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Human Salivary Thiocyanate Level of Adult Volunteers in Sokoto, Nigeria

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ABSTRACT

Salivary thiocyanate levels of 379 volunteers aged 18-55 years were estimated spectrophotometrically. The results obtained were analyzed on the basis of sex, smoking, kola nut eating and gari eating habits. The levels of salivary thiocyanate levels of smokers, smokers who eat gari, smoker who eat kola nut and smokers who eat gari and kola nut are 3.61±2.07mM, 3.27±1.96mM, 4.19±2.15mM and 4.18±2.39mM respectively. These values are significantly higher than those of non-smokers who, eats kola nut (1.35±1.05mM), eat gari (1.49±1.09mM), eat gari and kola nut (1.64±1.02mM) and neither eat gari nor kola nut (1.30±0.93mM). The values obtained were significantly higher for females. Significant percentage of smokers (70-80%) had salivary thiocyanate level >2.0mmol/l. Only about 20% of non-smokers had salivary thiocyanate levels >2.0mmol/l. Analysis of the results did not show any synergistic effect of these habits on salivary thiocyanate of the subjects combining these habits. No correlation was also found between salivary thiocyanate level and age. Cyanide exposure may therefore be another risk factor in tobacco smoking.

INTRODUCTION

Many plants and plant products contain cyanogenic glycosides. When hydrolyzed, either enzymatically or non-enzymatically, cyanogenic glycosides yield hydrogen cyanide (HCN), a sugar and a ketone or aldehyde (Conn, 1981). Plants containing cyanogenic glycosides include cassava, linseed, beans and peas, which are known to contain linamarin co-existing with it methyl derivative lotaustralin. Millet, sorghum, tropical grass and maize are sources of dhurin. Amygdalin is found in plums, cherries, pears, apple and apricots. These compounds are also reported to be present in plants such as rice, unripe sugar cane, several species of nuts and certain species of yam (Osuntokun, 1981; Oke, 1979). Significant number of these plants and their products are staple foods, especially in the tropics. One other source of cyanide is tobacco smoke (Osuntokun, 198l).

Cyanide, one of the products of hydrolysis of cyanogenic glycosides is a potent cytotoxic agent that kills the cell by inhibiting cytochrome oxidase of the electron transport chain (Okoye, 1992; Kouichiro *et al,* 2000). The cell dies as a result of energy deprivation.

Cyanide is introduced into human system through a number of ways. It could be through inhalation of hydrogen cyanide gas produced in the process of pyrolysis or combustion of nitrogen containing compounds, through ingestion of cyanide salt for the purpose of homicide or suicide, or ingestion of cyanogenic glycosides in meals especially in the tropics (Kouichiro *et al,* 2000).

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When ingested as cyanogenic glycoside, it is hydrolyzed producing cyanide, the ultimate cyanogen. This process is initiated only when the plant material containing them is crushed or otherwise disrupted. In addition to its toxicity related to the inhibition of cytochrome oxidase, it is also implicated in the aetiology of Tropical Ataxic Neuropathy (Osuntokun, 198l).

The ingestion of sub-lethal doses of cyanide activates the body own mechanisms of detoxification, that ensures the transformation of cyanide into a less toxic substance, thiocyanate (SCN). The principal detoxification pathway is that catalyzed by rhodanese. Rhodanese catalyses the transfer of sulphur atom, obtained from a suitable donor, thiosulphate, to a nucleophilic acceptor, cyanide (Bourdoux *et al,* 1980). Thiocyanate is excreted from the body mainly through the kidney (Bourdoux *et al,* 1980) but also through saliva and other body fluids (Osuntokun, 1981). The physiological role of salivary thiocyanate may be the antibacterial effect of the hypothiocyanate, which is produced by the action of salivary peroxidases from thiocyanate (Kouichiro *et al,* 2000).

The determination of toxic levels of blood cyanide (>5μmol/l) is important in the diagnosis of acute cyanide intoxication. Toxic levels of both blood cyanide and serum thiocyanate are important in the diagnosis of chronic exposure to cyanide in form of hydrogen cyanide gas or as cyanogenic glycoside in the diet. Estimation of physiological levels of both salivary and serum levels of thiocyanate is used to differentiate between smokers and non-smokers (Kouichiro *et al,* 2000).

Diets of the people in the study area is prepared from foodstuffs that contain cyanogenic glycosides. In addition, a sizeable number of them smoke tobacco.

There is dearth of information on salivary thiocyanate level of subjects in the study area. This work was therefore aimed at estimating salivary thiocyanate level of the population in Sokoto, Nigeria, since it is a good index for estimating the level of chronic exposure to cyanide. The results obtained were analyzed on the basis of smoking status, gari (fried, fermented cassava product) consumption, and kola nut eating habit and on the basis of gender. The effect of age on salivary output was also analyzed.

MATERIALS AND **METHODS**

All the chemicals and reagents used in this work were of analytical grade and purchased from BDH England.

Human Subjects

A total of 379 apparently healthy human subjects, aged 18 to 55 years, were recruited for this research. Sixty eight percent (68%) of them were male. The volunteers were asked to fill in questionnaire providing information about their age and sex, and whether or not they smoke, take kola nut, and eat gari.

The subjects were classified into 8 groups and each group further subdivided into two on the basis of gender. The groups were:

1. Smoker: Subjects in this group were smokers of tobacco who eat neither gari nor kola nut.
2. Kola nut: They are those that take kola nut but neither smoke tobacco nor eat gari
3. Gari: Those in this group neither eat kola nut nor smoke but take gari.
4. Smoking + kola nut: Those who smoke and eat kola nut but do not eat gari were placed in this group.
5. Smoking + gari: members of this group do not take kola nut but smoke and eat gari.
6. Kola nut + gari: This group consisted of those who take kola nut and eat gari but do not smoke.
7. Smoking + kola nut + gari: Members of this group smoke, eat gari and kola nut.
8. Control: Subjects belonging to this group do not smoke, eat gari or eat kola nut.

None of the groups were less than 30 subjects. In no group were the females less than 15% of the group total.

Sample Collection

About 5ml of saliva was collected from each of the subject by continuous spiting into plastic sample bottles, corked, transferred to the laboratory and stored in a refrigerator at +4^{0}C. Thiocyanate was estimated from the samples within two days of collection.

Preparation of Samples for Analyses

The samples were transferred into centrifuge tubes and centrifuged at 4,000rpm for 5 minutes in a bench top centrifuge. The supernatant of salivary samples was collected into clean labeled tubes for analysis of SCN.

Estimation of the Concentration of** SCN **in Saliva

Into clean labelled test tubes were placed 0.2ml samples and 4.8ml ferric nitrate reagent. The resulting solution was vortexed and the absorbance read spectrophotometrically against a blank at 447nm using a computer-based spectrophotometer (Pharmacia LKB Biochrom 4060 model). The blank contained distilled water instead of saliva sample. The concentration of SCN in the samples was calculated using the molar extinction coefficient of $FeSCN^{2+}$ (calculated to be 3.11 x10^3 $cm^{-1}M^{-1}$) according to the Beer-Lambert's law. The $FeSCN^{2+}$ is formed according to the reaction:

$$Fe^{3-}(aq) + SCN(aq) \rightleftharpoons FeSCN^{2+}(aq)$$

Statistical Analysis

All the results were expressed as mean ± standard deviation. Statistical difference between the various groups (at 5% level of significance) was determined using student's **t**-test. The level of SCN and age of subjects were correlated using linear regression. The result for each group was presented as frequency distribution table.

RESULTS AND DISCUSSION

The mean human salivary thiocyanate of the subjects studied is presented in Table 1.

Cyanide is a potential cytotoxic agent, whose toxicity is caused by its reaction with iron of haemoglobin (and possibly cytochrome) and copper of the cytochrome oxidase of the electron transport chain (Oke, 1979; Kouichiro *et al,* 2000). These significantly affect the functions of these molecules as oxygen carrier and oxidative enzyme respectively. The ultimate implication of these is that it poisoned and killed the cell by hypoxia.

As indicated in Table 1, the results of the current study showed a significant difference between the males and females in most of the groups with respect to the salivary thiocyanate output. It could be that, though the subjects of both sexes may be subjected to the same cyanide load, their ability to detoxified and disposed off the cyanide may differ. This may be partly due to differences in the activity (or absolute amount) of the detoxifying enzymes: rhodanese and β-mercaptopyruvate sulphur transferase. Measurement of rhodanese activity in human erythrocytes indicated that the mean control value for males is less than that of females (Vazquez *et al,* 1987).

Thiocyanate is derived endogenously as a detoxification product of the reaction between cyanide and thiosulphate/β-mercaptopyruvate catalyzed by rhodanese/β-mercaptopyruvate sulphur transferase.

Table 1. Mean Human Salivary Thiocyanate (mM) of Apparently Healthy Subjects on the Basis of Gender and Specific Habits

Habit	Male	Female	Total
Smoking	3.22±2.07^{a}	4.72±1.47^{b}	3.61±2.07^{a}
Gari	1.47±1.12^{c}	1.57±l.07^{c}	1.49±1.09^{c}
Kolanut	1.31±1.13^{c}	1.45±0.86^{c}	1.35±1.05^{c}
Smoking+kolanut	3.18±l.81^{a}	4.92±1.96^{b}	4.19±2.15^{ba}
Smoking+gari	3.16±1.89^{a}	3.89±2.4^{a}	3.27+1.96^{a}
Kolanut+gari	1.49±0.85^{c}	1.84±1.20^{c}	1.64±1.02^{c}
Smoking+ kola nut+ gari	3.78±1.93 ^{a}	4.91±3.03^{b}	4.18±2.39^{b}
Control	1.31±0.92^{c}	1.34±1.01c	l.30±0.93^{c}

a,b,c: values bearing different superscript horizontally and vertically differ significantly

Rhodanese is predominantly a liver mitochondrial enzyme (Saidu *et al,* 2000: Alqarawi *et al,* 2001). The reaction catalyzed by this enzyme is according to the general equation:

$$S_2O_3^{2-} + CN \xrightarrow{\text{Rhodanese}} SCN + SO_3^{2-}$$

Endogenously the major sources of sulphur used for the sulphuration reaction of cyanide are sulphur containing amino acids. One of the sulphur containing amino acids found in protein, methionine, is a nutritionally essential amino acid. Increased CN load may diminish methionine level thereby placing additional demand for this amino acid. This may explain why supplementation with methionine help ameliorate tropical ataxic neuropathy, a neurological condition characterized by lesions of the optic, auditory and peripheral nerves, common in the tropical countries where cassava and cassava products are dietary staples (Osuntokun, 1981). In a work to assess the exposure to cyanide intoxication in Nigerian patients suffering from tropical neuropathy, Osuntokun (1981) reported that sulphur containing amino acids were completely undetectable in the plasma of 60% of his patients and greatly reduced in the rest.

Table 1 also indicated that smoking significantly ($P<0.05$) increase the level of salivary thiocyanate compared to the value obtained for non-smoker. The concentration of salivary thiocyanate varies widely and depends on dietary and smoking habits. Earlier report (Markku *et al,* 1999) indicated that normal non-smokers usually have salivary SCN^- concentrations of 0.5-2mM; while smokers may have salivary [SCN^-] of up to 6mM. In the current work, mean salivary thiocyanate concentration of 3.61±2.07mM was obtained for smokers as against 1.30±0.93mM obtained for non-smokers (control subjects). Concentration of cyanide in tobacco and cigarette smoke of 500-1,600ppm has been reported (Osuntokun, 1981; Osborne *et al,* 1956). Cyanide is incorporated into human body through a number of ways, one of which is the inhalation of hydrogen cyanide gas produced in the process of pyrolysis or combustion of nitrogen containing compounds (Kouichiro *et al,* 2000). This is the most probable way in which cyanide in tobacco may get into human body.

The determination of fatal levels of blood cyanide (over 5μM) is important in the diagnosis of acute cyanide intoxication. Toxic plasma levels of both cyanide and thiocyanate are important in the diagnosis of chronic cyanide intoxication as found during hydrogen cyanide gas exposure in industries, involving habitual intake of cyanogenic glycosides in diets and in the diagnosis related to tobacco amblyopias (Kouichiro *et al,* 2000). Determination of physiological levels of plasma and salivary SCN is use to distinguished between smokers (Kouichiro *et al,* 2000; Markku *et al,* 1999; Pechacek *et al*, 1984; Callas *et al*, 1989; Olson *et al*, 1985).

The frequency distributions of salivary SCN^- levels of the subjects based on specific habits are presented in Table 2.

Kola nut consumption in the current study did not influence the salivary SCN^- level of the subjects. It could be possible that the concentration of cyanide containing compounds of kola nut may be so low that their metabolism did not affect the salivary out put of thiocyanate. This may explain why eighty percent (80%) of the subjects that consumed kola nut had salivary thiocyanate level less than or equal to 2.0mM.

Consumption of gari, a fermented product of cassava, did not affect the salivary thiocyanate level significantly ($P<0.05$) in the current work. Analysis of the results indicated that the majority of the subjects that take gari have salivary SCN level less than 2.0mmol/l (Table 2). This is similar to the result obtained for the control subjects. Nigerians use cassava for food in several ways. The commonest cassava product used for food through out the country is gari. Though fresh cassava root may contain up to 160mg% HCN, significant amount of this is lost during fermentation and frying (Osuntokun, 1981; Oke, 1979). As a result the concentration of HCN in gain could be as low as 1.1mg% (Oke, 1979). Thus the consumption of gari may not influence the cyanide load significantly and may explain the observed non-significant effect of gari consumption on the salivary thiocyanate level in the current work. It was reported that supplementing poultry feeds with 20% cassava diet did not affect rhodanese activity of developing *Gallus domesticus* (Saidu *et al*, 2000). In a related report, a diet of 50% gari was found not to cause any significant biochemical or haematological changes in adult female rats (Olusi *et al*, 1979).

Synergism is a concept that may influence metabolism of some compounds. The combined effect of smoking and kola nut eating; smoking and gari eating; kola nut and gari consumption; and smoking, gari and kola nut eating on the salivary SCN^- excretion were studied and frequency distribution tables are presented in Table 2. The results showed that the majority (76%) of the smokers who take kola nut had salivary SCN^- levels greater than 2.0mM. The result of smokers who take gari is similar to that of smokers who take kola nut. Majority of the subjects that eats gari and kola nut (78%) had salivary thiocyanate level less than 2.0mM. Eighty four percent (84%) of the subjects that smoke and consumed both gari and kola nut had salivary level of thiocyanate greater than 2.0mmol/l. Analysis of the results therefore did not show any synergism with any of the parameter studied. This observation further implicated tobacco as the sole factor that influences salivary SCN^- excretion of humans in the study area.

The current work also studied the effect of age on salivary excretion of SCN^- and found no significant ($P>0.05$) correlation between these parameters in all cases except two. This observation is similar to the report of Kouichiro and co-workers (2000). These researchers reported that no correlation was observed between age and blood cyanide or serum thiocyanate in both smokers and non-smokers. An expected positive correlation was however found between blood cyanide and salivary thiocyanate (Kouichiro *et al*, 2000).

Table 2. Frequency distribution of human salivary thiocyanate levels of subjects with specific habits

Habit	Male		Female		Total	
	<u><</u>2mM	>2mM	<u><</u>2mM	>2mM	<u><</u>2mM	>2mM
Smoking	9(33%)	18(66.7%)	2(15.4%)	11(84.6%)	11(27.5%)	29(72.5%)
Kola nut	22(88.0%)	3(18.0%)	6(60%)	4(40%)	28(80%)	7(20%)
Gari	40(72.7%)	15(27.3%)	21(75%)	7(25%)	61(73.5%)	22(26.5%)
Smoking + Gari	10(32.3%)	21(67.7%)	2(33.3%)	4(66.7%)	12(32.4%)	25(67.6%)
Smoking + Kola nut	4(33.3%)	8(66.7%)	6(20.7%)	23(79.3%)	10(24.4%)	31(75.6%)
Gari + Kola nut	28(82%)	6(17.6%)	18(72%)	7(28%)	46(78%)	13(22%)'
S + K + G	4(20%)	16(80%)	1(8.3%)	11(91.7%)	5(15.6%)	27(84.4%)
Control	42(77.8%)	12(22.2%)	9(90%)	1(10%)	51(79.7%)	**13(20.3%)**

REFERENCES

Alqarawi, A. A., Mousa, H. M. and Ali, B. H. (2001). Tissue and Intracellular Distribution of Rhodanese and Mercaptopyruvate Sulphur Transferase in Ruminants and Birds; *Vet. Res.* 32:63-70.

Bourdoux, P., Manita, M., Hanson, A., and Ermans, A. M. (1980). Cassava Toxicology: the role of linamarin; <u>In</u>: 'Role of Cassava in the Etiology of Endemic Goitre and Cretinism; IDRC, Ottawa, Canada.

Conn, E. E. (1981). Cyanogenic Glycosides; Biochemistry of Plants, 7:479-500.

Callas, P. W., Haugh, L. D. and Flynn, B. S. (1989). Effect of Long Term Storage on Salivary Thiocyanate Concentration. Addictive Behaviors 14:643-648.

Kouichiro, T., Kataoka, M., and Seto, Y. (2000). Cyanide and Thiocyanate Levels in Blood and Saliva of Healthy Adult Volunteers; *J.f Health Sci.* 46(5):343-350.

Markku, L., Juhani, V. and Jan, H. (1999). Spectrophotometric Determination of Thiocyanate in Human Saliva; *J. Chemical Edu.* 76(9):1281-1282.

Oke, O. L. (1979). Some Aspects of the Role of Cyanogenic Glycosides in Nutrition, *Wld Rev. Nutr. Diet.* 33:70-103.

Okoye, Z. S. C. (1992). Biochemical Aspect of Nutrition; Prentice-Hall of India, New Delhi; 118-123.

Osborne, J. S., Adamek, S., and Hobb, M. E. (1956). Some Components of Gas Phase of Cigarette Smoke; *Analyt. Chem.* **28:**211-215.

Olson, B. L. , McDonald, J. L. Jr., Gleson, M. J., Stookey, O. K., Schemehorn, B. R., Drook, C. A., Beiswanger, B. B. and Christen, A. G. (1985). Comparisons of Various Salivary Parameters in Smokers Before and After the Use of a Nicotine-Containing Chewing Gum. *J. Dent. Res.* **64(5):**826-830.

Olusi, S. 0., Oke, O. L., and Odusote, A. (1979). Effect of Cyanogenic Agents on Reproduction and Neonatal Development in Rat. *Biol.* Neonate; **36(5/6):233**-243.

Osuntokun, B. O. (1981); Cassava Diet, Chronic Cyanide Intoxication and Neuropathy in Nigerian Africans; *Wld Rev. Nutr. Diet.* 36:141-173.

Pechacek, T. F., Box, B. H., Murray, D. M., Luepker, R. V. (1984). Review of Techniques for Measurement of Smoking Behavior: <u>In</u>: Behavioral Health: A hand Book of Health Enhancement and Disease Prevention (Mataarazzo, J. D., Weiss, S. M., Herd, J. A., Miller, N. E. and Weiss, S. M. Eds.). Wiley, New York 729-754.

Saidu, Y., Bilbis, L. S., Sahabi, D. M., and Wasagu, R. S. (2000). Comparative Rhodanese Activity in Some tissues of a Day Old and 12Weeks Old Domestic Fowl(*Gallus domesticus*). *Nig. J. Biochem. Mol. Biol.* **15(2):** 173-174.

Saidu, Y., Temple, V. J., Sahabi, D. M., and Wasagu, R. S. (2000). Rhodanese Activity Profile in Some tissues of Developing Domestic Fowl(*Gallüs domesticus*) Fed 20% Cassava Supplemented Poultry Feeds,' *Nig. J. Biochem. Mol. Bio.* 15(1):

Vazquez, E., Buzaleh, A. M., Wider, E. and Alcira, M. D. C. B. (1987). Red Blood Cell Rhodanese: Its Possible Role in Modulating 8-amino LevulinateSynthetase Activity in Mammals; *Int. J. Biochem.* 19(2):217-219.