Incidence of endemic ataxic polyneuropathy and its relation to exposure to cyanide in a Nigerian community

O S A Oluwole, A O Onabolu, I A Cotgreave, H Rosling, A Persson, H Link

Background: The occurrence of ataxic polyneuropathy in an endemic area in south west Nigeria has been attributed to exposure to cyanide from cassava foods. However, it has been shown that the prevalence of ataxic polyneuropathy is not high in several communities in the tropics where exposure to cyanide from cassava foods is high.

Objectives: To determine the incidence of ataxic polyneuropathy in an endemic community, and to compare the intake of cassava foods, exposure to cyanide, and levels of thiols in cases and controls.

Methods: A cohort of 3167 healthy subjects aged 10 years and over in Ososa, Nigeria, was followed for two years, screened, and examined neurologically. Ataxic polyneuropathy was diagnosed if sensory polyneuropathy and sensory gait ataxia were both present. Controls were selected randomly within 10 year age groups of subjects who screened negative. Intake of cassava foods, exposure to cyanide, concentrations of thiols (glutathione, cysteine, and γ glutamylcysteine) in plasma, and visual evoked potentials were measured.

Results: Person-years of follow up were 6246 for 1469 male and 1698 female subjects in the cohort. The incidence of ataxic polyneuropathy was 64 per 10 000 person–years (31 for male and 93 for female subjects). Multivariate odd ratios were 0.78 (95% CI 0.23 to 2.61) for intake of the commonest cassava food, and 1.64 (0.56 to 5.09) for concentration of thiocyanate in plasma. The concentration of thiols was less than the reference limits in two controls, but in none of the cases. The latency of P100 was prolonged in 20 cases (69%) compared with 14 controls (42%) (p<0.05).

Conclusions: The incidence of ataxic polyneuropathy is high in Ososa, Nigeria, but the intake of cassava foods, exposure to cyanide, and levels of thiols, are not related to the occurrence. These findings do not suggest that cyanide is the cause of endemic ataxic polyneuropathy.

METHODS

Study site
The study was conducted in Ososa, a community in south western Nigeria, which is located on latitude 6.71 N and longitude 3.53 E. This semiurban community is within the area that was defined as endemic for ataxic polyneuropathy (fig 1). A de facto census that was done in 1998 showed that about 5000 people reside in the community. The prevalence of ataxic polyneuropathy was determined in Ososa in 1969 and 1998.

Definition of a case of ataxic polyneuropathy
In the 1960s ataxic polyneuropathy was defined as the presence of any two of the following features: sensory polyneuropathy, sensory gait ataxia, optic atrophy, and neurosensory deafness. During a prevalence study in Ososa in 1998, we observed, however, that several combinations of signs without sensory gait ataxia and sensory polyneuropathy would be diagnosed as ataxic polyneuropathy when the case definition of the 1960s was used. To...
prevent such misdiagnoses the definition of was made more stringent in this study by the requirement for the presence of both symmetrical sensory polyneuropathy and sensory gait ataxia in every case.

Establishment of the cohort and screening
In a previous study all subjects aged 10 years and above in Ososa were screened and examined for ataxic polyneuropathy, using the case definition of the 1960s. All subjects living in Ososa who were not diagnosed as having ataxic polyneuropathy or other neurological illness formed a cohort that was followed for two years in the present incidence study. All deaths and movements out of the cohort were recorded.

At the end of the follow up, the cohort was screened with a tandem-walk test, which was standardised from descriptions in textbooks. To undertake the tandem-walk test subjects were instructed to stand with both feet together, look straight ahead and not at the ground, and walk heel to toe in a straight line over a distance of three metres without hesitation or stopping, with both hands by the sides and not swinging. Inability to tandem-walk, or stepping out of a straight line consistently after three trials, was considered a positive test. Subjects who were positive on the tandem-walk test were invited for a neurological examination. The sensitivity of the tandem-walk test as a screening test for ataxic polyneuropathy was previously shown to be 100%, with a specificity of 58%.

A full medical history and a history of exposure to pesticides, industrial chemicals, drugs, smoking, and alcohol were obtained from each subject. A neurological examination was done as described in standard texts.

Cases and controls
Cases of ataxic polyneuropathy and subjects who screened negative on the tandem-walk test were grouped into 10 year age categories of each sex. Three controls were selected randomly for each case within the 10 year age categories of subjects who screened negative.

Blood and urine samples
Samples of venous blood, which were collected after an overnight fast, were centrifuged immediately, and the plasma kept on ice during transportation to the laboratory, where they were stored at −80°C until analysis. Four hour timed urine samples were collected between about 8.00 am and 12.00 noon. Subjects were given a jar in which to collect their urine after emptying their bladder under supervision. Final urine samples, which were collected at the end of four hours under supervision, were tested for sugar and protein. The volumes of urine were measured and aliquots were stored at −80°C until analysis.

The concentrations of thiocyanate in plasma and urine were determined using previously described methods. The concentrations of cysteine, glutathione, and γ-glutamylcysteine, the major low molecular weight thiols in the plasma, were determined using established methods. Briefly, low molecular weight thiols in the plasma were reduced with dithiothreitol and derivatised with monobromobimane. Protein was precipitated from samples with trichloroacetic acid. Samples were centrifuged, and 25 µl of the supernatant was applied to an HPLC column to determine the thiol-bimane adducts.

The concentrations of creatinine and of inorganic sulphate were determined using standard methods. The excretion of creatinine and thiocyanate in the urine per hour was calculated. The reference limits for thiocyanate, thiols, and inorganic sulphate were as previously published.

Food history and body mass index
A history of all foods eaten during the week preceding the day the urine and blood samples were collected was obtained from the subjects. The number of meals and quantities of gari, lafun, and fufu, the local foods that are processed from cassava roots, were counted from the diet history of each subject. As eba, another cassava food, is prepared by adding boiling water to gari, it was counted separately from the diet history. An intake of cassava foods of five or more times a week was considered high, based on the local consumption pattern, previous reports, and estimates of levels of cyanogenic compounds in gari sold in Nigeria.

Semiquantitative methods were used to estimate the amounts of protein in the foods. The amount of protein taken per day was calculated for each subject. The reference lower limit for intake of protein was 0.75 g/kg body mass per day. Body mass (kg) and height (m) were measured for each subject, and the body mass index (BMI) was calculated using the formula body mass/height². The reference lower limit for the BMI was 18.5.

Neurophysiological tests
Visual evoked potential (VEP) responses were examined using portable neurophysiology equipment (Medilec Synergy). The scalp electrodes were cleansed with abrasive cream or acetone until the resistance was less than 4000 Ω. The recording scalp electrode, which was placed 5 cm above the inion, was referenced to Fpz, and the ground electrode was placed at Cz. The stimulator, which was a 15 inch liquid crystal display personal computer monitor, was placed 1 m in front of the subject’s face in the sitting position. The pattern stimulus operated at two reversals a second. Subjects were instructed to gaze at a spot at the centre of checkers of size 16. Each eye was recorded separately in full field.

Three trials, each of 100 sweeps, were recorded for each subject. Latency was measured from departures from baseline, and amplitude from baseline to peak. Latency in one eye was considered abnormal. Visual acuity was tested at the bedside. Subjects with impairment of vision or cataracts were excluded. All recordings were done in a darkened room in the local primary health care clinic in the community. Nerve conduction tests were attempted, but not successful because of technical problems with the electrical
The subjects used for determination of reference values for VEP were selected from another Nigerian community, which is located in the non-endemic area in Nigeria where exposure to cyanide from cassava food is high, but where the prevalence of ataxic polyneuropathy is low.12 The controls were randomly selected from among persons aged 60 to 80 years in the community. The 95% interpercentile range for the P100 latency was 108 to 125 ms (n = 26), while the 95% interpercentile range for the P100 amplitude was 5 to 21 μV.

Statistical analysis
Subjects who died or moved out of the community during the follow up period were censored. Total follow up period was calculated by summing up the duration of follow up for each subject. The incidence rate was calculated per 10 000 person-years. Risk factors were compared between cases and controls using univariate analysis and a multiple logistic regression model. Univariate and multivariate odds ratios of the risk factors were calculated. P100 latency and amplitude of the visual evoked potentials for cases and controls were compared using the $\chi^2$ test. Statistical calculations were done using SPSS version 10.1, EPI info version 6, and Medcalc.

RESULTS
Incidence
There were 3167 subjects in the cohort, 1469 male and 1698 female. The distribution of age and sex of the subjects is shown in table 1. We censored 111 subjects—54 who moved out of the community and 57 who died. The mean (SD) age of subjects who moved out of the community during the follow up was 33 (18) years (median 28, range 10 to 80), and of those who died, 65 (21) years (median 70, range 10 to 105). Of 3056 subjects who were alive at the end of follow up, 52 were not available at the time of screening, and of the 3004 subjects who were screened, 271 who screened positive for ataxic polyneuropathy were invited for neurological examination. Twelve of these defaulted from the neurological examination. After the examination, ataxic polyneuropathy was diagnosed in 40 subjects (31 female and nine male). Seven of these had neurosensory deafness, but none had optic atrophy. None of the subjects had clinical or laboratory evidence of alcoholism, diabetes mellitus, or chronic renal failure, and none had been exposed to pesticides. No clinical features of malnutrition or vitamin deficiency were observed in any subject.

Person–years of follow up, distribution of the ages of subjects with ataxic polyneuropathy, and age and sex specific incidence rates are shown in table 2. Total person–years of follow up were 6246.2. The incidence of ataxic polyneuropathy was 64 per 10 000 person–years in people aged 10 years or more in Ososa (31 per 10 000 for male subjects and 93 per 10 000 for female subjects). The age specific incidence rate was highest in the 70 to 79 year age group for both sexes. The approximate cumulative incidence was 63 per 10 000 per year (31 per 10 000 per year for male subjects, and 91 per 10 000 per year for female subjects).

Nested case–control
All subjects who were selected for the case–control study agreed to participate, but only those who gave consent to venesection (23 (58%) of 40 cases of ataxic polyneuropathy and 60 (50%) of 120 controls) were included in the case–control study. Although the response rate was about 50%, there was no evidence that those who refused venesection were different from those who accepted with respect to their

### Table 1 Distribution of subjects in the cohort and subjects censored

<table>
<thead>
<tr>
<th>Age group (years)</th>
<th>Cohort Male</th>
<th>Cohort Female</th>
<th>Moved* Male</th>
<th>Moved* Female</th>
<th>Died Male</th>
<th>Died Female</th>
<th>Screened† Male</th>
<th>Screened† Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 to 19</td>
<td>563</td>
<td>504</td>
<td>9</td>
<td>7</td>
<td>1</td>
<td>2</td>
<td>533</td>
<td>495</td>
</tr>
<tr>
<td>20 to 29</td>
<td>220</td>
<td>350</td>
<td>3</td>
<td>11</td>
<td>0</td>
<td>1</td>
<td>203</td>
<td>338</td>
</tr>
<tr>
<td>30 to 39</td>
<td>184</td>
<td>259</td>
<td>3</td>
<td>4</td>
<td>2</td>
<td>0</td>
<td>167</td>
<td>255</td>
</tr>
<tr>
<td>40 to 49</td>
<td>155</td>
<td>175</td>
<td>1</td>
<td>4</td>
<td>7</td>
<td>2</td>
<td>143</td>
<td>169</td>
</tr>
<tr>
<td>50 to 59</td>
<td>126</td>
<td>130</td>
<td>2</td>
<td>4</td>
<td>3</td>
<td>1</td>
<td>119</td>
<td>145</td>
</tr>
<tr>
<td>60 to 69</td>
<td>124</td>
<td>119</td>
<td>3</td>
<td>1</td>
<td>6</td>
<td>2</td>
<td>115</td>
<td>116</td>
</tr>
<tr>
<td>70 to 79</td>
<td>62</td>
<td>74</td>
<td>1</td>
<td>0</td>
<td>5</td>
<td>9</td>
<td>56</td>
<td>65</td>
</tr>
<tr>
<td>80 to 89</td>
<td>27</td>
<td>58</td>
<td>0</td>
<td>1</td>
<td>3</td>
<td>8</td>
<td>24</td>
<td>49</td>
</tr>
<tr>
<td>90 to 99</td>
<td>7</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>≥100</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>1469</td>
<td>1698</td>
<td>22</td>
<td>32</td>
<td>30</td>
<td>27</td>
<td>1365</td>
<td>1639</td>
</tr>
</tbody>
</table>

*Subjects who moved out of the community during the follow up period.
†Subjects who were screened for ataxic polyneuropathy at the end of the follow up period.

### Table 2 Distribution of person–years of follow up and age specific incidence rates

<table>
<thead>
<tr>
<th>Age group (years)</th>
<th>Person-years of follow up</th>
<th>Cases Male</th>
<th>Cases Female</th>
<th>Age specific incidence (per 10 000 person–years) Male</th>
<th>Age specific incidence (per 10 000 person–years) Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 to 19</td>
<td>1116.9</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>20 to 29</td>
<td>430.8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>30 to 39</td>
<td>564.7</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>40 to 49</td>
<td>304.3</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>87</td>
</tr>
<tr>
<td>50 to 59</td>
<td>249.2</td>
<td>0</td>
<td>5</td>
<td>0</td>
<td>170</td>
</tr>
<tr>
<td>60 to 69</td>
<td>240.0</td>
<td>3</td>
<td>9</td>
<td>125</td>
<td>380</td>
</tr>
<tr>
<td>70 to 79</td>
<td>117.9</td>
<td>4</td>
<td>11</td>
<td>339</td>
<td>786</td>
</tr>
<tr>
<td>≥80</td>
<td>63.3</td>
<td>2</td>
<td>3</td>
<td>315</td>
<td>241</td>
</tr>
<tr>
<td>Total</td>
<td>2895.1</td>
<td>9</td>
<td>31</td>
<td>31</td>
<td>93</td>
</tr>
</tbody>
</table>
intake of cassava foods or exposure to cyanide. There were 26 male and 57 female subjects in the nested case–control study. The mean (SD) age of the cases was 69 (10) years (range 45 to 84), and the mean age of the controls was 65 (11) years (range 40 to 89).

The distribution of risk factors and their odds ratios are shown in table 3. The BMI values of six cases and 14 controls were below the reference limits (p > 0.05). Five cases (22%) compared with 16 controls (27%) took gari above the reference limit, while 15 cases (65%) compared with 41 controls (68%) took eba above the reference limit. There was no statistical difference in the intake of all cassava foods between cases and controls, and between male and female subjects (p > 0.05). Twelve cases (52%) compared with 24 controls (40%) had levels of thiocyanate above the reference limits. Exposure to cyanide was not significantly different between cases and controls (p > 0.05). The concentrations of cysteine and γ-glutamylcysteine in the plasma were less than the reference limits in two controls and in one case. The total thiol level was less than the reference limits in two controls and in none of the cases, while plasma glutathione was below the reference limit in four controls and in one case. The total thiol level was below the reference limit in two controls and in none of the cases; therefore thiols were not included in the multivariate model. The univariate odds ratio was 2.67 (95% confidence interval, 0.89 to 7.96) for the excretion of inorganic sulphate. The P100 latency was prolonged in 20 of 29 cases compared with 14 of 33 controls (p < 0.05). The P100 amplitude was reduced in 11 of 29 cases compared with 15 of 33 controls (p > 0.05).

**DISCUSSION**

Our study shows that new cases of ataxic polyneuropathy still occur in Ososa, a community in the endemic area for ataxic polyneuropathy in south west Nigeria. In 1969 the prevalence of ataxic polyneuropathy was 22 per 1000 in Ososa, while in 1998 a study using the same case definition of the 1960s found a prevalence of 60 per 1000 in the same area. The incidence of 64 per 10 000 person-years in this study suggests that the occurrence of ataxic polyneuropathy in Ososa, and probably in other communities in the endemic area, remains high, although several communities which were studied in the 1950s and 1960s have not been restudied. The absence of previous data on incidence, however, precludes firm conclusions about changes in the rate of occurrence.

Ataxic polyneuropathy has been described predominantly in the elderly population in prevalence studies. The fact that the highest age specific incidence rate was in the 60 to 79 year age range in our study provides further support for this being predominantly a disease of the elderly. It has been suggested that it may be a neurodegenerative disease, although exposure to toxins and deficiencies of micronutrients have been the focus of most studies.

The male to female incidence rate ratio of 1:3 in this study shows that the disease is more common in women. The predominance of women has also been reported in prevalence studies. The reason for a higher prevalence in women is not known, but it is unlikely to reflect a greater exposure to cyanide from cassava foods. In this study there was no difference in the intake of cassava foods or exposure to cyanide between male and female subjects, although the number of male cases was small. A study of 21 communities in Nigeria also showed similar intakes of cassava foods and similar exposure to cyanide in male and female subjects.

The cause of endemic ataxic polyneuropathy has been attributed to exposure to cyanide from cassava foods. This hypothesis is based largely on studies which showed high intakes of cassava foods and high levels of thiocyanate in plasma and urine of subjects in the endemic area in Nigeria. However, the level of intake of cassava foods in cases of endemic ataxic polyneuropathy and their family members was similar in another study. In the present study the level of intake of cassava foods was also similar in cases and controls. Studies of the intake of cassava foods in Nigeria have shown that variations in intake within specific ethnic groups are small, while variations in intake between ethnic groups are large.

Cassava roots, from which several foods are processed, contain cyanogenic compounds that are usually reduced during processing. Cyanogenic compounds, however, often remain in cassava foods because the methods of processing cassava roots are not 100% efficient. Although hydrogen cyanide is rarely detected in these foods, consumption of foods containing cyanogenic compounds has been shown to result in exposure to cyanide, though the amount of cyanide absorbed from a single meal is small.

Cyanide is metabolised to several compounds such as thiocyanate, cyanate, formate, and 2-iminothiazolidine-4-carboxylic acid, but the major metabolite is thiocyanate. Thiols, which are sources of sulphur, are required to detoxify cyanide to thiocyanate. It has been hypothesised that the detoxification of cyanide to thiocyanate may be impaired in cases of ataxic polyneuropathy because of a deficiency of thiols. The metabolism of cyanide to thiocyanate has not, however, been shown to be impaired, even in starvation.

This may reflect the continuous release of amino acids from the lean body mass during starvation. Experimental studies do not show impairment of detoxification of cyanide to thiocyanate in rats on low protein or a diet containing no sulphur amino acids.

In this study the finding of similar levels of thiols in cases and controls does not suggest deficiency of substrates needed to detoxify cyanide to thiocyanate in ataxic polyneuropathy. Studies that have shown high rather than low levels of thiocyanate in cases of ataxic polyneuropathy do not support the notion of impairment of detoxification of cyanide to thiocyanate in this disease.

Specific deficiencies of

**Table 3 Risk factors and odds ratios**

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Odds ratios</th>
<th>Univariate (CI)</th>
<th>Multivariate (CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (male, female)</td>
<td>0.36</td>
<td>(0.11 to 1.21)</td>
<td>0.33 (0.09 to 1.18)</td>
</tr>
<tr>
<td>Age (60 to 66 years, &gt;60 years)</td>
<td>2.56</td>
<td>(0.77 to 8.52)</td>
<td>3.79 (0.96 to 14.96)</td>
</tr>
<tr>
<td>BMI (&gt;18.5, &lt;18.5 kg/m²)</td>
<td>1.39</td>
<td>(0.42 to 4.62)</td>
<td>1.12 (0.33 to 3.85)</td>
</tr>
<tr>
<td>Intake of foods (0–4/day, &gt;5/day)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gari*</td>
<td>0.76</td>
<td>(0.24 to 2.40)</td>
<td>0.76 (0.21 to 2.78)</td>
</tr>
<tr>
<td>Eba*</td>
<td>0.87</td>
<td>(0.31 to 2.40)</td>
<td>0.78 (0.23 to 2.61)</td>
</tr>
<tr>
<td>Plasma thiocyanate (0–6 µmol/l, &gt;6 µmol/l)</td>
<td>1.64</td>
<td>(0.62 to 4.30)</td>
<td>1.69 (0.56 to 5.09)</td>
</tr>
</tbody>
</table>

*Gari and Eba are foods from cassava roots.

BMI, body mass index; CI, confidence interval.
vitamins and other micronutrients have not been found, except for the presence of low levels of riboflavin in one study. Assays of B vitamins’ and levels of methylnalonic acid in the urine were normal. No change in the clinical status of the subjects was observed in clinical trials with protein and vitamin supplements.

Although the clinical features of ataxic polyneuropathy seen in Nigeria suggest peripheral and central nervous system pathology, detailed neuropathological or neuroimaging studies have not been done to define the nature of the lesions clearly. In this study the delayed P100 amplitude of the VEP suggests demyelination of the optic nerves. The neuropathological investigations in our study were, however, too limited to allow any conclusions to be drawn on possible pathology in the neuroaxis and peripheral nerves.

There are several limitations to a study of the relation between exposure to cyanide from the intake of cassava foods and the occurrence of ataxic polyneuropathy. It is difficult to estimate exposure to cyanide from the frequency of intake of cassava foods because of the wide variation in levels of cyanogenic compounds in foods that are processed using different methods. Differences in the duration of storage, during which cyanogenic compounds are lost, also contribute to the difficulty in estimating exposure to cyanide from cassava food intake. In addition, the duration of exposure to cyanide from cassava foods in the endemic community is difficult to measure accurately because the intake of these foods starts in early childhood in such communities.

Thiocyanate levels have been used in several studies to measure exposure to cyanide quantitatively. It has been shown that thiocyanate concentrations in the urine correlate with the intake of gari, the most popular cassava food in Nigeria. However, it is only possible to assess the intake of cassava foods in the short term by thiocyanate levels. The most popular cassava food in Nigeria suggests peripheral and central nervous system pathology. The study was funded by the International Programme in Chemical Toxicology, Institute of Neurology, Huddinge University Hospital, Karolinska Institute

Competing interests: none declared

REFERENCES


A Persson, Department of Clinical Neurophysiology, Huddinge University Hospital, Karolinska Institute

H Link, Department of Neurology, Huddinge University Hospital, Karolinska Institute

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*J Neurol Neurosurg Psychiatry* 2003 74: 1417-1422
doi: 10.1136/jnnp.74.10.1417

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