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Preliminary seroprevalence study of neurotropic virus antibodies in Nodding syndrome

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ABSTRACT

Nodding syndrome (NS) is a mostly East African pediatric epileptiform encephalopathy of unknown etiology that shares some clinical features with measles-associated subacute sclerosing panencephalitis (SSPE) and progressive rubella panencephalitis. Two independent studies in northern Uganda identified an association between NS and prior measles infection, while an earlier study in South Sudan found an inverse association. We report preliminary serologic analyses of antibodies to measles (MV), rubella (RV), HSV-1, and CMV viruses in northern Ugandan children with NS and Household (HC) and Community (CC) Controls. Only MV-positive titers were significantly different (3-fold and > 2-fold) in NS relative to HC and HC + CC, respectively. While these results are consistent with greater prior measles infection in Ugandan persons with NS, further studies are needed to determine whether Measles virus (MV) plays any role in the etiology and pathogenesis of NS. Resolving this issue will be invaluable for the thousands of children at risk for this devastating yet often neglected condition.

1. Introduction

Nodding syndrome (NS) is a mostly East African pediatric epileptiform encephalopathy of unknown etiology [1] named for the characteristic vertical head nodding that appears early in the course of the disease. NS shares some clinical features with other tardive brain disorders such as measles-associated subacute sclerosing panencephalitis (SSPE) and progressive rubella panencephalitis [2]. Both NS and SSPE have an insidious afebrile onset in children, with behavioral changes and intellectual deterioration, followed by myoclonus, typically beginning with the head, and tonic-clonic and other generalized seizures [3–5]. Signs of sleepiness, stupor, catatonia, muscle weakness, speech disturbance and sudden falls are reported in both disorders [4,6–8]. With the advance of SSPE, myoclonus may decrease or disappear, spasticity with pyramidal and extrapyramidal signs becomes apparent, and a few develop ataxia, dystonia and dyskinesia [4]. Similarly, ataxia, hyperreflexia and parkinsonian signs are described in longstanding cases of NS [9]. Unusually cold lower extremities have been reported in children with NS [3,6] while, in advanced stages of SSPE, loss of thermoregulation causes marked core temperature fluctuations [4]. Progressive cognitive decline leading to dementia and mutism is described in both disorders [4,6,9]. The NS electroencephalogram, particularly the ictal phenomenon of repetitive head drops in clusters with associated electrodecrememt response, is consistent with late-onset epileptic spasms [10] which occur in SSPE and other disorders. Neuropathological analyses have either largely excluded [11] or pointed to some similarities with SSPE [12]. Pollanen and colleagues [11] did not find histologic evidence of viral or autoimmune encephalitis or cerebral parasitism. The overall pathological process was neurodegeneration with tau deposition. These authors concluded that if NS is caused by infection with a virus, the nexus is unlikely through the pathogenic mechanisms that cause a post-encephalitic state, as occurs in measles infections leading to SSPE. In contrast, the neuropathology in NS described by Hotterbeekx and colleagues [12] showed similarities to p-tau depositions in SSPE, although involvement of the hippocampus and the more diffuse distribution of tau deposits in all layers of the brain in

Abbreviations: MV, measles virus; RV, rubella virus; HSV-1, herpes simplex virus 1; CMV, cytomegalovirus; SSPE, subacute sclerosing panencephalitis; NS, nodding syndrome; HC, household controls; CC, community controls..

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SSPE were not present in NS. It has been suggested that deposition of p-tau neurofibrillary tangles is a secondary event in SSPE and this is likely also the case in NS.

Given that two independent studies of NS in Kitgum District, northern Uganda, identified a case association with prior measles infection [2], while an earlier study in then-southern Sudan found an inverse association between caregiver reports of prior measles infection and NS in children [3], it is imperative to either confirm or rule out the possibility that NS is a type of post-measles brain disorder. This is particularly relevant considering that children affected by NS were at high risk for infectious disease because of inactive vaccination and drug prophylaxis programs, severe shortages of food and medicine, prolonged civil disturbance, and displacement from their homes [2].

The preliminary serological analyses reported here demonstrate, for the first time, an association between measles virus (MV)-positive titers and NS among affected children and adolescents in northern Uganda. However, further studies are needed to assess whether early-age measles infection has any role in the etiology and pathogenesis of NS.

2. Materials and methods

2.1. Case-control study design and descriptive statistics

Study participants were drawn from the NS-affected Acholi community of Tumangu Village in Lamit Parish, Labongo Akwang Sub-County, Kitgum District, north central Uganda. Nodding syndrome cases (NS), household controls (HC) and community controls (CC) were randomly selected, individually screened, and matched, whenever possible, by both age and gender. NS was defined according to an international consensus definition [1]. Household controls (HC) were recruited from households with NS cases, and community controls (CC) were drawn from NS-free households in the same community. HC and CC had no history of head nodding or other seizures, including febrile seizures [2]. Weight was measured using a calibrated digital scale. Height was measured using a vertical stadiometer. Each child was measured clothed and barefoot.

2.2. Informed consent and human experimentation guidelines

The study design was approved by the Institutional Review Board (IRB) of Oregon Health & Science University (OHSU), Portland, Oregon, USA, in concert with the Research and Ethics Committee (REC) of the School of Medicine (SOM), College of Health Sciences, Makerere University, Kampala, Uganda. The university SOM-REC-approved research plan was reviewed and approved by the Uganda National Council for Science and Technology, the Office of the President of Uganda, and the Chairman, Local Council III, Kitgum District, northern Uganda [2].

The research team worked closely with the Tumangu Village Health Team Leader in carrying out the study in Tumangu Village. Parents and or guardians (caregivers) of children gave informed consent on behalf of all child participants. Informed consent for the interview was administered orally and data were collected by 5 Ugandan medical students who spoke with household caretakers in the Acholi language under the supervision of an academic physician who was born and nurtured near Kitgum Town. OHSU-IRB/SOM-REC-approved oral consent was employed because the caregivers could not read the documents. Oral consent was documented by placement of an inked thumbprint on the consent forms. One copy of the set of executed consent forms was deposited with the Department of Food Technology and Nutrition, College of Agricultural and Environmental Sciences, Makerere University, Kampala, Uganda [2].

2.3. Serology

Specific IgG-class antibodies for measles virus (MV), rubella virus (RV), herpes simplex virus-1 (HSV-1), and cytomegalovirus (CMV) were determined with commercially available Elisa kits (Euroimmun, Lubeck, Germany), according to the manufacturer's instructions. Results were evaluated semi-quantitatively by calculating the ratio of the absorbance of NS or control samples (HCs and CCs) over the absorbance of a cutoff calibrator. A ratio value of <0.8 was considered negative; a ratio value of $\geq 0.8 < 1.1$ was considered equivocal (borderline); and a ratio value ≥ 1.1 was considered positive. A chi-square test of independence was performed to examine the relationship of testing positive for MV, RV, HSV-1, and CMV IgG-class antibodies and having NS.

2.4. Study limitations

(a) The case-control study [2] that provided the samples for the current study relied heavily on the recall of caregivers, the accuracy of which is unknown. Self-report bias was however circumvented by the unbiased serology analyses reported here. (b) Unequal numbers of NS, HC, and CC samples were compared because some of the samples had been entirely spent in previous analyses [2]. Yet, such differences were small and sufficient statistical power was retained to draw accurate conclusions about the study populations. (c) The assumption that healthy siblings (HC) of a NS case were equally likely to have been immunized against measles during a measles vaccination campaign is unproven but probable. (d) No information was collected regarding the possibility of passive immunization acquired through physiologic transfer of maternal measles antibodies from mother to child. However, it is reasonable to assume that this potentiality would have evenly affected all three (NS, HC and CC) study populations. In sum, we anticipate that the study limitations outlined above did not differentially affect the evaluation of exposure among the cases and controls to significantly influence the outcomes and conclusions presented here.

3. Results

The average [mean ± SD] age, height, and weight of study participants were as follows: NS [15.6 ± 1.75 years, 151.5 ± 11.58 cm, 38.2 ± 10.30 Kg]; HC [13.0 ± 3.45 years, 144.3 ± 14.74 cm, 33.6 ± 10.44 Kg]; and CC [15.4 ± 1.42 years, 161.8 ± 9.97 cm, 47.4 ± 8.15 Kg].

We found differences in the prevalence of the measles virus (MV) serotype (but not of three other common neurotropic viruses) in a subset of northern Ugandan NS cases compared with healthy household controls (HC) and community controls (CC) matched to the extent possible for age, gender and family size (Table 1). We carried out IgG ELISA serology on 38 NS, up to 39 HC and up to 23 CC samples that had been frozen (-80 °C) with one brief thaw since collection in 2014 [2]. Antibody titers for MV, RV, HSV-1, and CMV were measured in two runs, and a 10% random sample of each of the three groups was re-run to confirm initial test results. In contrast to near universal serology evidence of prior RV, HSV-1, and CMV infection of NS, HC, and CC (with no significant association between testing positive for RV, HSV-1, and CMV IgG antibodies and having NS), the number of positive IgG-class MV antibodies was significantly higher [3-fold; χ^2 (1, $N = 68$) = 10.06, $p = 0.0015$] in NS than HC and twice as high in NS than HC + CC [2-fold; χ^2 (1, N = 89) = 10.29, $p = 0.0058$] (Table 1).

A detailed comparison of the demographic, clinical and exposure histories of NS cases and controls (HC and CC) is described elsewhere [2]. This study showed that NS cases were distinguished from controls by caregiver report of a significantly higher history of measles infection, dependence on emergency food supplies (including moldy maize) prior to onset of head nodding, and lower body weight on examination at a mean age of 15.5 years, 8 years after the mean age of onset of head nodding in most NS cases. Reported vaccination rates in NS and controls were similar (mostly >90%) for poliomyelitis, tetanus, diphtheria, pertussis, and measles. This was consistent with a 2003 emergency measles vaccination campaign in northern Uganda that preceded a wave of NS [2].

Table 1
Serology: Measles virus (MV), rubella virus (RV), herpes simplex virus-1 (HSV-1), and cytomegalovirus (CMV) specific IgG-class antibodies were determined in Nodding syndrome cases (NS), household controls (HC), and community controls (CC). There was a significant association between testing positive for MV IgG antibodies and having NS ($p < 0.05$) even when the borderline values were included in the calculation (data not shown). There was no significant association between testing positive for RV, HSV-1, and CMV IgG antibodies and having NS. Some samples were measles-positive and rubella-negative, and many rubella-positive samples were measles-negative. Data reproducibility of 10% samples per group was 100%.

IgG-class Titers		NS Cases	Household Controls (HC)	Community Controls (CC)	p
MV	n	38	39	22	$0.0015_{NS\ vs\ HC}$ $0.0058_{NS\ vs\ HC+CC}$
	+	**17 (45%)**	**6 (15%)**	**6 (27%)**	
	+/-	6 (16%)	3 (8%)	2 (9%)	
	-	15 (39%)	30 (77%)	14 (64%)	
RV	n	38	37	22	≥0.05
	+	33 (87%)	27 (73%)	19 (86%)	
	+/-	0 (0%)	1 (3%)	0 (0%)	
	-	5 (13%)	9 (24%)	3 (14%)	
HSV-1	n	38	39	23	≥0.05
	+	37 (97%)	38 (97%)	23 (100%)	
	+/-	1 (3%)	0 (0%)	0 (0%)	
	-	0 (0%)	1 (3%)	0 (0%)	
CMV	n	38	39	23	≥0.05
	+	36 (95%)	37 (95%)	23 (100%)	
	+/-	2 (5%)	1 (2.5%)	0 (0%)	
	-	0 (0%)	1 (2.5%)	0 (0%)	

n, total number of individuals; +, positive titers (qualitative); +/−, borderline titers (qualitative); −, negative titers (qualitative).

4. Discussion

These findings reveal an association between MV and NS in accord with the higher caregiver reported incidence of childhood measles prior to onset of head nodding compared with controls [2]. An earlier (2009) independent case-control study of NS in the same population found a significant difference for reported prior measles infection that was lost when data were adjusted for subject age [13].

The presence of demonstrable sera IgG-class antibodies to MV generally indicates immunity to measles acquired due to prior infection or via immunization. The low percentages of immunity to MV in all three groups (45% of positive titers in NS cases, 15% in HC and 27% in CC) suggest a) the unreliability of the 2014 self-reported data for prior measles infection/immunization [2], b) the 2003 emergency measles vaccination campaign reached only a small proportion of the population studied here, and/or c) genetic factors in the local population may have modified the response to the vaccine. Importantly, the serology data favor immunity acquired by prior measles infection since the 2003 measles vaccination campaign would have treated single-household NS and HC subjects alike and NS would have shared genetics with HC siblings. However, we cannot completely rule out that the differential MV-IgG serology between groups may have resulted from a different host-immune response between NS subjects and healthy controls. Indeed, a recent study in South Sudan found some differences in several autoantibodies among NS-cases and controls. An alteration in the HLA haplotype was noted, which may have led to inconsistent host-immune responses to pathogens [14].

Given that a) some of the clinical and neuropathological features of NS have striking parallels with measles-associated SSPE; b) the onset peak of Ugandan NS cases from 2003 to 2008 followed the onset peak of reported measles incidence in Uganda from 1998 to 2003 (>40,000 cases/year nationwide) [2]; c) two independent studies of NS in Kitgum District, northern Uganda, revealed a case association with reported prior measles infection; and d) the serology findings reported here confirm an association between NS and MV infection but not between NS and other neurotropic viruses, namely RV (*Paramyxoviridae*), HSV-1 and CMV (*Herpeviridae*), further research along these lines seems warranted. In addition, several other neurotropic virus families (*Anneloviridae, Flaviviridae, Herpeviridae, Hepadnaviridae, Papillomaviridae, Polyomaviridae and Virgaviridae)* have shown no connection with onchocerciasis-associated epilepsy, an entity that includes NS [15].

Notably, the present discovery of an association between NS and measles serology does not necessarily indicate a cause-effect relationship. Rather, MV might (like HIV) suppress immunity [16,17] and thereby increase risk for opportunistic infections, whether viral, bacterial, fungal or other organisms, including parasitic nematodes. A higher rate of nematode infection (*Onchocerca volvulus*, *Mansonella perstans)* was found in NS vs. CC in 2002 in Sudan [3], and onchocerciasis was subsequently associated with the neurological disorder in other East African countries [18]. However, some NS cases are negative and some controls are positive for evidence of *O. volvulus* infection. While the prevalence of nematode infection was not determined here, caregiver-reported use of the drug (ivermectin) administered to treat onchocerciasis showed some variability (64–76%) among NS, HC and CC subject groups. Interactions among multiple infectious agents (notably other neurotropic viruses) and their association with NS needs to be further evaluated in future studies, especially in the context of the pervasive malnutrition that has characterized outbreaks of NS [2,19]. The possibility that NS is related to progressive rubella panencephalitis, a disorder comparable clinically but evolving more slowly than SSPE several years after congenital or postnatal rubella infection [20], is unlikely based on present results (Table 1).

5. Conclusions

We report, for the first time, an association between measles virus (MV)-positive titers and NS among affected children and adolescents in northern Uganda. By contrast, there were no case-control differences in serum antibody titers for Rubella virus (RV), Cytomegalovirus (CMV) or Herpes simplex virus 1 (HSV-1). These results, coupled with the partly overlapping clinical and neuropathological features of NS and SSPE, including reports of head nodding in SSPE, merit further research to understand if Measles virus (MV) plays any role in the etiology and pathogenesis of NS.

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CRediT authorship contribution statement

Raquel Valdes Angues: Investigation, Methodology, Formal analysis, Validation, Writing – original draft. **Valerie S. Palmer:** Conceptualization, Resources, Data curation, Validation. **Rajarshi Mazumder:** Resources, Writing – review & editing. **Caesar Okot:** Resources. **Peter S. Spencer:** Conceptualization, Supervision, Writing – original draft, Writing – review & editing, Funding acquisition.

Declaration of Competing Interest

None.

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