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THE CONCENTRATIONS OF METHAEMOGLOBIN, CARBOXYHAEMOGLOBIN AND SOME HAEMATOLOGICAL PARAMETERS IN TOBACCO SNUFF ADDICTS IN IGBO OF NIGERIA

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Summary: Methaemoglobin, carboxyhaemoglobin concentrations and some haematological parameters were studied in fifty tobacco snuff addicts (40 males and 10 females) in some villages of Anambra State, Nigeria. The aim was to investigate possible adverse effects of tobacco snuff in addicts in Igbos of Anambra State. Fifty apparently healthy persons (25 males and 25 females) who do not inhale snuff were used as controls. The age range of tests and control subjects was 25-65 years. The results showed no statistically significant difference when the tests group was compared with the control group. A comparison of the results on the basis of sex, age and period of exposure, showed no significant differences. Blood picture in test and control groups was normocytic and normochromic. The results suggest that tobacco snuff may not have any adverse effect on haemoglobin metabolism and erythropoiesis.

Key Words: *Methaemoglobin, carboxyhaemoglobin, tobacco snuff and haematological parameters.*

Introduction

Methaemoglobin (Hi) is the form of haemaglobin in which the iron of the haemoglobin has been oxidized to the ferric state. It cannot bind and therefore does not transport oxygen. The production of methaemoglobin occurs at a slow but steady rate in the red cells. This is caused mainly by the fact that while oxygen is being transported, it gains an electron from the iron and is transported in the superoxide anion form whereas the iron is in the ferric state. Upon normal deoxygenation, the electron of the superoxide is given back to the iron so that molecular oxygen is released and haemoglobin remains in the functional state. However, this is not perfect and consequently a small proportion of the superoxide molecules are released which causes formation of methaemoglobin (Brewer and Prassad 1993). It is continuously reduced almost entirely by the reduced nicotine adenine dinucleotide (NADH) diphorase system (Coleman and Colernan 1996). About 1-3% of haemoglobin is converted into methaemoglobin on daily basis and without an effective reducing system, the erythrocycte becomes non-functional. Methaemoglobinaemia is a feature of unstable haemoglobins, congenital deficiency of NADH methaemoglobin reductase as well as exposure to oxidant drugs and chemicals (Beutler 1993).

Carboxyhaemoglobin (COHb) is a product of chemical combination between carbon monoxide and haemoglobin. Carbon monoxide is one of the most common toxins encountered in work settings: the gas being emitted in situations where there is incomplete combustion of carbon-containing substances. The body burden due to inhalation of carbon monoxide is measured by an individual's blood carboxyhaemogblobin level (Wickramatilake 1999). It does not transport oxygen and toxicity results from generalized anoxia. Carbon monoxide poisoning leads to a decrease in amount of oxygen transported by haemoglobin. Thus not only does the amount of oxygen not released for cellular respiration, the affinity of oxygen for haemoglobin is reduced. The affinity of carbon monoxide for haemoglobin is about 200 times as that of oxygen and as a result, it is not displaced from haemoglobin except at high oxygen tension. Accidental poisoning can occur even at low levels of the gas in the atmosphere with prolonged exposure and this can lead to cell death through apoptosis (Varon *et al* 1999).

Haematological parameters like packed cell volume PCV haemoglobin (Hb) and

reticulocytes are non-specific indicators of effective erythropoesis and erythrocyte functional viability. (Gilmer & Koepke 1976). They are affected to different degrees in some haematological/metabolic diseases and hence have become immensely useful in diagnosis, progrnosis and monitoring of treatment (Onwukeme 1993).

Tobacco is the dried and processed leaves of the plant Nicotine *Tobacum Linn* which is widely cultivated in America and some West African countries. It is yellowish brown in colour with a strong odor and bitter faste. The principal content of tobacco is nicotine. It has no use in Medicine though it is of value chiefly as an insecticide (Feyerabend and Russell 1979). Tobacco snuff is in powered form with potash and sweeteners as the main additives. In Igbo communities of Nigerian where it is utilized for cultural and traditional purposes, it is either inhaled through the nose or applied orally. Some addicts also chew the dried leaves. Nicotine has been associated with addiction in regular smokers and snuffers. Cryer *et al* (1976) stated that in small doses, nicotine has a stimulatory effect on the autonomic nervous system which causes raised blood pressure and pulse rates.

In view of the various pharmacological actions of nicotine, additives which are constituents of tobacco snuff and the wide use in many communities of Igbo tribe and possibly other communities; it is possible that haemoglobin the principal pigment in erythrocytes may be affected particularly by peroxidation. This can cause respiratory distress, which may be aggravated in respiratory disorders. This study was designed to investigate possible adverse effects of tobacco snuff on haemoglobin, its conversion products and some haematologiccal parameters.

Subjects and Methods

The subjects were drawn from fifty-tobacco-snuff addicts (40 males, 10 females) from some Igbo communities of Anambra State of Nigeria. Addiction was defined in this study as consistent and regular inhalation of tobacco snuff for a period ranging from 5 – 30 years. The controls consisted of fifty apparently healthy persons (25 males. 25 females) who do not take or have never inhaled tobacco snuff. The age range of both tests and control subjects was 25-65 years. Whole blood was collected from both test and control subjects after informed consent into EDTA specimen bottles for determination of methaemoglobin, haemoglobin concentration, reticulocyte count and blood picture. The method of Evelyn and, Malloy (1938) was used for methaemoglobin determination, while the method of Van Assendelft (1970) was adopted for carboxyhaemoglobin, Drabkin and Austin method (1932) was used for haemoglobin while the method of Bain (1995) was adopted for reticulocyte count and blood picture respectively. The mean results and standards deviation (SD) were compared using students “t” test.

Table 1: *Mean results of Hi, COHb, Hb, PCV and retics and sex comparison in test and control groups*.

Subjects:	Hi± SD %	COHi ±SD%	Hb±SDg/dI	PCV±SDL/L	Retics±SD%
Male N=40	1.5.5±0.29	0.98±0.28	1.3.5±1.30	0.40±02	0.60±0.20
Control					
Male n=25	1.52 ±0.24	0.96±0.28	1.3.7±1.10	0.39±0.03	0.72±0.26
P-value	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05
Females					
N=10	1.50±0.25	0.92±0.18	12.10±1.10	0.35±0.02	0.62±0.22
Controls					
N= 25	1.52±0.24	0.89±0.20	11.9±1.10	0.31±1.10	0.72±0.26
P-value	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05

Results

The mean values recorded in this study when compared with controls showed no significant differences (P>0.05) in all parameters measured. When compared on the basis of exposure/addiction period (5 - 20 and 21 years and above) sex and age, there were no significant differences. The blood picture of both test and control groups were all normochronic and normocytic. It also showed normal platelet morphology and number while the leucocytes appeared normal qualitatively and quantitatively in almost all test subjects. However a few cases showed leucocytosis and eosinophilia. The summary of results in test and control groups and comparisons on age and exposure are presented in Tables 1 and 2.

Table 2: *Mean Results of Age, and Exposure Basis*

Subjects: Age.	Hi±SD %	COHI ±SD %	Hb±SDg/dL %	PCV±SD L/L	Retics±SD %
Age (yrs.)					
25 –40 yr N=15	1.45±0.30	0.94±0.22	12.6±1.0	0.37±0.03	0.58±0.30
Control N=10	1.43±0.26	0.95±0.20	13.6±10	0.37±0.03	0.58±0.30
P-value	>0.05	>0.05	>0.05	>0.05	>0.05
41 – 65yrs. N=35	1.57±0.35	0.98±0.25	12.7±1.10	0.39±0.03	0.65±0.26
Control N=30	1.55±0.36	0.97±0.23	13.0±1.0	0.40 ±.0.28	0.67±0.28
Exposure (yrs)					
5-20 N=13	1.50±0.35	0.96±0.28	11.9±1.0	0.39±0.29	0.59±0.30
21 - 30 N=37	1.53±0.30	0.97±0.25	12.1±1.0	0.39±0.02	0.60±0.26
Control N=25	1.51±0.30	0.96±0.23	12.3 ±1.0	0.37 ±0.02	0.58±0.26
[P-value	NS	NS	NS	NS	NS

Discussion

Some diseases have long been associated with habits, culture and environments (Feyisitan *et al* 1997) Tobacco in particular has been linked to cancer of the mouth and alimentary canal (Sanghi *et al* 1955) The results of methaemoglobin and carboxyhaemoglobin obtained in this study did not suggest any adverse effects on the functional integrity of haemoglobin. There is an excellent system for the reduction of methaemoglobin to haemoglobin, which involves methaemoglobin reductase and NADH. It is probable that this process was effective and unaffected in subjects studied. It is only when the system is overwhelmed that methaemoglobinaemia and oxidant stress result. In general xenobiotics have been shown to induce methaemoglobinaemia, Heinz bodies and haemolysis (Carrell *et al* 1975) In another study, Hullinquist *et al* (1997) reported that methaemoglobin is one of the markers of oxygen free radical production during haemolysis. There seems to be paucity of data with regard to methaemoglobin concentration in snuffers to facilitate comparison. The results are suggestive of no adverse effects on haemoglobin metabolism.

Nicotine, an alkaloid contained in tobacco is absorbed in the oral or nasal cavity of humans. Russell *et al* (1980) reported that blood of tobacco smokers and snuffers contained significant concentration of nicotine. The authors also reported significant value for carboxyhaemoglobin in smokers and non-significant value in snuffers. The result of carboxyhaemoglobin recorded in this study agrees with the author already cited. This may be due to the fact that carbon monoxide is a product of incomplete combustion, which is not a feature of snuffing.

Oxygen free radicals and reactive oxygen species are entities that have been linked to peroxidation of biomolecules. (Michaelson 1986). The results of this study also suggest that these highly reactive entities might not have been generated in increased amounts in the subjects studied since methaemoglobin, an oxidation product of haemoglobin was not increased in test subjects. It can also be implied that the total antioxidant status (TAS) was effective qualitatively and quantitatively. A reduction in levels of plasma vit C and E and increased activity of erythrocyte superoxide dismutase and lipoperoxides have been reported in cigarette smokers (Zhou *et al*, 1997). In general total antioxidant status has been reported to be low in many diseases (Lantos, 1997). The statistically insignificant difference obtained in this study was supported by effective erythropoesis as indicated by the results of haematological parameters (PCV, Hb, reticulocyte and blood picture). This was strengthened by data obtained when results were compared on basis of sex, age and addiction periods. The implication is that tobacco snuff may not adversely affect the measured parameters in addicts even with long period of addiction. However lower values of some parameters have been reported in smokers (Isagar and Hagerup 1971, Galea and Davidson 1985) The leucocytosis and eosinophilia that were observed in a few subjects blood picture may be due to underlying bacterial and parasitic infection. The study showed that snuffing is more prevalent amongst men (80%). However

the absence of adverse effect was the same in both sexes while the age group of 41-65 years recorded the highest prevalence (70%). Carbon monoxide poisoning and increased carboxyhaemoglobin shave been reported to be a common feature in chronic cigarette smokers warranting the use of hyperbaric oxygen for clinical management (Weaver 1999). Although carbon monoxide poisoning is unlikely in snuff addiction considering the fact that there is no combustion, it is still necessary to determine its level in case the contents of snuff particularly the additives affect haemoglobin function. Moreover, tobacco snuffing is common among many Igbo communities in Nigeria and it does not appear as if similar study has been conducted in this area.

Conclusion

Although cancer of the mouth and alimentary canal have been reported in chronic tobacco snuffers and chewers, the results of this study do not suggest any adverse effect on oxygenation function of haemoglobin and erythropoesis. The study has not concluded that snuffing is totally wholesome. It is probable that methaemoglobinaemia and carbon monoxide poisoning which have been documented in chronic cigarette smokers, may not obtain in snuffing. It is concluded that tobacco snuffing does not affect the haematolpgical parameters studied.

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