Onchocerciasis and Epilepsy: A Matched Case-Control Study in the Central African Republic

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The occurrence of epileptic seizures during onchocercal infestation has been suspected. Epidemiologic studies are necessary to confirm the relation between onchocerciasis and epilepsy. A matched case-control study was conducted in dispensaries of three northwestern towns of the Central African Republic. Each epileptic case was matched against two nonepileptic controls on the six criteria of sex, age (±5 years), residence, treatment with ivermectin, date of last ivermectin dose, and the number of ivermectin doses. Onchocerciasis was defined as at least one microfilaria observed in iliac crest skin snip biopsy. A total of 561 subjects (187 cases and 374 controls) were included in the study. Of the epileptics, 39.6% had onchocerciasis, as did 35.8% of the controls. The mean dermal microfilarial load was 26 microfilariae per mg of skin (standard deviation, 42) in the epileptics and 24 microfilariae per mg of skin (standard deviation, 48) in the controls. This matched case-control study found some relation (odds ratio = 1.21, 95% confidence interval 0.81-1.80), although it was nonstatistically significant.
River). The subjects hailed from 66 villages in the region.

Inclusion

The medical personnel working in these towns had, in the weeks preceding the study, notified the village leaders, the epileptics, and their families about their participation in the study. The study was carried out under the auspices of the Central African Republic’s Ministry of Population and Public Health. Informed verbal consent was obtained from each included subject.

The epileptics were defined as subjects age 15 years and above who had had two or more unexplained seizures (10) (with all types of generalized or partial seizures included). The controls were age 15 years and above and did not have a neurologic illness.

Matching criteria

Two nonepileptic controls were matched with one epileptic for the factors that may affect the dermal microfilarial load (MFL). These included age (±5 years), sex, geographic zone of residence, whether or not they had been treated with ivermectin (i.e., treatment used in the annual mass treatment of onchocerciasis), the number of the doses, and the date of the last dose of ivermectin. After the matching was verified, each triplet, thus formed, was examined by a neurologist who verified the clinical inclusion and exclusion criteria.

Neurologic examination

A neurologist, helped by a local interpreter, confirmed the diagnosis after a thorough history taking (using a questionnaire designed by the Institute of Epidemiology and Tropical Neurology, Limoges, France (11), as recommended by the World Health Organization (12), in collaboration with the Pan African Association of Neurological Sciences) and a clear neurologic clinical examination and categorized the seizures according to the classification defined by the Commission on Classification and Terminology of the International League Against Epilepsy (10). Motor and sensory problems; cerebellar, pyramidal, and extrapyramidal syndromes; as well as all cranial nerves palsies were thoroughly evaluated in both the cases and the controls. The presence of abnormal physical signs led to exclusion of a subject. The neurologists looked for the dates of onset of seizure disorder, the date of the last seizure, whether the epilepsy was active, the seizure frequency before and after ivermectin therapy, the antiepileptic drugs used, the positive family history of epilepsy, and a positive personal history that could explain the occurrence of seizures (e.g., fetal or neonatal trauma, prematurity at birth, cranial trauma, meningitis, etc.).

Ophthalmologic examination

All subjects were examined by an ophthalmologist, who measured their visual acuity (using the Snellen chart test) and intraocular pressure (by Schiotz tonometry). Onchocercal-specific and nonspecific ocular lesions (e.g., conjunctivitis, punctate or sclerosing keratitis, uveitis, chorioretinitis, and optic atrophy) were checked for by using an ophthalmoscope and a slit lamp.

Dermatologic examination

Cutaneous lesions specific to onchocerciasis (e.g., onchocerca dermatitis, cutaneous atrophy, dyspigmentation, and subcutaneous nodules) were thoroughly searched for by inspection and palpation. These examinations were carried out in both the cases and the controls.

Parasitologic examination

Excisional skin biopsies (ESB) were taken from each iliac crest by using a Walzer sclerotomy skin snipper. A sample of 30 ESBs was weighed, and the mean weight was defined as 1 mg per ESB. Each ESB was left to stand in wells of a microplate containing 100 µl of sterile 0.9 percent sodium chloride. After 4 hours of incubation, the parasitologist performed a physical count of the *O. volvulus* microfilariae under light microscopy to determine the mean dermal MFL for each person. A subject was considered to be infested with onchocerciasis if at least one microfilaria was found in either of the two ESBs. For detection of the adult form of the parasite in the subjects who had nodules, eight nodulectomies were performed in the operating theaters at the dispensaries.

Serologic evaluation

A sample of 10 ml of blood as drawn from each subject by using nonanticoagulated Vacutainer tubes (Becton Dickinson, Franklin Lakes, New Jersey). Each sample was then centrifuged, and the sera were transferred into cryofreezing Nunc tubes (Nunc A/S, Roskilde, Denmark) and immediately put into liquid nitrogen containers. Then they were conserved in dry ice in an isothermal container ready to be flown to Limoges, France. Cysticercosis, human immunodeficiency virus (HIV), and toxoplasmosis serologies were
performed on all of the sera. The screening for cysticer-
cosis was done by enzyme-linked immunosorbent assay
method using a crude cysticercal antigen prepared as
described by Guerra et al. (13) and Chamouillet et al.
(14). The screening for HIV was done using enzyme-
linked immunosorbent assay Uniform II EIA Kit
(Organan, Fresnes, France). The confirmation of
seropositivity to the HIV-1 was done by Western blot
(Biotech/Dupont HIV-1 immunoglobulin G Western blot,
Ortho Diagnostic Systems, Roissy, France), and that of
HIV-2 was performed simultaneously in the New Lav-
Blot 2 (Diagnostic Pasteur, Marnes la Coquette,
France). The screening for toxoplasmosis was done
using the enzyme immunoassay method with a final
fluorescent detection (Vidas Toxo Immunoglobulin G,
BioMérieux, Marcy-l’Etoile, France).

Statistical analysis

Data was analyzed using Epi-Info 5.01 b software
(Centers for Disease Control and Prevention, French
version, National School of Public Health, Atlanta,
Georgia, 1992) and Statview 4.5 software (Abacus
Concept, Inc., Berkeley, California). Quantitative vari-
ables were given in the form of mean (standard devia-
tion). The frequency comparisons were made by
Pearson chi-square or Fisher exact tests. The mean
comparisons were performed by using the Student t
test, the Mann-Whitney U test, or the Kruskall-Wallis
test. The correlations were done by correlation coeffi-
cient calculation or by Spearman rank test. Exposure
proportions of the cases and the controls were calcu-
lated, and the matched odds ratios and their confidence
intervals were estimated. Each confidence interval was
estimated to the risk of 5 percent. The minimum num-
ber of subjects necessary for the study (two controls
for each case with an error risk α at 5 percent and a
power of 80 percent, in a region where the prevalence
of onchocerciasis in the controls was estimated at 70
percent) was 176 triplets and, hence, 528 subjects.

RESULTS

Demographic data

A total of 187 triplets were included, representing
187 epilepsy cases and 374 nonepileptic controls, for a
total of 561 subjects. Of these, 225 (40.1 percent) were
females and 336 (59.9 percent) were males. The mean
age was 25.6 years (8.6 standard deviation (SD)).

Onchocerciasis

Among the 561 subjects examined, 208 were posi-
tive for onchocerciasis. This corresponded to 37.1 per-
cent of the study population (38.4 percent were males
and 35.1 percent were females). The mean age was
26.3 (8.7 SD) years in those who had onchocerciasis
and 25.3 (8.5 SD) years in those who did not. The
mean dermal MFL in the subjects was 25 (40 SD)
microfilariae per mg of skin. Females had an MFL of
31 (51 SD) microfilariae per mg of skin, while males
had an MFL of 21 (42 SD) microfilariae per mg of
skin. The ivermectin therapeutic cover was 89.7 per-
cent. In the study population, 503 of 561 subjects had
taken ivermectin at least once and on average were
treated twice. The mean period between the last iver-
mection dose and the date of entry into the study was 5
months. This period varied between 15 days and 34
months. The mean dermal MFL was 26 (46 SD) in
those already treated with ivermectin microfilariae per
mg of skin and 36 (42 SD) microfilariae per mg of skin
in the nontreated cases (p < 0.01).

Cutaneous nodules were found in 87 of 561 exam-
ined subjects, representing 15.5 percent of the study
population. More than 75 percent of the nodules were
located at the level of the iliac crest, and no nodule was
found on the head. Sixty-six percent of the subjects
with nodules had onchocerciasis. The proportion of
odule carriers was higher in those who had onchocer-
ciasis (p < 0.001). Seven of eight nodules biopsied
were onchocercomas. The eighth was a mature lipoma
without microfilaria.

Blindness was found in 1.2 percent of the examined
subjects. Six of the seven blind persons were affected
in both eyes. The causes of blindness were multiple:
two subjects had onchocerciasis, one had glaucoma,
two had a sclerosing keratitis, and two had anterior
uveitis nonspecific for onchocerciasis.

Epilepsy

The mean age of epileptics was 25.0 (7.6 SD) years
(23.6 (6.3 SD) years for females and 26.1 (8.2 SD) years
for males). The distribution of the epileptic seizures was
95.1 percent generalized seizures, 1.2 percent simple
partial seizures, and 3.7 percent complex partial
seizures. In this study, 96.3 percent of the subjects had
active epilepsy. The mean evolution period for the
epilepsy was 9 years (7 SD). The mean period between
the last seizures and the first day of the study was 4
months (10 SD); this varied between 0 and 71 months. A
positive family history of epilepsy was found in 32.6
percent of the epileptic patients, 7.4 percent had suffered
measles with complications, 1.6 percent had suffered
from meningitis or encephalitis, and 3.7 percent had dif-
ficulties at birth; 10.2 percent of the mothers of the
epileptics had some problems during pregnancy.
However, 41 percent of the epileptics had no relevant
personal or family history that could explain their illness.
Relation between onchocerciasis and epilepsy

There was no significant difference between the cases and the controls on the matching criteria of age, sex, residence, treatment with ivermectin, number of ivermectin doses, and date of last ivermectin dose. Sixty-six percent of 187 triplets were perfectly matched for the six criteria, but all were perfectly matched for at least two matching criteria (sex and residence).

The matched odds ratio was 1.21 (95 percent confidence interval 0.81-1.80). Of the epileptics, 39.6 percent had onchocerciasis, as did 35.8 percent of the controls. The mean dermal MFL was 26 (42 SD) microfilariae per mg of skin in the epileptics and 24 (48 SD) microfilariae per mg of skin in the controls. Thus, there was no significant difference between the epileptics and the controls.

The number of matching criteria did not significantly modify the results of this study, since the matched odds ratio varied from 1.10 for the triplets matched for all six criteria to 1.21 for the triplets matched for at least two criteria. Screening for other factors that could explain epileptic seizures, such as HIV infection, cysticercosis, and toxoplasmosis infestation, did not reveal a significant difference between the cases and the controls (3 percent of the epileptics and 7 percent of the controls tested positive for HIV, 3.7 percent of the epileptics and 2.4 percent of the controls were seropositive for cysticercosis, and 15 percent of the epileptics and 18 percent of the controls were seropositive for toxoplasmosis).

DISCUSSION

Few studies on the prevalence of onchocerciasis in the Central African Republic have been published (15, 16). The NPFOB usually estimates this prevalence before the ivermectin mass treatment campaigns. To our knowledge, no study on epilepsy has been carried out in this country. Onchocerciasis and epilepsy represent two major health problems in the Central African Republic, particularly because of their serious medical, social, cultural, and economic implications. The local medical team believed that the prevalence of epilepsy was high. We studied these two diseases to discover if any causal relation exists between them.

The village leaders and families of the epileptics were notified in the weeks preceding this study. This allowed for sufficient inclusion of the cases and the controls to attain the minimal number of subjects. The mean sample age was 25.6 years (8.6 SD), which was comparable with the mean age of the general population (17). The sex ratio was 1.49. This sex disproportion at inclusion is probably related in part to the fact that women of marriage age are wary of confessing their epilepsy (18). The matching of cases and controls on the different factors that could affect the dermal MFL increases the study validity. The matched factors were as follows: age (as MFL increases with age) (16), sex (with MFL being higher in males than in females) (19, 20), residence (because the MFL is clearly correlated with the distance between the river and the homesteads) (20), treatment with ivermectin (21), and the dose and timing of treatment with ivermectin (22).

Onchocerciasis was defined in the cases and controls upon a positive ESB, and the mean dermal MFL was estimated. The latter method was used here for the first time to determine the link between epilepsy and onchocerciasis. The screening of onchocerciasis was performed by two ESBs taken at the two iliac crest (23) sites where the sensitivity of the technique for searching for microfilaria was 95 percent (24).

The prevalence of onchocerciasis of 37.1 percent in the controls was much lower than that found previously by NPFOB. This is probably linked to the excellent therapeutic cover, since 89.7 percent of subjects studied had at least had one cycle of ivermectin treatment with two doses of ivermectin on average. Testa et al. (16) and Diallo et al. (22) had shown during the ivermectin therapeutic trials that the MFL was reduced by more than 80 percent during the days that immediately followed a dose of ivermectin but remained detectable in some ESBs. Ivermectin causes intrauterine degeneration in the adult worms and temporary sequestration of unborn microfilariae (25). In the subsequent months, the adult worms resume microfilarial production, and the MFL increase again. In our study, the mean period of the last ivermectin dose was 5.5 months. One would suppose, therefore, that the majority of the people with treated onchocerciasis were positive during the screening. However, it was possible that some subjects classified as negative for onchocerciasis actually had onchocerciasis. In this case, the probability of finding false negatives should not have been different between the cases and the controls. Only in a single case did the ophthalmologic examination reveal microfilaria, which were few in number, in the anterior chamber of the eye when the iliac crest ESB was negative.

Cysticercosis, HIV, and toxoplasmosis infection were also screened for, but did not seem to explain the epilepsy cases in this study. In the epileptic subjects, the HIV prevalence was 6 percent, which is remarkably high for a rural area. The prevalence of cysticercosis was 2.9 percent, and toxoplasmosis was 17 percent. This results did not differ from the levels previously found in central Africa (26, 27).

This study had the aim of determining whether there is a relation between O. volvulus infestation and
epilepsy. This is the first matched case-control study to be carried out on this theme. Indeed, apart from only one previous case-control study (9), all of the previous studies have been cross-sectional (5-8). This matched case-control study in the Central African Republic did not show any relation between *O. volvulus* infestation and epilepsy. These results are in concordance with those of Kaboré et al. (7), who had examined 1,046 subjects in Burkina Faso who were above age 15. The prevalence of onchocerciasis in this region was 12.9 percent, and that of epilepsy was 1.5 percent; only two epileptics had onchocerciasis. This study, performed in an onchocerciasis hypoendemic area, where aerial fumigation and ivermectin mass treatment have been going on for several years now, had, like the present study, shown absolutely no relation between onchocerciasis and epilepsy.

However, several past studies have favored this relation. One study was done in Kyarusozi (Uganda) (5) in an onchocerciasis hyperendemic zone, where 231 persons were examined. Among the subjects who had onchocerciasis, 61 percent had epilepsy and 70 percent had growth retardation. The level of prevalence of epilepsy was 2.0 percent, but 91 percent of the population was below age 19 years. Another study was carried out in Uganda (6) in which authors compared the prevalence of epilepsy between two villages, one situated in an onchocerciasis hyperendemic zone and the other in an hypoendemic zone. In the first village, 8 percent of the subjects were classified as epileptics, and only 0.2 percent in the second village were. The relative risk for developing epilepsy, adjusted for age, sex, and ethnic background, was 6.5 times higher for the persons with onchocerciasis (95 percent confidence interval 3-15). These results were, however, disputed (28), since there seemed to be a high relation between the village and epilepsy, rather than between onchocerciasis and epilepsy. Other unexplained cofactors, such as isolated familial epilepsy, obstetric problems, nutritional, cerebral infections, or other parasitic infections, could probably be responsible for the epilepsy. Still, in the same country (Uganda), Kaiser et al. (8) carried out a study on 4,743 subjects in the parish of Kabende, district of Kabarole. The prevalence of onchocerciasis was determined in each of the 13 villages concerned (sampling about 30 subjects per village). The prevalence of onchocerciasis varied between 15 and 85 percent, depending upon the village. Sixty-one subjects (1.3 percent) were confirmed epileptics. There was a positive correlation between the prevalence of epilepsy and endemicity of onchocerciasis. Newell et al. (9) had, in a case-control study, examined 110 epileptics patients and 82 controls in two administrative zones with different endemicity for onchocerciasis (mesoendemic and hyperendemic). Epilepsy was more prevalent in the onchocerciasis hyperendemic zone. In the mesoendemic area, the difference in prevalence of onchocerciasis between epileptics and controls was not significant. In this study, there were fewer controls than epileptic cases. Moreover, the controls had received ivermectin more often than had the epileptics. Therefore, some controls could have been classified as negative for onchocerciasis, which could explain the difference between the prevalences of onchocerciasis among patients and controls.

It is difficult to establish a link between epilepsy and onchocerciasis. The methodologies and conclusions of the epidemiologic studies quoted in the literature differ. The etiologies of epilepsies are many and are frequently related to the sequelae of head injuries, neonatal head traumas, and infections. Epilepsy is described as a complication in other lymphatic filariasis, such as *Wuchereria bancrofti* or *Mansonella perstans*. The embryos of these filariae live in their adult stage within the vessels and may migrate anywhere in the body through the blood and thus penetrate the central nervous system (29). The neurologic complications may occur due to wandering of microfilariae in the brain tissue or meningeal spaces or to migration of adult worms in the neuromeningial spaces (30). The discovery of *O. volvulus* microfilariae in the cerebrospinal fluid by Mazzotti (31) was certainly accidental and possibly related to the cutaneous abrasion by the needle during the lumbar puncture, taking with it the microfilariae. In the onchocerciasis, there exists no microfilaraemia. The filariae live in the adult state buried in the dermis, preferably on the bony prominences and the microfilariae migrate subdermally, and thus, only adult filariae may end up in the central nervous system. Indeed, if any cause-effect relation exists between onchocerciasis and epilepsy, it is most certainly not a direct mechanical effect, and other pathophysiologic mechanisms must be explored.

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