inflammation and atherosclerosis. Circulation. 2002; 105: 1135-43.
6. Ross R. Atherosclerosis-an inflammatory disease. N Engl J Med. 1999;340:115–26.
7. Mannino DM, Buist AS. Global burden of COPD: risk factors, prevalence, and future trends. Lancet 2007;370:765–73.
8. Majka DS, Holers VM. Cigarette smoking and the risk of systemic lupus erythematosus and rheumatoid arthritis. Ann Rheum Dis. 2006;65:561–3.
9. Lakatos PL, Szamosi T, Lakatos L. Smoking in inflammatory bowel diseases: good, bad or ugly? World J Gastroenterol. 2007;13:6134–9.
10. Casas JP, Shah T, Hingorani AD. C-reactive protein and coronary heart disease: a critical review. J Intern Med. 2008;264:295–314.

11. Uhlar CM, Whitehead AS. Serum amyloid A, the major vertebrate acute-phase reactant. Eur J Biochem. 1999;265:501-23.
12. Kuller LH, Tracy RP, Shaten J, Meilahn EN. Relation of C-reactive protein and coronary heart disease in the MRFIT nested case-control study. Multiple Risk Factor Intervention Trial. Am J Epidemiol. 1996;144:537-47.
13. Ridker PM, Cushman M, Stampfer MJ, Tracy RP, Hennekens CH. Inflamation, aspirin, and the risk of cardiovascular disease in apparently healthy men. N Engl J Med. 1997;336:973–79.
14. Sato H, Miida T, Wada Y, Hasegawa H, Kuroda T, Narita I, et.al. Atherosclerosis is accelerated in patients with long-term well-controlled systemic lupus erythematosus (SLE). Clin Chim Acta. 2007;385:35-42.
15. Yudkin JS, Stehouwer CD, Emeis JJ, Coppack SW. C-reactive protein in healthy subjects: associations with obesity, insulin resistance, and endothelial dysfunction: a potential role for cytokines originating from adipose tissue? Arterioscler Thromb Vasc Biol. 1999;19:972-78.
16. Ridker PM. Clinical application of C-reactive protein for cardiovascular disease detection and prevention. Circulation 2003;107:363-69.
17. Hanyu O, Miida T, Kosuge K, Ito T, Soda S, Hirayama S, et al. Preheparin lipoprotein lipase mass is a practical marker of insulin resistance in ambulatory type 2 diabetic patients treated with oral hypoglycemic agents. Clin Chim Acta. 2007;384:118-23.
18. Lakka HM, Laaksonen DE, Lakka TA, Niskanen LK, Kumpusalo E, Tuomilehto J, et al. The metabolic syndrome and total and cardiovascular disease mortality in middle-aged men. JAMA. 2002;288:2709-16.
19. Kannel WB, McGee DL, Gordon T. A general cardiovascular risk profile: the Framingham Study. Am J Cardiol. 1976;38:46-51.
20. Wood D. Established and emerging cardiovascular risk factors. Am Heart J. 2001;141:49- 57.
21. Panagiotakos DB, Pitsavos C, Chrysohoou C, Stefanadis C, Toutouzas PK. Risk stratification of coronary heart disease through established and emerging lifestyle factors, in a Mediterranean population: CARDIO 2000 Epidemiological Study. J Cardiovasc Risk. 2001;6:329-39.
22. Tsujii S, Kuzuya H. The significance of lifestyle as a risk factor for the metabolic syndrome. Nippon Rinsho. 2004;62:1047-52.
23. Rahman I, MacNee W. Oxidant/antioxidant imbalance in smokers and chronic obstructive pulmonary disease. Thorax. 1996;51:348.
24. World Health Organization and World Heart Federation (April 2012): Cardiovascular harms from tobacco use and secondhand smoke. In Global gaps in awareness and implications for action. Ontario, Canada and Geneva, Switzerland: Waterloo; 2012.
25. Mendall MA, Patel P, Ballam L, Strachan D, Northfield TC. C reactive protein and its relation to cardiovascular risk factors: a population based cross sectional study. BMJ. 1996;312:1061-65.
26. Burns DM: Cigarettes and cigarette smoking. Clin Chest Med. 1991;12:631-42.
27. U.S. Department of Health E and Welfare: The heath consequence of smoking: a report to the surgeon general. 1971;71-7513, Washington DC, US Department of Health, Education, and Welfare, Public Health Service.
28. Muskat JE, Harris RE, Haley NJ, Wynder EL: Cigarette smoking and plasma cholesterol. Am Heart J. 1991;121:141-47.
29. Fusegawa Y, Goto S, Handa S, Kawada T, Ando Y. Platelet spontaneous aggregation in platelet-rich plasma is increased in habitual smokers. Thromb Res. 1999;93:271-78.
30. Bermudez EA, Rifai N, Buring JE, Manson JE, Ridker PM: Relation between markers of systemic vascular inflammation and smoking in women. Am J Cardiol. 2002;89:1117-19.
31. Facchini FS, Hollenbeck CB, Jeppesen J, Chen YD, Reaven GM. Insulin resistance and cigarette smoking. Lancet. 1992;339:1128-30.
32. Winternitz WW, Quillen D. Acute hormonal response to cigarette smoking. J Clin Pharmacol. 1977;17:389-97.
33. Oh SW, Yoon YS, Lee ES, Kim WK, Park C, Lee S, et al. Association between cigarette smoking and metabolic syndrome: the Korea National Health and Nutrition Examination Survey (Brief Report). Diabetes Care. 2005;28:2064–66.
34. Williamson DF, Madans J, Anda RF, Kleinman JC, Giovino GA, Byers T. Smoking cessation and severity of weight gain in a national cohort. N Engl J Med. 1991;324:739–45.
35. Shimokata H, Muller DC, Andres R. Studies in the distribution of body fat. III. Effects of cigarette smoking. JAMA. 1989;261(8):1169 –73.
36. Flegal KM, Troiano RP, Pamuk ER, Kuczmarski RJ, Campbell SM. The influence of smoking cessation on the prevalence of overweight in the United States. N Engl J Med. 1995;333(18):1165–70.

37. Huot I, Paradis G, Ledoux M. Quebec Heart Health Demonstration Project Research Group. Factors associated with overweight and obesity in Quebec adults. Int J Obes Relat Metab Disord. 2004;28(6):766– 74.
38. Molarius A, Seidell JC, Kuulasmaa K, Dobson AJ, Sans S. Smoking and relative body weight: an international perspective from the WHO MONICA Project. J Epidemiol Community Health. 1997;51(3):252–60.
39. Albanes D, Jones DY, Micozzi MS, Mattson ME. Associations between smoking and body weight in the U.S. population: analysis of NHANES II. Am J Public Health. 1987;77(4):439–44.
40. Dallosso HM, James WP. The role of smoking in the regulation of energy balance. Int J Obes. 1984;8(4):365–75.