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Asymptomatic malaria parasitaemia and seizure control in children with nodding syndrome; a cross-sectional study

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1 **Asymptomatic malaria parasitaemia and seizure control in** 2 **children with nodding syndrome; a cross sectional study**

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18 Abstract

19 Objective

20 *Plasmodium falciparum* is epileptogenic and in malaria endemic areas, it is a leading cause of
21 acute symptomatic seizures. In these areas, asymptomatic infections are common, considered
22 benign and not treated. The effects of such infections on seizures in patients with epilepsy is
23 unknown. This study examined the relationship between *P.falciparum* infection and seizure
24 control in children with a complex epilepsy.

25 Design

26 This cross-sectional study was nested in an ongoing trial ‘Doxycycline for the treatment of
27 nodding syndrome (NCT02850913)’. The hypothesis is that in patients with epilepsy,
28 asymptomatic *P.falciparum* increases the risk of seizures and impairs seizure control.

29 Setting and participants

30 Participants were Ugandan children with a complex epilepsy disorder – the nodding
31 syndrome, ages 8 years or older, receiving sodium valproate. All had standardized testing
32 including documentation of the number of seizures in the past month, a rapid malaria test and
33 if positive, the peripheral blood parasite density.

34 Outcomes

35 The primary outcome was the number of seizures in the past month (30 days).

36 Results

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2
3 37 A total of 164/240 (68%) had malaria. Asymptomatic infections were seen in 160/240 (67%)
4
5 38 and symptomatic infections in 4/240 (2.7%). In participants without malaria, the median
6
7 39 [IQR] number of seizures in the previous 30 days was 2.0 [1.0-4.0] and it was 4.0 [2.0-7.5] in
8
9 40 participants with malaria, $p=0.017$. The number of seizures in asymptomatic malaria persons
10
11 41 was 3.0[IQR 2.0-7.3] and it was 6.0[IQR 4.0-10.0] in symptomatic individuals, $p=0.024$.
12
13 42 Additionally, in asymptomatic patients, a positive correlation was observed between the
14
15 43 parasite density and number of seizures, $r=0.33$, $p=0.002$.
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19 44 **Conclusion**

20
21
22 45 In patients with nodding syndrome, both asymptomatic and symptomatic malaria are
23
24 46 associated with an increased risk of seizures and poorer seizure control. Similar effects
25
26 47 should be examined in other epilepsy disorders. Malaria prevention should be strengthened
27
28 48 for these patients and chemo-treatment and prevention studies considered.
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30

31 49 **Article Summary**

32 50 **Strengths and limitations**

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39 51 • Nodding syndrome is a poorly understood epilepsy disorder not representative of other
40
41 52 epilepsies. However, the disease offers the advantage of a uniform population of epilepsy
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43 53 patients, receiving the same antiepileptic drug, and a similar level of care in Uganda.
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45
46 54 • This was a cross sectional study that cannot ascribe causality; prospective studies should
47
48 55 be conducted to confirm the results.
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50
51 56 • The study also relied on parental recall of the number of convulsive seizures in the past
52
53 57 month and could have suffered from the shortfalls of recall bias. Again, a prospective
54
55 58 determination of study outcomes will be more appropriate.
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Introduction

Plasmodium falciparum is still a major public health problem in tropical countries and especially, in sub-Saharan Africa. Over 200 million cases are reported annually with several thousand deaths majorly among children younger than 5 years and in pregnant women[1]. *P. falciparum* presents with a spectrum of manifestations from asymptomatic infection, symptomatic but uncomplicated disease to severe or complicated malaria[2]. Asymptomatic infections are common especially in highly endemic areas[3–7]. These symptomless infections are generally considered benign and thought to be useful in maintaining immunity against severe disease[8]. Evidence is however emerging demonstrating that asymptomatic infections possibly have negative health effects (reviewed in Chen et al 2016)[9] including cognitive impairment[10], anaemia[11,12], co-infection with invasive bacterial disease[13] and increased maternal and neonatal mortality[14]

About 50% of children with acute severe falciparum malaria present with neurological involvement[15]. *P. falciparum* is known to be epileptogenic, and is a leading cause of acute seizures in children living in malaria endemic areas[16]. However, the effects of asymptomatic infections on the incidence and control of seizures in children with seizure disorders is unknown. This study examined the relationship between asymptomatic malarial

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3 81 infections and seizure control in patients with epilepsy using nodding syndrome as a model.
4
5 82 The hypothesis is that: in patients with epilepsy, asymptomatic *P. falciparum* infections are
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7 83 associated with; i) poorer seizure control; and that ii) seizure control is worse in patients with
8
9 84 higher parasitaemia.
10

11
12 85 Nodding syndrome (NS) is a poorly understood complex epilepsy disorder that affects
13
14 86 children and adolescents in some regions of Africa[17,18]. Northern Uganda, South Sudan
15
16 87 and southern Tanzania bear the greatest burden of this devastating disorder[19,20]. The
17
18 88 aetiology is unknown but cross reacting antibodies to *Onchocerca volvulus* have recently
19
20 89 been proposed to underlie the pathogenesis [18,21]. Symptoms develop in previously
21
22 90 normally developing children between the ages of 3-18 years[22,23]. Patients present with a
23
24 91 distinctive feature – clusters of head nodding – now defined as atonic seizures[21], with a
25
26 92 myoclonic element. The head nods present as repeated slow vertical head drops at a
27
28 93 frequency of 5-20/min most often, on presentation of food or in cold weather[24]. Over time,
29
30 94 the condition is complicated by multiple types of convulsive seizures (focal or multifocal,
31
32 95 atypical absence, myoclonic jerks and generalized tonic-clonic seizures), behaviour
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34 96 difficulties and psychiatric disorders, cognitive decline, and in many severe cases, physical
35
36 97 deformities and severe disability[22]. In Uganda, patients initiated on a specific symptomatic
37
38 98 treatment intervention including the provision of sodium valproate as antiepileptic therapy
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40 99 obtained a 75% reduction in the burden of seizures[25]. The current study examined the
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42 100 relationship between asymptomatic *P.falciparum* infection and seizure control in nodding
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44 101 syndrome.
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104 **Methods**

105 **Study design**

106 This was a cross-sectional study of the relationship between asymptomatic *P. falciparum*
107 malaria infections and seizure control in children with nodding syndrome. *The study was*
108 *nested within an ongoing trial 'Doxycycline for the treatment of nodding syndrome*
109 *(NCT02850913)'. This trial is testing the hypothesis that nodding syndrome is an *Onchocerca**
110 *volvulus* induced epileptic encephalopathy with antibodies to the parasite or its symbiotic
111 bacteria, *Wolbachia*, cross reacting with and damaging host neuron proteins[18,26]. Trial
112 participants are randomized to either oral doxycycline 100mg daily for 6 weeks or matching
113 placebo.

114 **Setting**

115 The trial is being conducted in the nodding syndrome affected districts of Kitgum, Pader and
116 Lamwo in Northern Uganda. This region is inhabited by the Acholi, a Luo speaking
117 community that is recovering from a decade-old civil war, high levels of poverty and psycho-
118 social problems. In 2016, the districts were served by 17 nodding syndrome treatment centres
119 where patients received clinical care and treatment according to national guidelines[27]. The
120 population prevalence of nodding syndrome in the affected age group in the region is 6.8
121 (95% CI 5.9-7.7) per 1,000[28]. The region is highly endemic to *P. falciparum* malaria and in
122 2015 and 2016 experienced a malaria epidemics.

123 **Study population**

124 The study recruited participants with a diagnosis of nodding syndrome per World Health
125 Organization criteria. All 240 were receiving sodium valproate (doses 12-35 mg/kg/day) as

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3 126 antiepileptic drug therapy plus nutritional, physical and psychological therapy[27] and had
4
5 127 been enrolled in the doxycycline for the treatment of nodding syndrome trial.
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8 128 **Procedures**

9 10 11 129 **Approvals**

12
13
14 130 Ethical approval for the trial was granted by Makerere University School of Medicine
15
16 131 Research and Ethics Committee (SOMREC) and University of Oxford Tropical Research
17
18 132 Ethics Committee (OxTREC). Uganda National Council of Science and Technology
19
20 133 (UNCST) and the National Drug Authority in Uganda provided regulatory approvals.
21
22 134 Consent was obtained from each participant's carer and assent from the participants (except
23
24 135 for cases with severe cognitive impairment).
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28 29 136 **Screening, recruitment and clinical assessments**

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31
32 137 Most children with nodding syndrome in Uganda live within a few Kilometres of the 17-
33
34 138 nodding syndrome treatment centres in the country. Patient registers from the selected centres
35
36 139 were accessed to identify potential participants. All patients with nodding syndrome in the
37
38 140 specific locations were invited to the nearest follow-up centre or a central location in a village
39
40 141 by the study field staff. Patients were then screened for eligibility and eligible participants
41
42 142 consented. The inclusion criteria included participants with confirmed nodding syndrome as
43
44 143 defined by the WHO; age 8 years or older (to avoid doxycycline toxicity) and written consent
45
46 144 by the parent or guardian. Females with a positive urinary HCG (pregnancy) test, known
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48 145 hypersensitivity to tetracycline, reported inability to swallow capsules, enrolled into another
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50 146 trial and suspected high likelihood of non-compliance with the study drug and follow-up
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53 147 schedule were excluded.
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3 148 As part of the requirements of the trial, all consenting participants and care givers were
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5 149 invited to and hospitalized in Kitgum General Hospital for about a week. During this period,
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7 150 all had detailed history, a full clinical and neurological assessment, an assessment of
8
9 151 functioning using the Gross Motor Function Classification System and Modified Rankins
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11 152 Score, Cognitive function on psychometric testing and the Cogstate (a computerized
12
13 153 cognitive test), Intellectual Disability using the Child and Adolescent Intellectual Disability
14
15 154 Screening Questionnaire (CAIDS-Q), Quality of Life with the Quality of Life in Childhood
16
17 155 Epilepsy Questionnaire and a diagnostic electroencephalogram (EEG) testing. The types of
18
19 156 seizures were described and the burden reported as the number of seizures in the past month.
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23 157 **Laboratory Procedures**

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25 158 All participants had 10mls of venous blood drawn for a complete blood count, liver and renal
26
27 159 function, and study specific tests. Malaria was tested using the *P. falciparum* malaria (HRP2)
28
29 160 rapid diagnostic test (CareStart™, 2016). Participants testing positive for malaria on the RDT
30
31 161 had thick Giemsa-stained blood smear slides prepared to determine the parasite density. Each
32
33 162 slide was examined by two observers, and any differences were reconciled by a third
34
35 163 observer. The number of asexual malaria parasites observed was reported per 200 white
36
37 164 blood cells (WBC) and the parasite density per microliter of blood estimated assuming 8000
38
39 165 WBC/ul of blood.
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44 166 **Definitions and study outcomes**

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47 167 a) Asymptomatic malaria was defined as *P. falciparum* parasitemia with no history of
48
49 168 fever in the past week and axillary temperature <37.5 °C.
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51 169 b) Symptomatic malaria was defined as *P. falciparum* parasitemia with history of fever
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53 170 in the past week or axillary temperature $\geq 37.5^{\circ}\text{C}$.
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3 171 c) Seizure burden was defined as the number of seizures in the past 30 days as reported
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5 172 by the caretaker.
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7 173 d) Good seizure control was defined as No seizures in the past 30 days as reported by the
8
9 174 caretaker.
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11 175 e) Poor seizure control was defined as one or more seizures in the past 30days as
12
13 176 reported by the caretaker.
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17 177 In this report, only the relevant clinical and laboratory testing, obtained at the enrolment
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19 178 screening before the interventions, is reported.
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23 180 **Patient and public involvement**

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25 181 The public was involved in developing the research questions of the overall study i.e.
26
27 182 understanding the aetiology and treatment of nodding syndrome but not with the design of
28
29 183 this sub-study. The main trial is however still ongoing and in addition to personal contact
30
31 184 with participant's families every six months, there are also community meetings every 3-6
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33 185 months. The study results will be fed back to the community at these meetings and on during
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35 186 the biannual Call in Local FM Radio broadcasts that the study conducts.
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43 188 **Data management and Statistical analyses**

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46 189 Data was entered in a Microsoft Access Database using Epi info version 7.1.5.2. All patient
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48 190 data was then exported and analysed with STATA version 12.0 (STATA CORP, Texas) and
49
50 191 Graph Pad Prism version 6.01 (GraphPad Software, Inc. California). Descriptive statistics
51
52 192 where used to explore the data and reported as proportions, percentages, means (SD) and
53
54 193 medians (IQR) as appropriate. Differences between the groups were tested by the Chi Square
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3 194 test (proportions), the student's t test for normally distributed data and the Mann Whitney test
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5 195 was used for skewed data. Participants were categorized into those with good seizure control
6
7 196 (no seizures in the past month) and those poor seizure control (one or more seizures in the
8
9 197 past month). A logistic regression model was used to assess the association between seizure
10
11 198 control and infection with plasmodium. Variables with a p value of 0.3 or less at bivariate
12
13 199 level were considered for multivariate analysis. Variables significantly associated with
14
15 200 infection by *Plasmodium falciparum* were further assessed for interaction with plasmodium
16
17 201 infection and seizure control. All variables that were not in the model because of a non-
18
19 202 significant p value were assessed for a confounding effect using a 10% difference between
20
21 203 the adjusted and unadjusted odds to show confounding. To examine if there is a direct
22
23 204 relationship between the malaria parasite density and seizure burden, Spearman's Rank
24
25 205 Correlation testing was performed between the peripheral blood parasite density (log 10
26
27 206 parasites/*ul*) and the number of seizures in the past month in patients with asymptomatic
28
29 207 malaria.
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36 209

210 Results

211 General description

212 Between September 2016 and August 2017, a total of 240 patients with nodding syndrome
213 were recruited into the trial and were included in this study. Of these, 140/240 (58.3%) were
214 male. The mean age was 15.6 (SD 2.0) years. The mean dose of sodium valproate was 22.6
215 (SD 7.2) mg/kg/day. A total of 164/240 (68.3%) participants tested positive for falciparum
216 malaria, most of whom had asymptomatic infections (160/164 [97.6%]).

217 A total of 159/240 (66.3%) reported experiencing at least one convulsive seizure in the past
218 year of whom 139 (57.9%) experienced at least one such seizure in the past 30 days. There
219 were no significant differences in the doses (mg/kg/day) of sodium valproate across the
220 groups. In patients without malaria, the median [IQR] number of seizures in the previous 30
221 days was 2.0 [1.0-4.0] and it was 4.0 [2.0-7.5] in patients with malaria, $p=0.017$, Mann-
222 Whitney test, **Figure 1**. Among the four symptomatic malaria cases, the median [IQR]
223 number of seizures in the past month was 6.0 [4.0-10.0]. However, because these were very
224 few, the group was excluded from all further analysis.

225 Among patients with asymptomatic malaria, the median [IQR] number of seizures
226 experienced in the past month was 3.0 (2.0-7.3). Generalized tonic-clonic and absence
227 seizures were the most common types of seizures described. Other seizure manifestations
228 were infrequent. Also, there were no differences in the manifestations of seizures in the three
229 groups, **Table 1**.

230

231

232 **Relationship between other patient characteristics and seizure control in patients with**
233 **nodding syndrome.**

234 Participants were categorized into those with good seizure control (no seizures in the past 30
235 days) and poor seizure control (one or more seizures in the past 30 days). In addition to
236 presences of malaria parasitaemia, sex (AOR 1.96 [95% CI 1.11-3.46], p value=0.03) and
237 dose of antiepileptic drug (AOR 1.04 [95% CI 1.00 - 1.13], p value=0.04) were significantly
238 associated with seizure control, **Table 2**. Furthermore, among children with asymptomatic
239 malaria, a positive linear correlation was observed between the number of seizures in the past
240 month and the peripheral blood parasite density $r=0.33$, (two tailed p value = 0.002). A linear
241 regression analysis gave the equation: $Y = 1.809X + 2.549$. **figure 1**.

242 **Discussion**

243 This study investigated the relationship between malaria infection and seizure control in
244 children with a complex epilepsy disorder, the nodding syndrome. The study found that
245 patients with plasmodium falciparum malaria infection experienced a significantly higher
246 number of seizures in the previous month compared to patients without malaria and, there
247 was a direct correlation between the peripheral blood parasite load and the number of
248 seizures. The study would suggest that in patients with seizure disorders on antiepileptic drug
249 treatment, malaria parasitaemia, whether asymptomatic or symptomatic, may increase the
250 risk of seizures and impair seizure control.

251 *Plasmodium falciparum* is epileptogenic[29]. In the malaria endemic regions of Africa,
252 falciparum malaria is a leading cause of acute seizures and convulsive status epilepticus. This
253 infection also contributes the largest fraction of seizure related hospitalizations in
254 children[16,29,30]. The seizures are not necessarily due to fever but are associated with
255 increasing parasitaemia possibly highlighting the pathological link between the presence of

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3 256 the parasites in the brain and the development of acute seizures[29,31] since the brain is a
4
5 257 preferential site for the sequestration of *P. falciparum* infected red cells. Already,
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7 258 asymptomatic malaria infections have been shown to affect cognition in healthy children [9]
8
9 259 but to the best of our knowledge, this is the first study to demonstrate a possible relationship
10
11 260 between asymptomatic infections and a higher burden of seizures in children with epilepsy.
12
13 261 The study would suggest that in children with epilepsy living in Africa, asymptomatic
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15 262 malaria infections may not be truly benign but may be a risk factor impairing seizure control.
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20 264 Apart from neurocysticercosis, the epileptogenic mechanisms of parasitic infections are not
21
22 265 well understood[32]. Plasmodium falciparum infection is characterized by sequestration of
23
24 266 the late stages of the intra-erythrocytic cycle, particularly in the brain. In the case of severe
25
26 267 malaria, acute seizures may potentially be induced through multiple pathways: i) indirectly
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28 268 through biochemical mechanisms associated with hypoglycemia, hyponatremia, or
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30 269 acidosis[29,31]; ii) a direct effect of the parasites (or parasite toxin) sequestered in cerebral
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32 270 vessels[29]; iii) an immunological mechanism since high titers of voltage gated cation
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34 271 channel antibodies have been observed in some children[33]; iv) compromised perfusion of
35
36 272 the brain due to cerebral microvascular parasite sequestration and raised intracranial pressure
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38 273 inhibiting substrate delivery[29] and; v) down-regulation of GABA receptors therefore
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40 274 decreasing the inhibitory effects on seizures[32]. It is possible that, in patients with low
41
42 275 seizure thresholds or a higher propensity to seizures such as children with epilepsy, even
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44 276 asymptomatic malaria infections may, by any of the above or other mechanisms, induce
45
46 277 seizures and impair seizure control. Therefore, poor seizure control maybe an unrecognized
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48 278 consequence of asymptomatic malaria infections in children with epilepsy in malaria endemic
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50 279 regions.
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3 281 Patients with malaria on average experienced twice the number of seizures experienced by
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5 282 those without malaria. The higher burden of seizures will not only impact on the clinical and
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7 283 antiepileptic drug needs of the patients but also on physician time, healthcare costs, patient's
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9 284 productivity, learning and achievements and on Quality of Life. There are an estimated 10
10
11 285 million people with epilepsy in Africa[34]. The prevalence of asymptomatic parasitaemia in
12
13 286 school age children in sub-Saharan Africa is 4-64% (reviewed in [35]). Assuming an average
14
15 287 10% prevalence, there may be over one million people with epilepsy in Africa who are at risk
16
17 288 of the potential adverse effects of asymptomatic malaria of poorer seizure control. Should
18
19 289 these findings be confirmed in other epilepsy disorders, it may be that patients with epilepsy
20
21 290 in the malaria endemic areas of Africa should be considered a special and may benefit from
22
23 291 enhanced malaria prevention. Already, children with sickle cell anaemia living in similar
24
25 292 settings are considered one such special group and in addition to the barrier methods of
26
27 293 malaria prevention, are offered malaria chemoprophylaxis[36]. Thus, this study should be
28
29 294 repeated in patients with other forms of epilepsy and if confirmed, trials should be conducted
30
31 295 to evaluate if children with epilepsy may also benefit from malaria chemo-treatment (of
32
33 296 asymptomatic cases) and chemoprophylaxis against infection in addition to current barrier
34
35 297 methods of malaria prevention.

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40 298 The study had some limitations. First, nodding syndrome is a poorly understood complex
41
42 299 epilepsy disorder that may not be representative of all other seizure disorders. However, the
43
44 300 syndrome offers the advantage of a uniform population with all patients receiving the same
45
46 301 antiepileptic drug and a similar level of care in Uganda[27]. Secondly, the study did not
47
48 302 conduct more sensitive assays such as PCR to identify sub-patent malaria infections. Third is
49
50 303 the possibility of recall bias in the determination of the number of seizures in the past month.
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52 304 However, we limited this bias by focusing on convulsive seizures that are less likely
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54 305 forgotten, limited to the past month, collecting the information using standardized tools, and
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3 306 by clinicians trained and experienced in the care of patients with epilepsy. Lastly, this was a
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5 307 cross sectional study with a limited sample size and so, a prospective study with a larger
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7 308 sample size should be conducted to confirm the results.
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9

10 309 **Conclusion**

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13
14 310 In conclusion, in patients with nodding syndrome, both asymptomatic and symptomatic
15
16 311 malaria infections are associated with an increased risk of seizures and poorer seizure control.
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18 312 Similar effects should be examined in other epilepsy disorders. Malaria prevention should be
19
20 313 strengthened for these patients and chemoprevention studies considered.
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3 447 **Author contributions**
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6 448 RI and RO conceived and designed the study, carried out initial analyses and drafted the
7
8 449 manuscript. RA, PA and AN designed the tools, collected data and critically reviewed the
9
10 450 manuscript. KM and CN designed the study and critically reviewed the manuscript.
11

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18
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35
36 461 Deborah Akol.
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38 462 **Competing interests**
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41 463 All the authors declare that they have no competing interests.
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44 464 **Data sharing statement**
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47 465 A data sharing plan for the trial is being developed to have the overall study data available on
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49 466 public websites.
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468 **Table 1: Summary of general characteristics in patients and without malaria**
 469 **parastaemia**

	Patients without malaria parasitaemia, N= 76	Patients with malaria parasitaemia, N= 160	P value
Mean (SD) Age, years	15.8 (1.5)	15.5 (2.1)	0.22 ^a
Sex, Male (%)	41 (54%)	94(58%)	0.48 ^b
Mean weight (SD), Kg	40.7(9.8)	41.9(8.9)	0.37 ^a
Mean (SD) axillary temp, °C	36.4(0.42)	36.5(0.4)	0.41 ^a
Parasite density (IQR), /ul	0 (0)	200 (20-460)	<0.0001 ^c
Mean (SD) dose of Valproate acid, (mg/kg/day)	24.1(8.2)	22.1(6.3)	0.06 ^a
Median (IQR) number of clusters of head nodding episodes in the last month (IQR)	3.0 [2.0-6.0]	4.0 [2.0-10.0]	0.38 ^c
Type of seizures in the past month			
Absence N (%)	12(15.7)	20(12.5)	0.62 ^b
Tonic N (%)	1(1.3)	0(0.0)	0.70 ^b
Clonic N (%)	1(1.3)	1(0.6)	1.00 ^b
Generalised tonic clonic N (%)	37(50)	73(45.6)	0.76 ^b
Myoclonic N (%)	1(1.3)	1(0.6)	0.54 ^b
Drop N (%)	2(2.6)	1(0.6)	0.24 ^b

470 ^a Un paired students t test

471 ^b chi square

472 ^c Mann Whitney test

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473 **Table 2: Factors associated with seizure control**

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		Seizure control		O.R (C.I)	P-Value	Adjusted odds	P Value
		Good	Poor				
Sex/ n (%)	Male	66(48.15)	71(51%)				
	Female	34(34.34)	65(65.6)	1.77(1.03-3.03)	0.04	1.96 (1.11-3.46)	0.02
Age Mean (S.D)		15.69(2.34)	15.73(1.66)	1.01(0.88-1.15)	0.88		
Body Temperature Mean (S.D)		36.46(0.47)	36.47(0.45)	0.98(0.55-1.71)	0.94		
Dose Mean (S.D)		23.42(7.87)	21.55(5.88)	1.04(0.99-1.08)	0.05	1.04 (1.00 - 1.13)	0.04
Weight/ Mean (S.D)		42.08(9.35)	41.5(9.34)	0.99(0.966-1.02)	0.6		
Duration with disease/ Mean (S.D)		8.44(2.68)	8.21(3.07)	1.02(0.93-1.12)	0.53		
log₁₀parasitemia / n (%)	0	66 (53.3)	57(46.3)				
	1 to 2.5	24 (38.71)	39(61.3)	1.80(0.93-3.36)	0.06	1.81(0.95-3.44)	0.07
	>2.5	10 (20.0)	40(80.0)	4.56(2.09-9.9)	<0.01	5.11(2.33-11.35)	< 0.01

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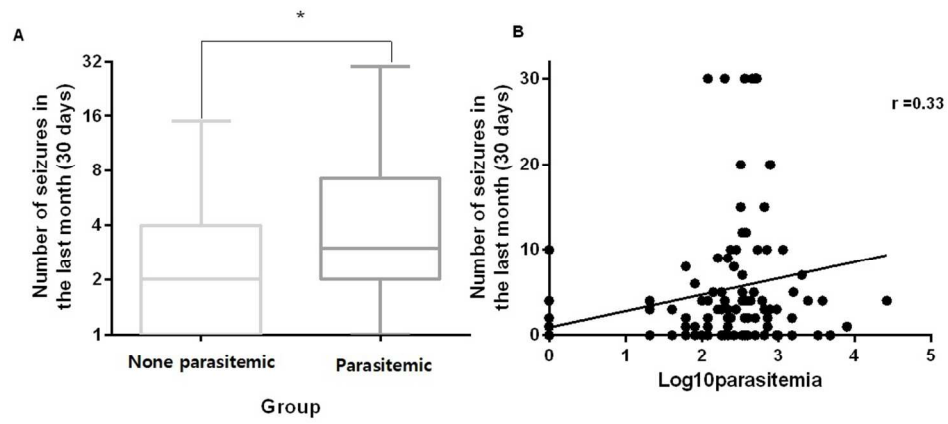
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5 476 **Figure 1:** A) Graph of seizure burden among participants who are non-parasitaemic and
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7 477 those who have asymptomatic parasitaemia. B) Graph showing the relationship between
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9 478 seizure burden and \log_{10} falciparum malaria parasite density among asymptomatic patients.
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15 480 **Figure legend**

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17
18 481 Figure 1 - compares the number (median) of convulsive seizures over the past month in
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20 482 nodding syndrome patients with and without falciparum malaria parasitaemia (A) and the
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22 483 relationship between the parasite density and the number of seizures (B).
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er review only

Reporting checklist for cross sectional study.

Based on the STROBE cross sectional guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

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In your methods section, say that you used the STROBE cross sectional reporting guidelines, and cite them as:

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		Reporting Item	Page Number
Title	#1a	Indicate the study's design with a commonly used term in the title or the abstract	1
Abstract	#1b	Provide in the abstract an informative and balanced summary of what was done and what was found	2-3
Background / rationale	#2	Explain the scientific background and rationale for the investigation being reported	5-6
Objectives	#3	State specific objectives, including any prespecified hypotheses	6
Study design	#4	Present key elements of study design early in the paper	6
Setting	#5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	7-9
Eligibility criteria	#6a	Give the eligibility criteria, and the sources and methods of selection of participants.	7-8

1		#7	Clearly define all outcomes, exposures, predictors, potential	9-10
2			confounders, and effect modifiers. Give diagnostic criteria, if	
3			applicable	
4				
5				
6	Data sources /	#8	For each variable of interest give sources of data and details of	7-10
7	measurement		methods of assessment (measurement). Describe	
8			comparability of assessment methods if there is more than one	
9			group. Give information separately for for exposed and	
10			unexposed groups if applicable.	
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14	Bias	#9	Describe any efforts to address potential sources of bias	8-10
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16				
17	Study size	#10	Explain how the study size was arrived at	6-7
18				
19	Quantitative	#11	Explain how quantitative variables were handled in the	9-10
20	variables		analyses. If applicable, describe which groupings were chosen,	
21			and why	
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24	Statistical	#12a	Describe all statistical methods, including those used to control	9-10
25	methods		for confounding	
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28		#12b	Describe any methods used to examine subgroups and	9-10
29			interactions	
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32		#12c	Explain how missing data were addressed	N/A
33				
34				
35		#12d	If applicable, describe analytical methods taking account of	N/A
36			sampling strategy	
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39		#12e	Describe any sensitivity analyses	N/A
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41	Participants	#13a	Report numbers of individuals at each stage of study—eg	11
42			numbers potentially eligible, examined for eligibility, confirmed	
43			eligible, included in the study, completing follow-up, and	
44			analysed. Give information separately for for exposed and	
45			unexposed groups if applicable.	
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49		#13b	Give reasons for non-participation at each stage	N/A
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52		#13c	Consider use of a flow diagram	N/A
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54	Descriptive data	#14a	Give characteristics of study participants (eg demographic,	11
55			clinical, social) and information on exposures and potential	
56			confounders. Give information separately for exposed and	
57			unexposed groups if applicable.	
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	#14b	Indicate number of participants with missing data for each variable of interest	N/A
Outcome data	#15	Report numbers of outcome events or summary measures. Give information separately for exposed and unexposed groups if applicable.	See note 1
Main results	#16a	Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	11; 22-24
	#16b	Report category boundaries when continuous variables were categorized	11
	#16c	If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	N/A
Other analyses	#17	Report other analyses done—e.g., analyses of subgroups and interactions, and sensitivity analyses	11-12
Key results	#18	Summarise key results with reference to study objectives	12-13
Limitations	#19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias.	14
Interpretation	#20	Give a cautious overall interpretation considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence.	13-15
Generalisability	#21	Discuss the generalisability (external validity) of the study results	15
Funding	#22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	21

Author notes

1. 11-12; 22-24

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BMJ Open

Asymptomatic malaria parasitaemia and seizure control in children with nodding syndrome; a cross-sectional study

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Primary Subject Heading:	Neurology
Secondary Subject Heading:	Infectious diseases, Global health, Paediatrics
Keywords:	Nodding syndrome, Malaria, Seizure control, children

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Manuscripts

1 **Asymptomatic malaria parasitaemia and seizure control in** 2 **children with nodding syndrome; a cross sectional study**

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18 **Abstract**

19 **Objective**

20 *Plasmodium falciparum* is epileptogenic and in malaria endemic areas, is a leading cause of
21 acute seizures. In these areas, asymptomatic infections are common but considered benign
22 and so, are not treated. The effects of such infections on seizures in patients with epilepsy is
23 unknown. This study examined the relationship between *P.falciparum* infection and seizure
24 control in children with a unique epilepsy type, the nodding syndrome.

25 **Design**

26 This cross-sectional study was nested in an ongoing trial ‘Doxycycline for the treatment of
27 nodding syndrome (NCT02850913)’. We hypothesised that, in patients with epilepsy,
28 infection by *P.falciparum*, including asymptomatic infections, increases the risk of seizures
29 and impairs seizure control.

30 **Setting and participants**

31 Participants were Ugandan children with nodding syndrome, age ≥ 8 years, receiving sodium
32 valproate. All had standardised testing including documentation of the number of seizures in
33 the past month, a rapid malaria test and if positive, the peripheral blood parasite density.

34 **Outcomes**

35 The primary outcome was the number of seizures in the past month (30 days).

36 **Results**

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3 37 A total of 164/240 (68%) had malaria. Asymptomatic infections (without fever) were seen in
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5 38 160/240 (67%) and symptomatic infections in 4/240 (2.7%). In participants without malaria,
6
7 39 the median [IQR] number of seizures in the past month was 2.0 [1.0-4.0] and it was 4.0 [2.0-
8
9 40 7.5] in participants with malaria, $p=0.017$. The number of seizures in asymptomatic persons
10
11 41 was 3.0[IQR 2.0-7.3] and 6.0[IQR 4.0-10.0] in symptomatic individuals, $p=0.024$.
12
13 42 Additionally, in asymptomatic patients, a positive correlation was observed between the
14
15 43 parasite density and number of seizures, $r=0.33$, $p=0.002$.
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19 44 **Conclusion**

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22 45 In patients with nodding syndrome, both asymptomatic and symptomatic malaria are
23
24 46 associated with an increased risk of seizures and poorer seizure control. Similar effects
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26 47 should be examined in other epilepsy disorders. Malaria prevention should be strengthened
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28 48 for these patients and chemo-treatment and prevention studies considered to improve seizure
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30 49 control.
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34 50 **Article Summary**

35 36 37 38 51 **Strengths and limitations**

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41 52 • Nodding syndrome is a poorly understood epilepsy disorder not representative of other
42
43 53 epilepsies. However, the disease offers the advantage of a uniform population of epilepsy
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45 54 patients, receiving the same antiepileptic drug, and a similar level of care in Uganda.
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48 55 • This was a cross sectional study that cannot ascribe causality; prospective studies should
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50 56 be conducted to confirm the results.
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3 57 • The study also relied on parental recall of the number of convulsive seizures in the past
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5 58 month and could have suffered from the shortfalls of recall bias. Again, a prospective
6
7 59 determination of study outcomes will be more appropriate.
8

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10 **Word count**

11
12 61 **Abstract** - 300
13

14
15 62 **Main text** - 2955
16

17
18 63 **Tables** - 2
19

20
21 64 **Figures** - 1
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23 65 **Key words** - Nodding syndrome, children, seizure control and malaria
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29
30 **Introduction**

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32
33 68 *Plasmodium falciparum* is still a major public health problem in tropical countries and
34
35 69 especially, in sub-Saharan Africa. Over 200 million cases are reported annually with several
36
37 70 thousand deaths majorly among children younger than 5 years and in pregnant women[1]. *P.*
38
39 71 *falciparum* presents with a spectrum of manifestations from asymptomatic infections (malaria
40
41 72 parasitaemia without fever), symptomatic but uncomplicated disease to severe or complicated
42
43 73 malaria[2]. Asymptomatic infections are common especially in highly endemic areas[3–7].
44
45 74 These symptomless malaria infections are generally considered benign and thought to be
46
47 75 useful in maintaining immunity against severe disease[8]. Evidence is however emerging
48
49 76 demonstrating that asymptomatic infections possibly have negative health effects (reviewed
50
51 77 in Chen et al 2016)[9] including cognitive impairment[10], anaemia[11,12], co-infection
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53 78 with invasive bacterial disease[13] and increased maternal and neonatal mortality[14].
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3 79 About 50% of children with acute severe falciparum malaria present with neurological
4
5 80 involvement[15]. *P. falciparum* is known to be epileptogenic, and is a leading cause of acute
6
7 81 seizures in children living in malaria endemic areas[16]. However, the effects of
8
9 82 asymptomatic infections on the incidence and control of seizures in children with seizure
10
11 83 disorders is unknown. This study examined the relationship between asymptomatic malarial
12
13 84 infections and seizure control in patients with epilepsy using nodding syndrome as a model.
14
15 85 The hypothesis is that: in patients with epilepsy, asymptomatic *P. falciparum* infections are
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17 86 associated with; i) poorer seizure control; and that ii) seizure control is worse in patients with
18
19 87 higher parasitaemia.
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23 88 Nodding syndrome (NS) is a poorly understood complex epilepsy disorder that affects
24
25 89 children and adolescents in some regions of Africa[17,18]. Northern Uganda, South Sudan
26
27 90 and southern Tanzania bear the greatest burden of this devastating disorder[19,20]. The
28
29 91 aetiology is unknown but cross reacting antibodies to *Onchocerca volvulus* have recently
30
31 92 been proposed to underlie the pathogenesis [18,21]. Symptoms develop in previously
32
33 93 normally developing children between the ages of 3-18 years[22,23]. Patients present with a
34
35 94 distinctive feature – clusters of head nodding – now defined as atonic seizures[21], with a
36
37 95 myoclonic element. The head nods present as repeated slow vertical head drops at a
38
39 96 frequency of 5-20/min most often, on presentation of food or in cold weather[24]. Over time,
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41 97 the condition is complicated by multiple types of convulsive seizures (focal or multifocal,
42
43 98 atypical absence, myoclonic jerks and generalized tonic-clonic seizures), behaviour
44
45 99 difficulties and psychiatric disorders, cognitive decline, and in many severe cases, physical
46
47 100 deformities and severe disability[22]. In Uganda, patients initiated on a specific symptomatic
48
49 101 treatment intervention including the provision of sodium valproate as antiepileptic therapy
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51 102 obtained a 75% reduction in the burden of seizures[25]. The current study examined the
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3 103 relationship between asymptomatic *P.falciparum* infection and seizure control in nodding
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5 104 syndrome.

8 105 **Methods**

11 106 **Study design**

15 107 This was a cross-sectional study of the relationship between asymptomatic *P. falciparum*
16
17 108 malaria infections and seizure control in children with nodding syndrome. *The study was*
18
19 109 *nested within and all the participants are enrolled in an ongoing trial 'Doxycycline for the*
20
21 110 *treatment of nodding syndrome (NCT02850913)'. This trial is testing the hypothesis that*
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23 111 nodding syndrome is an *Onchocerca volvulus* induced epileptic encephalopathy with
24
25 112 antibodies to the parasite or its symbiotic bacteria, *Wolbachia*, cross reacting with and
26
27 113 damaging host neuron proteins[18,26]. Trial participants are randomised to either oral
28
29 114 doxycycline 100mg daily for 6 weeks or matching placebo.

33 115 **Setting**

36 116 The trial is being conducted in the nodding syndrome affected districts of Kitgum, Pader and
37
38 117 Lamwo in Northern Uganda. This region is inhabited by the Acholi, a Luo speaking
39
40 118 community that is recovering from a decade-old civil war, with high levels of poverty and
41
42 119 psycho-social problems. The districts are served by 17 nodding syndrome treatment centres
43
44 120 where patients receive clinical care and treatment according to national guidelines[27]. The
45
46 121 population prevalence of nodding syndrome in the affected age group in the region is 6.8
47
48 122 (95% CI 5.9-7.7) per 1,000[28]. The region is also highly endemic to *P. falciparum* malaria
49
50 123 and in 2015 and 2016 experienced a malaria epidemic.

55 124 **Study population**

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2
3 125 The study recruited all the 240 participants who had been enrolled in the doxycycline for the
4
5 126 treatment of nodding syndrome trial. The diagnosis of nodding syndrome was made
6
7 127 according to World Organization Criteria. All were receiving sodium valproate (doses 12-35
8
9 128 mg/kg/day) as antiepileptic therapy plus nutritional, physical and psychological therapy.

129 **Procedures**

130 **Approvals**

131 Ethical approval for the trial was granted by Makerere University School of Medicine
132 Research and Ethics Committee (SOMREC) and University of Oxford Tropical Research
133 Ethics Committee (OxTREC). Uganda National Council for Science and Technology
134 (UNCST) and the National Drug Authority in Uganda provided regulatory approvals.
135 Consent was obtained from each participant's carer and assent from the participants (except
136 for cases with severe cognitive impairment).

137 **Screening, recruitment and clinical assessments**

138 Most children with nodding syndrome in Uganda live within a few Kilometres of the 17-
139 nodding syndrome treatment centres in the country. Patient registers from the selected centres
140 were accessed to identify potential participants. All patients with nodding syndrome in the
141 specific locations were invited to the nearest follow-up centre or a central location in a village
142 by the study field staff. Patients were then screened for eligibility and eligible participants
143 consented. The inclusion criteria included participants with confirmed nodding syndrome as
144 defined by the WHO; age 8 years or older (to avoid doxycycline toxicity) and written consent
145 by the parent or guardian. Females with a positive urinary HCG (pregnancy) test, known
146 hypersensitivity to tetracycline, reported inability to swallow capsules, enrolled into another

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3 147 trial and suspected high likelihood of non-compliance with the study drug and follow-up
4
5 148 schedule were excluded.
6
7 149 As part of the requirements of the trial, all consenting participants and care givers were
8
9 150 invited to and hospitalized in Kitgum General Hospital for about a week. During this period,
10
11 151 they had detailed history, a full clinical and neurological assessment, an assessment of
12
13 152 functioning using the Gross Motor Function Classification System and Modified Rankins
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15 153 Score, Cognitive function on psychometric testing and the Cogstate (a computerized
16
17 154 cognitive test), Intellectual Disability using the Child and Adolescent Intellectual Disability
18
19 155 Screening Questionnaire (CAIDS-Q), Quality of Life with the Quality of Life in Childhood
20
21 156 Epilepsy Questionnaire and a diagnostic electroencephalogram (EEG) testing. The types of
22
23 157 seizures were described and the burden reported as the number of seizures in the past month.
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27 158 **Laboratory Procedures**

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30 159 All participants had 10mls of venous blood drawn for a complete blood count, liver and renal
31
32 160 function, and study specific tests. Malaria was tested using the *P. falciparum* malaria (HRP2)
33
34 161 rapid diagnostic test (CareStart™, 2016). Participants testing positive for malaria on the RDT
35
36 162 had thick Giemsa-stained blood smear slides prepared to determine the parasite density. Each
37
38 163 slide was examined by two observers, and any differences were reconciled by a third
39
40 164 observer. The number of asexual malaria parasites observed was reported per 200 white
41
42 165 blood cells (WBC) and the parasite density per microliter of blood estimated assuming 8000
43
44 166 WBC/ul of blood. The laboratory technicians performing these tests were blind to the rest of
45
46 167 the clinical information, including the burden of seizures in the past month, which was
47
48 168 obtained earlier by the study clinical and nursing staff.
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52 169 **Definitions and study outcomes**

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3 170 a) Asymptomatic malaria was defined as *P. falciparum* parasitemia with no history of
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5 171 fever in the past week and axillary temperature $<37.5^{\circ}\text{C}$.
6
7 172 b) Symptomatic malaria was defined as *P. falciparum* parasitemia with either history of
8
9 173 fever in the past week or axillary temperature $\geq 37.5^{\circ}\text{C}$.
10
11 174 c) Seizure burden was defined as the number of seizures in the past 30 days as reported
12
13 175 by the caretaker.
14
15 176 d) Good seizure control was defined as No seizures in the past 30 days as reported by the
16
17 177 caretaker.
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19 178 e) Poor seizure control was defined as one or more seizures in the past 30days as
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21 179 reported by the caretaker.
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25 180 In this report, only the relevant clinical and laboratory testing, obtained at the enrolment
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27 181 screening before the interventions, is reported.
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32 183 **Patient and public involvement**

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34 184 The public was involved in developing the research questions of the overall study i.e.
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36 185 understanding the aetiology and treatment of nodding syndrome but not with the design of
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38 186 this sub-study. The main trial is ongoing and in addition to personal contact with participant's
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40 187 families every six months, there are also community meetings every 3-6 months. The study
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42 188 results will be fed back to the community at these meetings and during the biannual Local
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44 189 FM Radio broadcasts that the study conducts.
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53 191 **Data management and Statistical analyses**

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3 192 Data was entered in a Microsoft Access Database using Epi info version 7.1.5.2. All patient
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5 193 data was then exported and analysed with STATA version 12.0 (STATA CORP, Texas) and
6
7 194 Graph Pad Prism version 6.01 (GraphPad Software, Inc. California). Descriptive statistics
8
9 195 where used to explore the data and this is reported as proportions, percentages, means (SD)
10
11 196 and medians (IQR) as appropriate. Differences between the groups were tested by the Chi
12
13 197 Square test (proportions), the student's t test for normally distributed data and the Mann
14
15 198 Whitney test was used for skewed data. Participants were categorized into those with good
16
17 199 seizure control (no seizures in the past month) and those poor seizure control (one or more
18
19 200 seizures in the past month). A logistic regression model was used to examine the relationship
20
21 201 between seizure control and infection with plasmodium. Variables with a p value of 0.3 or
22
23 202 less at bivariate level were considered for multivariate analysis. Variables significantly
24
25 203 associated with infection by *Plasmodium falciparum* were further assessed for interaction
26
27 204 with plasmodium infection and seizure control. All variables that were not in the model
28
29 205 because of a non-significant p value were assessed for a confounding effect using a 10%
30
31 206 difference between the adjusted and unadjusted odds to show confounding. To examine if
32
33 207 there is a direct relationship between the malaria parasite density and seizure burden,
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35 208 Spearman's Rank Correlation testing was performed between the peripheral blood parasite
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37 209 density (log 10 parasites/*ul*) and the number of seizures in the past month in patients with
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39 210 asymptomatic malaria.
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213 Results

214 General description

215 Between September 2016 and August 2017, a total of 240 patients with nodding syndrome
216 were recruited into the trial. All were included in this study. Of these, 140/240 (58.3%) were
217 male. The mean age was 15.6 (SD 2.0) years. The mean dose of sodium valproate was 22.6
218 (SD 7.2) mg/kg/day. A total of 164/240 (68.3%) participants tested positive for falciparum
219 malaria, most of whom had asymptomatic infections (160/164 [97.6%]).

220 A total of 159/240 (66.3%) reported experiencing at least one convulsive seizure in the past
221 year of whom 139 (57.9%) experienced at least one such seizure in the past 30 days. In
222 patients without malaria, the median [IQR] number of seizures in the previous 30 days was
223 2.0 [1.0-4.0] and it was 4.0 [2.0-7.5] in patients with malaria, $p=0.017$, Mann-Whitney test,
224 **Figure 1**. There were no significant differences in the doses (mg/kg/day) of sodium valproate
225 across the groups. Among the four symptomatic malaria cases, the median [IQR] number of
226 seizures in the past month was 6.0 [4.0-10.0]. However, because these were very few, the
227 group was excluded from all further analysis.

228 Among patients with asymptomatic malaria, the median [IQR] number of seizures
229 experienced in the past month was 3.0 (2.0-7.3). Generalized tonic-clonic and absence
230 seizures were the most common types of seizures described. Other seizure manifestations
231 were infrequent. Also, there were no differences in the manifestations of seizures in the three
232 groups, **Table 1**.

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3 235 **Relationship between other patient characteristics and seizure control in patients with**
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5 236 **nodding syndrome.**
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8 237 Participants were categorized into those with good seizure control (no seizures in the past 30
9
10 238 days) and poor seizure control (one or more seizures in the past 30 days). In addition to
11
12 239 presences of malaria parasitaemia, sex (AOR 1.96 [95% CI 1.11-3.46], p value=0.03) and
13
14 240 dose of antiepileptic drug (AOR 1.04 [95% CI 1.00 - 1.13], p value=0.04) were significantly
15
16 241 associated with seizure control, **Table 2**. Furthermore, among children with asymptomatic
17
18 242 malaria, a positive linear correlation was observed between the number of seizures in the past
19
20 243 month and the peripheral blood parasite density $r=0.33$, (two tailed p value = 0.002). A linear
21
22 244 regression analysis gave the equation: $Y = 1.809X + 2.549$, **figure 1**.
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27 245 **Discussion**
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29 246 This study investigated the relationship between asymptomatic malaria infection and seizure
30
31 247 control in children with a complex epilepsy disorder, the nodding syndrome. The study found
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33 248 that patients with plasmodium falciparum malaria infection experienced a significantly higher
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35 249 number of seizures in the previous month compared to patients without malaria and, there
36
37 250 was a direct correlation between the peripheral blood parasite load and the number of
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39 251 seizures. The study would suggest that in patients with seizure disorders on antiepileptic drug
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41 252 treatment, malaria parasitaemia, whether asymptomatic or symptomatic, may increase the
42
43 253 risk of seizures and impair seizure control.
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47 254 The association between acute infections and an increase in seizures in children with epilepsy is a
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49 255 well a recognised phenomenon. In the context of this study, *Plasmodium falciparum* is even
50
51 256 thought to be epileptogenic[29]: In the malaria endemic regions of Africa, falciparum
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53 257 malaria is a leading cause of acute seizures and convulsive status epilepticus. This infection
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55 258 also contributes the largest fraction of seizure related hospitalizations in children[16,29,30].
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57 259 The seizures are not necessarily due to fever but are associated with increasing parasitaemia
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3 260 possibly highlighting the pathological link between the presence of the parasites in the brain
4 261 and the development of acute seizures[29,31]. Already, asymptomatic malaria infections have
5 262 been shown to affect cognition in healthy children [9]. It was however not clear whether
6 263 asymptomatic malaria parasitaemia would be associated with an increased frequency or severity of
7 264 seizures. The finding of a dose-response effect of increasing seizures associated with higher parasite
8 265 load and with recognized fever, makes the association more compelling. To the best of our
9 266 knowledge, this is the first study to demonstrate a relationship between asymptomatic malaria
10 267 infections and a higher burden of seizures in children with epilepsy raising questions on the
11 268 meaning of “asymptomatic malaria”. It may be that the symptoms of malaria do fall along a
12 269 spectrum from mild to severe, with the fever reported by parents being an imperfect surrogate
13 270 for pathophysiological disruption. Our study would therefore suggest that in children with
14 271 epilepsy living in Africa, “asymptomatic” malaria infections may not be truly benign but may
15 272 be a risk factor impairing seizure control.
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19 274 Apart from neurocysticercosis, the epileptogenic mechanisms of parasitic infections are not
20 275 well understood[32]. Plasmodium falciparum infection is characterized by sequestration of
21 276 the late stages of the intra-erythrocytic cycle, particularly in the brain. In the case of severe
22 277 malaria, acute seizures may potentially be induced through multiple pathways: i) indirectly
23 278 through biochemical mechanisms associated with hypoglycemia, hyponatremia, or
24 279 acidosis[29,31]; ii) a direct effect of the parasites (or parasite toxin) sequestered in cerebral
25 280 vessels[29]; iii) an immunological mechanism since high titers of voltage gated cation
26 281 channel antibodies have been observed in some children[33]; iv) compromised perfusion of
27 282 the brain due to cerebral microvascular parasite sequestration and raised intracranial pressure
28 283 inhibiting substrate delivery[29] and; v) down-regulation of GABA receptors therefore
29 284 decreasing the inhibitory effects on seizures[32]. It is possible that, in patients with low
30 285 seizure thresholds or a higher propensity to seizures such as children with epilepsy, even
31 286 asymptomatic malaria infections may, by any of the above or other mechanisms, induce
32 287 seizures and impair seizure control. Therefore, poor seizure control maybe an unrecognized

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3 288 consequence of asymptomatic malaria infections in children with epilepsy in malaria endemic
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5 289 regions.

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9 291 Patients with malaria on average experienced twice the number of seizures experienced by
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11 292 those without malaria. The higher burden of seizures will not only impact on the clinical and
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13 293 antiepileptic drug needs of the patients but also on physician time, healthcare costs, patient's
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15 294 productivity, learning and achievements and on Quality of Life. There are an estimated 10
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17 295 million people with epilepsy in Africa[34]. The prevalence of asymptomatic parasitaemia in
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19 296 school age children in sub-Saharan Africa is 4-64% (reviewed in [35]). Assuming an average
20
21 297 10% prevalence, there may be over one million people with epilepsy in Africa who are at risk
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23 298 of the potential adverse effects of "asymptomatic" malaria associated poorer seizure control.

24
25 299 Should these findings be confirmed with other epilepsy disorders, it may be that patients with
26
27 300 epilepsy in the malaria endemic areas of Africa should be considered a special or vulnerable
28
29 301 group and considered for enhanced malaria prevention. Already, children with sickle cell
30
31 302 anaemia living in similar settings are considered one such special group and in addition to the
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33 303 barrier methods of malaria prevention such as bed nets, are offered enhanced malaria
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35 304 prevention through malaria chemoprophylaxis[36]. Thus, this study should be repeated in
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37 305 patients with other forms of epilepsy and if confirmed, trials should be conducted to evaluate
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39 306 if children with epilepsy may also benefit from malaria chemo-treatment (of asymptomatic
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41 307 cases) and chemoprophylaxis against infection in addition to current barrier methods of
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43 308 malaria prevention.

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48
49 309 The study had some limitations. First, nodding syndrome is a poorly understood complex
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51 310 epilepsy disorder that may not be representative of all other seizure disorders. However, the
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53 311 syndrome offers the advantage of a uniform population with all patients receiving the same
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55 312 antiepileptic drug and a similar level of care in Uganda[27]. Secondly, the study did not

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3 313 conduct more sensitive assays such as PCR to identify sub-patent malaria infections. Third is
4
5 314 the possibility of recall bias in the determination of the number of seizures in the past month.
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7 315 However, we limited this bias by focusing on convulsive seizures that are less likely
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9 316 forgotten, limited to the past month, collecting the information using standardized tools, and
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11 317 by clinicians trained and experienced in the care of patients with epilepsy. Lastly, this was a
12
13 318 cross sectional study with a limited sample size and so, a prospective study with a larger
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15 319 sample size should be conducted to confirm the results.
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19 320 **Conclusion**

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22 321 In conclusion, in patients with nodding syndrome, both asymptomatic and symptomatic
23
24 322 malaria infections are associated with an increased risk of seizures and poorer seizure control.
25
26 323 Similar effects should be examined in patients with other epilepsy disorders. Malaria
27
28 324 prevention should be strengthened for these patients and chemoprevention studies considered.
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9 458 **Author contributions**
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12 459 RI and RO conceived and designed the study, carried out initial analyses and drafted the
13
14 460 manuscript. RA, PA and AN designed the tools, collected data and critically reviewed the
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16 461 manuscript. EK performed the data analysis and critically reviewed the manuscript. KM and
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18 462 CN designed the study and critically reviewed the manuscript.
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27
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44 473 Deborah Akol.
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47 474 **Competing interests**
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50 475 All the authors declare that they have no competing interests.
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52 476 **Data sharing statement**
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477 A data sharing plan for the trial is being developed to have the overall study data available on
478 public websites.

479 **Table 1: Summary of general characteristics of nodding syndrome patients with and**
480 **without malaria parastaemia**

	Patients without malaria parasitaemia, N= 76	Patients with malaria parasitaemia, N= 160	P value
Mean (SD) Age, years	15.8 (1.5)	15.5 (2.1)	0.22 ^a
Sex, Male (%)	41 (54%)	94(58%)	0.48 ^b
Mean weight (SD), Kg	40.7(9.8)	41.9(8.9)	0.37 ^a
Mean (SD) axillary temp, °C	36.4(0.42)	36.5(0.4)	0.41 ^a
Parasite density (IQR), /ul	0 (0)	200 (20-460)	<0.0001 ^c
Mean (SD) dose of Valproate acid, (mg/kg/day)	24.1(8.2)	22.1(6.3)	0.06 ^a
Median (IQR) number of clusters of head nodding episodes in the last month (IQR)	3.0 [2.0-6.0]	4.0 [2.0-10.0]	0.38 ^c
Type of seizures in the past month			
Absence N (%)	12(15.7)	20(12.5)	0.62 ^b
Tonic N (%)	1(1.3)	0(0.0)	0.70 ^b
Clonic N (%)	1(1.3)	1(0.6)	1.00 ^b
Generalised tonic clonic N (%)	37(50)	73(45.6)	0.76 ^b
Myoclonic N (%)	1(1.3)	1(0.6)	0.54 ^b
Drop N (%)	2(2.6)	1(0.6)	0.24 ^b

481 ^a Un paired students t test

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482 ^b chi square
483 ^c Mann Whitney test

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484 **Table 2: Factors associated with seizure control**

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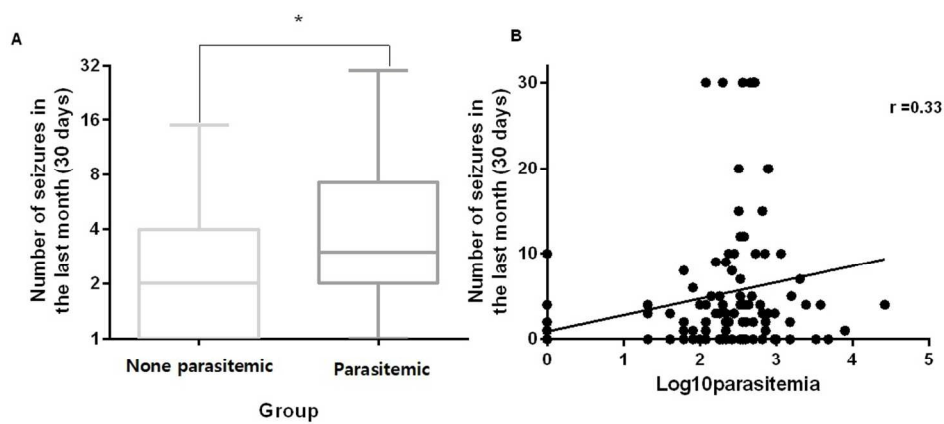
		Seizure control		O.R (C.I)	P-Value	Adjusted odds	P Value
		Good	Poor				
Sex/ n (%)	Male	66(48.15)	71(51%)				
	Female	34(34.34)	65(65.6)	1.77(1.03-3.03)	0.04	1.96 (1.11-3.46)	0.02
Age Mean (S.D)		15.69(2.34)	15.73(1.66)	1.01(0.88-1.15)	0.88		
Body Temperature Mean (S.D)		36.46(0.47)	36.47(0.45)	0.98(0.55-1.71)	0.94		
Dose Mean (S.D)		23.42(7.87)	21.55(5.88)	1.04(0.99-1.08)	0.05	1.04 (1.00 - 1.13)	0.04
Weight/ Mean (S.D)		42.08(9.35)	41.5(9.34)	0.99(0.966-1.02)	0.6		
Duration with disease/ Mean (S.D)		8.44(2.68)	8.21(3.07)	1.02(0.93-1.12)	0.53		
log₁₀parasitemia / n (%)	0	66 (53.3)	57(46.3)				
	1 to 2.5	24 (38.71)	39(61.3)	1.80(0.93-3.36)	0.06	1.81(0.95-3.44)	0.07
	>2.5	10 (20.0)	40(80.0)	4.56(2.09-9.9)	<0.01	5.11(2.33-11.35)	< 0.01

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6 487 **Figure 1:** A) Graph of seizure burden among participants who are non-parasitaemic and
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8 488 those who have asymptomatic parasitaemia. B) Graph showing the relationship between
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10 489 seizure burden and \log_{10} falciparum malaria parasite density among asymptomatic patients.
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1415 491 **Figure legend**

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18 492 Figure 1 - compares the number (median) of convulsive seizures over the past month in
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20 493 nodding syndrome patients with and without falciparum malaria parasitaemia (A) and the
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22 494 relationship between the parasite density and the number of seizures (B).
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Reporting checklist for cross sectional study.

Based on the STROBE cross sectional guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the STROBE cross sectional reporting guidelines, and cite them as:

von Elm E, Altman DG, Egger M, Pocock SJ, Gotsche PC, Vandenbroucke JP. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement: guidelines for reporting observational studies.

		Reporting Item	Page Number
Title	#1a	Indicate the study's design with a commonly used term in the title or the abstract	1
Abstract	#1b	Provide in the abstract an informative and balanced summary of what was done and what was found	2-3
Background / rationale	#2	Explain the scientific background and rationale for the investigation being reported	5-6
Objectives	#3	State specific objectives, including any prespecified hypotheses	6
Study design	#4	Present key elements of study design early in the paper	6
Setting	#5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	7-9
Eligibility criteria	#6a	Give the eligibility criteria, and the sources and methods of selection of participants.	7-8

1		#7	Clearly define all outcomes, exposures, predictors, potential	9-10
2			confounders, and effect modifiers. Give diagnostic criteria, if	
3			applicable	
4				
5				
6	Data sources /	#8	For each variable of interest give sources of data and details of	7-10
7	measurement		methods of assessment (measurement). Describe	
8			comparability of assessment methods if there is more than one	
9			group. Give information separately for for exposed and	
10			unexposed groups if applicable.	
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14	Bias	#9	Describe any efforts to address potential sources of bias	8-10
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17	Study size	#10	Explain how the study size was arrived at	6-7
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19	Quantitative	#11	Explain how quantitative variables were handled in the	9-10
20	variables		analyses. If applicable, describe which groupings were chosen,	
21			and why	
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24	Statistical	#12a	Describe all statistical methods, including those used to control	9-10
25	methods		for confounding	
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28		#12b	Describe any methods used to examine subgroups and	9-10
29			interactions	
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32		#12c	Explain how missing data were addressed	N/A
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34		#12d	If applicable, describe analytical methods taking account of	N/A
35			sampling strategy	
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38		#12e	Describe any sensitivity analyses	N/A
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41	Participants	#13a	Report numbers of individuals at each stage of study—eg	11
42			numbers potentially eligible, examined for eligibility, confirmed	
43			eligible, included in the study, completing follow-up, and	
44			analysed. Give information separately for for exposed and	
45			unexposed groups if applicable.	
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49		#13b	Give reasons for non-participation at each stage	N/A
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51		#13c	Consider use of a flow diagram	N/A
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54	Descriptive data	#14a	Give characteristics of study participants (eg demographic,	11
55			clinical, social) and information on exposures and potential	
56			confounders. Give information separately for exposed and	
57			unexposed groups if applicable.	
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1		#14b	Indicate number of participants with missing data for each variable of interest	N/A
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5	Outcome data	#15	Report numbers of outcome events or summary measures. Give information separately for exposed and unexposed groups if applicable.	See note 1
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10	Main results	#16a	Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	11; 22- 24
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17		#16b	Report category boundaries when continuous variables were categorized	11
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21		#16c	If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	N/A
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24	Other analyses	#17	Report other analyses done—e.g., analyses of subgroups and interactions, and sensitivity analyses	11-12
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28	Key results	#18	Summarise key results with reference to study objectives	12-13
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31	Limitations	#19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias.	14
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36	Interpretation	#20	Give a cautious overall interpretation considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence.	13-15
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41	Generalisability	#21	Discuss the generalisability (external validity) of the study results	15
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45	Funding	#22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	21
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Author notes

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