Field Trial Of A New Test Kit For Monitoring Urinary Cyanide Load And Diagnosis Of Cyanide Poisoning

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Abstract

A newly developed simple test kit for the assessment of urinary thiocyanate using a visual colour chart was compared with a standard spectrophotometric method. Results obtained from the determinations of the urinary thiocyanate levels of 104 adults using both methods did not differ significantly ($p > 0.05$). The results from both methods were also significantly positively correlated ($r = 0.90$; $p < 0.01$). These findings of significant agreement between the two methods further validate the field accuracy and reliability of the new simple visual colour chart method. The significance of the above results are discussed in relation to the relevance of such a simple test kit which can be used for the assessment of body cyanide load and confirmatory diagnosis of cyanide poisoning even in rural population settings where electric power supply and a laboratory equipped with a spectrophotometer may not be available.

Key word: Cassava, Cyanide poisoning, Diagnostic kit, Thiocyanate, Urine

Introduction

A presumptive diagnosis of cyanide poisoning is usually based on the spectacular symptoms and lesions of cyanide poisoning and the clinical history of intake of cyanide or its sources by the poisoned animal or man (Clarke & Clarke, 1975). There are, however, numerous diseases that manifest the same clinical signs and lesions observed in cyanide poisoning. It is therefore, usually necessary to confirm the diagnosis by carrying out chemical tests such as Steyn test (Buck, 1969), copper-guaic test (Coles, 1986) or picric acid test (Seifert, 1996), which can detect the presence of cyanide in gastrointestinal contents, fluids and body tissues. Reasonable arguments had however, been brought against these tests because all of them are "rough qualitative tests which can most times lead to miscarriage of justice in forensic chemistry" (Coles, 1986). Also, the qualitative colour changes that are usually associated with the presence of cyanide in tissues and gastrointestinal contents had been reported to occur in the presence of compounds such as halogens, ammonia and oxides of nitrogen (Buck, 1969). It is also argued that the examination of gastrointestinal contents alone is not sufficient because hydrocyanic acid is frequently found in the stomach of subjects that died from other causes, and in the case of cyanogenic plants, a great deal of hydrocyanic acid may be liberated only after death (Coles,

1986). Moreover, in populations that depend on cassava-based diets, the qualitative presence of cyanide in gastrointestinal contents and tissues may not be an indication of poisoning as subjects that consume these diets continually take in reasonable amounts of cyanogenic glycosides which release sublethal doses of cyanide that may not lead to acute toxic poisoning/death (Hahn, 1983; Oke, 1983). Further, tissues such as liver and muscle, which are usually analysed for the presence of cyanide, are known to lose their cyanide content rapidly after their removal from the body (Clarke & Clarke, 1975).

The above drawbacks of the existing methods for confirmatory diagnosis of cyanide poisoning led to the development of an accurate quantitative column method that utilizes a Bio-Rad AG3 – X4 anion exchange Econo-column for the determination of the thiocyanate content of urine of subjects suspected to be poisoned with cyanide (Lundquist *et al.*, 1995). The use of "thiocyanate in urine" as a quantitative indicator of the cyanide status of a subject is based on the fact that all cyanide absorbed by the body is converted to thiocyanate and excreted in urine, and thiocyanate in urine had been found to be relatively very stable – no losses in thiocyanate content were reported when urine samples were kept at 30 °C for 7 days, 4 °C for 14 days, and – 20 °C for 6 months (Lundquist *et al.*, 1995; Haque & Bradbury, 1999).

However, the accurate column method is sophisticated and difficult, and also requires expensive chemicals, resins and equipment which are not usually readily available for use in developing countries where cyanide poisoning due to ingestion of cyanogenic glycosides derived from cassava mostly occur (Haque & Bradbury, 1999). This constraint led to the development of a simpler method of quantitatively determining thiocyanate in urine, which was based on the quantitative oxidation of thiocyanate with permanganate at room temperature in a closed vial with the liberation of cyanide that reacts with a picrate paper colouring it (Haque & Bradbury, 1999). Under field conditions the thiocyanate content of the urine can then be obtained by matching the coloured picrate paper with a visual colour chart graded from 0 – 100ppm thiocyanate; but in a laboratory equipped with a spectrophotometer, the thiocyanate content can be obtained by elution of the coloured complex in water and measurement of its absorbance at 510nm (Haque & Bradbury, 1999).

In the laboratory where the new test methods were developed, "satisfactory agreement" had been found between results obtained from the simple visual colour chart method and the spectrophotometric method (Haque & Bradbury, 1999). There was then the need for a field trial and comparison of the two methods in population settings where there is continual exposure to cyanogens derived from cassava (*Manihot esculentum*, Crantz) based diets. This present study investigated the field accuracy, effectiveness and reliability of the visual colour chart method when compared with the spectrophotometric method in the assessment of the levels of thiocyanate in the urine of adults in a cassava consuming community, in Nsukka, Nigeria.

Material and Methods

The components of the "thiocyanate in urine" test kit were a protocol paper that gave the detailed stepwise method of thiocyanate analysis in urine, clear flat-bottomed plastic bottles with screw lids (25mm

diameter, 50mm height), small graduated 1ml plastic pipette, Whatman 3mm filter paper discs that contain thiocyanate controls equivalent to 4 and 40ppm thiocyanate, picrate papers glued to strips of clear plastic with PVA hobby glue, thiocyanate colour chart containing ten entries from yellow to brown representing 0 to 100ppm thiocyanate, a small vial containing potassium permanganate, and 1M H_2SO_4.

The urine samples used for the study were collected from 104 adults of both sexes (58 males and 46 females) in Nsukka whose informed consent were sought for and obtained before commencement of the study. On collection of the individual urine samples, a questionnaire was administered on each of the volunteer experimental subjects eliciting primarily information on all the food items such an individual consumed within the previous three days before the sample was collected, including breakfast, lunch, dinner and any others for each of the three days before sample collection. Information on the meals consumed within the previous three days was collected because the thiocyanate level in the urine of an individual had been found to indicate the amount of cyanide ingested over the previous three days (Lundquist *et al.*, 1995). Each individual's urine sample was identified with a number that corresponded with the one on his/her returned questionnaire.

Each of the urine samples was analyzed for thiocyanate immediately after collection. To 1ml of each individual's urine sample in a flat-bottomed plastic bottle was added three drops of 1.0 M sulphuric acid using a plastic pipette. The solution was mixed. Three drops of 0.13 M potassium permanganate was added to the solution and immediately a picrate paper attached to a plastic strip was inserted into the bottle making sure that the picrate paper does not come into physical contact with the solution. The bottle was immediately closed with the screw-capped lid and gently mixed. Distilled water (1ml) was used in place of 1ml of urine to serve as a blank for each batch of samples analyzed. Also, a filter paper disc loaded with 4ppm thiocyanate and another loaded with 40ppm thiocyanate immersed in 1ml of distilled water served as the controls.

The bottles of urine sample solutions, the blanks and the controls were allowed to stand on the laboratory bench for 16 hours at room temperature (28 – 30 °C). After the 16-hour period, the bottles were opened and the colour of the picrate papers were matched against the thiocyanate visual colour chart to determine the thiocyanate contents in parts per million (ppm). The blanks and controls were also checked to correspond to zero and the expected values (4ppm and 40ppm) respectively. For the spectrophotometric method, after the 16-hour period, the picrate paper in each of the sample bottles was eluted in 5.0ml of water for 30 minutes with gentle occasional stirring, and the absorbance of the resulting solution was read against a blank at 510nm using an SP8-100 ultraviolet spectrophotometer (Pye Unicam). The thiocyanate content of each urine sample and that of the controls was calculated using the equation: Thiocyanate content (ppm) = 78 X Absorbance (Haque & Bradbury, 1999).

Results of the visual colour chart method was compared with that of the spectrophotometric method and tested for significant difference using the Student's *t* test. The correlation coefficient of the two results was also calculated and tested for significance.

Table 1: Distribution of the different cassava meals* consumed by 104 adults (58 males and 46 females) within three days before the urine samples of the study subjects were collected for thiocyanate content analysis, and their corresponding urinary thiocyanate contents

	Cassava meals*					
	Fufu	*Fufu & Gari*	*Gari*	*Gari & Tapioca*	*Tapioca*	*None*
Number of subjects who consumed specific meals (% in brackets).	14 (13.5%)	10 (9.6%)	56 (53.8%)	14 (13.5%)	2 (1.9%)	6 (5.8%)
Mean (SE) number of cassava meals consumed in three days	3.00 (0.00)	3.21 (0.14)	2.71 (0.11)	2.57 (0.21)	1.00 (0.00)	NA
Mean (SE) urinary thiocyanate content (ppm) of subjects that consumed the different types of cassava meals.	4.44 (0.95)	3.12 (0.23)	2.08 (0.16)	3.13 (0.45)	1.73 (0.17)	0.69 (0.12)
Mean (SE) urinary thiocyanate (ppm) of subjects who consumed three meals of the different forms of cassava within three days [no. of subjects in square brackets].	4.44 (0.95) [n = 14]	3.06 (0.30) [n = 8]	2.13 (0.19) [n = 28]	2.62 (0.10) [n = 4]	- [n = 0]	NA

* *Cassava meals: Fufu = pounded cassava mash; Gari = toasted cassava granules; Tapioca = abacha*

Results and Discussion

Ninety-six (96) out of the 104 study subjects (92.3%) admitted eating at least one form of processed cassava meal within the three days before submission of their urine sample for study. The different cassava meals consumed by the study subjects included pounded cassava mash (*fufu*), toasted cassava granules (*gari*) and tapioca (*abacha*). The distribution of cassava meal preferences for the study subjects and their corresponding urinary thiocyanate output are presented in Table 1. Sixty-one and half percent (61.5%) of the study subjects (64 out of 104) consumed at least three cassava meals within the three days before urine sample submission (i.e., at least one cassava meal daily). The overall mean ± SE number of cassava meals consumed by the subjects within the three days before submission of urine sample for study was 2.59 ± 0.10 meals. This implied that on the average, each individual consumed between two and three cassava meals within the three days. This finding confirms the severally documented indispensable and important role that cassava and its processed forms play in securing nutrition in Africa specifically and developing countries in general (Philips, 1983; IITA, 1990; Nweke, 1996; Scott *et al.*, 2000).

A comparison of the descriptive statistics of the results of the visual colour chart method of urinary thiocyanate determination with that of the spectrophotometric method is presented in Table 2. The mean ± SE urine thiocyanate of all the study subjects as determined by the visual colour chart method was 2.85 ± 0.29ppm while that of the spectrophotometric method was 2.69 ± 0.18ppm. These two means were not found to differ significantly ($p \nless 0.05$). Results of the urinary thiocyanate of the individual subjects determined by the two methods were found to be significantly positively correlated ($r = 0.90$; $p < 0.01$) – this implied that the results obtained from visual colour

Table 2: Comparison of the descriptive statistics of the urinary thiocyanate values obtained using the visual colour chart method with that obtained using the spectrophotometric method for the 104 urine samples analyzed

Descriptive statistic	Result from visual colour chart method	Result from the spectrophotometric method.
Mean*	2.85	2.69
Standard error	0.29	0.18
Median	2	2.24
Mode	3	2.85
Standard deviation	2.95	1.88
Range	14.50 [0.5 – 15]	12.65 [0.37 – 13.02]
Minimum	0.5	0.37
Maximum	15	13.02
Sum	296	278.89
Count	104	104

** There was no significant difference between the means ($p > 0.05$). [Student's t test]*

chart method compared favourably and was in good agreement with that obtained from the spectrophotometric method in this trial. The above findings show that the visual colour chart method is reasonably accurate and reliable, and can be effectively used for the confirmatory assessment of the urinary thiocyanate levels and thus body cyanide load. These findings are consistent with the results of laboratory trials of the test kit, which showed "satisfactory agreement" between the results obtained from the visual colour chart method and the spectrophotometric method (Haque & Bradbury, 1999). The validated field accuracy and reliability of the visual colour chart method is of great importance because the test procedure can be carried out without electric power supply and standard laboratory facilities. It is of great relevance in developing-country settings where cyanide overload due to consumption of cassava-based diets mostly occur, and where most often electric power supply is either inconsistent or absent and well-equipped laboratories are rarely found. The simple test kit shall therefore be of immense help in monitoring of body cyanide load and confirmation of the diagnosis of cases of cyanide poisoning especially in rural population settings where standard laboratories are usually not available. It shall also facilitate the early prediction/determination of the possible onset of cassava-cyanide related diseases/disorders such as konzo (Essers *et al*, 1992; Howlett, 1994) and degenerative neuropathy (Osuntokun, 1981; 1994); and enable easy confirmation of the diagnosis of reported acute poisonings following cassava-based meals in humans and animals (Essers *et al.*, 1992; Mlingi *et al.*, 1992; Akintonwa & Tunwashe, 1992; Akintonwa *et al.*, 1994).

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