Working together to eliminate cyanide poisoning, konzo, tropical ataxic neuropathy (TAN) and neurolathyrism

CCDN News

Cassava Cyanide Diseases & Neurolathyrism Network

(ISSN 1838-8817 (Print): ISSN 1838-8825 (Online)

Issue Number 33, June 2019

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EDITORIAL

On the prevention of Konzo, lessons learned from Neurolathyrism

In line with the ideas of the editorial in the previous issue, no reports on new neurolathyrism cases were found while several new konzo areas are reported in this issue. A new Indian study demonstrates that small amounts of grass pea even during long periods cause no harm.[1] Indian authorities remain indecisive concerning the ban on the sale and storage of grass pea, while Spanish authorities did lift the ban on grass pea consumption with the advice to mix with cereals.[2] On the other hand, research on Konzo is still focusing on prevention and screening of populations at risk. Apparently there is a need for better methods for screening cyanide content in cassava foods as well as thiocyanate in the urine of people at risk. In Mozambique, a mapping and advocacy exercise is underway, led by Netherlands Leprosy Relief, and even before it started the Ministry of Health decided to include konzo in the health information system. June 28, a symposium on Konzo was held at Zurich University, Switzerland, including a round table discussion on its prevention (see report in this issue). As we were unable to attend, we summarized our views for circulation among the participants as copied below:

"The clinical symptoms of konzo and neurolathyrism are so similar that only the history of the patient can differentiate between these two socio-economic diseases. Konzo patients in DR Congo (Pokokabaka surrounding villages) or neurolathyrism patients in Ethiopia or Bangladesh have the same scissor gate and contraction of the calf muscles. In some cases the toe nails are worn from dragging their bare feet over the ground. Protective factors from the epidemiology of neurolathyrism may help to design strategies for the prevention of Konzo. Both these socio-economic diseases have a sudden onset

The socio-economic conditions of areas with high incidence of konzo and those where, historically, there was a high incidence of neurolathyrism are very similar. Both diseases occur in poor rural areas, mostly among illiterate people who grow cassava (*Manihot esculenta*)/grass pea (*Lathyrus sativus*) for their own consumption. During extreme poverty or drought-triggered famine, the diet consists mainly of Cassava in the case of konzo or grass pea in the case of neurolathyrism. When a diet of mainly grass pea is maintained during two months, up to 6% of the population, mainly young males can become irreversibly crippled. But when the diet contains at least 20% of cereals richer in sulphur amino acids, or when antioxidant-rich condiments such as onions or ginger are added in the preparation, these seem to protect against neurolathyrism.[3]

The common feature of both cassava roots and grass pea seeds is the very low content of sulphur amino acids methionine and cysteine. Deficiency of these amino acids can disturb the redox homeostasis by depletion of glutathione, creating oxidative stress. Heavy physical labour has been mentioned as risk factor for neurolathyrism. Interestingly, in the cassava growing areas in Africa, the women do most of the labour. To link this with the higher incidence of Konzo among young women, while the incidence of neurolathyrism is higher among young men seems an attractive hypothesis.

When young experimental animals are put under stress, the incidence of neurolathyrism-like symptoms increases 4.6-fold in rat pups.[4] There should be little doubt that during famines, people are under considerable stress to feed their family and themselves. The highest incidence of neurolathyrism in history occurred in a WWII labour camp at Vapniarca where the Jewish inmates had considerable stress.

In very young animals, the blood/brain barrier is not fully developed and also under heavy stress the blood/brain barrier may lose its integrity. Under such conditions toxic or neuro-excitatory molecules can enter into the spinal cord. In the experiment with rat pups, using β-ODAP, this occurred in the lower spinal cord along with transient haemorrhage.

The cyanogenic glucosides in cassava roots give rise to the mitochondrial toxin cyanide that can be detoxified by rhodanase to thiocyanate and eliminated in the urine. This is analysed as a measure of intake of insufficiently processed cassava. The transformation of cyanide into thiocyanate needs sulphur from methionine or cysteine, and these happen to be deficient in cassava-based diet. In case of deficiency of sulphur amino acids, the more toxic cyanate is formed that can affect the nervous system. Whether stress can influence these effects would be important information to help understanding the pathway to the development of konzo.

As mentioned above, konzo and neurolathyrism are two socio-economic diseases. The economic improvement of India and Ethiopia has made this disease almost a problem of the past. The market has also contributed to this because grass pea is now more expensive than rice and no longer the cheapest food available to the poor. In contrast to this development, the areas where konzo is rampant have slower economic development, if any.

A retired volunteer worker with extensive experience in konzo-afflicted areas in DR Congo told me: “in villages with only cassava consumption one can find konzo patients, but not when the villagers grow corn (Zea maïs) to mix with the cassava. When the cultivation of onions is successful, that year there are no new patients, but when the onions fail, there will be new cases”. While this needs confirmation by trained epidemiologists, these findings come very close to those of neurolathyrism, where cereals richer in sulphur amino acids or condiments rich in antioxidants are found to be protective factors.

Conclusion

-Alleviation of stress and improving the socio-economic conditions are beyond the power of scientific researchers. But stressing the description of konzo as a socio-economic disease might be a wake-up call for responsible authorities.

-Screening susceptible people for high levels of thio-cyanate in the urine should be accompanied by simple information on better processing methods for cassava roots such as the wetting method of Bradbury. Of equal importance would be dietary information for a better balanced diet, especially with a better balance of essential amino acids and richer in antioxidants.”

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ARTICLES

Neurolathyrism, Konzo and sulphur metabolism

Introduction: Early research leading to the topic

Early in my career I was employed in the research laboratory of a British food company. There I worked under a demanding analytical chemist subsequently with an 'ideas man', who became a major influence. He (alas, no more) unwittingly guided me to areas of nutritional and biochemical science that still affect my thinking.

Prior to my joining the laboratory, the analyst had begun developing the then new column chromatographic technique for amino acid analysis, invented by Moore and Stein (1954) - Nobel Laureates in Chemistry in 1972 - and the project became a major part of my work. This remarkable method, arduous in the manual form that I used, was subsequently automated and became the basis of modern HPLC. Then it was an excellent method for analysing amino acid mixtures or protein hydrolysates – but only at the rate of one per week! As a back-up to our work I would be sent to London's Patent Office Library where, supposedly looking up specific papers related to the project, I would stray into the biochemical literature. It was there that I discovered the Moore and Stein technique had been used to analyse the content of free amino acids in human and cat brains (Tallen et al., 1958). Cystathionine proved to be 10 times higher in concentration in human brain compared to cat brain. I wondered why – and I am still wondering!

My 'ideas man' once remarked that there was an interesting relationship between the plasma concentration of amino acids in rats, analysed while the animals were ingesting a purified protein, and the amino acid pattern of the pure protein that had been fed to them. I didn't realise the significance of that statement then, but the idea stayed with me. My 'ideas man' was no doubt referring to the work of Longnecker and Hause (1959) who examined this problem in a pair of remarkable papers. The mechanism of this phenomenon is explained later in this article. In a paper that I presented at the meeting on neurolathyrism and konzo, organised by Fernand Lambein, "Workshop on Toxico-Nutritional neurodegenerations Konzo and Neurolathyrism" in Gent in 2009 (Nunn et al., 2011), we showed that feeding methionine-deficient sources of protein to human volunteers caused a rapid fall in the plasma concentration of methionine to about one-half its normal post-absorptive concentration. Of course, we used the most up-to-date automatic amino acid analyser available for the analyses, each of which took just over 1 hour. Thus, I brought together those two powerful influences from my earliest days in science – amino acid analysis and protein metabolism.

The cystathionine / methionine story

Citations of the paper by Tallen et al. (1958) only just exceeded 200 by 2019 (Google Scholar), but the work is much more important than that number suggests. Subsequent research showed that the concentration of cystathionine in human plasma and in cerebrospinal fluid was very low, so most of the human brain content must be intracellular, but in which cells? And why not in cat brain? To my knowledge that topic has never been investigated. Cystathionine is an amino acid that contains sulphur (it is a condensation product of the amino acids serine and homocysteine, itself a sulphur-containing amino acid derived from methionine). We know that sulphur-containing amino acids enter the brain from blood plasma as methionine (via a transport system shared with other amino acids), as cystine by exchange with glutamate, a mechanism that is inhibited by ODAP (the neurotoxin from grass-pea) and the more recently discovered transport system for the important derivative of methionine, S-adenosylmethionine (Reichel, et al., 1997), that is shared with nucleosides, which are also taken up by the brain from blood plasma.

Methionine metabolism in humans

Subjects suffering from neurolathyrism or konzo develop the signs and symptoms of these diseases after having eaten grass pea or cassava, respectively, as a dietary staple for extended periods prior to the incidence of their illness; these food sources are both deficient in sulphur amino acids (Rudra and Chowdhury, 1950; Cliff et al, 1985). Nutritionists usually group methionine and cystine together as a common source of sulphur, but it is methionine that is the nutritionally essential amino acid as it can be converted into cystine (or its reduced form cysteine) by metabolic reactions that occur mainly in the liver, but also in other tissues too including the brain. This process is not reversible, so that cystine cannot be converted to methionine in animals. An essential amino acid is one that cannot be biosynthesised by humans and other animals and, when the content of food is deficient in an essential amino acid, the growth rate of the young is reduced and loss of bodily tissue (mainly skeletal muscle) occurs in adults. The physiological mechanism works like this. When one eats and digests protein, amino acids are released and taken up in part by the gastrointestinal tract into the hepatic portal vein and subsequently the concentration of amino acids in the peripheral blood

rises. Probably to avoid osmotic disturbances, these amino acids are then incorporated by generalised protein synthesis into body protein, mainly in skeletal muscle and liver. During this time, if an amino acid is deficient in the diet (termed the limiting amino acid), and the incoming concentration of dietary amino acids does not match the needs of the body for the biosynthesis of protein, the concentration of that amino acid in plasma will fall. The experiments of Longnecker and Hause (1959) demonstrated this process long before a physiological understanding of the phenomenon was known. The effect was demonstrated for methionine in our experiments (Nunn et al, 2011) after our volunteers had consumed 200g each of cooked Lathyrus sativus or Lens culinaris seeds, both of which are deficient in methionine. The reduction in the plasma concentration of methionine was seen for up to six hours following each feed. Following repeated feeding of Lens culinaris seed (to imitate a daily feeding cycle) the methionine plasma concentration was maintained at about one-half of the normal post-absorptive concentration throughout the day and recovered only marginally overnight.

Upon this evidence we suggested that consuming a methionine-deficient diet for an extended period, as do subjects suffering from neurolathyrism and konzo, would have the same effect. Indeed, as the methionine deficiency progressed, the plasma concentration of methionine might be expected to fall even further.

Methionine and the redox homeostasis controlling glutathione

How might these changes affect the central nervous system? The kinetics of methionine flow across the blood-brain barrier into the cerebrospinal fluid suggest that halving the plasma concentration would reduce the entry of methionine into the brain by this route by about one-half. Data are not available for the kinetics of flow of S-adenosylmethionine into the brain, but the plasma concentration of this compound would be expected to fall roughly in parallel with that of the free methionine concentration. In our experiments the plasma cystine concentrations remained constant in the short term, but we might expect that would be reduced also on progressive methionine and cystine deprivation. A major result of reduced sulphur amino acid flow into the central nervous system is likely to be a lowered concentration of intracellular glutathione, a major intracellular anti-oxidant (Kusama-Eguchi et al., 2011; Nunn et al., 2011) and of hydrogen sulphide, a potent neuromodulator (Panthi et al., 2018).

Methionine and motor neurons

Methionine is involved in many biochemical reactions, but it came as a great surprise to discover that rat motor neurones contain elevated concentrations of the free amino acid (Amara et al., 1995) compared to other cell types in the rat central nervous system. What is even more surprising is that, since its publication, this paper had been cited just 12 times (Google Scholar, 2019) mostly by research collaborators of F. Lambein, who was sent the paper by a colleague! No transport system specific for the concentration of methionine is known that might produce and maintain this relatively high concentration of free methionine in motor neurones and no-one appears interested in investigating whether human motor neurones also contain a similarly high concentration of this amino acid. What if methionine were an essential factor for some specific cellular function in these cells, which, when deficient, leads to their demise? The cellular mechanisms of apoptosis and necrosis are well known, but current knowledge concerning the triggers that set these processes in motion is poor. Were high concentrations of methionine also present in human motor neurones, a reduction might occur in subjects suffering from idiopathic neurodegenerative motor systems diseases from a loss of the (presently unknown) systems by which that concentration is maintained. In neurolathyrism and konzo the loss of the putative high concentration of methionine in motor neurones would be expected to result from the reduced inflow of methionine into the central nervous system due to the suboptimal concentration of plasma methionine.

Determining the possible accumulation of methionine by human motor neurones is experimentally approachable, as brain banks in many Western countries contain tissues from subjects who died from motor neurone diseases (and appropriate controls) and the materials required to apply the histological techniques used by Amara et al. (1995) are readily to hand, using commercially-available antibodies against methionine. That work on sulphur amino acid metabolism has to be applied to human or other primate tissue is obvious from the results on cystathionine (Tallen et al., 1958); Diwaker and Ravindranath, (2007) later found that the enzyme that cleaves cystathionine into serine and cysteine (cystathionine-γ-lyase) is one-hundred times more active in human brain than in mouse brain. Clearly there is something distinct about sulphur amino acid metabolism in the human brain that is not shared with that of lower animals, but the present picture is incomplete. However, that is not to say that the central nervous system of lower animals does not suffer from methionine deprivation. Day-old chicks feed a ration high in grass pea were protected from neurological signs by supplementing their diet with

methionine (Fikre et al., 2010). Studies of the effect of β-ODAP on a rat primary motor neurone cell culture and in a rat motor neurone cell line showed that the cells became increasingly sensitive to the neurotoxin as the methionine concentration of the medium was reduced, an effect that was attributed to a reduction in the intracellular concentration of glutathione (Kusama-Eguchi et al., 2011). With this experimental support it is particularly unfortunate that attempts by F. Lambein to attenuate an outbreak of neurolathyrism in Ethiopia by distributing tablets providing 500mg methionine/day to subjects at risk was frustrated by Ethiopian customs officers!

The question has been asked by Rao (2001): "Do we need more research on neurolathyrism'? Unfortunately, further research on the biological/biochemical background of neurolathyrism and konzo will not help subjects who suffer the nutritional deprivations that lead to these diseases and assistance must come from other sources. But it is unfortunate that these conditions are not regarded as being of interest to clinical researchers in motor systems diseases because, they claim, neurolathyrism and konzo are not spontaneously progressive, as is sporadic motor neurone disease/amyotrophic lateral sclerosis (MND/ALS). Specifically, in the instance of neurolathyrism, available evidence indicates that, once victims are transferred to a diet free from grass pea or have their existing diets supplemented by other foods that contain adequate amount of methionine, the signs and symptoms of the disease do not progress. Presumably the same is true of konzo. However, such a view is at odds with the scientific basis of this article: that cells of the human central nervous system utilise sulphur amino acids by mechanisms that cannot be explained satisfactorily at present and which may be deficient in sporadic neurodegenerative diseases.

Essential Sulphur amino acids and motor neuron diseases

So, what steps might be taken to transfer the knowledge gained from the study of neurolathyrism and konzo to sporadic human neurodegenerative diseases? It is more than 50 years since I first began working in this research area but, during that time, and despite increasing research effort, little progress has been made in alleviating the signs and symptoms of these disorders. Those with long memories will recall the treatment proposed by Plaitakis et al. (1988) for patients suffering from MND/ALS of daily dietary supplements of branched-chain amino acids (valine, leucine and isoleucine). Subsequent expensive trials in the field did not substantiate the early pilot study, which was not surprising to some because the scientific basis for the treatment was exceptionally weak. Based upon somewhat stronger science a Food and Drugs Administration-approved Phase-2 clinical trial involving the feeding of serine to subjects suffering from MND/ALS is presently under way in the United States, the outcome of which is anticipated impatiently! However, no-one, it seems, has yet proposed a clinical trial of methionine supplementation in such patients, despite nearly 70 years of scientific effort since the proposal by Rudra and Chowdhury (1950), regarding neurolathyrism, and almost 45 years since Cliff et al. (1985), regarding konzo, drew attention to the backdrop of these diseases being a low dietary intake of sulphur-containing amino acids. Who can say whether a clinical trial of methionine supplementation in MND/ALS could be justified? The scientific evidence is much stronger for that course of action than for feeding supplements of branched-chain amino acids or serine. If the Phase-2 trial now under way for serine is successful, we still will have little indication of the mechanism of its action. But hold the front page - isn't cystathionine derived from serine and homocysteine, which is itself derived from methionine? Could it be that in a well-nourished person suffering from MND/ALS, but with a more than adequate intake of methionine, serine supplementation might (depending upon the regulation of the relevant metabolic pathways), stimulate cystathionine formation in the central nervous system and hence that of glutathione, hydrogen sulphide and other goodies? And what if our dietary supplement contained both serine and methionine? We'll never know – not unless someone tries it, but please acknowledge the source of this idea!

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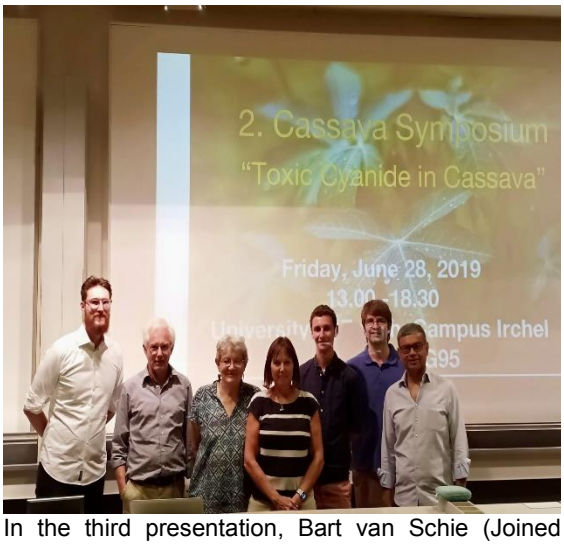
Conference Report on The "2. Cassava Symposium"

The "2. Cassava Symposium" took place on Friday, 28th of June 2019 at the University of Zurich, Switzerland. The main goal of this international meeting was to join forces and expertise for fighting *konzo*. This neglected disease is caused by cyanide intoxications from improperly processed cassava, a staple food in Africa. The meeting consisted of six talks from the fields of medicine, biology, chemistry, food engineering and financial studies and were presented by speakers from academia and industry.

In the first inspiring talk, Julie Cliff (*Eduardo Mondlane University, Maputo Mozambique*) presented results of monitoring of cyanogens in flour and thiocyanate in urine in *konzo* afflicted areas of Mozambique. Sporadic new cases of konzo are still appearing in these areas and monitoring shows that patients, their families, and apparently healthy schoolchildren still suffer from cyanide intoxication at the time of the cassava harvest each year. The Netherlands Leprosy Relief in Mozambique have recently expanded their focus to include the disabling neglected tropical diseases (NTDs) of konzo and lymphatic filariasis and have obtained funding for a mapping exercise through the Coalition for Operational Research on NTDs (COR-NTD). This exercise should provide more data and a platform for advocacy on prevention.

In the second talk, Ros Gleadow (*Monash University, Melbourne Australia*) reported on new systems for predicting the toxicity of cassava in a changing climate. She showed that all parts of the cassava plant are cyanogenic. The cyanogenic glucosides (mostly linamarin) are almost exclusively synthesised in the shoot tips and transported to the roots, with some synthesis in the periderm. The concentration of these cyanogens varies with the environment. For example, concentrations are higher in plants that are drought stressed. The concentration goes down again when watering of the plants is resumed; at least it does in pot studies. This makes it difficult to predict the bitterness level, and the degree of processing required to make the cassava safe to eat. Simple and cheap testing systems are required. Their group compared three different systems to test the cyanogen level of foods bought in Australian supermarkets: 1-the laboratory system used routinely in the Gleadow lab, based on the König reactions, 2-picrate papers developed by the late Howard Bradbury and 3-a new system using a corrin-based chemosensor technology developed by CyanoGuard. There was a high correlation between the results obtained using each system and this showed that many cassava products for sale are well above the 10ppm HCN level legislated in Australia. Each system has strengths and weaknesses, depending on whether the work is being done in the field or in the lab, cost and ease of use.



In the third presentation, Bart van Schie (Joined Dadtco-PhilAfricaFoods) presented results of extracting high quantities of linamarase from cassava leaves using either fresh, lyophilized or composting leaves underscoring the excellent stability of this enzyme. Using this enzyme extract during the wet processing of cassava tubers shows

that within 30 minutes all linamarin was converted to cyanohydrin and free cyanide. This represents enormous progress compared to the traditional processing routes where it can take up to 24 hours to remove linamarin. Enzymatic removal of cyanogenic glucosides has the potential to become the preferred process route in future. Field trials are momentarily ongoing in Mozambique in a joined project with master students at Eduardo Mondlane University and Lurio Universities. Bart van Schie made aware that linamarase extraction from cassava leaves is now the only route to obtain large quantities of this enzyme and proposed the development of bacterial expression of β-glycosidases. He estimated the cost of cloning, expression and upscaling of linamarase roughly at 3 Million Euro.

Felix Zelder (University of Zurich, Switzerland) described in the fourth presentation his serendipitous findings on detecting toxic cyanide with vitamin B_{12} and related molecules. The stepwise improvement of this method which resulted in the commercialisation of a test-kit and a smartphone-based detection system was discussed. The latter was developed together with the Karlen group from ETH Zurich (Switzerland).

Mathias Cherbuin (CyanoGuard, Switzerland), drew the attention of the audience to the very short shelf life of cassava due to its rapid deterioration. The resultant economic losses underscore the need for processing to get a storable and high-value crop. Currently, throughout Africa processing techniques and methods vary widely and the reduction of cyanide in the resulting products with it. CyanoGuard has developed an app that can calculate the adequate processing times to reach a safe product. Through online connectivity, obtained data can be used to give recommendations to communities and to warn regions in case of high cyanide levels.

After a coffee break, Deep Kapur (Australian Centre for Financial Studies, Melbourne Australia) described his way from working for investment industries to his current academic affiliation. Inspired by the achievements of the late Howard Bradburry, he got interested in solving the konzo problem and sees his potential role in management and fundraising.

The presentations were followed by stimulating round table discussions in which the participants decided to establish a working group. Main goal is fighting *konzo* and the first joined projects are in preparations. The working group will be initially headed by Felix Zelder until Ros Gleadow will take over in the beginning of 2020.

The symposium was a great success and finished with a conference dinner at the beer garden of *Linde Oberstrass*, a traditional Zurich brewery.

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Chronic Exposure to Cyanogens in Cassava: Evidence from Zambezia Province, Mozambique (Based on presentation at XV1 Jornadas de Saúde, Maputo, September 2018)

Introduction

In Mozambique the first cases of konzo were identified in 1981 in an epidemic with more than 1100 cases in northern Nampula Province. Since then the disease has been both epidemic and endemic in Nampula and neighbouring Zambézia Province. Monitoring in Nampula has shown continued cyanide exposure from cassava in both patients with demonstrated konzo, but also in asymptomatic schoolchildren. This study presents the results of recent measurements of the concentration of cyanogens in cassava flour and of thiocyanate in urine in patients and their families in Zambézia Province, with the aim of determining if cyanide exposure has continued.

Methods

In October 2017, at the end of the cassava harvest period, we collected samples of urine from 40 individuals residing in three localities of Mocuba, Gilé and Ilé Districts. Fifteen were known konzo patients and twenty-five were family members. A cassava flour specimen was collected from fourteen of the fifteen households. The concentrations of urinary thiocyanate and cyanogen in flour were measured using kits based on the picric acid method. Normal maximum values were considered to be 40 μmol/L in urine and 10 ppm in flour.

Results

The 15 konzo patients had a mean urinary thiocyanate concentration of 613.5 μmol L^{-1} (±404.5 SD), versus 392.2 μmol/L (±234.3 SD) in those without konzo (p=0.07). Two patients had high outlying values (1,290 and 1,720 μmol L^{-1}) and were consuming flour with cyanogen concentrations of 30 and 40 ppm respectively. Thirteen of the 14 flour samples had concentrations between 15 and 50 ppm. In 8 samples, the concentrations were between 30 and 50 ppm.

Conclusion

Almost 40 years after the identification of konzo in Mozambique, konzo patients and their families continue to be exposed to cyanogens from bitter cassava. The disease affects the poorest of the rural poor, dramatically reducing their quality of life. A lack of response from local health authorities compounds the situation. Effective implementation of methods to detoxify bitter cassava is urgent, along with education and physical rehabilitation. Efforts are under way to increase konzo awareness at the central level of the Ministry of Health.

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Konzo Disease-Democratic Republic Of Congo: Cassava Poisoning

Konzo, a food-borne illness that paralyzes the legs and back, has been raging in Feshi territory in southwestern Democratic Republic of the Congo (DRC)'s Kwango province for several months, the ACP Director general of the Higher Pedagogical Institute (ISP) of Feshi, Professor Simon Masaki, staying in Kinshasa, said here Tuesday [23 Apr 2019].

This disease, he said, particularly affects children and adolescents aged 4 to 18 years. Many children are paralyzed and abandoned to their plight, he lamented. Many families are plunged into distress because of this situation. The Feshi Hospital Center, which is not well equipped, is not able to take care of these many cases of Konzo, he said. Professor Masaki makes a strong appeal to the provincial and national authorities and partners of the DRC to come to the rescue of the population of Feshi, which is landlocked and very difficult to access. Feshi is located 385 km [239 mi] from Kenge, capital of the Kwango province, and more than 700 km [435 mi] from Kinshasa, capital of the DRC.

Konzo is a disease attacking the lower limbs and spine, causing a deplorable and often irreversible physical deformity. This disease is caused by the consumption of a very bitter cassava variety that would normally remain soaked in water for at least 4 days to remove cyanide before being consumed. But many people do not respect this deadline. The territory of Kahemba, in the same province of Kwango, was hit in 2018 by Konzo.

Dr. Sadiki Ngeleza of the School of Public Health of the University of Kinshasa conducts field studies on Konzo disease, funded by the National Institutes of Health through the Oregon Health and Science University (OHSU), to determine the biological risk factors associated with Konzo and the role of nutritional factors in susceptibility to Konzo.

-Communicated by: ProMED-mail Rapporteur Mary Marshall

Outbreak of Cyanide Poisoning Caused by Consumption of Cassava Flour — Kasese District, Uganda, September 2017

Reprinted with permission from Morbidity and Mortality Weekly Report (*MMWR*)
April 5, 2019 / 68(13);308–311

Summary

In September 2017, an outbreak of suspected cyanide poisoning, involving 98 cases with two deaths, occurred in western Uganda. Epidemiologic and laboratory investigation identified consumption of a cassava flour dish made from wild cultivars of cassava with high cyanogenic content as the cause of the outbreak.

Materials

Cassava (*Manihot esculenta*), an edible tuberous root that is resistant to drought, diseases, and pests, is a major source of carbohydrates in tropical areas, the second most widely grown and consumed food in Uganda after bananas, and a staple in the diet for approximately 57% of the Uganda population.[1] On September 5, 2017, a funeral was held in Kasese District in western Uganda. Following the funeral, 33 persons with symptoms that included diarrhoea, vomiting, and abdominal pains were admitted to Bwera Hospital in Kasese District. On September 8, the Uganda Ministry of Health received notification from the Kasese District health team regarding this outbreak of suspected food poisoning. An investigation to determine the cause of the outbreak and recommend control measures revealed that the outbreak resulted from consumption of a cassava dish made by combining hot water with cassava flour. The implicated batch of cassava flour was traced back to a single wholesaler and found to contain high cyanogenic content. Informed by the investigation findings, police confiscated all cassava flour from retailers identified as the patients' source of the flour. Health education about cyanide poisoning from cassava and the need to adequately process cassava to reduce cyanogenic content was conducted by public health officials.

Epidemiologic Investigation

An investigation into the outbreak was conducted by fellows of the Uganda Public Health Fellowship Program and their supervisors. A probable case was defined as sudden onset of vomiting or diarrhoea with one or more of the following signs or symptoms in a resident of one of three Kasese District subcounties during September 1–9, 2017: myalgia, tachycardia, tachypnea, headache, dizziness, lethargy, convulsions, or syncope. Medical records at Bwera Hospital, which has a catchment area covering the three subcounties, were systematically reviewed. Active case-searching was conducted with the help of community leaders.

The investigation identified 98 probable cases, with two deaths (case-fatality rate = 2%). The median patient age was 10 years (range = 11 months–75 years). Reported signs or symptoms included vomiting (95%), diarrhoea (87%), malaise (60%), dizziness (48%), tachypnea (27%), syncope (16%), and tachycardia (10%); 6% of patients reported fever. These signs and symptoms suggested cyanide poisoning.[3] Although the recommended

treatment for acute cyanide toxicity is hydroxocobalamin (injectable vitamin B12),[4] persons who went to health care facilities were managed on intravenous antibiotics and oral rehydration salts.

The outbreak affected all age groups; the attack rate was similar in males and females, and in all three subcounties, but was lower in persons aged 19–44 years (5.5 per 10,000 population) than in younger or older persons (≤18 years, 15.1 and ≥45 years, 12.1) (p = 0.003). Illness onset began a few hours after the funeral on September 5, and continued through September 8. Among funeral attendees, a peak in cases occurred a few hours after the evening meal at the funeral; among nonattendees, three successively diminishing peaks occurred, each a few hours after the evening meals on September 6, 7, and 8.

A case-control study was conducted to identify the likely source of the outbreak. Two age-matched (within 5 years) controls for each case-patient were selected from among neighbours of case-patients who had eaten cassava during September 1–9 but did not develop vomiting or diarrhoea. A total of 88 case-patients and 176 controls were interviewed in person regarding potential exposures. To account for the matched design, Mantel-Haenszel odds ratios (ORs) and the associated 95% confidence intervals (CIs) were computed, where the stratification variable was the match-set. Analyses were performed using CDC's Epi Info software.

Case-patients were more likely than were controls to have attended the funeral (OR = 40; 95% CI = 5.4–298) and to have purchased their cassava flour from retailers that were supplied by wholesaler A (OR = infinity; 95% CI = 5.6–infinity). When the data were stratified by funeral attendance, all funeral attendees were noted to have eaten cassava purchased from a retailer supplied by wholesaler A. Among nonattendees, 100% of case-patients and 79.2% of controls bought cassava flour from retailers supplied by wholesaler A during the outbreak period (OR = infinity; Fisher's exact 95% CI = 4.3–infinity).

Traceback and Laboratory Investigations

The Uganda Public Health Fellowship Program investigators conducted interviews with area retailers and wholesalers regarding their sources of cassava, and the implicated product was further traced back to its source. Two primary sources were identified. Farmers grew their own cassava, known as "sweet" cultivars. Residents also bought cassava from retailers, especially for serving at communal gatherings when a large quantity was needed. The retailers bought their cassava flour from wholesalers, who mainly bought from cassava mills in Kasese town, approximately 31 miles (50 km) away. During the outbreak period, wholesaler A was the main supplier to retailers in the three subcounties. Wholesaler A reportedly bought the implicated batch from a town bordering Uganda and Tanzania, approximately 174 miles (280 km) from Kasese; the implicated batch was further traced back to Tanzania. Because this batch cost less than other batches for sale at the time, investigators speculated that it might have been from "wild" cultivars. This suspicion was corroborated by funeral attendees, who described the cassava flour dish served at the funeral as pure white, which is typical of flour from wild cultivars, instead of the creamy-colored flour from sweet cultivars.

Cassava flour samples were obtained for visual inspection and spectrophotometric cyanide testing by the Government Analytical Laboratory in Uganda. The five samples obtained from the implicated batch were pure white in color and contained cyanogenic glycoside that was equivalent to an average of 88 ppm of cyanide (range = 85–90), more than eight times the recommended safe level of 10 ppm.[2]

Informed by findings of this investigation, police in Kasese District confiscated all sacks of cassava flour from retailers where affected families had purchased the product. Health education was conducted in the communities about cyanide poisoning from cassava and the need to adequately process cassava to reduce the cyanide content.

Discussion

The epidemiologic, traceback, and laboratory investigations indicated that this outbreak of cyanide poisoning resulted from eating cassava with a high cyanogenic content. Patients' signs and symptoms included dizziness, vomiting, tachypnea, syncope, and tachycardia and were consistent with acute cyanide poisoning,[3,5] the absence of fever made infectious etiology unlikely. Symptoms occurred a few hours after meals during which a cassava flour dish was served. This finding was consistent with previous reports, with symptoms typically starting 4–6 hours after ingesting a meal, as the cyanide is released upon digestion of the cyanogenic glycosides.[6] The case-control study strongly linked the outbreak to cassava flour supplied by wholesaler A, and the traceback investigation suggested that the implicated cassava might have originated in Tanzania. The laboratory investigation found high levels of cyanogenic glycosides in the implicated cassava flour.

Cassava crops are resistant to drought, pests, and diseases, making cassava invaluable for food security, especially in areas plagued by food shortages.[7] Approximately 600 million tropical residents, half of whom live in Africa, rely on cassava as their main food source.[8] Acute cyanide poisoning, often with fatal consequences, can occur

after eating a large amount of cassava, especially in communities dependent on a monotonous cassava diet.[9] Recurrent exposure to nonlethal concentrations through a monotonous cassava-based diet leads to long-term effects, including paralytic diseases such as tropical ataxic neuropathy and konzo, a neurologic disease characterized by sudden onset of irreversible, nonprogressive spastic paralysis.[2] In sub-Saharan Africa, particularly Uganda, Tanzania, and the Democratic Republic of the Congo, thousands of persons might have experienced cyanide poisoning from cassava,[7,8] but the full extent of the problem remains unknown because reliable data are lacking.

Although wild cassava cultivars have greater yield, higher resistance to pests, and longer storability in the soil than do sweet cultivars, they are bitter, and hence, have a lower market value. In addition, the cyanogenic content of wild cultivars is as high as 2,000 ppm of dry weight,[1] 200 times the safe level (<10 ppm) recommended by the World Health Organization.[2] Therefore, wild cultivars are not recommended for human consumption. However, some farmers still plant wild cultivars because of their resilience and high yield.[1]

Although the cyanogenic content of sweet cassava is substantially less than that of wild cultivars (up to 100 ppm),[1] the sweet cassava cultivars still require detoxification before they are consumed; this involves peeling the tubers, soaking them in water for 4–6 days, and sun-drying or roasting them. The outer layer is then scraped off and the remainder ground into flour. This process promotes enzymatic degradation of cyanogenic glycosides. If the soaking or drying time is too short, enzymatic degradation will be inadequate, and cyanogenic glycosides remain high.[5] During droughts, cassava traders sometimes fail to follow recommended procedures, which can result in a product with high levels of cyanogenic glycosides that can lead to cyanide poisoning.[1]

A rapid, semiquantitative, colorimetric test that is free to workers in developing countries can be used by relatively untrained persons to quickly determine the cyanogenic potential of cassava flour.[10] Wholesalers and government food inspectors can use this method to routinely measure cyanogenic content of commercial cassava flour. Farmers and consumers in areas that depend upon cassava should be warned about cyanide poisoning caused by eating improperly processed or wild-cultivar cassava, and instructed to strictly adhere to the established processing methods to degrade cyanogenic glycosides.

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Acknowledgments

Kasese district health office and police department; Uganda Government Analytical Laboratory.

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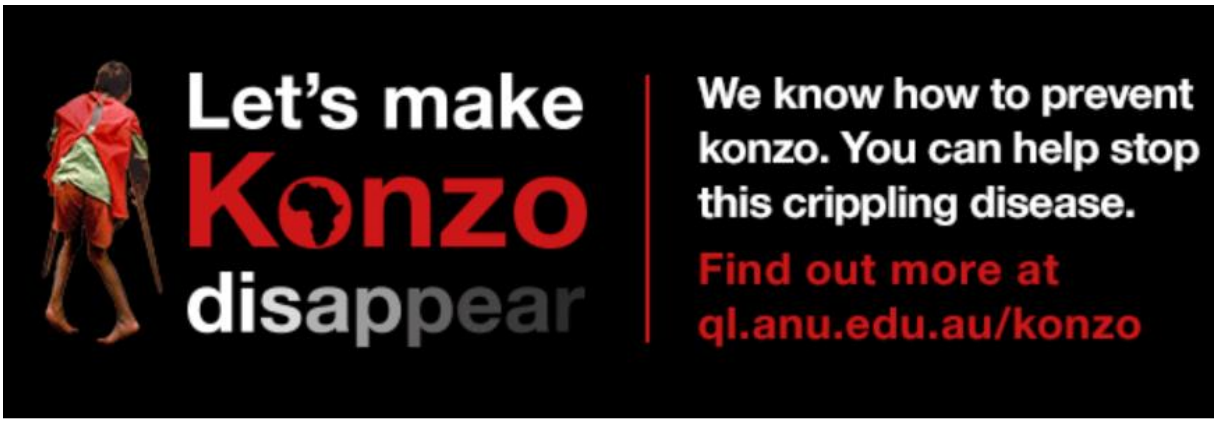

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