

Short Communication

# Differential Effects of Alcoholic Beverages and Cigarette Smoke on Humoral Immunity

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**ABSTRACT:** Cigarette smoking and alcohol consumption are among social practices of some Nigerian youths. These practices have adverse health consequences but the basis of which is yet to be elucidated. This study was designed to provide information on humoral immune responses in Nigerians that smoke cigarettes, consume alcohol or combine cigarette smoking with alcohol use. The serum levels of IgG, IgA, IgM, alpha-2-macroglobulin, caeruloplasmin and transferrin were determined in 13 male Nigerians that smoke cigarettes, 15 male Nigerians that consume alcohol, 16 male Nigerians that combine both cigarette smoking and alcohol use, and 14 sex/age matched controls by immunodiffusion method. The result shows that immunoglobulin classes were increased in all test subjects compared with controls. IgA was significantly increased in subjects that combined cigarette smoking alcohol consumption compared with the controls. IgG and IgM were significantly increased in cigarette smokers compared with the controls while IgG was significantly increased in those that consume only alcohol compared with the controls. All the three acute phase proteins were reduced in subjects that consume alcohol compared with the controls while these acute phase proteins were raised in cigarette smokers compared with controls. In subjects that combine the consumption of alcohol with cigarette smoking, the level of caeruloplasmin and transferrin were raised while alpha-2-macroglobulin was reduced compared with the controls. The study shows that alcohol consumption or cigarette smoking affects different aspects of humoral immunity. Raised levels of immunoglobulin classes in the three groups of test subjects hypothesised the development of auto-immune disease in long term alcohol- and cigarette-users.

**Key Words:** Immune-responses, cigarettes, alcohol, Nigeria.

## INTRODUCTION

Immune responses and resistance to opportunistic infections are affected by tobacco smoke. The precise mechanism(s) by which smoking affects immune responses and the components of cigarette smoke responsible for the effects have not been clarified (Hecht, 2002). In a study carried out by McMillan et al (1997), a decrease in IgM level was observed in cigarette smokers. In addition to this, evidence of lower

levels of IgG with increased smoking has been reported (Mill et al, 1991; McMillian et al., 1997). Adnan et al (2001) observed that serum levels of IgG, IgA, IgE and IgM were significantly lower in smokers compared with non-smokers. Sera of cigarette smokers contained high concentrations of alpha-2-macroglobulin, C3, alpha-1-glycoproteins and caeruloplasmin while transferrin was lower in cigarette smokers (Galdston et al 1987., Tappia et.al, 1995).

One of the characteristics of the immune aberrations in chronic alcoholics is the elevated level of serum immunoglobulins particularly IgG and IgA (Roselle, 1992). Considering that immunoglobulins are produced by cells of B lymphocyte lineage, the elevated immunoglobulin levels in alcoholics indicate B cell dysfunctions. In a murine model of acute alcohol intake, Kawakami et al (1990) showed increased mutagen-induced immunoglobulin production in the alcohol-treated group. While the

functions of B-lymphocytes are impaired in alcoholics, the absolute number of B cells is not different from that in non-alcoholic individuals (Roselle, 1992). Lippi et al (1992) reported that serum levels of mucoproteins, alpha-1-acid glycoprotein, haptoglobin and fibrinogen were significantly increased in alcohol consumers independent of liver damage. In another study, the serum levels of Cu and caeruloplasmin were insignificantly raised in male alcoholics (Wheeler et al, 2001a).

There are several studies on effects of cigarette smoke or alcohol consumption as a major health risk factor in many diseases such as pulmonary and cardiovascular pathologies (Shinton et al., 1989), respiratory infections (Sopori and Kozak, 1998) and impaired immune response (McMillan et al., 1997). However, studies evaluating the levels of immunoglobulins and acute phase proteins as indices of impaired immune response in cigarette smokers and or alcohol users have produced contradicting results. Also, none of the studies examined the combined effects of cigarette smoking and alcohol consumption on immunoglobulin classes and inflammatory responses.

The aim of this study is to measure the levels of three immunoglobulins classes (IgG, IgA and IgM) and acute phase proteins (caeruloplasmin, transferrin and alpha-2 macroglobulin) in cigarette smokers with or without alcohol consumption. This will determine the possible long-term effects of combining alcohol consumption with tobacco smoking on immune status of Nigerian males.

## MATERIALS AND METHODS

A total of 58 males aged between 23-64 yrs of age ( $37.8 \pm 10.5$  yrs) were recruited. Informed consent was obtained before sample collection. They were divided into 4 groups, viz those that smoke cigarette alone (n = 13), males that consume alcoholic beverages alone (n = 15), males that combine alcohol consumption with cigarette smoking (n = 16) and males that neither smoke cigarette nor consume alcoholic beverages (n = 14). The brand of beer commonly consumed are Star, Gulder, 33 export, Guinness stout and Heinekens. The brands of cigarette smoked included Rothmans, Benson and Hedges, London Brown and White, and St Moritz. Exclusion criteria are those with history of malignant diseases, metabolic disorders, and apparent respiratory dysfunction/diseases. Others excluded were those with high parasite densities, abnormal liver functions, pathogenic infections and abnormal renal functions as presented by blood, urine and stool tests as described in a standard text (Cheesbrough, 1991). The strict selection criteria lead to low number of subjects.

Five milliliters of venous blood was collected aseptically from each subject into plain bottles for the collection of serum after clotting. The serum was stored at  $-20^{\circ}\text{C}$  till analysed for the concentrations of Ig classes (IgA, G and M) and acute phase protein using single radial immuno-diffusion method (Arinola et al, 2007).

## RESULTS

The levels of all the three Ig classes were raised in the three groups of test subjects compared with the controls. IgA was significantly raised in subjects that consume alcohol alone or in those that combine cigarette smoking with alcohol use compared with controls. IgM was significantly raised in subjects that consume alcohol or in those that smoke cigarettes compared with the controls (Table 1).

**Table 1:**

The levels of immunoglobulin levels (mean  $\pm$  SD) in test subjects and controls

Subjects	S+A	A	S	C
Number	16	15	13	14
IgA(g/I)	5.26 $\pm 5.87^*$	3.31 $\pm 2.56^*$	2.68 $\pm 3.00$	2.14 $\pm 1.48$
IgG(g/I)	19.67 $\pm 8.46$	21.83 $\pm 16.54$	28.94 $\pm 24.42$	17.53 $\pm 8.57$
IgM(g/I)	2.38 $\pm 0.94$	3.14 $\pm 3.04^*$	3.44 $\pm 3.16^*$	1.52 $\pm 1.18$

\*Statistically different from controls

S+A = Cigarette smokers and alcoholic beverages consumers

A = Those that consume alcoholic beverage alone

S = Cigarette smokers alone

C = Controls

**Table 2:**

The levels of acute phase proteins (mean  $\pm$  SD) in test subjects and controls

Subjects	S+A	A	S	C
Number	16	15	13	14
A2MG(g/I)	1.79 $\pm 0.63^*$	1.49 $\pm 0.35^*$	2.14 $\pm 1.05^*$	2.01 $\pm 0.98$
CLP(g/I)	0.99 $\pm 0.59$	0.78 $\pm 0.52$	1.34 $\pm 1.49$	0.87 $\pm 0.66$
TRF(g/I)	3.00 $\pm 1.42$	2.10 $\pm 0.47^*$	3.33 $\pm 3.57^*$	2.76 $\pm 1.15$

\*Statistically different from controls

S+A = Cigarette smokers and alcoholic beverages consumers

A = Those that consume alcoholic beverage alone

S = Cigarette smokers alone; C = Controls

A2MG = Alpha-2-macroglobulin

CLP = Caeruloplasmin; TRF = Transferrin

The levels of alpha-2-macroglobulin and transferrin were significantly raised in cigarette smokers compared with the controls while the levels of these two acute

phase reactants were significantly reduced in those that consume alcohol alone compared with controls. Alpha-2-macroglobulin was significantly reduced in subjects that combine cigarette smoking and alcohol consumption compared with controls. (Table 2)

## **DISCUSSION**

Few and contradicting reports are documented on effects of either cigarette smoking or alcohol consumption on immune functions. Studies on effects of alcohol and cigarette on immune system are immunosuppression and impaired host defence. (Mc Millan et al, 1997) It has been observed that a high percentage of those who consume alcohol also smoke cigarettes (Anderson et al, 1991). It is therefore necessary to examine the combined effects of both cigarette smoking and alcohol consumption on immune responses.

In this present study, mean plasma IgG, IgA and IgM levels in smokers were higher compared with controls. This is in contrast to previous studies, which showed decreases in levels of these immunoglobulins (McMillian et al., 1997). Mean IgA level was highest in subjects that consume alcoholic beverages and smoke cigarettes compared with the other three groups. This observation reflects the response of the immune system to irritation of both the respiratory and gastrointestinal mucosa by cigarette smoke and alcohol as IgA has been associated with seromucous membranes. IgA protects these membranes against myriads of soluble antigens by inhibiting their adherence to surface of mucosal cells (Salmonu, 2003). Thus the presence of either tobacco smoke or alcohol on these membranes results in increased production of this immunoglobulin. In the other groups, higher mean IgA levels were also observed but these increases were not statistically significant.

The mean plasma IgM levels in smokers show a statistically significant increase when compared with the level in controls. Smokers are highly susceptible to opportunistic bacteria infection (especially tuberculosis) and IgM have been known to provide a defence against bacteremia. (Alberg, 2002) Hence, it is likely that smokers considered for this study were harbouring some opportunistic bacteria, though a bacteriological analysis was not done on them. The higher mean IgG level in test subjects compared with controls also reflects a degree of secondary infection since IgG is the principal antibody in secondary antibody response. (Salimonu, 2003) It may be suggested that continuous exposure to components of cigarette and alcohol have stimulatory effects on immunoglobulin production, thus the increased levels

of immunoglobulins in all test subjects. The possible long term effect of alcohol consumption and cigarette smoking is development of autoimmune diseases due to polyclonal activation of B cells. Meadows et al (1992) in his study observed high levels of these immunoglobulins classes in alcoholics with liver disease. It is possible that the liver damage in our subjects that consume alcoholic beverages is on the onset.

Acute phase proteins as components of humoral immunity were also examined in this present study. Smoking has been shown to provoke an inflammatory response through generation of enormous amounts of free radicals. The immune system responds rapidly by increased synthesis of acute phase proteins to scavenge oxygen radicals generated, minimize injury and repair of inflammatory lesion. The increased levels of the acute phase proteins especially caeruloplasmin is consistent with the findings of Patcht and Davis (1988). Apart from the actions of these free radicals in stimulating production of acute phase proteins, endotoxins found in tobacco smoke have been shown to do the same. These endotoxins increase the levels of IL-1 and TNF- $\alpha$  produced by macrophages, which are potent stimulators of acute phase proteins production and this may account for the observed increases.

The mean levels of acute phase proteins are lower in subjects that consume alcohol alone compared with controls and other test subjects. This observation suggests a decrease production of acute phase proteins as a result of compromised liver function or gradual liver damage. Alternatively, it is possible that the production of IL-1, IL-6 and TNF- $\alpha$  are compromised in Nigerians consuming alcoholic beverages, thus reducing the levels of acute phase proteins in individuals that consume alcoholic beverages compared with controls. The levels of the three acute phase proteins were higher in subjects that combine alcohol use and cigarette smoking compared with those that consume alcohol alone. This is an indication that cigarette stimulates production of acute phase proteins since these proteins are reduced in our subjects that consume only alcoholic beverages.

This study shows that cigarette smoke and alcohol consumption affects different aspects of the humoral immune status, thus provide additional information on reasons for increased susceptibility of either cigarette smokers or consumers of alcoholic beverages to infections.

## **REFERENCES**

**Adnan Y, Esen A, Ferah E, Zuhail K, Ates B, Birol B. (2001).** Evaluation of circulating immune complexes in

- healthy smokers. *Turkish Respiratory Journal* Vol 2, Number 3, pp 32-35.
- Alberg, A. (2002).** The influence of cigarette smoking on circulating concentrations of antioxidant micronutrient. *Toxicology* 15;180(2):121-37
- Arinola O.G and Ezeh C.C. (2007):** C1 inhibitor, C3 activator, IgG, IgA, IgM titers in Nigerian sickle cell patients with *P. falciparum* parasitemia. *Iranian J. Immunol.* 4(1): 44-49.
- Cheesbrough M. (1991).** Medical Laboratory Manual for tropical Countries, Vol. 1, 2<sup>nd</sup> Ed. Trop. Health Tech/Butterworth – Heinemann (ELBS).
- Galdston M., Feldman J.G., Levystka V. and Magnusson B. (1987).** Antioxidant activity of serum caeruloplasmin and transferrin available iron-binding capacity in smokers and non-smokers. *Am Rev Respir Dis* 135(4):783-7.
- Hecht S.S. (2002).** Cigarette smoking and Lung cancer: chemical mechanisms and approaches to prevention. *Lancet Oncol* 3: 461-469
- Kawakami M., Meyer A.A. DeSerres S. and Peterson H.D. (1990).** Effects of acute ethanol ingestion and burn injury on serum immunoglobulin. *Journal of Burn Care and Rehabilitation* 11: 395-399.
- Lippi G., Fedi S., Grassi M., Rosi E., Liotta A.A., Fontanelli A., Cellai A.P., Mazzanti R. (1992).** Acute phase proteins in alcoholics with or without liver injury. *Ital J.Gastroenterol* 24(7):383-5.
- Meadows G.G., Wallendal M., Kosugl A., Wunderlich J. and Singer D.S. (1992).** Ethanol induces marked changes in lymphocyte populations and NK cell activity in mice. *Alcoholism: Clinical and experimental research* 16, 474-479.
- McMillian S.A. Douglas J.P, Archbold G.P., McCrum E.E. and Evans A.E. (1997).** Effects of low to moderate levels of smoking and alcohol consumption on serum immunoglobulin concentration. *J.Clin Pathol* 50(10):819-22.
- Flanders WO, Boring J.R. Annest D.L. and Destefand F. (1991).** The association of race, cigarette smoking and smoking cessation to measures of the immune system in middle aged men. *Clin immunopathol* 59:187-200.
- Pacht E.R. and Davis W.B. (1988).** Decreased caeruloplasmin ferroxidase activity in cigarette smokers. *J.Lab Clin Med* 111(6):661-8.
- Roselle G., Mendenhall C.L. Grossman C.J. (1993).** Effects of alcohol on immunity and cancer. In *Alcohol, Immunity and Cancer*. Yirmiya, R. and Taylor, A.N. eds, pp. 3-21. CRC Press, Boca Raton, FL.
- Salimonu L.S., Mohammed I, Oyeyinka G.O., Arinola O.G. (2003).** Basic Immunology for students of Medicine and Biology. 2<sup>nd</sup> Edition College Press, Nigeria
- Shinton R. and Beeven G. (1989).** Meta analysis of relation between cigarette smoking and stroke. *BMJ* 298:789-794.
- Sopori M.L. and Kozak W. (1998).** Immunomodulatory effects of cigarette smoke. *J Neuro Immunol* 83(1-2):148-56.
- Tappia P.S., Troughton K.L., Langley-Evans S.C., Grimble R.F. (1995).** Cigarette smoking influences cytokine production and antioxidant defenses. *Clin Sci (Lond)* 88(4):485-9.