Serum Amyloid – A protein and serum Rheumatoid Factor as serological surrogate markers for smoking risk factor in Saudi population

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Abstract: Tobacco smoking represents major national and international health hazard that interferes with wide range of physiological functions and biomarkers. In the current study we have investigated the influence of tobacco smoking on some biological markers such as serum amyloid protein-A, rheumatoid factor, serum glucose level and lipid profile in Saudi population. The fore mentioned parameters were investigated in a total of 275 cases in 3 different age categories (less than 20 years old, 20-40 years old and older than 40 years old). Long term survey was adopted in all cases; yet, lightly smoking and heavily smoking groups were compared to never smoking healthy population. Results obtained showed significant increase in serum amyloid protein-A and rheumatoid factor in correlation to the degree of smoking nonetheless in the age category older than 40 years old. Serum glucose, triglyceride, and total cholesterol was not affected by smoking in all studied age categories; however serum LDL-cholesterol was elevated and serum HDL-cholesterol was depressed in correlation to the degree of smoking in all age categories. In conclusion, tobacco smoking represents major cardiovascular risk factor in Saudi population in all age categories and serum amyloid protein-A and rheumatoid factor might be used as a serological surrogate marker for such risk.

Keywords: Tobacco, amyloid A protein, rheumatoid factor, lipid profile, Saudi population.

INTRODUCTION

Cigarette smoking is manageable risk factor for morbidity and mortality worldwide (Gerace *et al*., 1991). Currently smoking is highly associated with young age, poor income, weak educational achievement, and unpleasant neighborhood environment (Choi *et al*., 1997; Peto *et al*., 1992). Regular smokers exhibit higher risk factor for cardiovascular, respiratory malignant disorders than nonsmokers (Howard *et al*., 1998; Massion and Carbone, 2003; Murin and Inciardi, 2001; Wiencke *et al*., 1999). Approximately 50% of healthy adults with invasive pneumococcal infection are tobacco smokers (Nuorti *et al*., 2000). Cigarette smoking is a significant risk factor for heart disease and stroke subsequent to disturbed lipid profile and progressive atherosclerosis (Howard *et al*., 1998).

Atherosclerosis is directly related to lipids peroxidation within the extracellular environment of vascular subendothelial spaces. These peroxidized lipids, nonetheless LDL-cholesterols, initiate inflammatory cascade which in turn, leads to the generation of the fatty plaques. The progressive nature of these lesions is associated with arterial calcification, changes the mechanical characteristics of the arterial wall, and predisposing plaque rupture, monocytic infiltration and thrombus formation (Berliner *et al*., 1995). Strong interrelationship between several inflammatory biomarkers and other atherosclerosis risk factors was reported in various populations (Bell *et al*., 1996; Coulie and Van Snick, 1985).

Serum amyloid protein-A (SAA) is an acute phase stress protein primarily synthesized by hepatocytes due to variety of tissue reactions to infections, traumatic lesions, surgical procedures, burns, tissue vascular infarction, inflammatory disorders, neoplasic diseases and stress (Malle and De Beer, 1996). SAA synthesis is induced primarily by interluken-6, interluken-1 and tumor necrosis factor-α, which are multifunctional cytokines produced by several cell types of body (Olsson *et al*., 1999; Westermark *et al*., 1990). The use of SAA as disease marker has been proved effective in a variety of diseases (Cunnane, 2001; Dinarello *et al*., 1988; Lindhorst *et al*., 1997).

Rheumatoid Factor (RF) is an autoantibaody against IgG that is primarily correlated with rheumatic diseases such as rheumatoid arthritis, osteoarthritis and rheumatic fever (Coulie and Van Snick, 1985; Harris, 1990). RF is widely used marker for the diagnosis and follow-up of these rheumatic diseases (Schellekens *et al*., 2000). Several reports indicated positive correlation between tobacco smoking and the course of rheumatoid arthritis (Hutchinson *et al*., 2001).

Herein, we are studying the possibility of using the inflammatory markers, SAA and RF, as early surrogate

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markers for the severity assessment of tobacco smoking risk factor in Saudi population in relation to their lipid profile.

SUBJECTS AND METHODS

Subjects

A total of 275 adult male divided into three groups depending on their ages [less than 20 years, 20-40 years and above 40 years], 91 of whom were current light cigarette smokers (< 20 cigarettes/day), 91 of whom were heavy smokers (> 20 cigarettes/day) and 93 who had never smoked (control). All participants were clinically investigated and found free from rheumatoid arthritis and from other clinical diseases such as, diabetic, hypertension, liver or kidney disorders coronary heart diseases and major ECG abnormalities. The study design was approved by the clinical ethics committee in Ministry of Health, Kingdom of Saudi Arabia.

Clinical examination

Body mass index (BMI) was calculated as follow: body weight (in kg) was divided by squared height (in meters). The procedure for the measurements of weight, height, systolic and diastolic blood pressure was according to the standard procedures within Saudi health care institutes.

Inclusion/exclusion criteria

Patients included in this study are clinically diagnosed as RA for more than two year according to the standards of the American Association of Rheumatology (AAR); do not suffer any other chronic illness such as DM, hypertension etc., had not performed any major surgery in the last two years. Light smokers groups are defined as those who are smoking less than twenty cigarettes daily for more than one year. Heavy smokers are defined as those who are smoking more than twenty cigaresste per day for more than one year. None smokers are those who did not smoke any cigarette or live in close contact with any smokers for the past five years. Any heavily caffeine consuming patient (consuming more than five cups of coffee per day or equivalent) are excluded form the study.

Collection and analysis of blood samples

Venous blood samples were withdrawn from peripheral vein while the patient is sitting, after fasting for 12-14 hours. Blood samples were allowed to clot, centrifuged and the sera were 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). Specific polyclonal (SAA) or monoclonal (RF) antibodies coated polystyrene particles were used for SAA and RF analysis (Dade Behring). SAA determinations were run in batch using separate cuvette rotors.

Serum total cholesterol was measured using the method of Rautela and Leidtke, triacylglycerol, high-density lipoprotein cholesterol (HDL-c), and low -density lipoprotein cholesterol (LDL-c) was calculated as previously reported (Friedewald *et al*., 1972; Rautela and Liedtke, 1978).

STATISTICAL ANALYSIS

Data is expressed as mean ± SD. Analysis of variance (ANOVA) with Tukey's post hoc test was used for testing the significance of parametric data using SPSS® for windows, version 17.0.0. $P<0.05$ was taken as the cut off value for significance.

RESULTS

Sociodemographics of the population in the current study covered all the age range of smoking habit in Saudi population (from 18 to 68 years old). Selected sample of the population were divided with equal sample size into three smoking categories; never smokers, light smokers and heavy smokers. All cases did not show any clinical sign of smoking related major illness such as cardiopulmonary, malignant or diabetic diseases. BMI was not significantly different between all groups in the current study, yet, within the normal range of Saudi population (table 1). Former smokers manifested higher BMI than current smokers; however, current smokers showed higher caffeine consumption than never smokers (unpublished clinical observation).

SAA and RF are classic serological markers for diagnosis and follow up of several tissue traumatic and inflammatory diseases. SAA and RF did not show any significant change in serum level in relation to age in the never smokers' group. SAA showed steadily increased serum level in relation to the degree of smoking. The change in SAA level in response to smoking was more significant in higher age category. RF level showed significant increased serum level in heavy smokers older than 40 years old (table 2). On the other hand, former smokers were more frequently RF seropositive than never smokers.

Lipid profile represents classic and robust risk factor assessment marker for tobacco smoking (Yarnell, 1996). In the current study, no significant difference in lipid profile was detected between different age categories of never smoking group in Saudi population. In moderately smokers group, TG and HDL-c showed increasing and decreasing trends, respectively, in older age category. However, the fore mentioned trend did not reach the significance level of our study design. In contrast to moderately smokers' group, older than 40 years old age category of heavily smokers' group showed significant

increase in TC, TG and LDL-c; and significant decrease in HDL-c level (table 3).

DISCUSSION

Tobacco smoking represents major socioeconomic national and international problem. The absence of classic risk factors for smoking other than debilitating illness such as, cardiovascular, respiratory and malignant disorders intensifies our suggested use of SAA and RF as early sensitive surrogate marker for smoking in Saudi population.

The different BMI of Saudi population from other populations, due to food habits and life style, adds some ambiguity to the use of BMI as cardiovascular risk factor due to smoking in Saudi population (El Mouzan *et al*.). This in turns shows the importance of using SAA and RF as early surrogate markers for the predicted debilitating cardiovascular effects of smoking in such kind of populations.

In current work, SAA was more sensitive marker to smoking compared to RF. There is accumulating evidence that higher level SAA is predictor of coronary heart disease, and may possess an important role in the different stages of atherosclerosis (Chen *et al*., 2010; Coetzee *et al*., 1986). The predictive value of SAA was reported in both apparently healthy persons and in patients with well established coronary heart disease or following myocardial infarction (Malle and De Beer, 1996; Westermark *et al*., 1990). The mechanism responsible for this acute phase response in coronary heart diseases is not yet fully understood (Westermark *et al*., 1990). To the best of our knowledge, this is the first prospective study analyzing the effects of tobacco smoking on RF in rheumatoid free subjects. The interrelation between tobacco smoking and rheumatic diseases and/or their serological markers have been reported before albeit to a lesser extent (Hutchinson *et al*., 2001). Whether the elevated RF factor in tobacco smoking is very early predictive of rheumatic disease or serological marker *per-se*, is not fully ruled out which warrants thorough future investigation. We and others have previously observed increased joint damage and/or decreased joint functional status in tobacco smokers with long-standing rheumatic diseases (Hutchinson *et al*., 2001).

In the current study, no significant difference in lipid profile was observed in all age categories of never and moderate smoking group in Saudi population. Significant differences were only detected in subjected over 40 years of heavily smokers group. In Middle East populations such as Saudi population, lipid profile might be different from other populations due to unique food habits and life style (El Mouzan *et al*.). Yet, the use of other serological markers would be crucial for the assessment of smoking induced atherosclerotic disorders. The reported

Table 1: Sociodemographic characteristics of Saudi cases under investigation

	Control			Light smokers			Heavy smokers		
Age category	< 20 (n=31)	20-40 (n=31)	> 40 (n=31)	< 20 (n=30)	20-40 (n=30)	> 40 (n=31)	< 20 (n=30)	20-40 (n=30)	> 40 (n=31)
Age	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
BMI	26±0.49	25.9±0.53	26.4±0.46	25.6±0.37	27.1±0.62	25±1.9	25.1±0.6	26.7±0.4	25.1±0.3

Table 2: Serum Amyloid A protein (SAA) and Rheumatoid factor (RF) profile in Saudi smokers

	Control			Light smokers			Heavy smokers		
Age category	< 20 (n=31)	20-40 (n=31)	> 40 (n=31)	< 20 (n=30)	20-40 (n=30)	> 40 (n=31)	< 20 (n=30)	20-40 (n=30)	> 40 (n=31)
SAA	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
RF	7.15±0.95	.28±1.5	7.63±1.2	7.18±0.8	7.5±0.06	7.6±0.96	.51±1.8	7.5±1.33	8.1**±1.19

*: Significantly different from corresponding age category in control group ($p<0.05$).
**: Significantly different from corresponding age category in light smokers group ($p<0.05$).

Table 3: Serum Lipid Profile in Saudi smokers

	Control			Light smokers			Heavy smokers		
Age category	< 20 (n=31)	20-40 (n=31)	> 40 (n=31)	< 20 (n=30)	20-40 (n=30)	> 40 (n=31)	< 20 (n=30)	20-40 (n=30)	> 40 (n=31)
TG	115 ±7.05	117.6±7.6	120±0.06	117.9 ±6.8	119.4±7.4	125 ±7.3	119.3 ±7.5	120 ±7.5	157**±4
TC	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
LDL-c	117 ±5.9	118±8.6	117±9.9	115 ±10.5	119±6.9	117±8.6	118 ±11	117 ±8	137**±13
HDL-c	39.6 ±0.9	38±0.62	37.2±0.55	37.6 ±1.01	37.5±0.7	36.5±0.5	37 ±1.5	37. ±0.98	34.6**±0.47

**: Significantly different from corresponding age category in light smokers group ($p<0.05$).

association between SAA and astherscleroticstatus in CHD strongly supports our hypothesis for using SAA serum level as a predictive risk factor assessment parameter (Jylhava *et al*., 2009; Lewis *et al*., 2004; Shainkin-Kestenbaum *et al*., 1996; Yamada and Miida, 1997). The early inflammatory status of subendothelial layer of arterial walls might be the underlying reason for the elevated SAA level in relation to tobacco smoking that warrant further experimental investigations.

CONCLUSION

In conclusion, we have provided preliminary evidence for the clinical use of SAA and RF serum levels in the risk assessment of tobacco smoking in Saudi population. SAA showed higher predictive sensitivity; however, RF has long term retrospective assessment for smoking risk factor in Saudi population.

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REFERENCES

Bell AW, Chan SL, Marcantonio D and Ali-Khan Z (1996). Both murine SAA1 and SAA2 yield AA amyloid in alveolar hydatid cyst-infected mice. *Scand. J. Immunol.,* **43**: 173-180.

Berliner JA, Navab M, Fogelman AM, Frank JS, Demer LL, Edwards PA, Watson AD and Lusis AJ (1995). Atherosclerosis: basic mechanisms. Oxidation, inflammation, and genetics. *Circulation*, **91**: 2488-2496.

Chen GL, Liu LW, Xie S, Liu H, Liu YQ and Li YS (2010). High-density lipoprotein associated factors apoA-I and serum amyloid A in Chinese non-diabetic patients with coronary heart disease. *Chin. Med. J.,* (Engl.) **123**: 658-663.

Choi WS, Pierce JP, Gilpin EA, Farkas AJ and Berry CC (1997). Which adolescent experimenters progress to established smoking in the United States. *Am. J. Prev. Med.,* **13**: 385-391.

Coetzee GA, Strachan AF, van der Westhuyzen DR, Hoppe HC, Jeenah MS and de Beer FC (1986). Serum amyloid A-containing human high density lipoprotein 3. Density, size, and apolipoprotein composition. *J. Biol. Chem.,* **261**: 9644-9651.

Coulie PG and Van Snick J (1985). Rheumatoid Factor (RF) production during anamnestic immune responses in the mouse. III. Activation of RF precursor cells is induced by their interaction with immune complexes and carrier-specific helper T cells. *J. Exp. Med.,* **161**: 88-97.

Cunnane G (2001). Amyloid precursors and amyloidosis in inflammatory arthritis. *Curr. Opin. Rheumatol.,* **13**: 67-73.

Dinarello CA, Cannon JG and Wolff SM (1988). New concepts on the pathogenesis of fever. *Rev. Infect. Dis.,* **10**: 168-189.

El Mouzan MI, Foster PJ, Al Herbish AS, Al Salloum AA, Al Omer AA, Qurachi MM and Kecojevic T (2010). Prevalence of overweight and obesity in Saudi children and adolescents. *Ann. Saudi Med.,* **30**: 203-208.

Friedewald WT, Levy RI and Fredrickson DS (1972). Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin. Chem.*, **18**: 499-502.

Gerace TA, Hollis J, Ockene JK and, Svendsen, K., 1991. Smoking cessation and change in diastolic blood pressure, body weight, and plasma lipids. MRFIT Research Group. *Prev. Med.,* **20**: 602-620.

Harris ED, Jr (1990). Rheumatoid arthritis. Pathophysiology and implications for therapy. *N. Engl. J. Med.,* **322**: 1277-1289.

Howard G, Wagenknecht LE, Burke GL, Diez-Roux A, Evans GW, McGovern P, Nieto FJ and Tell GS (1998). Cigarette smoking and progression of atherosclerosis: The Atherosclerosis Risk in Communities (ARIC) Study. *JAMA*, **279**: 119-124.

Hutchinson D, Shepstone L, Moots R, Lear JT and Lynch MP (2001). Heavy cigarette smoking is strongly associated with rheumatoid arthritis (RA), particularly in patients without a family history of RA. *Ann. Rheum. Dis.,* **60**: 223-227.

Jylhava J, Haarala A, Eklund C, Pertovaara M, Kahonen M, Hutri-Kahonen N, Levula M, Lehtimaki T, Huupponen R, Jula A, Juonala M, Viikari J, Raitakari O and Hurme M (2009). Serum amyloid A is independently associated with metabolic risk factors but not with early atherosclerosis: The cardiovascular risk in young finns study. *J. Intern. Med.,* **266**: 286-295.

Lewis KE, Kirk EA, McDonald TO, Wang S, Wight TN, O'Brien KD and Chait A (2004). Increase in serum amyloid a evoked by dietary cholesterol is associated with increased atherosclerosis in mice. *Circulation,* **110**: 540-545.

Lindhorst E, Young D, Bagshaw W, Hyland M and Kisilevsky R (1997). Acute inflammation, acute phase serum amyloid A and cholesterol metabolism in the mouse. *Biochim. Biophys. Acta.,* **1339**: 143-154.

Malle E and De Beer FC (1996). Human serum amyloid A (SAA) protein: a prominent acute-phase reactant for clinical practice. *Eur. J. Clin. Invest.,* **26**: 427-435.

Massion PP and Carbone DP (2003). The molecular basis of lung cancer: Molecular abnormalities and therapeutic implications. *Respir. Res.,* **4**: 12.

Murin S and Inciardi J (2001). Cigarette smoking and the risk of pulmonary metastasis from breast cancer. *Chest,* **119**: 1635-1640.

Nuorti JP, Butler JC, Farley MM, Harrison LH, McGeer A, Kolczak MS and Breiman RF (2000). Cigarette smoking and invasive pneumococcal disease. Active Bacterial Core Surveillance Team. *N. Engl. J. Med.,* **342**: 681-689.

Olsson N, Siegbahn A and Nilsson G (1999). Serum amyloid: A induces chemotaxis of human mast cells by activating a pertussis toxin-sensitive signal transduction pathway. *Biochem. Biophys. Res. Commun.,* **254**: 143-146.

Peto R, Lopez AD, Boreham J, Thun M and Heath C Jr (1992). Mortality from tobacco in developed countries: indirect estimation from national vital statistics. *Lancet,* **339**: 1268-1278.

Rautela GS and Liedtke RJ (1978). Automated enzymic measurement of total cholesterol in serum. *Clin. Chem.,* **24**: 108-114.

Schellekens GA, Visser H, de Jong BA, van den Hoogen FH, Hazes JM, Breedveld FC and van Venrooij WJ (2000). The diagnostic properties of rheumatoid arthritis antibodies recognizing a cyclic citrullinated peptide. *Arthritis Rheum.,* **43**: 155-163.

Shainkin-Kestenbaum R, Zimlichman S, Lis M, Lidor C, Pomerantz M, Knyszynski A, Preciado-Patt L and Fridkin M (1996). Effect of serum amyloid A, HDL-apolipoprotein, on endothelial cell proliferation. Implication of an enigmatic protein to atherosclerosis. *Biomed. Pept. Proteins Nucleic Acids,* **2**: 79-84.

Westermark GT, Sletten K, Grubb A and Westermark P (1990). AA-amyloidosis. Tissue component-specific association of various protein AA subspecies and evidence of a fourth SAA gene product. *Am. J. Pathol.,* **137**: 377-383.

Wiencke JK, Thurston SW, Kelsey KT, Varkonyi A, Wain JC, Mark EJ and Christiani DC (1999). Early age at smoking initiation and tobacco carcinogen DNA damage in the lung. *J. Natl. Cancer Inst.,* **91**: 614-619.

Yamada T and Miida T (1997). Serum amyloid A remains at physiological concentrations in coronary atherosclerosis. *Clin. Chem.,* **43**: 193.

Yarnell JW (1996). Smoking and cardiovascular disease. *QJM,* **89**: 493-498.