EPILEPSY AND NEUROCYSTICERCOSIS IN NORTHWEST CAMEROON: A SEROLOGICAL STUDY

Summary

Rationale: The prevalence of epilepsy in Cameroon is higher than that of the industrialized world and other developing countries. Neurocysticercosis that arises from Taenia solium infestation appears to be an important cause of epilepsy in some parts of Cameroon although there are some conflicting data in this regard. The prevalence of epilepsy appears to be especially high in the Momo Division of the Northwest Province of Cameroon. We hypothesized that those patients in Momo who have epilepsy have a higher percentage of seropositivity to Taenia solium (*T. solium*) than a matched control population.

Methods: A case control study was conducted in the Momo sub-division of Ngie which has nineteen villages. Those with epilepsy were recruited from the health clinics in Ngie and control subjects were randomly selected from 19 Ngie villages. A screening questionnaire was applied by trained field workers to identify potential cases of epileptic seizures to be included in the study. Blood samples were taken from all consenting individuals by finger prick, stored in StabilZyme Select, and assayed for antibodies to *T. solium*.

Key Findings: We accrued 249 patients with epilepsy, of whom 237 met criteria for inclusion, and 245 age-matched controls. There was no significant difference between the control and seizure populations in seropositivity to *T. solium* which was 4.9% in the control group vs. 5% in the epilepsy group.

Conclusion. These data do not support the hypothesis that those individuals in the Ngie sub-division of Momo who have epilepsy have a higher percentage of seropositivity to *T. solium* than

a matched control population. The data make it highly unlikely that neurocysticercosis plays a causative role in the increased prevalence of epilepsy in this region of Cameroon.

Key words: epilepsy, neurocysticercosis, Cameroon

Similar to many countries in Africa, the prevalence of epilepsy in Cameroon is higher than that in industrialized countries and has been reported to vary from 5.5 to 136 per thousand population (Boussinesq et al., 2002, Kamgno et al., 2003; Prischich et al., 2008). Ngie, a subdivision of the District of Momo in Northwest Cameroon, contains 19 villages. Local health care providers have reported a high incidence of epilepsy in the adult population [J. Ambanibe & A. Kourosh, personal communication]. Epilepsy in Ngie appears to be an early childhood onset disorder that lasts well into adulthood, being life-long in most cases. Unconfirmed reports indicate that in most individuals the seizures appear to be primary generalized, but in some cases appear to be focal in onset (e.gs. blinking, staring, visual phenomena, etc.), with evolution to generalized convulsions. There are many anecdotal accounts of severe seizure-related injuries in Ngie including burns and falls, as well as deaths from uncontrolled status epilepticus and drowning.

The cause of the high rate of epilepsy in this region has not been determined. Neurocysticercosis arising from *Taenia solium (T. solium)* infection has been postulated to be the major cause of epilepsy in some parts of Cameroon, as it is in other developing counries (Del Brutto, 2005; Garcia and Del Brutto, 2005; Nicoletti et al., 2005). Zoli et al. (2003), in an uncontrolled study in three rural localities in the West and Northwest provinces of Cameroon, used an enzyme-linked immunosorbent assay for both circulating antigen (Ag-ELISA) and antibody (Ab-ELISA) detection in individuals who had epilepsy. These authors concluded that *T. solium* cysticercosis appeared to be an important cause of epilepsy in Cameroon; however, this

finding has not been confirmed in all studies (Dongmo et al., 2004), suggesting that the etiology of epilepsy may vary by region.

Pigs are prevalent throughout the North west region of Cameroon, including the Momo division, where they are often kept in close proximity to houses. As neurocysticercosis is endemic in less developed countries where pigs are raised as a food source (White, 2000; Garcia et al., 2003), this factor raises the issue of whether neurocysticercsis has a causative role in the epilepsy in the in the District of Momo in Cameroon. The purpose of this study was to test the hypothesis that neurocysticercosis was responsible for the increased incidence of epilepsy. More specifically, the primary objective was to compare the serological status to *T. solium* in individuals with epilepsy with matched, unrelated, case controls in the sub-district of Ngie. A secondary objective was to obtain a demographic profile of those individuals identified with epilepsy in this region.

Methods

Ethics Approvals and Consent:

This study was carried out under the ethical precepts found in the World Medical Association Declaration of Helsinki (http://www.wma.net/e/policy/b3.htm). Ethics approval was obtained from the Research Ethics Board (REB) of the Hospital for Sick Children, Toronto, Canada, the National Ethics Committee, Cameroon, and the local district ethics committee, Alpha Royal Clinic REB. Community consent was first obtained by having medical health volunteers explain the goal of the study to village traditional and administrative and health authorities. Community information sessions were then conducted, the purpose of which was to allow individuals to discuss their questions and concerns. Village coordinators, who helped study

participants to complete the questionnaires, were trained on how to obtain informed consent. In addition, the coordinators determined who could read. Ngie language is not written. Therefore, to make things clear a translated version of the informed consent was made on an audiotape and played where necessary. Following these community meetings, potential subjects and controls were provided further information and if they agreed to participate, consent for the administration of the questionnaire and blood sampling was obtained.

Participants

Individuals with epilepsy were recruited from two health clinics in the subdivision of Ngie, Momo District, Northwest Cameroon. Persons with epilepsy had been attending these health centers for distribution of antiepileptic medication (phenobarbital). Those with epilepsy were defined as having had at least two unexplained unprovoked seizures occurring within a time range of > 24 hours. This was to include all types of partial and/or generalized seizures (Nsengiuumva et al., 2003). In order to eliminate neonatal seizures and febrile seizures, individuals with seizure onset under four years of age were excluded. Similarly, in order to eliminate cerebrovascular disease and dementing illnesses, individuals in whom seizure onset occurred over 60 years of age were also excluded.

Controls with no history of epilepsy were randomly selected from the 19 villages in Ngie sub-division by village coordinators and were matched for age and sex with each subjects in the epilepsy group. Exclusion criteria for controls were a history of epilepsy in the subject or in the family. It should be noted however, that it was difficult to be completely sure whether controls were distantly related to subjects with epilepsy.

Questionnaires

An adapted form of a previously validated epilepsy screening questionnaire (Placencia et al., 1992a) was applied by trained field workers to identify potential cases with epilepsy. This questionnaire was developed for the detection of epileptic seizures in epidemiological studies in developing countries. This questionnaire has been validated on a population of 72,121 people, and has been shown to have a sensitivity of 79.3%, a specificity of 92.9%, a positive predictive value of 18.3% and a negative predictive value of 99.6% (Placencia 1992a,b).

Following completion of this validated epilepsy questionnaire, participants completed a second, more comprehensive, demographic/medical questionnaire that has been developed, validated and used by the Pan African Association of Neurological Sciences in Limoges France, and in many African countries, including Cameroon (Prischich et al., 2008). Except for the demographic and epilepsy-related data, the results from this questionnaire are beyond the scope of the current paper.

Procedure

Individuals in 19 villages were designated as coordinators and trained to administer the screening questionnaire. Training was completed by a local investigator (JA) and a member (IE) of the research team from Canada.

Potential participants from each village in Ngie were screened door to door by the village coordinators to identify individuals who had epilepsy and randomly select controls. Those consenting to participate265 individuals with epilepsy with an equal number of controls were convened to the nearest local Health Clinic, where a local physician (SAA) reviewed each individual's medical history, and further confirmed those with a history of epilepsy and the respective controls. Some individuals who initially presented themselves as controls were

examined and discovered to be persons with epilepsy and were consequently recruited as subjects. Others presented themselves as having epilepsy, but had only experienced either one epileptic seizure or were deemed not to have had epileptic seizures, and were thus excluded from the study. In either case they were referred to the nearest health facility for assessment.

Blood sampling/serology

Supplies for blood samples were brought to Cameroon by a research team member (IE) from Canada. The drawing of blood for serologies, storage of serology samples, and transfer of all serology samples for analysis to the Centers for Disease Control in Atlanta (CDC) was coordinated by the local team of investigators (IT, JA, AN). The method used for collecting measured whole blood with quantitative recovery of antibody to *T. solium* was that described by Handali et al. (2007). Blood samples were obtained by a qualified medical technician under sterile conditions by finger stick, and were collected on filter paper, which was immediately immersed in extraction/preservative (Stabilizyme TM) and frozen. Blood samples from both the epilepsy and control groups were obtained at one of two health clinics. The samples were frozen and stored at the Catholic Medical Health Centre, Mambu Bafut until shipped in cold packs via DHL to the CDC, where the EITB assay for diagnosis of human neurocysticercosis using lentil lectin affinity-purified *T. solium* metacestode glycoprotein antigens (Montano et al., 2005; Tsang et al., 1989; Tsang & Wilson, 1995) was carried out. The sensitivity of this assay is 98% and its specificity reaches 100% in patients with two or more viable brain lesions as demonstrated by neuroimaging (Tsang et al., 1989; Tsang and Wilson, 1995). Newly developed quantitative ELISA employing synthetic diagnostic antigens was also employed (Greene et al 2000, Hancock et al 2003, 2004, 2006).

Results

Demographics.

We accrued 249 individuals with epilepsy and 245 age-matched controls. Of those with epilepsy, 12 reported an age of onset of 4 years or less and were thus excluded. The mean age of control subjects was 17.5 years and that of the epilepsy group was 18.3 years ($t = 1.56$, $p > 0.1$). There were 53% male and 47% female in the epilepsy group and 57% male and 43% female in the control group ($\chi^2 = 1.25$, $p >. 0.1$). Mean age of seizure onset was at 12.2 years. Sixty-eight percent of the epilepsy group had generalized convulsive seizures with 24% having localization-related epilepsy with secondary generalized seizures. Sixty percent of the individuals with epilepsy reported having relatives with epilepsy. The majority (7.7%) of the individuals with epilepsy began to have seizures at age 12 years or younger; 26.8% had onset in the teen years, 4.1% between the ages of 20 and 29, and 1.4% over the age of 40. Table 1 provides further details of the demographic and seizure-related variables.

Serology. Of the 482 samples tested, only 24 samples were positive on EITB for cysticercosis. There were 7 glycoprotein bands on the LLGP blot strips that indicated a positive sero-sample: gp50, 42, 24, 18, 14, and 13. The appearance of any or a combination of the set bands was considered to be a positive sample. Further, there was no significant difference in sero-positivity to *T. solium* between those with epilepsy (n = 11; 4.4%) and the controls (n = 13; 5.3%).

Discussion

There is an increased prevalence of epilepsy in Ngie, a subdivision of the Momo district in the northwest province of Cameroon, as in other areas of Cameroon (Boussinesq et al., 2002;

Kamgno et al., 2003; Prischich et al., 2008). In addition to the health implications of the epilepsy itself, there are other associated health consequences. In Ngie, as has been reported elsewhere in Cameroon (Kamgno et al., 2003), there are accounts of severe seizure-related injuries (falls, burns, etc.) as well as death from uncontrolled status epilepticus, SUDEP and drowning. The relative risk of dying with epilepsy compared with controls in the central province of Cameroon has been reported to be 6.2 (Kamgno et al., 2003).

Our data do not support the hypothesis that individuals with epilepsy from Ngie have a higher percentage of seropositivity to *T. solium* than a matched control population. These data make it highly unlikely that neurocysticercicosis plays a causative role in the increased prevalence of epilepsy in this district in Cameroon. Interestingly, other recently published data (Nkouawa et al., 2010) indicate that cysticercicosis is very rare in rural areas of southwest Cameroon, agreeing with our finding of a very low incidence of serological positivity to *T. solium* in the area we sampled in the northwest.

Given our negative findings, the cause of the increased rate of epilepsy in Ngie remains unknown. In the sample with epilepsy it was found that the age of seizure onset varied widely, although the majority (almost 70%) had their first seizure before the age of 12 years. Most (68%) also had generalized seizures, and 59% had at least one family memory with epilepsy. These figures raised the possibility of a genetic etiology (Prischich et al., 2008). A second candidate for causation would be other parasitic diseases. One possibility would be Onchocerca volvulus, the causative agent in onchocerciasis (river blindness). A number of studies have documented an association between onchocerciasis and epilepsy in other regions of Cameroon as well as in other countries in west, central and east Africa (Boussinesq et al., 2002; Nkouawa et al., 2010; Pion et al., 2009). Local health workers recently confirmed that onchocerciasis is

common in Ngie [J. Ambanibe, personal communication], and it remains to be determined whether this parasite could account for the high rate of epilepsy.

In this study ,l initiated it, sorted for the principal investigator who recruited other co-investigators and funding .l equally coordinated ,trained local coordinators(paramedical staff) ,contribute material for the building of the protocol ,consent and assent forms, ,tested the questionnaire for twenty patients before commencement of study, got two ethics approvals one from Alpha Royal and the other one from the National Ethics committee, managed the funds for the study and supplies, transported blood sample on a motorcycle for storage in a refrigerator in Bamenda (62km) from study site, shipped blood samples to CDC Atlanta USA and questionnaires to Canada for assay and reviewed paper for publication with editors.

For full article see Seizure ;European journal for Epilepsia Irene et al 2013.

As shown above, nurses can contribute immensely to clinical research and the support that they need range from Education and Training, funding, access to internet, means of movement, and many research studies for them to take part in.

Nurses in Cameroon for example are not given the funds to take part in research and the paradox is that most of them head the health centres at the rural areas where they meet many patients in difficulty where they could easily initiate research projects and carry them on if they were trained.

Interaction with experience nurses abroad to tap experience from them is another factor that needs to be encouraged and our infrastructure in Cameroon needs to be improved upon so that tests and blood samples can easily be assayed.

Price awards ceremonies and small funding needs to be organized so that nurses will be triggered to work harder as they compete for these prizes and to crown it papers/journals that publish the research work of nurses need to be put in place so that they know that they belong and doctors should be made to understand that working as collaborators with nurses in research will yield more fruits.

At moment, lam currently coordinating a clinical trial study (Clinical research nurse) on Darunavir/ritonavir(Antiretroviral drugs) for second line HIV patients at the Yaounde Central Hospital and my functions are summarised here.

1. Promote Good Clinical Practice in the conduct of the Manet Study by adhering to protocol requirements, protecting the rights and welfare of recruited subjects, assuring the integrity of data generated and conducting clinical investigations according to regulations, standard operating procedures and guidance documents
2. Ensure that potentially eligible patients are promptly identified for screening when they attend clinic
3. Ensure that potentially eligible patients are promptly approached for participation in the Study
4. Ensure that patients recruited in the Study do so after giving written informed consent
5. Provide support and information to patients recruited to the Study, following strictly the guidelines and regulations for consent to a clinical trial, and the trial protocol
6. Carry out the clinical procedures required by the Study including (but not limited to) the assessment of patient well-being and adherence, the pill count, the collection of samples for testing, pregnancy test, urinalysis, and arrange appropriate follow-up
7. Liaise closely with Pharmacy concerning dispensation of medication to recruited patients, including antiretroviral drugs and any concomitant medication
8. Where relevant, ensure medication and treatment is administered according to the protocol reporting any clinical outcomes, as appropriate, maintaining the highest standards of nursing care appropriate to the subject's co-existing health needs
9. Collect appropriate samples from patients at the appropriate study time points
10. Contribute to the arrangements required for the collection of intense PK samples
11. Ensure the prompt transport of samples from the clinic to the laboratory
12. Ensure that planned and unplanned activities involving recruited patients are promptly and accurately documented and followed-up
13. Establish and maintain a positive relationship with Study patients.
14. In conclusion,nurses have a pivotal role to play especially in countries like Cameroon where research needs to be developed if they are adequately trained and financed.