Full Length Research Paper

Serum amyloid A protein, rheumatoid factor and lipid profile in relation to tobacco smoking in Saudi subjects

Al-Sieni A.* and Al-Abbasi F.

Department of Biochemistry, Faculty of Science, King Abdulaziz University P. O. Box 80203, Jeddah, 21589, Saudi Arabia.

Tobacco smoking is a major cause of many diseases, including lung cancer, cardiovascular disease, osteoporosis, aging and death. The associations between tobacco smoking, serum amyloid A (SAA) protein, rheumatoid factor (RF) and lipid profile were examined in 275 men that were divided into three groups according to their age (less than 20 years, 20 to 40 years and above 40 years), of which 91 were currently light cigarette smokers (less than 20 cigarettes/day), 91 were heavy smokers (20 cigarettes or more/day) and 93 had never smoked (control). As such, all men were part of a long-term survey and it was obtained that, heavy smokers had significantly higher SAA levels than light smokers or those who had never smoked at all (p < 0.01 and <0.001 respectively). Mean serum level of RF was statistically and significantly higher in heavy smokers of over 40 years age group, whereas serum glucose, triacylglycerol and total cholesterol levels were not affected by smoking in the different age groups when compared with the control group. However, serum LDL-c was significantly elevated and HDL-c level was decreased in heavy smokers (p < 0.001) and light smokers (p < 0.05) as compared to the control groups. Tobacco smoking is a risk factor for many diseases, related to SAA and RF (coronary heart diseases and Alzheimer rheumatoid arthritis), and these parameters can be used as prognostic markers in surveying the hazardous effect of tobacco smoking.

Key words: Tobacco smoking, serum amyloid A protein, lipid profile, Saudi Arabia.

INTRODUCTION

Serum amyloid protein A (SAA) is an acute phase protein produced by hepatocytes and secreted into the serum whose level is elevated in the blood during infection, trauma, surgery, burns, tissue infarction, inflammation, neoplasia and stress (Masdottir et al., 2000). SAA pro-duction is induced mainly by interleukine (IL-6, IL-1) and tumor necrosis factor (TNF-_) that are multifunctional cytokines produced by many cell types (Saag et al., 1997; Wolfe et al., 2000).

Epidemiologic studies have shown that cigarette smoking is associated with higher rates of myocardial infarction and death from coronary artery disease (Dallongeville et al., 1998). Habitual cigarette smokers have lower unadjusted mortality rates following acute myocardial infarction (AMI), a phenomenon often termed as 'smoker's paradox'. Some investigators have shown

that cigarette smokers, suffering from AMI, tend to be younger with less diffuse coronary artery disease and fewer co-morbidities compared to nonsmokers and these differences have been invoked to explain many of the differences in early mortality (Kenford et al., 2005).

Rheumatoid arthritis (RA) has a very heterogeneous course, ranging from a mild transient disease to a destructive arthritis with persistent inflammation, but the underlying pathogenic mechanisms are largely unknown. Tobacco smoking is the environmental factor that has most consistently been identified to have adverse effects on RA (Michnovicz et al., 1986). As seen in previous studies, it was established whether tobacco smoking has a direct effect on the immunopathogenic mechanisms of RA or whether the association is secondary to smoking associated lifestyle factors (Garrett, 1978), hormonal balance (Eadington et al., 1991) or the direct toxic effect of tobacco (Mattey et al., 2002; Harrison et al., 2001). The association between the inflammatory makers and other risk factors of chronic heart disease (CHD), such as age, gender, smoking

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*Corresponding author. E-mail: alabassif@hotmail.com.

Table 1. Sociodemographic characteristics (age and body mass index) of all studied groups (mean ±SD).

Group parameter	(Control)			(Light smokers)			(Heavy smokers)		
	<20 years n=31	20-40 years n=31	>40 years n=31	<20 years n=30	20-40 years n=30	>40 years n=31	<20 years n=30	20-40 years n=30	>40 years n=31
Age Mean±SD	18 ± 1.3	33 ± 3.4	60 ± 8.3	19 ± 1.9	36 ± 5.4	58 ± 5.7	17 ± 1.4	32 ± 6.2	68 ± 1.62
P*	-------	-------	-------	N.S	N.S	N.S	N.S	N.S	N.S
P**	-------	------	-----	------	-------	-------	N.S	N.S	N.S
BMI Mean±SD	26.0 ± 0.49	25.9±0.53	26.4±0.46	25.6±0.37	27.1±0.62	25.0±1.9	25.1±0.6	26.7±0.4	25.1±0.3
P*	------	------	------	N.S	N.S	N.S	N.S	N.S	N.S
P**	------	------	------	------	------	------	N.S	N.S	N.S

P*: t test and significance between light and heavy smokers vs. control. P**: t test and significance between light smokers vs. heavy smokers. N.s: non significant. n = number of cases.

habits, obesity, diabetes mellitus, education level and social class, low and high- density lipoprotein cholesterol, high levels of triglycerides, insulin resistance and physical activity (inverse) supports the idea that the inflammatory markers might be the expression of intermediate mechanisms (Teitsson et al., 1984). The reduction of cardiovascular risk by lifestyle changes, such as diet, weight loss, exercise and smoking cessation has been established (Bukhari et al., 2002) by the idea that it might be possible to reduce cardio-vascular risk and the progression of atherosclerosis by reducing circulating levels of inflammatory markers (Meyer et al., 2003).

There is no definitive evidence for this suggestion. Regular physical activity reduces in both sexes and at all ages in coronary and cardiovascular morbidity and mortality independently from other risk factors (Tuomi et al., 1990). This study focused on the association between light or heavy tobacco smoking and inflammatory markers as SAA and RF. Also, possible association was seen between these markers and risk factors of CHD (age, total cholesterol), high and low density lipoproteins (HDL-c, LDL-c), triacylglycerol, body mass index (BMI) and smoking habits in Saudi subjects.

MATERIALS AND METHODS

Subjects

The study was approved by the Saudi ethics committee and a written consent was obtained from each subject. A total of 275 adult male were divided into three groups according to their ages (less than 20 years, 20 to 40 years and above 40 years), of which 91 were currently light cigarette smokers (less than 20 cigarettes/day) since 9 years, 91 were heavy smokers (20 cigarettes or more/day) since 10 years and 93 had never smoked (control). All participants were apparently healthy and free from clinical diseases as diabetic, hypertension, liver or kidney disorders CHD and major ECG abnormalities. Clinical examination of the body mass index (BMI) was calculated as body weight (kg) divided by the squared height (m). The procedure for the measurements of weight, height, waist circumference and hip circumference, systolic and diastolic blood pressure was similar to the methods described (Jonsson et al.,

1998). As such, collection and analysis of blood samples (12 to 14 h fasting) were drawn from all individuals at the time of their clinical examination. Blood was allowed to clot, centrifuged and the supernatant serum was kept frozen at -20°C until analysis. Serum amyloid A protein (SAA) and rheumatoid factor (RF) were measured by fixed-time immunonephelometry on a BN II analyzer (Dade Behring, Marburg, Germany). However, reagents containing specific polyclonal (SAA) or monoclonal (RF) antibodies were coated to polystyrene particles (Dade Behring) (Barlow, 1994; Hashimoto et al., 1997).

The serum's total cholesterol was measured using the method of Pepys et al. (1978), while triacylglycerol (Cushman et al., 2005), high-density lipoprotein cholesterol (HDL-c) (Cao et al., 2003) and low–density lipoprotein cholesterol (LDL-c) were calculated (Li et al., 1998) . Statistical analysis results were reported as mean ± SD and they were compared using Student's t test for continuous variables and chi-square analysis for discontinuous variables. As such, a p value _0.05 was considered significant. However, one-way ANOVA was additionally used as a confirmatory test and Spearman rank correlation was also used.

RESULTS

The sociodemographic data in Table (1) revealed that, the age of the selected subjects in this study between 16 and 68 years were divided according to smoking status into light, heavy and non smokers. As such, non significant changes were seen in the mean of BMI for all studied groups. Table 2 revealed that, there was a significant elevation in the levels of serum SAA in light (over 4 years) and heavy smokers (over 20 years) (p < 0.01) as compared with the control. In addition, non significant changes were not seen in light or heavy smokers in less than 20 years and 20 to 40 years groups as compared with the control group. Heavy smokers were more often RF sero-positive than light or non smokers and as such, this difference reached statistical significance for RF (P < 0.01).

Table 3 showed that, there was no statistically signifi-cant difference in the levels of serum triacylglycerol, total cholesterol, LDL-C and HDL-C in light and heavy smokers of all ages (less than 20 years). However serum levels of triglycerides, total cholesterol and LDL-c were

Table 2. Serum amyloid A protein (SAA) and rheumatoid factor (RF) in all studied groups (mean ± SD).

Groups parameters	(Control)			(Light smokers)			(Heavy smokers)		
	<20 years n=31	20-40 years n=31	>40 years n=31	<20 years n=30	20-40 years n=30	>40 years n=31	<20 years n=30	20-40 years n=30	>40 years n=31
SAA (mg/dl) Mean ± SD	3.44 ±0.07	3.56 ±0.11	4.72±0.14	3.48 ± 0.11	4.82±0.11	6.72 ±0.2	3.53 ±0.12	5.8 ± 0.24	8.92 ± 0.14
P*	-------	-------	------	N.S	N.S	<0.01	N.S	<0.01	<0.01
P**	-----	------	------	------	------	-----	N.S	N.S	<0.01
RF (IU/ml) Mean± SD	7.15 ±0.95	7.28 ± 1.5	7.63 ±1.2	7.18 ± 0.8	7.5±0.06	7.6±0.96	7.51±1.8	7.5±1.33	8.1± 1.19
P*	------	------	-------	N.S	N.S	N.S	N.S	N.S	<0.01
P**	-----	------	-------	-------	-------	-------	N.S	N.S	<0.01

P*: t test and significance between light and heavy smokers Vs control. P**: t test and significance between light smokers Vs heavy smokers.
N.s: non significant; n = number of cases.

Table 3. Serum lipid profile in all studied groups (mean ± SD).

Groups parameters (mg/dl)	(Control)			(Light smokers)			(Heavy smokers)		
	<20 yrs n=31	20-40 yrs n=31	>40 yrs n=31	<20 yrs n=30	20-40 yrs n=30	>40 yrs n=31	<20 yrs n=30	20-40 yrs n=30	>40 yrs n=31
Triacylglyerol Mean± SD	115.0 ±7.05	117.6±7.6	120.0±0.06	117.9 ±6.8	119.4±7.4	125.0±7.3	119.3 ±7.5	120.0 ±7.5	157.0±4
P*	-------	-------	------	N.S	N.S	N.S	N.S	N.S	<0.01
P**	-----	------	------	------	------	-----	N.S	N.S	<0.01
TC Mean± SD	165.1 ±7.1	167±7.5	165±6.2	168 ±7.3	165±6.2	171.5±7	165.3 ±6.5	169±6.1	180±3.9
P*	------	-------	------	N.S	N.S	N.S	N.S	N.S	<0.05
P**	-----	------	------	------	------	-----	N.S	N.S	<0.05
LDL-c Mean±SD	117 ±5.9	118±8.6	127±9.9	115 ±10.5	119±6.9	117±8.6	118 ±11	117 ±8	137±13
P*	-------	-------	------	N.S	N.S	N.S	N.S	N.S	<0.001
P**	-----	------	------	------	------	-----	N.S	N.S	<0.001
HDL-c Mean± SD	39.6 ±0.9	38.0±0.62	37.2±0.55	37.6 ±1.01	37.5±0.7	36.5±0.5	37.0 ±1.5	37.0 ±0.98	34.6±0.47
P*	-------	-------	------	N.S	N.S	N.S	N.S	N.S	<0.01
P**	-----	------	------	------	------	-----	N.S	N.S	<0.01

P*: t test and significance between light and heavy smokers Vs control. P**: t test and significance between light smokers Vs heavy smokers. N.s: non significant; n= number of cases.

significantly elevated (p < 0.01, <0.05 and <0.001 respectively), while HDL-c level was significantly decreased (p < 0.01) in the over 40 years age group as compared with other studied groups. Nonetheless, serum SAA was positively correlated with total cholesterol, LDLc and triglycerides (r = 0.56, 0.68 and 0.61 respectively) and negatively correlated with HDL cholesterol (r = - 0.66).

DISCUSSION

Epidemiological studies have focused on the deleterious effects of smoking on human health. Of particular interest, the study of a number of epidemiological reports implies that cigarette smoking is a serious risk factor for cardiovascular diseases and amylodosis (De Beer et al.,

1982).

This, to our knowledge, is the first prospective study that analysed the effects of tobacco smoking on the incidence of amylodosis and rheumatoid. It shows that tobacco smokers have higher disease incidence in the over 40 years age group. This effect is most evident in heavy smokers and is not limited to light smokers. The only previously published prospective study on the effects of tobacco smoking on disease activity in early polyarthritis did not reveal such association (Fu and Borensztajn, 2002). As such, we examined the impact of light or heavy smoking in different ages on SAA, RF and lipid profile.

There is increasing evidence that higher level of SAA is a predictor of coronary heart disease (CHD) and may play an important role in the different stages of the development of atherosclerosis (Stewart et al., 2005). The predictive value of SAA is found apparently in both smokers and non smokers of different ages, but its level is higher significantly in heavy and light smokers of over 40 years age group. This indicated that, tobacco smoking is responsible for this acute phase response that leads to CHD.

Although both proteins function in innate immunity, these pentraxins are quite different. RF is a major acute phase protein, whereas SAA is only mildly affected by acute inflammation (Casserly and Topol, 2004). Both proteins differ in lipid-binding functions as well. SAA binds HDL-c and the very low density lipoprotein, but did not bind the unmodified LDLc. Similarly, RF binds unmodified and oxidized LDL-c, thereby mediating metabolism, clearance and deposition of LDLc. RF may thus initiate foam cell formation and early atherosclerosis.

However, SAA binding on amyloid-like structures in oxidized LDL blocks macrophage uptake of modified LDL serves to prevent atherosclerosis. RF and SAA may therefore represent distinct aspects of inflammation. Although SAA and RF were independent predictors of CVD events in this study and as such, exhibited some differences in associations with CHD risk factors, there were no apparent synergistic effects on CHD event prediction when the two biomarkers were combined (Rolph et al., 2002; Peri et al., 2000). SAA will have unique clinical potential in monitoring atherosclerotic progression before acute events are associated with the elevated RF. Nonetheless, the potential for SAA will be necessary to further explore these relationships in other cohorts, particularly in younger men and women with less advanced atherosclerosis.

Fundamentally, these studies lack reliable information on important confounders known to be associated with tobacco smoking including anxiety, depression, educational level, caffeine consumption and use of anti-rheumatic medica-tions. In this study, none of these confounding factors were found to be associated with disease incidence. Thus, the study's findings suggested that the observed impact of tobacco smoking on disease incidence is immunologically mediated.

Conclusion

This study suggests that tobacco smoking is associated with increased SAA and RF production particularly in the over 40 years age group. This confirms that RF does not predict adverse prognosis in RA while RF seropositivity may be an independent risk factor for more active disease and increased RA.

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REFERENCES

Masdottir B, Jonsson T, Manfredsdottir V, Vikingsson A, Brekkan A, Valdimarsson H (2000). Smoking, rheumatoid factor isotypes and severity of rheumatoid arthritis. Rheumatology, 39:1202–5.

Saag KG, Cerhan JR, Kolluri S, Ohashi K, Hunninghake GW, Schwartz DA (1997). Cigarette smoking and rheumatoid arthritis severity. Ann. Rheum Dis., 56: 463–9.

Dallongeville J, Marecaux N, Fruchart JC, Amouyel P (1998). Cigarette smoking is associated with unhealthy patterns of nutrient intake: a meta-analysis. J. Nutr., 128:1450–7.

Kenford SL, Wetter DW, Welsch SK, Smith SS, Fiore MC, Baker TB. (2005). Progression of college-age cigarette samplers: what influences outcome. Addict Behav., 30: 285–94.

Michnovicz JJ, Hershcopf RJ, Naganuma H, Bradlow HL, Fishman J (1986). Increased 2-hydroxylation of estradiol as a possible mechanism for the anti-estrogenic effect of cigarette smoking. N Engl. J. Med., 315: 1305–1309.

Garrett RJ (1978). Collagen and non-collagen protein synthesis in developing lung exposed to tobacco smoke. Environ Res., 17: 205–15.

Eadington DW, Patrick AW, Frier BM (1991). Association between connective tissue changes and smoking habit in type 2 diabetes and in non-diabetic humans. Diabetes Res. Clin. Pract., 11:121–5.

Mattey DL, Hutchinson D, Dawes PT (2002). Smoking and disease severity in rheumatoid arthritis: association with polymorphism at the glutathione S-transferase M1 locus. Arthritis Rheum, 46: 640–646.

Harrison BJ, Silman AJ, Wiles NJ, Scott DG, Symmons DP (2001). The association of cigarette smoking with disease outcome in patients with early inflammatory polyarthritis. Arthritis Rheum, 44: 323–30.

Teitsson I, Withrington RH, Seifert MH, Valdimarsson H (1984). Prospective study of early rheumatoid arthritis. I. Prognostic value of IgA rheumatoid factor. Ann Rheum Dis., 43: 673–8.

Bukhari M, Lunt M, Harrison BJ, Scott DG, Symmons DP, Silman AJ (2002). Rheumatoid factor is the major predictor of increasing severity of radiographic erosions in rheumatoid arthritis: results from the Norfolk Arthritis Register Study, a large inception cohort. Arthritis Rheum, 46: 906–1012.

Meyer O, Labarre C, Dougados M (2003). Anticitrullinated protein/peptide antibody assays in early rheumatoid arthritis for predicting five year radiographic damage. Ann. Rheum Dis., 62:120–6.

Tuomi T, Heliovaara M, Palosuo T, Aho K (1990). Smoking, lung function, and rheumatoid factors. Ann. Rheum Dis., 49: 753–6.

Jonsson T, Thorsteinsson J, Valdimarsson H (1998). Does smoking stimulate rheumatoid factor production in non-rheumatic individuals? APMIS, 106: 970–4.

Barlow WE (1994). Robust variance estimation for the case-cohort design. Biometrics. 50: 1064 –1072.

Hashimoto S, Katou M, Dong Y, Murakami K, Terada S, Inoue M (1997). Effects of hormone replacement therapy on serum amyloid P component in postmenopausal women. Maturitas, 26: 113–119.

Pepys MB, Dash AC, Markham RE, Thomas HC, Williams BD, Petrie A (1978). Comparative clinical study of protein SAP (amyloid P component) and C-reactive protein in serum. Clin. Exp. Immunol., 32: 119 –124.

Cushman M, Arnold AM, Psaty BM, Manolio TA, Kuller LH, Burke GL, Polak JF, Tracy RP (2005). C-reactive protein and the 10-year incidence of coronary heart disease in older men and women: the cardiovascular health study. Circulation, 112: 25–31.

Cao JJ, Thach C, Manolio TA, Psaty BM, Kuller LH, Chaves PH, Polak JF, Sutton-Tyrrell K, Herrington DM, Price TR, Cushman M (2003). C-reactive protein, carotid intima-media thickness, and incidence of ischemic stroke in the elderly: the Cardiovascular Health Study. Circulation, 108: 166–170.

Li XA, Yutani C, Shimokado K (1998). Serum amyloid P component associates with high density lipoprotein as well as very low density lipoprotein but not with low density lipoprotein. Biochem. Biophys. Res. Commun.,; 244:249 –252.

de Beer FC, Soutar AK, Baltz ML, Trayner IM, Feinstein A, Pepys MB (1982). Low density lipoprotein and very low density lipoprotein are selectively bound by aggregated C-reactive protein. J. Exp. Med.. 156: 230 –242.

Fu T, Borensztajn J (2002). Macrophage uptake of low-density lipoprotein bound to aggregated C-reactive protein: possible mechanism of foam-cell formation in atherosclerotic lesions. Biochem. J., 366: 195–201.

Stewart CR, Tseng AA, Mok YF, Staples MK, Schiesser CH, Lawrence LJ, Varghese JN, Moore KJ, Howlett GJ (2005). Oxidation of low-density lipoproteins induces amyloid-like structures that are recognized by macrophages. Biochemistry, 44: 9108 –9116.

Casserly I, Topol E (2004). Convergence of atherosclerosis and Alzheimer's disease: inflammation, cholesterol, and misfolded proteins. Lancet, 363: 1139 –1146.

Rolph MS, Zimmer S, Bottazzi B, Garlanda C, Mantovani A, Hansson GK (2002). Production of the long pentraxin PTX3 in advanced atherosclerotic plaques. Arterioscler Thromb Vasc Biol., 22: e10 –14.

Peri G, Introna M, Corradi D, Iacuitti G, Signorini S, Avanzini F, Pizzetti F, Maggioni AP, Moccetti T, Metra M, Cas LD, Ghezzi P, Sipe JD, Re G, Olivetti G, Mantovani A, Latini R (2000). PTX3, A prototypical long pentraxin, is an early indicator of acute myocardial infarction in humans. Circulation, 102: 636–641.