British Journal of Medicine & Medical Research
3(4): 2054-2068, 2013

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Nodding Syndrome (NS) in Northern Uganda: A Probable Metabolic Disorder

David Lagoro Kitara[1*], Amos Deogratius Mwaka[2], Denis Arony Anywar[3], Gilbert Uwonda[3], Bernard Abwang[4] and Edward Kigonya[4]

[1]Department of Surgery, Gulu University, Faculty of Medicine, P.0. Box166, Gulu Uganda.
[2]Department of Internal Medicine, Makerere University, College of Health Sciences, Medical School, P.0. Box 7072, Kampala, Uganda.
[3]Department of Biochemistry and Biostatistics, Gulu University, P.0. Box 166, Gulu, Uganda.
[4]Uganda Ministry of Health, Headquarters, P.0. Box 7272, Kampala, Uganda.

Authors' contributions

This work was carried out in collaboration between all authors. Author DLK designed the research protocol, conducted the literature search, supervised the data collection, analyzed the data, and prepared the manuscript for publication. Author ADM analyzed the data, did literature search and proof read the manuscript. Author DAA participated in data collection and literature search and proof read the manuscript. Author EK was involved in data analysis and interpretation of results. Author GU participated in the data analysis in preparation for manuscript presentations. Author BA participated in the collection of blood and skin samples for both cases and controls of the study. All authors read and approved the final manuscript

Research Article

Received 3rd April 2013
Accepted 19th June 2013
Published 4th July 2013

ABSTRACT

Aims: To conduct an epidemiological study to establish the association between malnutrition**,** metabolic disorder and *Onchocerciasis* to Nodding Syndrome (NS) in Northern Uganda.
Study Design: Case-control study design.
Place and Duration of Study: Odek and Atiak sub counties in Gulu and Amuru districts between 10th to 20th June 2012.
Methodology: We recruited consecutively 101 children with probable NS in the 2 sub counties in Gulu and Amuru districts. Controls were from the same population but without symptoms of NS and were matched by age, sex and residents. History and physical examinations were conducted; anthropometry, blood samples and skin snips

**Corresponding author: Email: klagoro@gmail.com;*

were obtained from cases and controls. Researchers were pediatricians, psychiatrists, nurses, laboratory scientists and epidemiologists. The research proposal was approved by the Ministry of Health and the IRB of Gulu University.
Results: There was a statistically significant association between NS with malnutrition (t=0.142; p=0.044), *Onchocerciasis* (X^2 = 152.74, p<0.001; OR 7.025 95% CI 3.891, 12.682) and High Anion Gap (X^2=146.752, p<0.001; OR 6.313 95%CI 4.027, 9.895).
Conclusion: Nodding syndrome is associated with metabolic disorder in young children who are malnourished and infected with *Onchocerciasis*.

Keywords: Nodding syndrome; Onchocerciasis; malnutrition; metabolic disorder; Gulu; Northern Uganda.

DEFINITIONS

Anion Gap: *The difference in the measured cations and measured anions in serum and plasma.*

Malnutrition: *This term was used to mean under-nutrition and was defined as a BMI for age for a child between 5 to 19 years less than -2SD and for the serum albumen, a level of less than 38g/dl for a child was considered undernourished.*

1. INTRODUCTION

"Nodding syndrome" is an unexplained neurologic disorder that has recently been reported among children in several sub-Saharan Africa countries and primarily among internally displaced persons [1,2,3,4]. The primary and characteristic feature is a paroxysmal "spell" in which the head bobs forward repeatedly over a period of minutes; in most cases the child appears unresponsive during the episode [3,4]. The illness has a clustering of onset between ages of 5 and 15 years [4, 6]. The nodding episodes are thought to be the initial feature of a more progressive neurological illness, in which there is neurological deterioration progressing to further seizures and cognitive impairment [1,4]. Although an illness descriptively similar to NS has been reported from southern Tanzania since the early 1960's; Liberia (1983), Britain (1909) [5] and Western Uganda (2001), the syndrome has more recently been reported, and in epidemic proportions, from southern Sudan and Northern Uganda since the mid-1990's [1,3,4]. To date, NS has not been identified outside these areas. The reasons for the geographic and temporal clustering of the illness are still unknown.

1.1 Nodding Syndrome in Northern Uganda

In 2009, an illness descriptively consistent with NS was reported by Kitgum district in northern Uganda [3,6]. In response, an investigation was jointly conducted by the Uganda Ministry of Health (MOH), the World Health Organization (WHO), and the U.S. Centers for Disease Control and Prevention (CDC) [3,6]. The multifaceted investigation included a case series on 23 cases with NS and a case-control study involving NS cases and age-matched controls, assessing various exposures and epidemiologic risk factors and laboratory testing for over 30 possible infectious, toxic, and nutritional etiologies [3,6].

The case series involved detailed neurologic history, physical and neurologic examination, cognitive evaluation, and, in a subset of cases, electroencephalography (EEG), Brain Magnetic Resonance Imaging (MRI), and cerebrospinal fluid (CSF) analysis [3]. This case series established that the nodding episodes were due to atonic seizures, and that children with NS suffered from multiple different seizure types, resulting in a form of epileptic encephalopathy [3]. The estimated prevalence of the syndrome in the area was many-fold higher than would be expected for similar types of epilepsy [3]. The CSF findings did not show pleocytosis or protein elevation, and MRI demonstrated diffuse cerebral and cerebellar atrophy, disproportionate to age [3]. Eight months later, 12 of these children were re-assessed with interim history and physical/neurological examination; most had experienced worsening of frequency and intensity of head nodding and other seizures, and none had improved [3].

Results from the case-control study excluded a number of suspected causes including nutritional factors, heavy metal poisoning, use of traditional herbs and foods, as well as wild meat among others [3,6].The investigation did however; find that NS cases were significantly more likely to have antibodies against *Onchocerca volvulus microfilariae* (the cause of “river blindness”) than controls, indicating prior infection with the parasite, or something cross-reacting with antigens on the parasite. The population as a whole was also found to be significantly deficient in serum vitamin B6 (pyridoxine) [3,6]. The significance of these findings is continuing to be further investigated. While these preliminary studies from Kitgum have provided considerable additional information on the nature and possible non-causes of NS, there are still numerous important unanswered questions including poverty, food insecurity and indigenous beliefs of the affected community [6,7,8].

Anecdotally, some residents of Kitgum district suggest that NS is a progressive and invariably fatal disease, with all children afflicted with the syndrome progressing to physical wasting, growth retardation, cognitive impairment, and death within months to years [3,6,8]. However, the true progression of the illness, the frequency of fatalities, the prognostic features, and causes of death are not known. The illness has been described as beginning insidiously, with nodding being the sentinel event, with progression to further seizures, cognitive decline, and physical changes [3,6]. However, the pattern of physical, neurologic and cognitive decline is not known, and it is unclear whether subtle neurologic, biochemical, or nutritional changes may precede nodding [3,5,8,9].

Nodding has been reported to be brought about by specific stimuli, particularly presentation of hot food, cold weather or water [6,8,9,10]. However, the peculiar association with these specific stimuli, as well as the unusual clustering of such a stereotypic presentation of a seizure, remains to be explained [3,6,11,12]. It has also been reported that some families have several children affected while others have only one of several children in the family affected [3,6,8,11,12]. What possible exposures caused such clustering in families is worth investigation. Although follow up of some cases after 8 months found worsening of the symptoms, it was not clear if the children had been consistently taking anti-epileptic medicines [3,6].

Through several weeks of careful observation of children with NS in their environment, these authors noted that the clinical features of NS and the peculiar association with specific stimuli of nodding pointed towards a metabolic disorder and one of such disorder that have never been investigated for exclusion over the period of investigation by the Uganda Ministry of Health was metabolic disorder. One of such metabolic disorder is caused by mitochondrial disease which commonly diagnosed by exclusion.

Until recently, a broad range of diseases that may be caused by mitochondrial dysfunction was not well understood or appreciated [13]. This changed with the discovery that mutations of the mitochondrial DNA could cause certain diseases. For the first time, scientists showed that a single nucleotide change in mitochondrial DNA of a mouse led to the development of muscle weakness and progressive heart disease and metabolic derangement [13].

The clinical presentations of children with nodding syndrome have most features of metabolic disorder such drooling of saliva, muscle weakness, bone deformities, seizure is provoked by physical exercises, temperature lability, stunting and failure to thrive and perhaps mitochondrial disease might be one of the causes of the metabolic disorder which could clearly give a clue to the discovery of the cause of NS.

In this study we carried out a systematic epidemiological case-control study with the interest of measuring the body's biochemical parameters in the cases and compared it to the controls in order to ascertain whether nodding occurred as a result of or in association with biochemical derangements.

2. MATERIALS AND METHODS

2.1 Setting

This study was conducted in Gulu and Amuru districts in Northern Uganda in June 2012. Gulu and Amuru was originally one district which is about 343km north of the capital Kampala. This region is just recovering from over 20 years of civil war. Gulu is one of the regional centers for northern Uganda and draws largely rural population; many of whom lived in the internally displaced people camps (IDPS) for the past 10 to 12 years for safety from the insurgency. According to the Gulu and Amuru district Development Plans 2009/2010, the 2 districts have a total population of about 650,000 people. It is estimated that over 300 children in the 2 districts have NS and that over 20 children have so far died of diseases related to NS. This area where the study was conducted was not covered by the CDC study of 2009 and these NS children had not received medication for control of seizures since the outbreak of the disease in the area in 2002.

2.2 Design

We conducted a case-control study involving children with probable cases of NS and their controls.

2.3 Population

The study population (probable cases and their controls) was selected consecutively from the 2 sub counties in Gulu and Amuru districts. The selection of the study population was conducted by a team of experts drawn from the fields of paediatrics, psychiatry, nursing, food science, vector control and epidemiology. The WHO epidemiologic surveillance case definition for probable case of NS was used for screening and recruiting the cases [16].

A probable case of NS was defined using the WHO epidemiological surveillance case definition as a child who is at least 2 years old or an adolescent who was previously growing well and presents with an epileptic disorder characterized by two or more episodes of recurrent head nodding that occur spontaneously, consequent to the sight of food or while

eating or on exposure to coldness and head nodding occurred 15–20 times per minute; with or without other types of seizures, neurological signs, regression in growth and cognitive decline or mental retardation [16].

A control was defined as a child 2 years and above who was living normally without any symptoms and signs of NS and had lived with the cases in the IDP camps. Following recruitment of each case, a control from the same residential area and having a birth date of not more than 12 months of the case's birth date and of the same sex were recruited. All the children were from the villages were the cases were recruited in Odek and Atiak sub counties during the same study period.

The sample size was calculated using preliminary data on NS obtained from the District Health offices of Gulu and Amuru. Substitutions into this formula for comparative study [17]; yielded 101 cases and 101 controls.

2.4 Inclusion Criteria for NS Cases

Children 2 years and above with probable NS; Consent from parents and assent for children 14 years and above.

2.5 Exclusion Criteria for NS Cases

Children 2 years and above but with reported history of abnormal physical, cognitive and social development before the onset of nodding. Refusal to consent to the study was another exclusion factor.

2.6 Inclusion Criteria for Controls

Healthy children who were 2 years and above and lived in the same camps with NS cases and were of the same age, sex and residents with NS cases. Consent from the parents and assent for the controls above 14 years were required.

2.7 Exclusion Criteria for Controls

Healthy children who had not lived in the camps with NS cases and those that had gross physical, cognitive and social impairment. None-responsive children who were 14 years and above were excluded from the study.

2.8 Data Collection

Questionnaire was used to collect data on the socio-demographic characteristics, anthropometry, prenatal, natal, post-natal and childhood experiences of both cases and controls. The physical and family social history of the parents of the children with NS and their controls were also explored during the interviews. Anthropometric measurements included the measurements of weight (Kg), Height (Cm), and Mid Upper Arm circumference (MUAC) and head circumferences of cases and controls. The Body Mass Index (BMI) for each of the cases and controls were calculated using the formula (weight/Height (M^2) and the BMI for age (z-scores) read on the 2007 WHO reference guidelines [18] to determine the nutritional status of the cases and control. Those with a BMI of –2SD or less were considered to be malnourished. Similarly the serum albumen levels were also analyzed for cases and controls and a level less than 38g/dL for a child was considered low and therefore

malnourished. The other part of the blood sample was used for hematological and clinical chemistry.

2.9 Procedures

Blood sample (5mls) was obtained from the cubital fossa of each of the children and stored in 2 separate bottles (EDTA and Plain bottles) for hematological, biochemical and clinical chemistry analysis respectively and kept for safe transportation in a cool box. Tourniquets were not used to identify the veins of the patients as this was likely to interfere with the electrolyte profile of the patients.

Two skin samples (Skin snips) from each participant were removed from the posterior iliac crest region and stored in normal saline for 12 hours and examined under a microscope for the presence of the *OV* microfilariae.

Four trained research assistants, with the authors supervising, collected data using questionnaires and pre-tested checklists. The research assistants (two males and two females) were medical officers with experience in study on non-communicable diseases. Consent and Assent were obtained from the children and parents/Guardians. Assent was obtained from children 14 years and above in the presence of their parents or Guardians. Those children who were above 14 years and were non-responsive were excluded from the study. Procedures on children less than 14 years were conducted after obtaining consent from their parents.

Data collection took 10 days and removal of blood for the investigations were conducted mainly in the afternoon and evening hours (2:00pm and 6:00pm) as that was the hours the participants were examined. The blood samples were transferred in a cool box to Gulu Regional Referral Hospital and Gulu University laboratories where tests were conducted on the same day of the sample collection. The remaining samples were stored in a refrigerator and temperature maintained at -80ºC.

2.10 Dependent Variable

Probable cases of NS and their controls.

2.11 Independent Variables

These were sex, age, level of education, schooling status, anthropometric measurements (Weight, MUAC, head circumference and Height), Pre, post and natal experiences of the mothers and children, hematological and biochemical indices (serum K+, Na+, HCO_3-, Cl- levels, serum albumen levels) and skin snips for *OV microfilariae*.

2.12 Analysis

SPSS statistical software package version 15.0 was used for the univariate analysis of the socio-demographic characteristics and other variables. Bivariate analysis was used to test the associations between the dependent and independent variables. Odds ratios (**OR**) with a 95% confidence interval **(CI)** was calculated. Categorical variables were analyzed using a Chi square tests and Fisher's exact t–test was used where cell numbers were expected to

be less than five. T-test was used to compare continuous variables that were normally distributed. For variables that were not normally distributed, they were log transformed before being subjected to a multiple logistic regression analysis. Variables found to have a border line statistical significance association with NS at bivariate level were entered into the multiple logistic regression models using conditional logistic regression to identify the independent predictors for NS controlling for other factors. The Cox regression procedure was used to fit the Conditional Logit regression model. This was done by creating a censoring indicator variable. A strata variable was also created to specify the variable that determined the stratification. The enter method was used to get the final model of independent risk factors for NS using a p-value of less than 0.05 as the cut off for the level of statistical significance.

2.13 Ethical Consideration

The study was approved by the Research and Ethics Committee of Gulu University, Faculty of Medicine and Ministry of Health of Uganda and was conducted in accordance with Good Clinical practice standards. All parents/Guardians of the children gave a written informed consent and confidentiality of information was maintained throughout the study and follow-up of the cases and controls.

3. RESULTS

During the 10 day study period in June 2012, One hundred and one (101) NS cases and 101 controls were enrolled consecutively into this study. All the children were recruited from the study areas of Aromowanglobo and Pacilo parishes in Odek and Atiak sub counties in Gulu and Amuru districts respectively. Among the children, there were 63 males and 38 females with M: F ratio of 1.7:1 for cases and controls respectively. The mean age for cases was 11.38 years (SD$\pm$ 0.5) and similarly the mean age for the controls was 11.53 (SD$\pm$ 0.6). This reflected a closely matched case and control children by age. The head of the household were exclusively peasant farmers for both cases and controls.

3.1 Nutritional Status

The nutritional status of the children was measured using the BMI for age (z-score) and serum albumen levels. The BMI for age for a child between 5 to 19 years lying between -2SD to -3SD was considered malnourished while for the serum albumen, a serum level of less than 38g/dL for a child was considered malnourished (X^2=2.935, p=0.087; **OR** 1.635 95%Ci 0.930, 2.875).Thus there was no statistical significant difference between the nutritional status of NS cases and control at bivariate analysis but at multivariate logistic regression level, a positive correlation and a statistically significant difference was observed between the nutritional status of NS cases and controls (t=0.142; p=0.044). Further to this, in 80% of the cases the head circumference of the cases were smaller (20-30%) compared to the normal child for age and this was statistically significant. The difference in head circumference depended on the duration of the illness and the age of onset of the Nodding Syndrome. Those whose nodding started at an earlier age had a more severe deficiency in head circumference compared to those that had a late onset.

3.2 Serum Electrolytes

Normal serum potassium level was found to be statistically significant between the cases and controls (X^2 =7.846, p=0.005; **OR** 11.361 95%CI 1.401, 92.137). This means that, it was more likely to find a normal serum potassium level in controls than in NS cases. Low serum sodium level were found to be statistically and significantly different between the NS cases and their controls (X^2 =5.127, p<0.001; **OR** 0.049 95%CI 0.023, 0.102). This indicated that most NS were in a state of hyponatraemia (<135mEq/L) compared to their controls. Over 80% of the cases had a serum concentration of between 130–133 mEq/L. Hyponatraemia alone could in part explain some of the symptoms and signs found in NS children such as seizures, deformed bones and floppy muscles. Similarly, it was observed that it was more likely to find a normal serum sodium level (135-145mEq/L) in the controls than the cases and this was statistically significant (X^2 =75.093, p<0.001; **OR** 19.225 95%CI 9.177, 40.275) (Table 2).

As regards the other electrolytes; serum bicarbonate and chloride levels had a similar pattern of statistically significant difference between NS cases and controls (Table 2). The serum bicarbonate was specifically noted to be very low, a point below the critical clinical limits. Because of these observed differences between the concentration of cations and anions among cases and controls, the Anion Gap (AG) was calculated in order to determine any clinically detectable anion difference. It was found that there was a high AG which was statistically and significantly different between cases and control (X^2=146.752, p=0.000; **OR** 6.313 95%CI 4.027, 9.895). A high AG was defined as the difference between the measured cations and anions in the cases compared to the controls and any value more than 28mEq/L was considered significantly high.

The other variables such as prior treatment with Ivermectin, a positive skin snip for *Onchocerciasis*, positive skin feature for *OV*, school drop-out, the presence of two or more children with NS in the same family, children having been reported to have suffered from severe malaria or other severe childhood illness before the onset of Nodding were found to be statistically significant (Table 2).

The Table 3 shows that most children with NS had normal childhood development (physical, cognitive and social) but compared to their controls less than 50% of cases had an abnormal/delayed physical, cognitive and social childhood development. The difference in the pattern of childhood development between the cases and control was statistically significant (Table 3).

Table 1. The socio-demographic characteristics of case-control samples. Chi square test

Characteristics	Cases	Controls	Chi Square tests
Number of subjects	101	101	
Gender			
Male	63(62.38%)	63(62.38%)	p=0.557
Age			
Mean	11.38 yrs	11.53 yrs	
Age Range	2.5–17 yrs	2.5–17 yrs	
Peasant Farmer as Head of Household	101(100.00%)	101(100.00%)	p=0.500

Table 2. Experience of the mothers, NS cases and Controls

Variables	Cases	Controls	p-value	Odds Ratio (OR)	95% CI
Sex (F:M)	38(37.62%)	63(62.38%)	p=1.000	1.000	0.804,1.245
School dropout	72(71.29%)	0(0.00%)	**P<0.001**	**3.225**	**1.005,5.056**
Pregnancy carried to term	96(95.04%)	100(99.00%)	p=0.097	1.701	1.157,2.501
Mother had a major illness during pregnancy	2(1.98%)	0(0.00%)	p=0.155	0.890	0.953,1.008
Mother reported medication with adverse effects during pregnancy	2(1.98%)	0(0.00%)	p=0.155	0.890	0.953,1.008
Child suffered severe Malaria	16(15.84%)	2(1.98%)	**p=0.001**	**0.107**	**0.024,0.480**
Child suffered severe childhood illness	9(8.91%)	0(0.00%)	**p=0.002**	**0.477**	**0.411,0.553**
Skin feature for *Onchocerciasis*	16(15.84%)	0(0.00%)	**P<0.001**	**2.188**	**1.871,2.559**
Other children with NS in the same family	34(33.66%)	18(17.82%)	**p=0.001**	**1.464**	**1.122,1.910**
Positive skin snips for *Onchocerciasis*	78(77.22%)	10(9.90%)	**P<0.001**	**7.025**	**3.891,12.682**
Prior treated with Ivermectin	29(28.71%)	46(45.54%)	**p=0.013**	**1.416**	**1.083,1.852**
Laboratory investigations					
Low serum bicarbonate level	101(100.00%)	0(0.00%)	**p<0.001**	**8.500**	**0.400,23.060**
Normal serum potassium level	61(60.39%)	77(76.24%)	**p=0.005**	**11.361**	**1.401,92.137**
Low serum Sodium level	88(87.13%)	25(24.75%)	**p<0.001**	**0.049**	**0.023,0.102**
Normal serum sodium level	13(12.87%)	71(70.03%)	**p<0.001**	**19.225**	**9.177,40.275**
Low serum Chloride level	17(16.83%)	2(1.98%)	**p<0.001**	**0.104**	**0.026,2.456**
High Anion Gap	101(100.00%)	16(12.87%)	**p<0.001**	**6.313**	**4.027,9.895**
Malnourished Children	48(47.52%)	36(35.64%)	p=0.087	1.635	0.930,2.875
Lived in the Camps	101(100.00%)	101(100.00%)	**p<0.001**		

Electrolyte ranges: Low bicarbonate (<10.0mEq/L); Normal potassium level (3.5-4.5mEq/L); Low sodium (<135mEq/L); Normal serum Sodium (135-145mEq/L); Low serum Chloride (<95mEq/L); High Anion Gap (>28mEq/L); Severe malnutrition (-3SD and -2SD); Normal bicarbonate level (22.0-29.0mEq/L); Normal Anion Gap (8.0 – 16mEq/L); High Anion Gap (> 28mEq/L)

Table 3. The reported pattern of childhood development for the cases and controls. Chi square test

Characteristics	Cases	Controls	Chi Square tests
Normal social childhood development	58(57.43%)	101(100.00%)	
Delayed social childhood development	20(19.80%)	0(0.00%)	
Abnormal social childhood development	23(22.77%)	0(0.00%)	$p<0.001$ $X^2=54.62$
Normal physical childhood development	56(55.44%)	101(100.00%)	
Delayed physical childhood development	26(25.74%)	0(0.00%)	
Abnormal physical childhood development	19(18.82%)	0(0.00%)	$p<0.001$, $X^2=57.89$
Normal cognitive childhood development	53(52.48%)	101(100.00%)	
Delayed cognitive childhood development	24(23.76%)	0(0.00%)	
Abnormal cognitive childhood development	24(23.76%)	0(0.00%)	$p<0.001$, $X^{2=}62.96$

As for the parents of children with Nodding syndrome, the physical and family social history did not reveal any disease that could suggest a hereditary condition. For example, none of the parents reported to have suffered from diseases such as Migraine or motion sickness before or during the onset of the nodding among their children. None of the mothers of the children reported specific illness during the antenatal, natal and postnatal periods. None of the parents of NS children have clinical features of Nodding Syndrome.

4. DISCUSSION

4.1 Socio-demographic Characteristics

The socio-demographic of the participants were comparable to previous studies conducted in Southern Sudan and Northern Uganda respectively [3,9,11,12]. However, this case-control study had an advantage of having closely matched participants in age, gender and Residents (Table. 1). Secondly, the cases had not received medications for symptomatic management of NS. Furthermore, the required sample size of the study participants (101 cases versus 101 controls) was calculated using a known statistical formula (McNemar's, 1947) [17] for determining the study sample for this case control study. There was however, no evidence of gender predisposition to the syndrome (p=0.557). All cases and controls were from poor families with the head of the households being exclusively peasant farmers and all NS children lived in the IDP camps (Table 1).

4.2 Maternal Exposures and Childhood Illnesses

Most mothers had an uneventful pregnancy with little or no complications reported to have been experienced before, during and after delivery. All the mothers delivered by spontaneous vaginal delivery (SVD) at their homes and not in the health centers. The

maternal exposures & experiences during and after pregnancy were reported not to have affected the birth outcomes but keen to note was that most mothers reported that their children who developed NS had normal physical, cognitive and social developments before the onset of the NS (Table 3). The mothers themselves had not exhibited any symptoms or signs of Nodding Syndrome and this perhaps rules out the possibility of inherited/genetic disease. They reported no illnesses such as migraine or motion sickness which would possibly give credibility to a hypothesis of a possibility of an inherited/genetic medical disease. There are further no reported cases of nodding syndrome that have occurred among parents of children with nodding syndrome.

4.3 Families with Nodding Syndrome

More cases of NS were observed in families where there was already one sibling with the syndrome in comparison with their control families (Table 2). This may suggest a clustering of exposure factor within these families. This, we suggest may be environmental or dietary factors among others that have not yet been identified.

4.4 Skin Snip Results

Children with NS were more likely to have a positive skin snip for *Onchocerciasis* than controls and it was observed that most children with NS had not been treated with Ivermectin prior to the onset of the syndrome and this was statistically significant (Table 2). It is the opinion of these researchers that the occurrence of *Onchocerciasis* in this population seems not to be a probable risk factor to NS but rather a confounding factor. This is because *Onchocerciasis* occurs at endemic levels in many Sub Saharan African countries but there are limited or no reported cases of NS in those areas. Besides, the community in Northern Uganda reported that they have had a long experience (over 100years) with *Onchocerciasis* and that no such disease as NS had ever been experienced or reported in their communities [8]. These communities in Northern Uganda have long had a local name for *Onchocerciasis as* “ajonga miya” which confirms the local knowledge about *Onchocerciasis*. Perhaps if this time round a new strain of *Onchocerca volvulus* may have been introduced in the ecosystem during the prolonged civil war, this could perhaps make a plausible case for *Onchocerciasis* having an association with NS.

4.5 Treatment for *Onchocerciasis* with Ivermectin

As regards prior treatment for *Onchocerciasis*, nearly half of the controls (45%) had prior treatment for *Onchocerciasis*; majority (55%) though did not have prior treatment. As for the cases, 29% received treatment for *Onchocerciasis* while the majority (71%) did not receive treatment prior to the onset of Nodding. At the time of the study we found that 77.2% of the cases were skin snip positive for OV as compared to 9.9% of the controls. In our view, the occurrence of OV was perhaps a confounder or incidental to the syndrome since most controls (55%) had no prior treatment for OV yet they did not develop the syndrome while for the cases that had treatment (29%) still developed the syndrome and continued to nod.

4.6 Malnutrition

Approximately half of the NS children were severely malnourished compared to their matched controls. This finding is perhaps not surprising because there have been several researches and reports about the high prevalence of malnutrition in the population of

Northern Uganda [7,8]. This may still confirm other previous studies that showed that nutritional deficiency may be a factor that is playing a part in the epidemiology of Nodding syndrome.

4.7 Laboratory Investigations

4.7.1 Serum electrolytes

Cases had in general a lower level of serum electrolytes compared to controls. Serum potassium, sodium, chloride and bicarbonates were specifically lower in cases compared to controls and these were statistically significant (Table 2). The NS children were not physically in cardiac or liver failure to perhaps explain the low levels of serum sodium. Similarly, the corresponding values for the serum creatinine and blood urea nitrogen were however within the normal ranges for both cases and controls. From these findings, it is probable that these NS children did not have intrinsic renal disease to explain a possibility of a renal cause of hyponatraemia observed. The CDC studies that were conducted on children with NS in Northern Uganda also reported the absence of intrinsic renal diseases [3]. Because of the observation that there was a variation in the electrolyte profile between the cases and controls, an Anion Gap (AG) was calculated using the formula: AG = [Na+] – [Cl-] – [HCO3-] with the normal value being 8–16 mEq/L [14,15]. Anion Gap (AG) was therefore defined as the difference in the measured cations and measured anions in serum and plasma.

The magnitude of this difference ("gap") in the serum is often calculated in medicine when attempting to identify the cause of a metabolic acidosis. If the gap is greater than normal (>28mEq/L), then a high anion gap metabolic acidosis is diagnosed [15]. We calculated the AG using the traditional equation of AG = [Na]–[Cl]–[HCO3-] rather than the adjusted AG using the Figge equation; where AGAdjusted = ({4.4 – (observed serum albumen (g/dl)] x 2.5} + AG) – [serum lactate (mmol/L)/L] which would remove the effect of hypoalbuminaemia which may coexist in these patients [15]. Children with NS had a higher AG compared to their controls and this observation was positively correlated and statistically significant (Table 2). A high AG in a person indicates a state of acidosis [15].This may perhaps indicate a metabolic disorder which is typified by several metabolic and endocrine effects in humans [14,15]. Such high AG may have implications with medicines that have the potential of increasing further this gap. If indeed the high Anion Gap was related to the symptoms of NS, then Sodium Valproate may only temporarily improve the symptoms and signs of the syndrome but in the long run will further widen the AG. Conditional logistical regression analysis and correlation tests were conducted on AG among the cases and controls which indicated a statistically significant difference ($t=-0.896$, $p<0.001$).

4.7.2 State of acidosis

This study showed that children with NS were in a state of acidosis. Blood tests from limited number of cases and controls indicated a high level of lactic acid and pyruvate in cases than controls. This Acidosis could be hypothesized to have developed from abnormal metabolism [14,15] of food substances which led to production of excess acid as by-products of abnormal metabolism. This could occur as a result of chronic malnutrition due to long term starvation in which the body resorts to the production of energy through an alternative energy production pathway [14,15]. This could perhaps be the case because majority of NS cases were born and lived in the IDP camps where there was inadequate food supply to the children and mothers during and after the IDP camps [7,8]. The prevalence of malnutrition in

the general population of Gulu and Amuru was found to be much higher than the national average [7].

Alternatively, the source of the acidosis could be a result of defective mitochondrial function due to mainly in parts as a result of a mutation in its genes for energy production thus leading to the production of excessive acids [13]. Mitochondrial disease ties well with the clinical presentation of NS in that they present with brain developmental delays, neuro-psychiatric disturbances, mental retardation, seizures, dysautonomia (temperature instability and other dysautonomic problems); muscle weakness, cramping, dysmortility, hypotonia and muscle pains [13]. Stress and physical exercises have also been demonstrated to stimulate seizures in children with NS and which occurs as well in mitochondrial disorder [13]. These symptoms and signs in a mitochondrial disease are very similar to the clinical findings in children with Nodding Syndrome and it is at these observed findings that these authors present a case for a possibility of a mitochondrial disease in these NS children. These anecdotal observations may further suggest a defect in the energy production pathways and a mitochondrial disorder which is such a major culprit [13]. It is not immediately known what may have differentially led to the mitochondrial disorders in cases and not in these controls since they all lived in the same IDP camps and fed on the same food from World Food Program (WFP). However, specific family exposure such as in environmental contaminations or specific food ration which is contaminated can equally provide a clustered exposure to a group living in the same IDP camp and this will be exhibited by some exposed persons developing the condition while the others remain free of the illness. No confirmatory tests however have been made to conclusively point to this hypothesis that the acidosis is a result of mitochondrial disorder. It is thought that the mitochondrial disorder was not probably inherited but developed as a result of an exposure to a specific agent not yet identified.

Some literatures have further suggested that there is a form of epilepsy described as Devastating Epileptic Encephalopathy in school-aged children (DESC) [19] which have similar presentation with nodding syndrome. These authors have since reviewed several literatures on DESC and have observed that whereas Nodding syndrome affects children of the school going age just like DESC, it varies contrastly with the DESC in that Nodding syndrome exhibits no specific features of an encephalopathy because cerebro-spinal fluid analysis revealed no pleocytosis and the onset of nodding is not preceded with a febrile illness or intractable epilepsy, status epilepticus and does not persist as intractable perisylvian epilepsy once it develops. However, it has been observed that Nodding Syndrome is similar to DESC in that they both affect school going-age children who have seizures and eventually develop severe cognitive deterioration.

The authors would wish to state that a case-control study design is prone to information and selection bias as well as bias resulting from confounding factors. However, we minimized bias by training our research assistants on how to administer the questionnaire and collect samples appropriately. Blinding of the authors and research assistants were not possible because the clinical picture of NS cases were evidently different from their controls. Selection bias was minimized by restricting cases and controls that were living within the same villages. Confounding was minimized by matching cases and controls by age, sex and the area of residents. We wish to state that it is possible that some controls may have eventually developed NS. We have not identified these because we have not conducted a follow-up study on this study population.

5. CONCLUSION

Nodding syndrome is associated with metabolic disorder in young children who are malnourished and infected with *Onchocerciasis*.

CONSENT

All the authors declare that written informed consent/ Assent was obtained from each of the children with the cases and their controls in the presence of the guardian/parents.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the Faculty of Medicine institutional review committee, which is the appropriate ethics committee and the approval have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. The Ethical clearance reference number is GU/IRC/02/01/13 and find attached the approval letter.

ACKNOWLEDGEMENTS

We acknowledge the roles played by members of the investigation team especially the staffs from the ministry of Health, Gulu Regional Referral Hospital, Odek HC III and Gulu District Health office. We are greatly indebted to the families of the cases and controls for accepting willingly to participate in this study.

COMPETING INTERESTS

The authors report no conflict of interest.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=205&id=12&aid=1613