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Full Length Research Paper

Prevention of konzo in the Democratic Republic of Congo (DRC) using the wetting method and correlation between konzo incidence and percentage of children with high urinary thiocyanate level

Jean Pierre Banea[1], J. Howard Bradbury[2]*, Chretienne Mandombi[3], Damien Nahimana[1], Ian C. Denton[2], Matthew P. Foster[2], N'landa Kuwa[1] and Desire Tshala Katumbay[4]

[1]Programme National de Nutrition (PRONANUT), Kinshasa, Democratic Republic of Congo (DRC).
[2]Evolution, Ecology and Genetics Research School of Biology, Australian National University, Canberra, ACT 0200, Australia.
[3]Hospital General de Reference, Zone de Sante de Popokabaka, Democratic Republic of Congo (DRC).
[4]Centre Neuropsychopathologique, Universite de Kinshasa, Democratic Republic of Congo (DRC).

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There were 68 konzo cases in three villages in Boko Health Zone, Bandundu Province, Democratic Republic of Congo (DRC), where yearly incidence had increased greatly since 2009. The nine months long intervention to prevent konzo started in the wet season. Village women were taught the wetting method, after which there were no new konzo cases and urinary thiocyanate levels were low. Using data from four different interventions in March 2010, July 2011, September 2013 and the present intervention in October-November 2012, a correlation was found between the monthly cyanide intake (measured by percentage of children with high urinary thiocyanate level) and the monthly konzo incidence. This dose response relationship between cyanide intake and konzo incidence, together with the prevention of konzo in many villages using the wetting method to reduce cyanogen intake, shows that konzo is very likely to be due to high cyanide/low sulfur amino acid intake in a diet of bitter cassava.

Key words: Dose-response relation, konzo prevalence, urinary thiocyanate, cyanide, cassava flour, wetting method.

INTRODUCTION

In the Democratic Republic of Congo (DRC) the roots of cassava are peeled, soaked (retted) in water for 3-4 days, and then dried in the sun. After drying, the roots are pounded and sieved. If the time of retting is reduced to 1-

*Corresponding author. E-mail: Howard.Bradbury@anu.edu.au. Tel: +61-2-6125-0775.

2 days (called short soaking), because of the families need for food or fear that the roots may be stolen or pressure to sell the flour (Banea et al., 1992; Tylleskar et al., 1992), then cyanogens are incompletely removed from the flour and village people may become poisoned by cyanide and konzo may occur. Konzo is an irreversible paralysis of the legs that occurs particularly in children and young women after childbirth who live on a monotonous diet of high cyanide cassava, and is associated with high cyanide and low sulfur amino acid intake (Ministry of Health Mozambique, 1984; Cliff et al., 1985; Howlett et al., 1990). This association is supported by many studies at an aggregated level and is strengthened by the recent prevention of konzo by greatly reducing the intake of cyanide from cassava flour, by use of the wetting method (Banea et al., 2012a, 2013). Further support for the hypothesis is given in this paper by showing a month by month correlation between the percentage of school children with high urinary thiocyanate levels and incidence of konzo.

Konzo occurs amongst poor village people in DRC, Mozambique, Tanzania, Cameroon, Central African Republic and Angola. Konzo was first reported by Trolli (1938) in Popokabaka Health Zone in Bandundu Province, DRC and was controlled for the first time in Kay Kalenge village in the same Health Zone in 2010, through the use of the wetting method by village women (Banea et al., 2012a). Fourteen months after the intervention ceased, Kay Kalenge was visited again and it was found that there were no new cases of konzo, the wetting method was still being used by the women and its use had spread by word of mouth to three adjacent villages (Banea et al., 2014).

One way to get rid of cyanogens from cassava flour would be to ensure that short soaking of cassava (Banea et al., 1992; Tylleskar et al., 1992) does not occur, but this would be difficult to achieve in practice because of the reasons given above. Therefore, a better procedure would be to use the wetting method on cassava flour as an additional reliable method to make sure that cyanogens are removed, just before the flour is cooked in the traditional way in boiling water to make a stiff porridge called fufu. The wetting method consists of mixing the flour with water, spreading the wet flour in a thin layer and standing it in the sun for 2 h or in the shade for 5 h to allow hydrogen cyanide gas to escape (Bradbury, 2006; Cumbana et al., 2007; Bradbury and Denton, 2010). The advantages of using the wetting method to remove cyanogens from flour, rather than attempting to increase the time of retting are that (1) it is an additional processing method that removes cyanogens and makes the flour safe to eat, (2) it is fast (2-5 h) when compared with several extra days of retting and (3) being located inside the house or just outside in the sun, it is much more secure from thieves than retting cassava roots in the river (Banea et al., 2013). The wetting method is popular because it is simple to use, requires only a bowl, a knife and a mat and the fufu produced from it can be stored for up to three days, when compared with only one day for fufu made from untreated flour. Its taste is much superior to that of fufu prepared from untreated flour, which is bitter because of the bitter linamarin that it contains (King and Bradbury, 1995).

In this paper, we reported the prevention of konzo using the wetting method in three villages and we use the data, along with that from three other interventions, to obtain a month by month correlation between incidence of konzo and the urinary thiocyanate content of school children.

MATERIALS AND METHODS

Study area

In September 2012, the team visited the Kwango District health team and nutrition coordinator in Kenge, who want to expand interventions to control konzo, which is increasing in this district. A further visit was made to the Boko Health Zone office where two possible sites were proposed for further work. After visiting the two sites, three villages in the savanna were chosen, Tsakala Mbewa, Tsakala Kenia and Mabaka (collectively called Boko 2) and located north of three previous villages (Figure 1) in which konzo was successfully prevented in 2011-2012 (collectively called Boko 1, Banea et al., 2013). Tsakala Mbewa is on a main road, has a primary and secondary school, a health centre and a maize mill. Tsakala Kenia has a primary and secondary school. Mabaka is 8 km from the main road, has a primary school and a small health centre. Water is available about 1-4 km away.

Agriculture and processing

Bitter (high cyanide) cassava is the main crop, along with sweet (low cyanide) cassava which is often stolen. Fields are located in the forest and are now fallowed for 3-5 years, when compared with 10 years previously. There is a shortage of fields to grow cassava because now only women are engaged in agriculture, and it is not carried out like before. Cassava, corn, peanut and cowpea are major crops and there are chickens, goats and pigs. Traditional varieties of cassava are bomba, mbengi and kayayi which are resistant to plant diseases and there are new varieties mwuazi, musanzu and F-100 which give roots after six months. Cassava roots are retted (soaked) for 2 days and nights instead of the 3-4 days recommended to remove cyanogens and the soaked roots are dried for only 0.5-1 day, because of problems of food security. Cassava is processed into cossettes or chickwangue (Banea et al., 1992). Traders visit these villages mostly during the dry season (May-September), at corn harvest and caterpillar season.

Second visit to the three villages

On the second visit by the full team (Banea team, Caritas team and Boko Health Zone team) to the villages in October-November 2012, a population census was made and suspected konzo cases examined in their homes by community health workers following a standardised WHO protocol. The four diagnostic criteria for konzo are as follows: (1) a spastic visible walk, (2) sudden onset within a week in a formerly healthy person, (3) exaggerated bilateral patellar

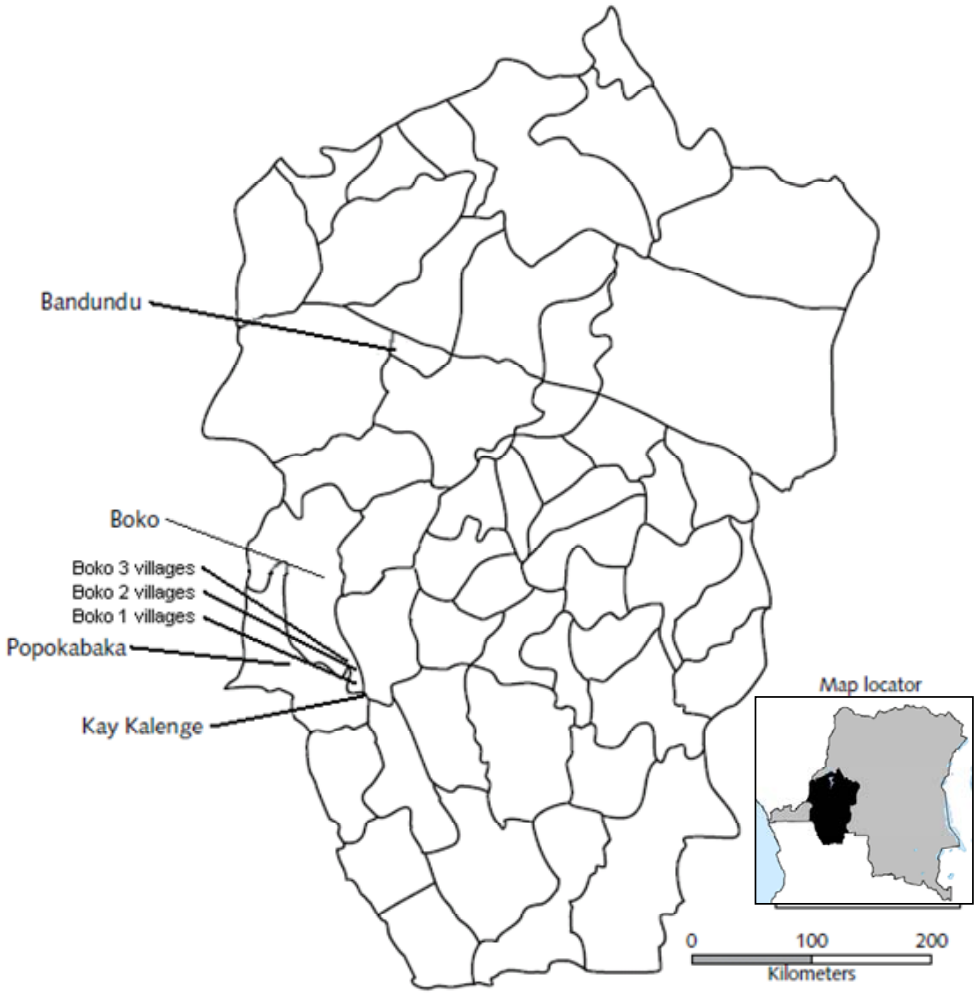

Figure 1. Map showing the health zones of Bandundu Province, DRC, including the location of the Boko 2 villages (this study), Kay Kalenge village (Banea et al., 2012a), three Boko 1 villages (Banea et al., 2013), and the six Boko 3 villages.

or achillian reflexes and (4) non-progressive evolution of the disease (WHO, 1996). Konzo cases were given multivitamins and antiinflammatory drugs. A food consumption survey was conducted among 58 households with one or more konzo cases. Cassava flour samples were collected from households and urine samples were collected from school children, about 85 from boys and 85 from girls with about 120 from 6-9 years old and 50 from 10-14 years old, with permission from their parents. All samples were analysed on site using cassava cyanide kit B 2 and urinary thiocyanate kit D1 supplied from Australia.

The wetting method was taught to 10-12 of the leading women in each village and they in turn each taught 10-15 others in the village. The training reached a total of 379 women in the three villages and a committee was formed to ensure follow up. Each woman received a plastic bowl, knife and a mat, but the delivery of the mat was delayed to a later visit. The wetting method involved adding cassava flour to a bowl and marking the level of the flour on the inside of the bowl. Water was added and the level of the wet flour initially decreased and then increased as more water was added with mixing, until it reached the mark. The wet flour was spread in a thin layer about 1 cm thick on a mat or a basket and allowed to stand for 2 h in the sun or 5 h in the shade to allow hydrogen cyanide gas to escape (Bradbury, 2006; Cumbana et al., 2007; Bradbury and Denton, 2010). The damp flour was then cooked in boiling water in the traditional way to make a thick porridge called fufu. Fufu was normally eaten with pounded, boiled cassava leaves or something else to give it flavour.

Subsequent visits to the three villages

Every month after the second visit, the three villages were visited by the Caritas team to check on use of the wetting method and in March 2013 the full team visited for the third time. Nine focus groups were conducted to check on the continued use of the wetting method during the wet season. Training sessions on use of the wetting method were held for untrained women. Difficulties raised by the women were lack of sun, houses made of straw which oozed water during rain, and a shortage of mats. During this period, people ate cassava flour, cassava leaves, mushrooms and some hunting families ate meat. Cassava flour and urine samples were collected and analysed on site. The fourth and final visit of the full

Table 1. Population, number of konzo cases and konzo prevalence (%) in Boko 2 villages in October-November 2012.

Village	Population	Number of konzo cases	Konzo prevalence (%)
Tsakala Mbewa	752	26	3.5
Tsakala Kenia	314	21	6.7
Mabaka	972	21	2.2
Total	2038	68	3.3

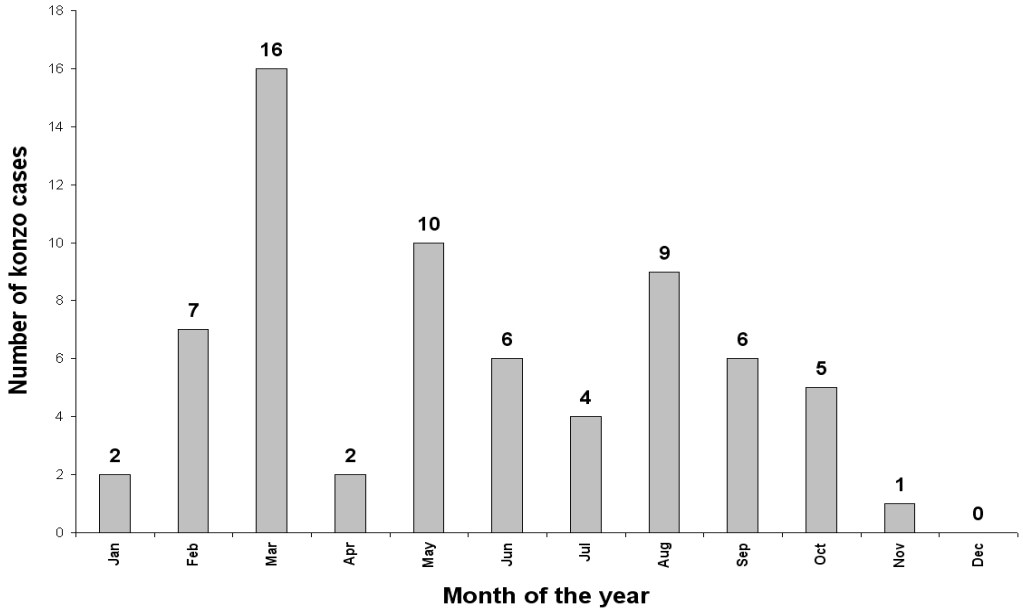

Figure 2. Monthly distribution of konzo cases in the Boko 2 villages.

team occurred in June 2013 and the same procedure was followed as in the third visit, with nine focus groups, training sessions for untrained women to use the wetting method, and collection and analysis of flour and urine samples. Bowls, knives and mats were given to women after training to encourage them to continue using the wetting method.

Urinary thiocyanate analysis

Urine samples (normally 55-60 but 45 in one case) were collected randomly from school age children in each village with consent of their parents and a record was made of their age and sex. These samples were analysed on site using the simple picrate thiocyanate kit D1 (Haque and Bradbury, 1999; http://biology.anu.edu.au/hosted_sites/CCDN/). A colour chart with 10 shades of colour from yellow to brown was used, which corresponded to 0 to 1720 µmole thiocyanate/L.

Total cyanide analysis

Thirty samples of cassava flour about to be used to prepare fufu were collected randomly from households in each village before teaching the wetting method in October-November 2012 and at the subsequent visits in March and June 2013. Analyses for total cyanide content were made on site using a simple picrate kit B2 (Egan et al., 1998; Bradbury et al., 1999; http://biology.anu.edu.au/hosted_sites/CCDN/). A colour chart was used with 10 shades of colour from yellow to brown corresponding to 0-800 mg HCN equivalents/kg cassava flour (ppm).

Ethics statement

Prior to the study, the protocol was approved by the DRC Ministry of Public Health Ethics Committee. The day before the collection of cassava flour and urine samples, each household gave their free oral consent for the sampling to be carried out.

RESULTS

In Table 1, the distribution of population, number of konzo cases and konzo prevalence (%) in the three villages are shown. There are 68 konzo cases, 42 females (62%) and 26 (38%) males, in a population of 2038 with a mean konzo prevalence of 3.3%. The monthly occurrence of konzo cases is shown in Figure 2 and the years in which they occurred in Figure 3. Onset of konzo occurred in less than one day in 91% of cases. Two people with konzo (3%) were unable to walk, 3% used two sticks,

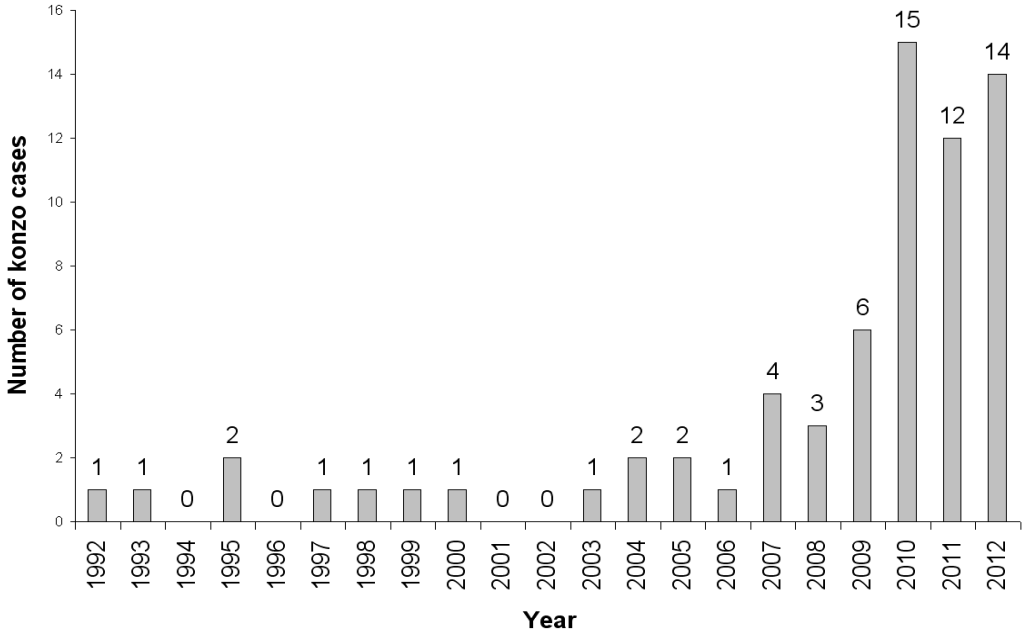

Figure 3. Annual distribution of konzo cases in the Boko 2 villages.

Table 2. Mean urinary thiocyanate level (µmole/L) of school children in Boko 2 villages before introducing the wetting method (October-November, 2012) and during the intervention[a].

Village	Mean urinary thiocyanate level (µmole/L)		
	October-November 2012	March 2013	June 2013
Tsakala Mbewa	51(60)	104(81)	100(96)
Tsakala Kenia	224(129)	161(128)	121(96)
Mabaka	240(171)	139(151)	137(110)
Mean	172(105)	135(29)	119(19)

[a]Mean values based on sample sizes of 45-60 with most sample sizes of 60. Standard deviation shown in brackets.

12% one stick and 82% limped but did not need a stick. Speech disorders were observed in 12% of patients and 16% had impaired vision. Six patients (9%) had goitre, determined by palpation. Patellar and achillian reflexes were exaggerated bilaterally in all cases.

A food consumption survey in October-November of 58 households with at least one konzo case showed that the mean number of days that foods were consumed per week were as follows: cassava flour 7, collection products (caterpillars, roots) 5, vegetables 4, cooking oil 2.8, fruits (bananas, pineapple, orange, avocado) 2.5, cereals (maize, rice, wheat flour) 1.9, sugar and sugar products 1.9, meat and fish 1.3, milk 0.5, legumes (beans, cowpeas) 0.4. The people of Tsakala Mbewa consumed more maize flour than those of the other villages because there was a maize mill in their village. Many people made fufu from a mixture of cassava flour and corn flour. In March 2013, people consumed mainly cassava flour, cassava leaves and mushrooms and hunting families (mainly in Mabaka) ate some meat. In June 2013, there was trade from Kinshasa and Kikwit through Tsakala Mbewa and these people had a better diet.

No new case of konzo occurred in the Boko 2 villages after the intervention commenced in October-November 2012. A total of 37 women gave birth over the nine month intervention and none of them contracted konzo after child birth. In March 2013, only 71% of women were using the wetting method partly due to heavy rains, but by June 2013 this had risen to 95%.

The mean urinary thiocyanate levels of school children are shown in Table 2. There were no significant differences between the mean urinary thiocyanate levels of boys and girls or between the two age groups, 5-9 and

Table 3. Percentage of school children in Boko 2 villages with urinary thiocyanate level of >350 µmole/L.

Village	Percentage of children with urinary thiocyanate level of > 350 µmole/L		
	October-November 2012[a]	March 2013	June 2013
Tsakala Mbewa	0	0	0
Tsakala Kenia	1	0	0
Mabaka	4	2	0

[a]Before introducing the wetting method.

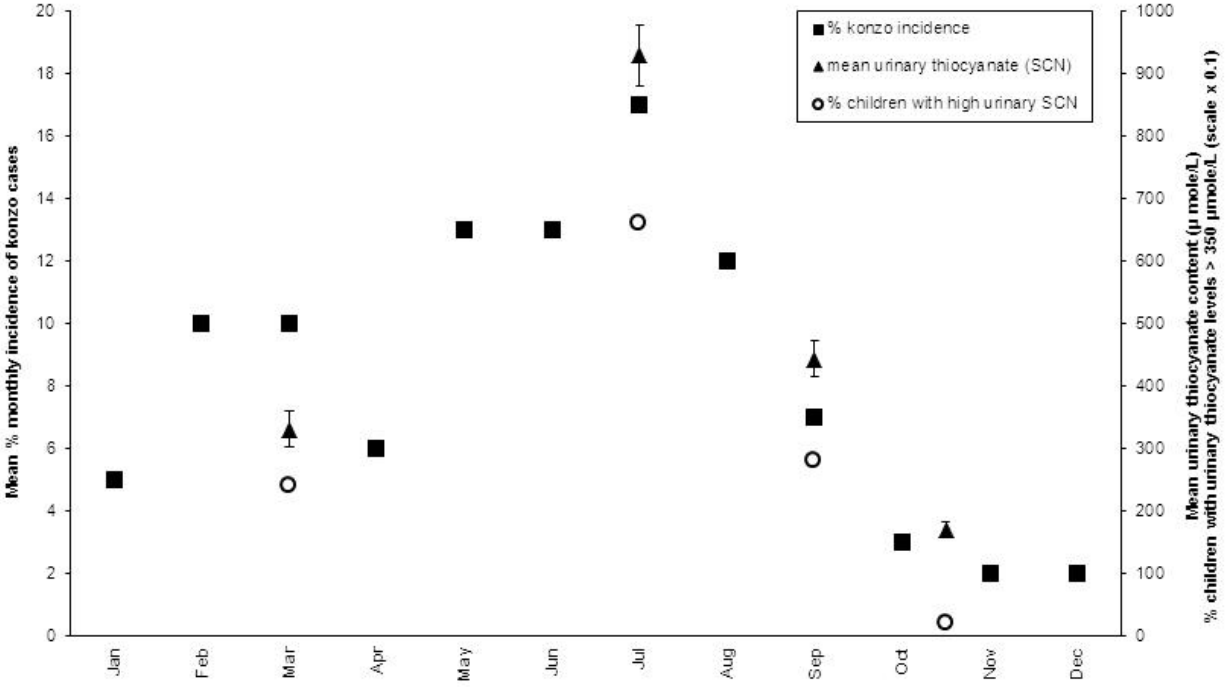

Figure 4. Graphs of the mean percentage monthly incidence of konzo cases (■), the mean urinary thiocyanate levels of school children with standard error bars (▲) and the percentage of children with urinary thiocyanate levels >350 µmole/L (○) for the four interventions made in March 2010, July 2011, September 2013 and the current intervention in October-November 2012.

10-14 years. In Table 3, the percentage of school children with a high urinary thiocyanate level of >350 µmole/L is given. The mean total cyanide content for Tsakala Mbewa, Tsakala Kenia and Mabaka villages before teaching the wetting method in October-November 2012 were 9 (19), 12 (14) and 7 (12) ppm, in March 2013 they were 9 (10), 9(8) and 8 (6) ppm and in June 2013, they were 6 (5), 6 (5) and 7 (9) ppm, respectively. Standard deviations are shown in brackets. In Figure 4, the mean percentage monthly incidence of konzo averaged over four interventions, viz. Kay Kalenge village in March 2010 (Banea et al., 2012a); three villages, Boko 1, in July 2011 (Banea et al., 2013); six villages, Boko 3, in September 2013 and this study in three villages in October-November 2012 is plotted. On the same graph, is plotted the mean urinary thiocyanate level of school children before intervention with standard error bars included and the percentage of children with mean urinary thiocyanate level >350 µmole/L. A correlation analysis showed that the mean percentage monthly incidence of konzo is significantly correlated with the percentage of children with mean urinary thiocyanate level >350 µmole/L, with P = 0.037 and correlation coefficient of 0.963. A weaker correlation was found between mean percentage monthly incidence of konzo and mean urinary thiocyanate content of school children, because the latter is a poorer measure of children in danger of contracting konzo than is the percentage of children with very high urinary thiocyanate levels (Banea et al., 2013).

DISCUSSION

As shown in Table 1, there were a total of 68 konzo cases in the three villages with a konzo prevalence rate ranging from 2.2% in Mabaka to 6.7% in Tsakala Kenia. There were 3% severely disabled patients (unable to walk), 15% moderately disabled (needing one or two sticks) and 82% mildly disabled, who walked without a stick. This data was combined with that from five previous konzo studies (Banea et al., 2012a, 2013) and the mean percentage results (standard deviations in brackets) were as follows: severely disabled 8 (5), moderately disabled 26 (14), mildly disabled 66 (13).

Figure 2 shows a maximum incidence of konzo in March (near the end of the wet season) with smaller peaks in May and August. This is the first time that konzo incidence has peaked in the wet season. Perhaps the retted cassava could not be dried sufficiently in the wet conditions and short drying times of about one day, could produce elevated residual acetone cyanohydrin content (Banea et al., 1992; Tylleskar et al., 1992). This was not observed in the mean total cyanide content of cassava flour samples, which were low before the wetting method was taught in October-November 2012 and did not significantly exceed the WHO safe level of 10 ppm (FAO/WHO, 1991). However, detailed inspection showed that 20% of the total cyanide results obtained before the wetting method was taught were ≥ 20 ppm and gave rise to much higher standard deviations from the mean (see above) than with the later results. This showed that 20% of the village women were not processing their flour correctly before the wetting method was taught.

In Figure 3 it is shown, that konzo cases occurred every year for ten successive years from 2003-2012, with large numbers since 2009, as with the Boko 1 villages (Banea et al., 2013) and Kay Kalenge (Banea et al., 2012a). The total number of konzo cases in these three locations with incidence since 1992 was 150, of which 102 (68%) occurred from 2009 to 2012. This confirmed anecdotal evidence from Kwango District Health Team (see above) that konzo was getting worse in this district. There are two reasons for this. Before 2009, the production of cassava was higher than it is now because of the present shortage of fields to grow cassava (see above), hence there was more food available and they sold more cassava and were able to buy other food which reduced their total intake of cyanogens. The second reason is that they are still obliged to sell some cassava and when they sell cassava they reduce the soaking time. They also eat the short-soaked cassava with increased cyanide content, which increases greatly their intake of poisonous cyanogens, and leads to konzo.

In Figure 4, are the results of four interventions in the same geographic region (Figure 1) at Kay Kalenge in March 2010 (Banea et al., 2012a), Boko 1 in July 2011 (Banea et al., 2013), Boko 3 in September 2013 and the present intervention in October-November 2012. In Figure 4, in October-November, the small mean urinary thiocyanate level and the small percentage of children with urinary thiocyanate level >350 µmole/L before intervention is consistent with the low recorded incidence of konzo, whereas in July, there is a high urinary thiocyanate level consistent with a large incidence of konzo (Banea et al., 2013). The monthly konzo incidence curve would be expected to change in different regions and countries, being dependent on the seasons of the year and other factors, which would explain the wide range of urinary thiocyanate levels reported in the literature from children with konzo.

There is a significant correlation between the percentage of children with urinary thiocyanate levels >350 µmole/L and the mean percentage monthly incidence of konzo. Therefore, there is a dose response relationship (Tylleskar, 1994) between cyanide intake (measured by percentage of children with urinary thiocyanate content >350 µmole/L) and the incidence of konzo. A dose-response relationship is further evidence of the correctness of the high cyanide, low S-containing amino acid association as an explanation of the occurrence of konzo (Banea et al., 2012b). Thus, the best measure of the likely incidence of konzo in a population are urinary thiocyanate measurements on school children, but because these values are dependent on the seasons of the year (Figure 4), which vary from one region and country to another, it is necessary to make comparisons in the same region. This has been done here, since the four studies are all located geographically close to one another (Figure 1).

The low mean urinary thiocyanate level before the intervention started was due to the fact that it commenced in the wet season, when cyanogen intake from cassava and konzo incidence was very low (Figure 4). For this reason the mean urinary thiocyanate level actually increased in Tsakala Mbewa from October-November 2012 to March 2013, but decreased for the other villages (Table 2), but most importantly was low in all three villages in the dry season when the level is usually high (June 2013). Furthermore, the percentage of children with urinary thiocyanate content >350 µmole/L had reduced to zero in the three villages in June 2013 (Table 3). The total time of intervention was progressively reduced from 1.5 years in the first intervention (Banea et al., 2012a) to one year in the second intervention (Banea et al., 2013) to nine months in this intervention. The progressive reduction in intervention time from eighteen to nine months still produced good results, reduced the cost of the intervention per person and allowed better use of limited funding.

Conclusion

This intervention to control konzo was commenced in the wet season when konzo incidence was low, and therefore

gave much lower urinary thiocyanate and cassava cyanide values than in previous interventions (Banea et al., 2012a, 2013). The intervention was completed satisfactorily in 9 months when compared with previous interventions of 18 and 12 months and this reduced the cost per person in the village. The yearly records of konzo incidence in all three interventions showed that konzo cases had greatly increased since 2009, in agreement with anecdotal evidence that konzo was increasing in this area.

It was found that the mean monthly incidence of konzo was significantly correlated with the percentage of children with very high urinary thiocyanate levels, which is a measure of cyanide intake. This represents a dose response relation between konzo incidence and cyanide intake and shows that konzo is very likely to be due to high cyanide intake from bitter cassava combined with low sulfur amino acid intake (Tylleskar, 1994). Since detoxification of ingested cyanide occurs by formation of thiocyanate which is removed in the urine, the best measure of children in danger of contracting konzo is by urinary thiocyanate determinations, but the results should be related to the season of the year. It is good to make urinary thiocyanate measurements during the dry season when cassava consumption is high and urinary thiocyanate levels of >350 µmole/L in individual children indicate a child in danger of contracting konzo (Banea et al., 2012a, b, 2013).

Conflict of Interests

The author(s) have not declared any conflict of interests.

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