SERUM TITERS OF ACUTE PHASE PROTEINS AND IMMUNOGLOBULIN CLASSES IN NIGERIAN CIGARETTE SMOKERS

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Abstract

Background: Smoking remains the cause of preventable death but the cause(s) of adverse effect(s) of cigarette smoking is yet to be completely revealed.

Objectives: To determine the levels of immunoglobulin classes (IgG, A and M) and acute phase proteins (alpha 2-macroglobulin, transferrin and caeruloplasmin) in 13 Nigerian male cigarette smokers and 14 non – smokers as control by single radial immuno-diffusion method.

Methods: Immuno-diffusion method was used to determine the levels of immunoglobulin classes and acute phase proteins.

Setting: Students and staff of University of Ibadan, Nigeria.

Results: The mean levels of IgM, transferrin and alpha 2-macroglobulin were significantly raised while IgA, IgG and caeruloplasmin were not significantly raised in cigarette smokers compared with the control. There were no significant correlation between duration of smoking, nicotine inhaled, and tar inhaled with any of the humoral factors.

Conclusion: This study detected abnormalities in certain aspects of humoral immune responses in Nigerian smokers and this could lead to development of auto-antibodies and cardiovascular diseases in long time cigarette smokers.

Key words: Cigarette smoke, immunoglobulin, acute phase reactants, immuno-diffusion.

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Introduction

Cigarette smoking is a major health risk factor and significantly increases the incidence of cardiovascular disease, cancers of various organs, acute and chronic respiratory tract infections[1], but the basis of these observations is still a subject of intense research. Concentrations of immunoglobulin classes in serum are affected by tobacco smoke but the results vary. The precise mechanism(s) by which smoking affects serum immunoglobulin levels and the components of cigarette smoke responsible for the effects have not been clarified[2]. Mean IgG and IgM levels were significantly lower, and IgE and C3 levels were significantly higher in the smokers than in the non-smokers, but the level of IgD remain uncharged [3]. Evidence of smoke responses relationship with lower level of IgG with increased smoking has been presented.[3] Smoking reduced serum level of IgG1 but did not affect the concentration of IgG2 and IgG3. Adnan et al[4] observed that serum levels of IgA and IgG were lower while the levels of IgE and IgM were similar in smokers compared with non-smokers.

Due to proxidant-oxidant imbalance and inflammation in cigarette smokers, the smokers have 14% decreases in serum caeruloplasmin ferroxidase activity.[5] Galdston et al

observed similarity in the levels of transferrin of smokers and non-smokers.[5] In another study, plasma concentration of alpha-2-macroglobulin, alpha-1-antitrypsin and caeruloplasmin were increased in smokers than non-smokers.[6] None of the previous studies reported above was carried out on Nigerian cigarette smokers. The present study estimated the serum levels of immunoglobulin classes (IgG, A and M) and acute phase proteins (alpha-2-macroglobulin, caeruloplasmin and transferrin) in male Nigerian cigarette smokers. This will be the first time that information will be provided about humoral immune status of cigarette smokers in Nigeria.

Materials and Methods

Twenty-seven (27) males aged between 23-64 yrs of age (37.8 + 10.5 yrs) were recruited. Informed consent was obtained before sample collection. They were divided into 2 groups those that smoke cigarette alone (n = 13) and males that do not smoke cigarette or consume alcoholic beverages (n = 14). Exclusion criteria are those that combine both alcoholic beverages and smoke cigarette, those that consume alcoholic beverages, history of malignant diseases, metabolic disorders, and apparent respiratory dysfunction/diseases. The strict selection criteria resulted to low number of subjects. Five milliliters of venous blood was collected aseptically from each subject into plan bottles for the collection of serum after clotting. The serum was stored at -20C till analysed for the concentrations of Ig classes (IgA, G and M) and acute phase protein using single radial immuno-diffusion method (11). The brand of cigarette and the number of sticks consumed per day by smoker and duration of smoking were determined based on the questionnaire given to the participants. The approximate tar and nicotine contents of the different brands of cigarette were obtained from the manufacture's statement on the content of their products. The nicotine and tar inhaled per day by an individual was calculated thus:

Tar inhaled = Tar content on the packet x number of sticks of cigarette smoked per day
Nicotine inhaled = Nicotine content x number of sticks of cigarette smoked\day

Data Analysis: Data were presented as mean and standard deviation. Student t-test was used to test the significance of differences between mean values. Pearson's correlations was used to correlate the levels of IgA, IgG, IgM, transferrin, caeruloplasmin and alpha-2-macroglobulin with tar inhaled per day or nicotine inhaled per day. The probability value (p) greater than 0.05 was considered insignificant.

Results

Based on the information obtained from questionnaires, the average number of stick of cigarettes smoked daily was 10.2 + 2.9, the duration of cigarette smoking was 19.77 +

11.30 years and the mean level of nicotine in cigarette smokers was 15.22 + 7.97mg while the mean level of tar was 185.94 + 97.37mg. The levels of immunoglobulin classes (Table 1) and acute phase proteins (Table 2) were elevated in Nigerian cigarette smokers compared with non-smokers but only IgM and alpha 2-macrglobulin showed significant increases. There were no significant correlations between the levels of immunoglobulin classes or acute phase proteins with duration of smoking of tar inhaled daily or nicotine inhaled daily.

Table 1: The titers of mmunoglobulin classes (mean + SD) in test subjects and control

	Smokers (n=13)	Control (n=14)	t-values	p-values
IgA(g/L)	2.68 ± 3.00	2.14 ± 1.48	0.56	>0.05
IgG(g/L)	28.9 ± 24.4	17.5 ± 8.57	1.59	>0.05
IgM(g/L)	.44 ± 3.16	.1.52 ± 1.88	2.38	<0.05

Table 2: The levels of acute phase proteins (mean + SD) in test subjects and control

	Smokers (n=13)	Control (n=14)	t-values	p-values
A2MG(g/L)	2.14 ± 1.05	2.01 ± 1.48	4.33	<0.05
CLP(g/L)	1.34 ± 1.49	0.87 ± 0.66	1.00	>0.05
TRF(g/L)	3.33 ± 3.57	2.76 ± 1.15	0.54	<0.05

A2MG = alpha-2-macroglobulin,   CLP = Caeruloplasmin,   TRF = Transferrin

Table 3: Pearson's correlation coefficient between the tar inhaled, nicotine inhaled and duration of smoking (DS) with IgA, IgG, IgM, A2MG, CLP and TRF in the smokers

		IgA	IgG	IgM	A2MG	CLP	TRF
Tar	r-values	-0.09	0.02	-0.06	0.02	-0.36	-0.47
(n = 13)	p-values	0.77	0.94	0.81	0.94	0.22	0.11
Nicotine	r-values	-0.09	0.00	-0.08	-0.01	-0.37	-0.46
(n = 13)	p-values	0.77	0.99	0.79	0.99	0.22	0.12
DS	r-values	.27	-0.35	0.12	-0.38	-0.19	-0.05
(n = 13)	p-values	0.37	0.25	0.70	0.20	0.54	0.87

Discussion and conclusion

The complex cellular interactions involving cells of immune system, inflammatory and haematopoietic systems evolved to protect vertebrates from foreign invaders. Cigarette smoke has been shown to affect both the cellular immunity in various ways.[7] It is possible that these effects dependents on the race, duration and intensity of exposure to components of cigarette smoke.

In this present study, mean plasma levels of immunoglobulin classes in smokers were higher compared with controls. This is in contrast to previous studies which showed decreases in the levels of these immunoglobulin classes.[8] Raised level of immunoglobulin classes may be due to polyclonal activation of B cells by components of cigarette. This thereby raises the possibility of auto-immune

diseases and production of auto-antibodies in long time cigarette smokers.

IgA in smokers reflect the response of the immune system to irritation of both the respiratory and gastrointestinal mucosa by cigarette smoke, as IgA has been associated with protection of sero-mucous membranes. IgA protects these membranes against myriads of soluble antigens by inhibiting their adherence to surface of mucosal cells. Thus the presence of tobacco smoke on these membranes results in increase production of this immunoglobulin class A (IgA). Raised levels of IgM and IgG could not be explained by the authors but the presence of sub-clinical infections may be suggested since cigarette smokers have been known to be highly susceptible to opportunistic infection (especially tuberculosis).

Acute phase proteins as components of humoral immunity were also examined in this study. Smoking has been shown to provoke an inflammatory response through generation of enormous amounts of free radicals. Free radicals stimulate the production of acute phase proteins, some of which functions as antioxidants in certain conditions.[1] The immune system responds rapidly by increased synthesis of acute phase proteins to scavenge free radicals. The increased levels of the acute phase proteins especially alpha 2-macroglobulin is consistent with the findings of Patcht and Davis.[9] Also, endotoxins found in cigarettes, increase the levels of IL-1 and TNF-alpha production by macrophages, which are potent stimulators for the synthesis of acute phase proteins and this may account for the observed increases.

Alpha 2–macroglobulin (A2MG) is a large plasma glycoprotein that binds many proteases which are released from damaged tissue as well as from phagocytic cells.[10] Thus significantly raised level of A2MG in cigarette smokers may be one of the attempts by immune system to bind and reduce proteases in cigarette smokers.

Concentration of caeruloplasmin was found to be 12% higher in the plasma of smokers compared with non-smokers.[6] Caeruloplasmin is also known to be linked to the risk of developing cardiovascular diseases.[12] The presence of measurable amount of caeruloplasmin and other acute phase proteins, in human artherosclerotic lesions have been reported.[13] It has been reported that individuals with angiographically documented coronary artery disease are characteristics of elevated IgA levls.[14] The present findings has detected significantly elevated levels of IgM, transferrin and A2MG, but non-significantly raised levels of IgG, IgA and caeruloplasmin in Nigerian Cigarette smokers and this may account for possible auto-immune diseases and cardiovascular diseases in long time heavy smokers.

Acknowledgements

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