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High Anion Gap Metabolic Acidosis among Children with Nodding Syndrome (NS) in Northern Uganda: Case Series

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Authors' contributions

All the authors contributed in design of the research, data collection, analysis and manuscript writing. All the authors read and approved the manuscript.

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ABSTRACT

Aims: To conduct a hormonal and biochemical studies on 10 patients with diagnosis of probable Nodding Syndrome (NS).
Study Design: A cross-sectional study
Place and Duration of Study: Atanga Health Center III in Pader District in Northern Uganda in September 2012.
Methodology: We recruited consecutively 10 children with probable Nodding Syndrome who had been admitted for symptomatic management of seizures, injuries resulting from falls and nutritional rehabilitation. History, physical examinations, biophysical measurements (anthropometry) and blood investigations including serum electrolytes, liver function tests, thyroid hormones and vitamin D assays. Ethical approval was obtained from Gulu University Institutional Review Committee.
Results: All children had severely low serum calcium and bicarbonate levels and a high Anion Gap. Thyroid hormones and vitamin D assays were largely normal.
Conclusion: Children with Nodding Syndrome undergoing treatment for seizure control and nutritional rehabilitation have high Anion Gap metabolic acidosis.

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Keywords: Nodding Syndrome; metabolic acidosis; high Anion Gap; hormones; Pader; Northern Uganda.

DEFINITIONS

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 [1].

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 (>20mEq/L), then a high anion gap metabolic acidosis is diagnosed [14,15,17].

1. INTRODUCTION

Nodding syndrome (NS) is an unexplained neurologic disorder that has recently been reported among children in some sub-Saharan African countries, primarily among internally displaced persons or those formerly displaced and later returned to their villages [1,2,3]. Nodding Syndrome is a clinical constellation of symptoms that most begins with head nodding and later sometimes results in progressive neurological deterioration [2,3,4].

Majority of these Children with Nodding Syndrome have severe malnutrition requiring nutritional rehabilitations [3,5,6,7]. 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 [4,5,6,8]. Although this illness is descriptively similar to NS reported from Britain in 1909 [9], southern Tanzania in early 1960’s and Liberia in 1983, the syndrome has more recently been reported at epidemic proportions from Southern Sudan and Northern Uganda since the mid-1990’s [5,6,8,10].

The illness has a clustering of onset, mainly between ages of 5 to 15 years [6,8,10,11,12] and 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 [8,10,11,12,13].

Up to now NS has not been identified outside these areas and the reasons for the geographic and temporal clustering of the illness are still unknown.

1.1 Nodding Syndrome in Northern Uganda

The etiology of NS still remains unknown. A case-control study conducted by United States Centre for Disease Control and Prevention (CDC) 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 [4,6]. 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 or concurrent infection with the parasite, or something cross-reacting with antigens on the parasite [4]. The population of the research subjects as a whole was also found to be significantly deficient in serum vitamin B6 (pyridoxine) [4,6].

Anecdotal report from residents of Kitgum District in Northern Uganda suggests that NS is a progressive and invariably fatal disease however, researchers from CDC could not confirm this [4]. It has been reported that most children afflicted with the syndrome have been observed to progress through states of physical wasting, growth retardation, cognitive impairment to death within months to years when left without support [4,6,8]. The natural course of the disease, prognostic features, case fatality rate and and exact causes of death remain unknown [10,12,13,14]. The illness has been described as beginning insidiously, with nodding being the sentinel event, with progression to further seizures, cognitive decline, and physical changes [11,12,13,14]. However, the pattern of physical, neurologic, and cognitive decline is not known, and it is unclear whether subtle neurologic, hormonal, biochemical or nutritional changes may precede nodding [12,13,14]. Nodding has been reported to be brought about by specific stimuli, particularly presentation with hot food, emotional stress, physical exercises and cold weather or water [1,12]. 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 [12]. Follow up of some 12 cases after 8 months observed that several cases had worsened [4], however, it was not clear if the children had been consistently taking their anti-epileptic medicines.

1.2 Metabolic Derangements during Chronic Starvation

The majority of the children suffering from Nodding syndrome have exhibited growth failure, growth retardation and stunting [13,14]. We suspected that the chronic lack of adequate food and balanced nutrition for NS children and their mothers during the IDP camps could have been in part responsible for the stunted growth and growth failure. These observations were intriguing and these led these researchers to hypothesize that there could be some metabolic derangements with acidosis and hormonal imbalances among the NS children.

Previous studies have shown that chronic starvation can lead to keto-acidosis due to the increase in counter-regulatory hormones and a decrease in insulin secretion; a balance which promotes fatty acid oxidation, gluconeogenesis and ketone production [15,16,17]. The clinical presentation of children with NS have shown most features of metabolic disorder such as drooling of saliva, muscle weakness, bone deformities, stunting, wasting and seizures which are provoked by stressful activities such as physical exercises and temperature changes [1].

In this study we performed hormonal and biochemical analysis, and calculated Anion Gap on serum samples from children diagnosed as probable NS patients and who were undergoing symptomatic seizure control and nutritional rehabilitation with the goal to determine whether there were any derangements in hormonal and electrolyte profiles.

2. METHODOLOGY

2.1 Study Setting

The study was conducted in Pader district in Northern Uganda in September 2012. This region is just recovering from over 20 years of civil war between the Government of Uganda and Lord's Resistance Army (LRA). The populations of Pader are largely rural; many of whom were displaced into camps infamously known as the internally displaced peoples camps (IDPS) for safety from insurgency. It is estimated that over 3,000 children in the 2 districts of Kitgum and Pader have NS and that over 200 children have so far died of complications related to the NS [4,6,12]. The site where the study was conducted was largely not covered by the CDC study of 2009 or Kitara et al study of 2013. These NS children investigated had been receiving food supplementations, multivitamins, sodium valproate or/and carbamazepine and other medicines for symptomatic management of the syndrome from the beginning of the year 2012 (about 6 months of treatment). These 10 patients were consecutively selected out of the eighteen patients who were admitted to the Atanga Health Center III for inpatients' management. Atanga Health Center III is a governmental Health centre with a special nodding syndrome ward that was opened in March 2012 for the treatment and rehabilitation of these children. The children with NS were all from areas less than 10km from the center and therefore these where people from the same community. Most of the children (60%) had another sibling with Nodding Syndrome and all were born and/or raised in the IDPS camps normally and the onset of the nodding came later. The initial part of their lives before the onset of nodding was described by their mothers and guardians focusing on their physical, emotional and cognitive childhood development as normal. The patients were diagnosed using the WHO epidemiological surveillance case definition for the diagnosis of probable Nodding syndrome. Those that had NS plus meaning (Nodding syndrome with epilepsy or other neurological conditions) and those that did not consent to the study were excluded.

2.2 Study Design

We conducted a cross-sectional study involving 10 probable cases of NS in Atanga Health Center III in Pader District.

2.3 Selection of Study Participants

We consecutively recruited children undergoing treatment and rehabilitation at the Atanga NS treatment and rehabilitation center in Pader. These children were independently diagnosed by the team of experts from Uganda Ministry of Health (MOH) and World Health Organization (WHO) surveillance team using the WHO epidemiologic surveillance case definition of probable Nodding Syndrome [1]. Those patients that did not consent or had Nodding syndrome plus were excluded from the study.

2.4 Data Collection

Questionnaire was used to collect data on the socio-demographic characteristics; prenatal, natal and postnatal history and physical examination of the NS patients from their parents/or guardians. The questions included the number of siblings in the same family with NS, the prenatal, natal and postnatal development process for each of the children (physical, cognitive and emotional development), when nodding was first observed in the child and

what had been done so far by the parents. The physical measurements such as weight (Kg), Height (cm), MUAC (cm) and head circumference (cm) was conducted and included in the questionnaire. Blood samples were drawn from each individual patient for hematological, biochemical and hormonal analysis. Venous blood (5mls) was obtained from the cubital fossa of each child and safely delivered in 2 separate bottles (EDTA and Plain bottles) and stored for safe transportation in a cool box.

Blood sample collection took a day and the blood drawn was in the morning hours (8:00am to 10:00am) as those were the hours the patients were seen. Blood samples were transferred in a cool box to the Gulu University and Mulago National Referral Hospital laboratories where the hormonal and biochemical tests were conducted. The remaining samples were stored in a refrigerator and temperature maintained at - 80^0 C.

2.5 Dependent Variables

Hematological tests (WBC, Hb and platelet counts), hormonal profiles (TSH, T_4, T_3, Prolactin, Osteocalcin, Parathyroid hormone), Vit_D_3, and biochemical tests (serum K+, Na+, HCO_3-, Cl- levels, C-reactive protein, complements, rheumatoid factors, serum albumen levels) were the dependent variables.

2.6 Independent Variables

Gender and age.

2.7 Data Analysis

SPSS statistical software package version 15.0 was used for the univariate analysis of socio-demographic characteristics, hematological, hormonal, and biochemical tests. We used descriptive statistics - means, standard deviations, the minimum and maximum values of the variable analyzed to explain the pattern of these variables in the patients.

2.8 Ethical Consideration

The study was approved by the Institutional review committee (IRC) of Gulu University GU/IRC/02/01/13. Informed consent and Assent was obtained from each patient in the presence of the parents/guardians. Confidentiality of information was adhered to for all the participants of this research.

2.9 Anthropometric Measurements

All the NS children had a BMI for age lying between -2SD and -3SD and the corresponding results from the MUAC and head circumference shows a deficiency (20%) which is comparable with the results of the BMI for age.

Table 1. Hematological and clinical chemistry results

Characteristics	Mean values	STDEV	Lowest Highest
Total number of subjects	10		
Age	13.5yrs	1.86	10 -17
Blood Parameters (means)			
Total white blood cell count (WBC)	$7.6x10^3$/µL	1.60	4.4– 0.3
Haemoglobin concentration (Hb)	11.87g/dL	0.69	10.90-13.20
Platelet counts	$290x10^3$/µL	81.11	127.0 – 442.0
Renal Function tests			
Serum Creatinine	0.7mg/dl	0.1	0.6 - 1.1
Serum blood urea nitrogen (BUN)	17.0mg/dl	3.4	10.0–50.0
Serum Albumin and Total Protein			
Serum albumin	28.9g/l	4.27	38.0-51.0
Total Protein	70.7g/l	2.69	60.0-80.0
Liver Function Tests			
AST	46.6U/L	13.67	34.0-78.0
ALT	28.5U/L	11.93	20.0- 57.0
ALP	185.9U/L	69.67	110.0-323.0
LDH	548.9U/L	78.25	427.0-628.0
Complement ©			
C3	0.44mg/L	0.33	0.2 – 1.3

Reference values: White blood count=4.0-11.0x10^3 /µL; Platelet counts = 150 – 400 x 10^3 /µL; Haemoglobin concentration (Hb) =12.0 -18.0g/dL; serum creatinine = 0.6–1.1mg/dl; Blood urea nitrogen =10.0- 50.0 mg/dl; serum Albumin= 38-51g/l; Total Protein=60–80g/l; AST= <33U/L; ALT= <26U/L; ALP =<936U/L; LDH=<279U/L; complement 3=0.9-1.8g/L

3. RESULTS

3.1 Hematological Tests

The total white blood cell counts (WBC) were all within normal ranges with a mean of 7,600SD± 1,599/µL and result was similar to previous studies conducted in this region [1,4]. Most patients had a low hemoglobin concentration (Hb); Mean of 11.87SD ± 0.69g/dl. The Platelet count was within the normal range for all the patients with mean of 290,000SD ± 81,000.11/µL (Table 1).

3.2 Renal Parameters

These were all within the normal ranges (serum creatinine and serum urea levels) (Table 1).

3.3 Liver Parameters

Particularly serum albumin was very low although total protein and alkaline phosphatase were within normal ranges (Table 1). AST and ALT were all within the normal ranges. However, LDH levels were at the upper limit of the normal ranges. The complement 3 value was within the normal range (Table 1).

3.4 Electrolyte Pattern

Result shows an abnormal distribution of the electrolyte pattern in these cases. The concentration of Sodium (mean 141.1 ± 1.6mmol/L) was normal, phosphates (2.9 ± 0.8mmol/L) and potassium (4.9 ± 0.7mmol/L) were above the normal ranges whereas the concentration of bicarbonate (mean 13.6 ± 3.0mmol/L) and calcium (mean 2.1± 0.1mmol/L) were below the critical clinical levels (Table 2).

3.5 Anion Gap (AG)

The Anion Gap was calculated using the formula: AG = [Na+] – [Cl-] – [HCO_{3-}]. Values between 8 to 16mEq/L were considered normal but values more than 20 were considered significantly high above the critical clinical levels and this indicated high Anion Gap metabolic acidosis. Anion Gap was (mean 23.8±2.3mmol/L with minimal value of 20 and maximum 27). These children were in state of metabolic acidosis.

3.6 Hormonal Assays

The hormones tested included Thyroid stimulating hormone (TSH) with mean 2.4SD±1.0μIU/mL; Thyroxin (T_4) with mean 7.2SD±1.5μg/dL; T_3 with mean, 15.2SD±41.3 μg/dL; Prolactin with mean 25.9SD±41.6ng/ml; Vitamin D_3 with mean 45.5SD±15.1ng/ml; Osteocalcin with a mean of10.0SD±0.0ng/ml; Parathyroid hormone with mean 26.5 SD±19.3pg/ml and C-reactive protein (cRP) with mean11.5SD± 22.1mg/L; Rheumatoid factor (RF) with mean 1.6SD ±1.6IU/ml. There were normal levels of thyroid stimulating hormone (TSH), thyroxin (T4), (T_3), and parathyroid hormone while the serum osteocalcin was 50% normal and 50% low in these patients. VitaminD_3 levels were high for most patients (60%) while normal in 40% of the cases (Table 2). Serum prolactin was high in a 15 years old girl who was breast feeding a baby of 2 months old at the time of sample draw. Overall the hormones of the children were within the normal ranges (Table 2)

4. DISCUSSION

4.1 Socio-demographic Characteristics

The socio-demographics of the ten patients studied were comparable to those reported in other studies among NS children in northern Uganda and Southern Sudan [1,3,4] which showed that most children with the syndrome were in the young age group. These reflected similar findings of children with nodding syndrome and who were generally from poor families and were malnourished [1,4,7]. The patients studied had been receiving medications for symptomatic management of NS: Anticonvulsants such as sodium valproate, phenobarbitone and carbamazepine, multivitamins, Ivermectin, Folic Acid and albendazole from Atanga Health Center III for over six months.

Table 2. The table shows the hormonal and biochemical results for the 10 nodding syndrome patients

Patient ID	Age	Gender	Phosph	Mg^{2+}	Ca^{2+}	Na^{+}	K^{+}	Cl^{-}	HCO_3^{-}	RF	cRP	Prolactin	TSH	T_4	T_3	PTH	Osteocalcin	vit_D_3	Anion Gap
WOD	15	M	3.3	0.9	2.2	142.0	4.8	102.0	14.0	3.0	32.8	6.6	1.1	9.2	1.5	24.0		41.0	26.0
APV	12	F	2.7	0.8	2.1	143.0	5.7	107.0	16.0	1.6	2.7	4.3	1.3	3.8	0.9			70.0	20.0
ODB	13	M	2.0	0.8	2.2	140.0	3.8	105.0	11.0	0.0	1.8	8.2	2.0	7.4	2.0	43.0	16.0	54.0	24.0
APB	15	F	2.0	0.7	1.9	141.0	4.3	104.0	15.0	0.0	0.8	147.6	4.0	7.4	1.3	20.0	8.0	30.0	22.0
OCR	10	M	2.0	0.9	2.2	142.0	4.5	102.0	18.0	0.0	2.4	8.8	2.3	7.6	1.9	38.0	43.0	35.0	22.0
ODM	14	M	3.9	0.8	2.1	139.0	6.2	105.0	7.0	0.0	0.6	9.6	2.2	5.7	1.5	2.0		70.0	27.0
LAJ	12	F	3.8	0.8	2.3	141.0	5.2	102.0	14.0	1.5	1.2	38.5	3.6	7.7	1.6	12.0	29.0	52.0	25.0
ABS	14	F	2.3	0.7	2.0	138.0	5.1	103.0	14.0	1.2	1.1	16.3	2.3	8.1	139.0	62.0		46.0	21.0
OCC	13	M	3.4	0.9	2.1	142.0	4.4	101.0	16.0	3.5	71.4	8.5	1.3	6.4	1.1	5.0	10.0	31.0	25.0
ADR	17	M	3.9	0.8	2.3	143.0	5.2	106.0	11.0	4.8	0.4	10.2	3.9	8.5	1.6	30.0	10.0	26.0	26.0
Mean	13.5		2.9	0.8	2.1	141.1	4.9	103.7	13.6	1.6	11.5	25.9	2.4	7.2	15.2	26.5	10.0	45.5	23.8
STDEV	1.86		0.8	0.1	0.1	1.6	0.7	1.9	3.0	1.6	22.1	41.6	1.0	1.5	41.3	19.3	0.0	15.1	2.3
Lowest	10		2.0	0.7	1.9	138.0	3.8	101.0	7.0	0.0	0.6	4.3	1.1	3.8	0.9	2.0	8.0	26.0	20.0
Highest	17		3.9	0.9	2.3	143.0	6.2	107.0	18.0	4.8	71.4	147.6	4.0	9.2	139.0	62.0	43.0	70.0	27.0

Reference values: N-MID Osteocalcin=11.0- 43.0ng/ml; Parathyroid hormone (PTH) =15.0-65.0pg/ml; T_4= 5.13-14.06µg/dl; T_3=0.80-2.00µg/dl; TSH=0.27-4.20µIU/mL; Vit_D_3=20.0-40.0ng/ml; c-reactive protein (crp)=0.5-5.0mg/L; Rheumatoid factor (RF)=0.0-14.0IU/ml; Mg^{2+} =0.65-0.90mmol/L; Ca^{2+}= 2.30-2.75mmol/L; Na^{+} =132.0-141.0mmol/L; K^{+}=3.3–4.6mmol/L; Cl^{-} =98.0-106.0mmol/L; Bicarbonate (HCO_3-)= 20.0-28.0mmol/L; Phosphate=1.1-2.0mmol/L

4.2 Laboratory Investigations

4.2.1 White blood cell counts (WBC)

Total white blood cell counts and its differential were largely within normal ranges. None of the children had evidence of febrile infections at the time of blood draw. However, a 14 years old male child had several non-septic wounds resulting from frequent injuries associated with the convulsive episodes. The film report on WBC did not indicate any toxic granules to suggest current active infective process. These findings may however, not exclude the possibility of post infectious etiology of Nodding Syndrome which were similarly found in previous studies conducted in the same region [1,4]. The hemoglobin concentration (Hb) among the patients was generally mildly low and typically, the anaemia was a normocytic normochromic type similarly seen in the case-control study conducted by Kitara *et al,* 2013. The low hemoglobin found in all these children is consistent with the low serum albumin level, malnutrition and the general wasting of the patients.

4.2.2 Renal parameters

All patients had a normal serum creatinine and blood urea nitrogen levels. This may perhaps indicate a normal renal function but most importantly is perhaps that these patients were unlikely to have any intrinsic renal diseases that could account for the abnormality in serum electrolytes profiles observed. The CDC studies conducted in Northern Uganda in 2009 also confirmed the absence of intrinsic renal diseases similarly observed by Kitara et al (2013). This perhaps rules out the possibility that the high Anion Gap metabolic acidosis is the end result of renal malfunction or disease.

4.2.3 Serum electrolytes

Serum potassium, sodium and phosphates for these children were found above their normal limits for age while bicarbonates, calcium and magnesium were specifically below the critical clinical limits (Table 2). Most patients had a very high serum phosphate level with a remarkably high value above the critical clinical limits (Table 2). This could be explained in part as a result of a high rate of active bones resorption which may be as a result of inadequate food intake thus leading to excessive loss of phosphate from bones and thus an increased phosphate serum levels. The mildly high serum concentration of sodium could be explained by the addition of sodium through the medication of Sodium Valproate that were being taken daily by NS children for the control seizures. It was not clear to the researchers why there was an elevated serum level of potassium (Table 2) except perhaps as a result of an on-going process in the metabolic acidosis. There was no evidence of haemolysis of the red cells during transportation of samples to laboratories and this was an unlikely case scenario to explain the rise in potassium concentration because we separated the serum from the whole blood by centrifuging and keeping the samples separately. We were unable to perform serum levels of insulin like growth factor (IGF) to establish whether or not the relatively high K+ levels could be accounted for by low blood levels of insulin and therefore more K+ were in the extracellular compartment. This may perhaps be one of the limiting factors for the result of this study although it provides an avenue for further research and in-depth analysis.

Furthermore, the extremely low bicarbonate, calcium and magnesium levels could be a result of a factor that continuously depletes them from the circulation. In a case–control study conducted by Kitara et al, 2013, they suggested a possibility of a metabolic disorder

resulting from dysfunctional mitochondria which continuously produces acids as end products of metabolism thereby depleting bicarbonate from the circulation [1]. These authors would suggest that the hypothesis that the high Anion Gap metabolic acidosis is a result of a mitochondrial dysfunction needs to be studied further so as to reach a decisive conclusion.

4.2.4 Liver parameters

The intrinsic liver enzymes AST and ALT were found to be within their normal ranges however, the enzyme Lactic Dehydrogenase (LDH) levels were found to be at the upper margin of normal ranges which may perhaps indicate an increased activity of the enzyme in the presence of lactic acidosis, increased cells turnover or cell destruction. The increased enzymes may perhaps still confirms the increased activities of these enzymes in a state of metabolic acidosis as an homeostatic process in order to process the lactic acid and clear it from the circulation.

4.2.5 Hormonal measurements

The investigations showed that the entire hormone tested were within normal ranges especially hormones involved in growth and development (Table 2). What was intriguing was the observed stunted growth [12,13], growth retardation [13,14] among the NS children in spite of these normal hormonal levels. The researchers concluded that perhaps the failure of NS children to grow could be attributable to the chronic metabolic acidosis and that acidosis prevented the binding of the hormones to their respective receptors [14,17]. Several studies have been conducted on the effects of metabolic acidosis on the body's growth and development and similar findings were observed [14,17]. Several researches have shown and concluded that acidosis decreased the body's ability to absorb minerals and other nutrients, decreased energy production in the cells, decreased body's ability to repair damaged cells, decreased body's ability to detoxify heavy metals and these make the body susceptible to fatigue and illnesses [15,16,17]. These researchers therefore have the view that the chronic acidosis may in part be responsible for the failure in growth and development of these NS children since it was anecdotally observed in Tumangu in Kitgum district that showed that children with NS who were drinking cows' milk everyday gained weight and height compared to their counterparts who did not [1]. Similarly, observation from Southern Sudan by Tumwine et al, 2013 have indicated that NS were found mainly among the Moru tribe yet none was observed among the Dinkas who live in the same community with the Moru [10]. The eating habits of the two tribes were different in that the Dinkas were mainly pastoralists and drank a lot more milk while the Moru who are predominantly crop growers and rarely consumed milk [10,13]. Furthermore, cows' milk is known to contain a relatively high quantity of bicarbonate and calcium which in this case of metabolic acidosis could act as a source of buffer to the metabolic acidosis in children with Nodding Syndrome.

4.3 High Anion Gap

All NS children had a high Anion Gap; far higher than the critical clinical limits. It is not clear to what extent treatment with sodium valproate could have contributed to attainment of normal levels of Na+ concentration among these NS children. In a previous case-control study conducted among NS children who had not yet been on treatment with Sodium valproate, the mean serum Na+ concentration were 133.6mmol/L and 143.1mmol/L between cases and controls respectively however, with the introduction of Sodium valproate as medication for the control of seizures among children with NS, the cases have an average Na^+ concentration of 141.1mmol/L which was within the normal limit. The serum sodium

concentration was consistently normal among these 10 NS cases compared to those found in the previous case-control study conducted in the region [1]. This high Anion Gap acidosis in these children with Nodding Syndrome indicated a state of metabolic acidosis. We suspect that indeed this high Anion Gap metabolic acidosis was related to the symptoms and signs of NS which was similarly observed in a case-control study conducted in Northern Uganda [1].

4.4 State of Acidosis

This investigation finding showed that children with NS were in a state of acidosis. Blood tests from these children indicated a very high Anion Gap (Metabolic Acidosis). This acidosis could have perhaps developed from abnormal metabolism of food or dysfunctional mitochondria. The possible resultant effect of these was the production of excessive acid as by-products of metabolism. This could occur as a result of chronic malnutrition such as in prolonged starvation where the body resorts to energy production through alternative energy pathway including metabolism of fat with resultant keto-acidosis [15]. In stressful situations or starvation, the insufficient NAD+ would inhibit the aerobic metabolism via the Kreb's cycle [15]. This along with the release of catabolic hormones such as growth hormones, catecholamines and glucagons would stimulate the lipolytic and ketogenic pathways [15]. In the usual ketone formation, aceto-acetic acid and B-hydroxybutyrate (BOHB) exist in a state of equilibrium, the balance of which is determined by the NAD^+/NADH ratio which would drive the equilibrium from the aceto-acetate towards BOHB. The results would be in higher fraction of the BOHB being present in the circulation [15].

Chronic starvation could be the case in NS because the majority of the NS cases were born and raised in IDP camps where there was inadequate food supply to children and their mothers during and after the IDP camps [7]. The prevalence of malnutrition rate in the general population of Northern region was much higher than the national average [7]. However true this may be, these authors observe that Nodding syndrome should have equally occurred on all the children that were born and raised in IDP camps of Northern Uganda because they faced the same camp life but fortunately, this is not the case because most children who were born and raised in the IDP camps did not develop NS and therefore the need for further exploration to link the missing link in this Syndrome development. There were only selected children that developed the syndrome indicating perhaps that there were probably other factors in play for the development of this syndrome.

Alternatively, the source of the metabolic acidosis could be a result of defective mitochondrial function due to mainly in parts as a result of a mutation in genes responsible for energy production thus leading to production of excessive acids [15,16]. Mitochondrial disease/disorder may occur as a result of environmental contamination during the childhood period of NS children. Mitochondrial disease ties well with the presentation of NS in that mitochondrial diseases present with brain developmental delays, neuro-psychiatric disturbances, mental retardation, seizures, dysautonomia (temperature instability and other dysautonomic problems); muscle weakness, cramping, dysmotility, hypotonia and muscle pains [15] which have equally been observed in children with Nodding Syndrome. Stress and physical exercises have been demonstrated to stimulate seizures in children with NS [1] perhaps a sign that there was an increasing acidosis resulting from lactic acidosis as a result of stress and exercises. These anecdotal observations may further suggest a defect in the energy production pathways and a mitochondrial disorder/disease which is such a major culprit [1,15,16]. It was not immediately known by these researchers what may have differentially led to the mitochondrial disorders in these NS Children which have been

similarly observed in a study conducted by a multidisciplinary team of researchers from Gulu University in Northern Uganda [1]. That case-control study found that all the children with Nodding Syndrome had high Anion Gap acidosis as compared to the controls that had none. This indicated that whereas all other blood and physical measurements among the cases were similar to the controls, the high anion gap acidosis was the major difference between the 2 groups with a clinically and critically low serum bicarbonate level [1].

This proof of concept study is not without limitation. The sample size of just 10 patients with probable Nodding Syndrome would preclude any hard conclusions to be drawn from the findings from this study. It is relieving however; that similar findings were observed in a larger sample of over 101 children with probable NS investigated in recent study in the region and the findings on electrolytes were similar to the ones observed in this study. Therefore, although the sample size for this study was small, largely determined by the large amount of money involved in performing the hormonal and biochemical studies, the information obtained from this study provides a tremendous clue to the biochemical abnormalities and probable patho-physiological pathway and the possible causes of Nodding Syndrome in this region [16,17].

5. CONCLUSIONS

Children with Nodding Syndrome included in this study were in state of high Anion Gap metabolic acidosis. All the children were undergoing treatment for seizure control with sodium valproate and nutritional rehabilitation with various high energy and high protein nutritional supplements. Chronic starvation and/or mitochondrial disorders affecting energy production pathways among children within the age range expected to have high growth rate could account for the development of NS in populations that have been depleted of food supply for long. The growth retardation and failure could also be in part a result of the chronic acidotic state experienced by the Children with Nodding Syndrome.

CONSENT

All the authors declare that written informed consent/ Assent was obtained from each of the children with Nodding Syndrome and in the presence of their parents or Guardian.

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.

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COMPETING INTERESTS

The authors have declared that no competing interests exists.

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