Metal Binding Proteins and Immunoglobulin Classes in the serum of Nigerian Cassava Processors

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Abstract: Cassava processing is an economically viable venture in tropical Africa where cassava is consumed in various forms, but cyanogenic glycosides that are contacted during cassava processing is known to have tremendous effects on nervous system but the effect of cyanogenic glycosides on immune functions of cassava processors is largely unknown. The objective of this study is to find out if aspects of humoral immune system are affected in cassava processors. This was achieved by measuring the levels of three immunoglobulin classes (IgG, IgA and IgM) and metal binding proteins (Transferrin, Caeruloplasmin, Alpha-2- Macroglobulin and Haptoglobin) in Nigerian cassava processors using principle of single radial immunodiffusion in immunoplates. The result shows that only the mean serum level of IgM was significantly increased in cassava processors compared with the controls (P=0.02). There were no significant changes in the mean levels of IgA, IgG, alpha-2 macroglobulin, caeruloplasmin and transferrin in cassava processors compared with the controls. It is the opinion of the authors that cassava processing has no tremendous adverse effect on some aspects of humoral immunity of cassava processors. [Nature and Science 2010;8(11):27-34]. (ISSN: 1545-0740).

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Introduction

Cassava *(Manihot esculenta crantz)* is a perennial herbaceous shrub which is extensively cultivated as an annual crop in tropical and subtropical regions for its edible starchy tuberous root (a major source of carbohydrates (Nwokoro et al, 2005). Nigeria is the world's largest producer of cassava and has been reported that Africa produced 72.7 million of the world's 158.1 million tuber tones (FAO/WHO. 1991).

Cassava contains potentially toxic compounds called cyanogenic glucosides (primarily Linamarin and small amount of Lotaustralin), oxalic acid and acetone. Therefore the food value of cassava is greatly compromised by the endogenous presence of these cyanogenic glucosides. The glucosides are hydrolyzed to hydrocyanic acid (HCN) by endogenous linamarase (a naturally occurring enzyme in cassava) when cassava tissues are disrupted by cutting, grafting, bruising or by other mechanical means (Bradbury et al, 1991). HCN is a potent poison, one dose of pure cassava cyanogenic glucoside (40mg) is sufficient to kill a cow (Aregheore and Agunbiade, 1991). The cyanide compound produces hydrogen cyanide gas and the usual route of absorption is inhalation of this gas. It is also absorb through the skin as an aqueous solution. Inhalation of HCN or its absorption through the skin over a long period results in increased blood cyanide levels (ATSD, 2006). High cyanide intake from the consumption of insufficiently processed cassava has been advanced as a possible aetiologic factor in some disease such as tropical ataxic neuropathy (Osutokun 1981), iodine deficiency disorder and "Konzo" or upper motorneuron disease (Tylleskar et al, 1992).

Akinrele (1986) and Okafor et al (2001) reported that large scale cassava processing could be hazardous, not by consuming residual cyanide in food, but the discharge of hydrocyanic acid into the air, and natural water sources in areas near large scale" garri" processing. Possible occupational exposures of humans to cyanide poisoning during large scale cassava processing have been reported (Okafor et al 2001). Results obtained by Okafor (2004) also showed high exposures to hydrocyanic acid among cassava processors in Nigeria.

There is no study on the effect of cyanide on immune functions of humans, and such study on experimental animals is limited. In fishes, sub-lethal concentrations of cyanide in fresh water environment lead to development of infectious diseases and raised level of cortisol (Carballo et al 1995). In rats, high cyanide causes increased lipid peroxidation, raised free radical and superoxide anion (Santy et al 2000). Muller and Krieglestein (1995) also observed the induction of lipid peroxidation by cyanide in cultured neurons from chick embryo telencephalon. The induction of oxidative stress by cyanide may involve increase in reactive oxygen species (Mills et al 1996). Cyanide, inhibition of antioxidant system (Ardelt et al 1989) and inhibition of mitochondrial function (Way 1984) which can result in production of superoxide anion (Carella et al 1988). Oxidative stress has been shown to participate in the carcinogenesis and

destruction of immune cells (Guyton and Kensler 1993), suggesting a possible adverse effect of cyanide or HCN on human immune functions. Thus investigations on the effect of cyanide will not be conclusive if cassava processors who are exposed to it daily (during the process of peeling, hand grating, washing of raw cassava), are not investigated as test subjects.

The aim of this study is to find out if humoral immune system is affected in cassava processors and possibly suggest a reason for the observation. Attempt to achieve this, is by measuring the levels of three immunoglobulin classes (IgG, IgA and IgM) and metal binding proteins (Transferrin, Caeruloplasmin, Alpha-2- Macroglobulin) in Nigerian cassava processors. This will help to determine the long-term effects of cyanide exposure on the immune status of Nigerian cassava processors.

Materials and Methods

A total of sixty subjects aged between 25 and 64 years (38.50 ± 9.90 years) were recruited for the study. These subjects included thirty four (34) cassava processors (test subjects) working in a cassava processing industry along Old-Ife Road, Onipepeye, Ibadan North East Local Government Area of Ibadan, Nigeria. Twenty six (26) control subjects within the same age range were recruited from members of staff of the University College Hospital, Ibadan, Nigeria. Informed consent was obtained from them before sample collection and the need for the study was explained in local language when necessary. The test subjects have been processing cassava tubers for between 3 years to 15 years (8.90 ± 5.13 years) and do process about 3 tonnes of cassava in a day. Based on clinical observation and responses to questionnaire, the following groups of subjects were excluded. The subjects excluded were those with

(i) History suggestive of malignant disease

(ii) History of metabolic disease e.g. diabetes mellitus

(iii) On special medication (including contraceptives) or diet.

(iv) Pregnancy.

Five milliliters of venous blood was collected from the antecubital vein without venous stasis from each subject and was dispensed into plain bottles. The blood samples were allowed to clot and retract after which the serum was separated into plain bottles for analysis of serum immunoglobulins and metal binding proteins. The serum was stored at -20^0C until time of analysis which was done within two weeks of sample collection.

Immunoglobulin classes and metal binding proteins were quantified by the single radial immuno-diffusion method, which is based on the principle of antigen-antibody precipitation reaction in agar gel (Akinosun et al 2006).

Data were presented as mean ± standard deviation. Student t-test was used to test the significance of difference between mean values. The probability value (p) greater than 0.05 was considered insignificant.

Results

The table 1 shows that the mean level of IgA was slightly reduced while mean serum level of IgG was slightly raised in cassava processors compared with the controls. The mean serum level of IgM was significantly increased in cassava processors compared with the controls (P=0.02). Also in Table 1, there were non significant increases in the values of alpha-2 macroglobulin, Caeruloplasmin and transferrin in cassava processors compared with the controls.

Table 1: The serum levels (means ± SD) of immunoglobulin classes, acute phase proteins and trace metals in test subjects compared with control subjects.

Subjects	CP (n=34)	NCP (n=26)	t-value	p-value
IgA (mg/dL)	69.53±24.99	78.00±27.51	1.23	0.23
IgG (mg/dL)	1096.18±341.30	1037.96±278.60	0.71	0.48
IgM (mg/dL)	188.76±178.99	99.44±54.74	2.41	0.02*
A2MG (g/L)	1.94±0.77	1.93±0.85	0.06	0.95
CLP (g/L)	0.26±0.20	0.23±0.12	0.63	0.53
TRF (g/L)	7.06±4.49	6.86±3.27	0.19	0.85

* P is significant at $\leq$ 0.05 (2 tailed)
n=number of subjects
CP=cassava processors
NCP=Non Cassava Processors (controls)
A2MG=alpha-2 macroglobulin
CLP=caeruloplasmin
TRF=transferrin

Figure 1: Freshly harvested cassava tubers

Figure 2: Showing the process of peeling cassava tubers.

Figure 3: The cassava tubers are being washed thoroughly after peeling.

Figure 4: This shows how peeled cassava tubers are stored ready to be grated.

Figure 5: This shows the process of grating cassava white pulp using a grinding machine

Figure 6: The grated produce is put in a jute sack and the sacks are stacked up on each other and allowed to ferment for days.

Figure 7: The dried fermented is sieved to remove large particles and fibres.

Figure 8: This shows the process of frying garri until it becomes crisp.

Discussion

Large scale cassava processing has been reported to be hazardous, not only by consuming residual cyanide in food but also by the discharge of hydrocyanic acid into the air and natural water sources in areas near large scale "garri" processing (Akinrele 1986 and Okafor et al 2001). A high exposure to hydrocyanic acid has also been observed among cassava processors in Nigeria (Okafor 2004). The effects of hydrocyanic acid (cyanide) on immune status of cassava processors have not been established and also available indirect data on experimental animals are few (Carballo et al 1995, Santy et al 2000, and Muller and Kriegistein 1995). The induction of oxidative stress, inhibition of antioxidant system, and production of superoxide anion and adverse effects on the nervous system by cyanide has also been reported (Mills et al 1996, Ardelt et al 1989, Way 1984). It is therefore necessary to examine the effect of cassava processing on human immune parameters.

In this present study, the mean serum IgM level in cassava processors was significantly raised compared with controls. IgM activates complement system which is necessary for phagocytosis, lysis of microorganisms and neutralization of toxins (Lisa et al 2002). Cyanide and few other components of cassava are known to be toxic and the half life of hydrogen cyanide in the atmosphere is about 1-3 years (Mills et al 1996, Ardelt et al 1989, Carella et al 1988). Therefore, raised level of IgM in cassava processors may be a mechanism to neutralize toxic components of cassava via complement activation. Results (significantly raised IgM and insignificantly different IgG and IgA) similar to that of present study were obtained in another group of Nigerians (petrol attendants) that are occupationally exposed to chemicals (Akinosun et al 2006).

IgG comprise approximately 80% of the serum antibody. IgG is responsible for most anti-bacterial, anti-viral and anti-toxic activity. The level of IgG is increased in liver diseases and infections (e.g. malaria). Moreover, IgM is the most primitive and largest immunoglobulin of the five classes (IgG, IgA, IgM, IgD and IgE). Apart from efficient complement fixation, it has an important function in complement dependent bacteriolysis (especially of Gram-negative organisms) (Salimonu 2004). The level of IgM is increased in liver diseases (Primary biliary cirrhosis, early hepatitis) and infections (malaria, trypanosomiasis). Thus slightly raised IgG and significantly raised IgM in cassava processors might be due to *Plasmodium* malaria infection because they are constantly exposed to *Anopheles* mosquito bites.

IgA is the predominant immunoglobulin found in mucosal secretion. The role of serum IgA is unclear, but was found to have antimicrobial activity. IgA deficiency is more frequent in adult subjects with chronic lung disease, recurrent sino-pulmonary infection, viral infections, autoimmune conditions, gastro-intestinal tract infection and disorders (Salimonu 2004). It is possible that slightly reduced IgA in cassava processors is caused by sub clinical (non-manifested) sino-pulmonary infection as a result of respiratory tract stimulation by inhaled smoke from burning fire-wood during frying.

Slight increases in the mean levels of metal binding proteins were observed in cassava processors compared with controls. Muller and Krieglestein (1995) and Santy et al (2000) observed that cyanide causes increased lipid peroxidation, raised free radicals and stimulated production of acute phase proteins in experimental animals. The stimulatory effect of free radicals on increased production of acute phase proteins may account for slightly raised metal binding proteins in cassava processors.

This study shows that cassava processing has slight adverse effect on humoral immune system in Nigerian processors.

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