Rising trends of neurocysticercosis: A serological report from tertiary-care hospital in South India

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KEY WORDS
Cysticercus cellulosae, enzyme-linked immunoelectro transfer blot, neurocysticercosis, seroprevalence, Taenia solium

ABSTRACT
Introduction: Taenia solium is a common two-host parasitic cestode, residing in both humans (definitive) and pigs (intermediate). Invasion of this parasitic cyst into central nervous system leads to a condition known as neurocysticercosis (NCC). The World Health Organization (WHO) considers NCC as one of the “most neglected” tropical zoonotic diseases. The disease is presented with pleomorphic clinical manifestations, of which epilepsy is the most common. Diagnosis of NCC is carried out by serological tests and imaging methods. Only a few studies from Andhra Pradesh, Kerala, Tamil Nadu, and Pondicherry are available regarding the seropositive levels of NCC in South India. Materials and Methods: A descriptive analysis was carried out on NCC suspected patients attending outpatient or inpatient department of different clinics majorly from neurology, medicine, pediatrics, ophthalmology, and skin at Jawaharlal Institute of Postgraduate Medical Education and Research, Puducherry, a tertiary care hospital in South India. A total of 391 patient samples (either serum or cerebrospinal fluid or urine) for 5 years from January 2011 to December 2015 were taken into the study. Serological investigations such as enzyme-linked imunosorbent assay and enzyme-linked immunoelectro transfer blot were performed for assessing the seropositivity levels of NCC. Results: The overall seropositive cases of NCC in the study population were found to be 32.5% of which positive male cases (59.1%) exceeding females (40.9%). The frequency of adult positive cases (77.2%) was more than that of pediatrics cases (22.8%) with an average of 30.9 years of age. Conclusions: NCC seropositive levels show an increasing trend with the study period. This necessitates a proper attention to the unnoticed spread of the parasitic disease, which affects the quality of life in the community. Quality screening and diagnostic strategy should be implied along with proper awareness for preventive measure practices have to be set up to reduce the impact of morbidity caused by NCC.

INTRODUCTION
Taenia solium the pork tapeworm can cause two forms of disease, namely, taeniasis and cysticercosis in humans. The
former is an asymptomatic to mild intestinal disease caused by the ingestion of improperly cooked pork infected with cysticerci, which develop into adult tapeworms within the intestine. Although teniasis being a mild condition it has more significance as it has crucial roles in transmission of the cysticercosis. Neurocysticercosis (NCC), a severe form of cysticercosis affecting the central nervous system (CNS), is a potentially dangerous systemic infection caused by Cysticercus cellulosae, the larval stage of T. solium.[11] Humans acquire this infection by ingestion of T. solium eggs from fecal contaminated soil, water or improperly cleaned, raw or undercooked vegetables.[12,13] The infective embryos (oncosphere) enter the intestinal mucosa, to reach the peripheral blood stream and after a brief migration, it matures into cysticerci in the sites of localization.[14] CNS is the most predominant site, and other less common regions include subcutaneous tissue, eye, muscles, or liver.[5] NCC presents with a wide range of varying clinical spectrum depending on the location and number of the cystic lesions among the infected individuals. Epilepsy is the most common presentation among the clinical manifestations of NCC. Approximately 29% people with epilepsy are diagnosed with NCC in endemic countries.[6]

Cysticercosis is considered to be transmitted predominantly from humans affected with teniasis who act as carriers. It is an endemic disease with majority of distribution in developing countries such as Latin America, China, Southeast Asia, Indonesia, Haiti, and Sub-Saharan Africa.[17,18] In addition, deaths due to cysticercosis are being reported in nonendemic developed countries like the United States, where immigration and travel from endemic regions are reported as the sole reason.[10,11] The global frequency of NCC is undetermined; a study suggests that 1.7–3.0 million people were estimated to have acquired epilepsy resulting from NCC that contributes to the major morbidity.[12] Extrapolated estimates suggest that around 1 million patients in India presenting with active epilepsy possibly have NCC.[13] NCC is also one of the most commonly occurring parasitic zoonoses in India, which occurs all throughout the country with varying incidence. The major reasons for India being endemic to this disease are, the lack of proper personal hygiene, unclean environmental practices, less accessibility for sanitized defecation, uncontrolled free-roaming scavenging pigs among the residential areas, consumption of undercooked pork meat.[6,14,15]

Only a few reports available on the seropositivity of NCC in southern parts of India; therefore, more awareness of the disease indices among the community is needed.[16] Thus, this study was carried out on clinically suspected NCC patients attending Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER), Puducherry, a tertiary care teaching and research hospital in Southeastern India for 5 years (2011–2015). This study would help to shed light on the unclear magnitude of the neglected tropical parasitic disease, NCC.

MATERIALS AND METHODS

Ethical considerations

Ethical approval for the study was obtained from the Institute Ethical Committee (IEC/SC/2012/4/142). The patient’s identity was kept confidential and all data were used solely for the purpose of study.

Study settings and subjects

The descriptive study was carried out from January 2011 to December 2015 (n = 391 cases) at JIPMER, Puducherry, India. It is a tertiary care hospital located in Southeast India, Puducherry, and delivers a wide range of services to patients from different regions of Tamil Nadu and other parts of the country.

The study population includes the clinically suspected cases of NCC presented with seizures, persistent headaches, focal neurologic signs, aphasia, Todd’s palsy, altered sensorium, cognitive dysfunction, Intracranial Space Occupying Lesions, cerebral granuloma, mobile subcutaneous nodules, retinal edema, and uveitis. Serological screening of the clinically suspected cases was carried out. This study includes all the subjects from different age groups, and both genders were documented during the period analyzed.

Preparation of native Cysticercus antigens

T. solium infected pork meat was obtained from the abattoir and the cysts (C. cellulosae) were dissected out and washed thoroughly to remove the tissue fibers using phosphate-buffered saline (PBS) pH 7.2 containing 1 mM a serine protease inhibitor, phenylmethylsulfonyl fluoride. To prepare the native whole cyst somatic antigen (WCA), the cysts are homogenized using a glass tissue homogenizer followed by sonication (Vibra-Cell, Sonics and Materials Inc., USA) at 4°C. The suspension is sonicated for eight times at 12 kHz with a pulse of 1 min and cooling interval of 30 s per cycle. It is then centrifuged at 18,625 × g for 30 min at 4°C, and the clear supernatant is collected and stored in aliquots at −20°C until further use.

Serological screening

The serological analysis was performed for determination of NCC seropositive from the clinically suspected cases from different clinics. Clinical specimens such as serum or cerebrospinal fluid or urine based on the clinical presentation are collected as directed by the physician.

Enzyme-linked immunosorbent assay

A commercially available enzyme-linked immunosorbent assay (ELISA) kit for T. solium IgG (cysticercosis) (DRG® International Inc., USA) was used for the detection
of anti-\textit{Cysticercus} antibodies in serum samples. This kit was chosen as it makes uses of \textit{T. solium} cyst fluid antigen (CFA) coated in the microtiter wells and detects reactive circulating antibody in patients’ sample. The test was performed as per the manufacturer’s instruction, in brief initially the 1:64 diluted patient’s sera and control sera are incubated with the antigen coated wells followed by the enzyme conjugate and the chromogen tetramethylbenzidine with subsequent washes after each incubation steps and finally read at 450 nm using microplate reader (680 XR, Bio-Rad Laboratories, USA).

**Enzyme-linked immunoelectro transfer blot**

Enzyme-linked immunoelectro transfer blot (EITB), the more specific and sensitive assay recommended by Centres for Disease Control and Prevention (CDC, USA) is carried out. The following protocol was carried out with slight modifications from earlier study.\(^{(10)}\) In brief, a 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis gel is run using mini-PROTEAN (Bio-Rad Laboratories, USA) to resolve 50 µg of the WCA per well. The antigen strips are prepared after it is electroblotted to 0.22 µm nitrocellulose membrane (NCM; Pall Life Sciences, USA) using semi-dry apparatus (Trans-Blot, Bio-Rad Laboratories, USA). The efficiency of protein blotting onto the NCM is verified using a rapid, reversible staining method with Ponceau S (Hi-Media, India). The strips are then blocked using 3% BSA for an hour and followed by incubation with primary antibody 1:100 diluted patient sample and secondary antibody 1:1000 diluted anti-human IgG horseradish peroxidase conjugate (GeNei, India) followed by subsequent washes with PBS (pH 7.2) containing 0.1% Tween 20 (PBS-T), after each incubation. Finally, diaminobenzidine (Sigma-Aldrich Corp., USA) is used as chromogen along with hydrogen peroxide to visualize the reactive protein bands. The major immunoreactive protein bands encountered in our study were 55 kDa, 50 kDa, 39–42 kDa, and 20–25 kDa. The presence of two or more bands is considered to be of positive serology [Figure 1].

**Statistical analysis**

The data were analyzed using Microsoft Excel 2011 and the rate of seropositivity are presented in percentages, and the significance for year-wise calculation was carried by unpaired \(t\)-test processed using Statistical Package for Social Sciences version 19 (IBM Corp., Inc, Armonk, NY, USA) and \(P < 0.05\) was considered to be statistically significant.

**RESULTS**

**Seropositivity of study population**

The study encompassed a total of 391 cases, investigated over a period of 5 years from January 2011 to December 2015 in JIPMER [Table 1]. The clinically suspected cases of NCC were majorly from following clinical departments; Medicine, Pediatrics, Ophthalmology, Neurology (Neuromedicine, Neurosurgery), and followed by other departments as depicted in Figure 2. The seasonal variation of the positive cases with more than 35% of seropositive cases occurred during February, April, May, August, September, November, and December as observed through the study period all round the year.

Among the analyzed samples, 32.5% (127 out of 391) with an average of 25 cases per year were found to be seropositive for NCC by EITB during 5 years, with a varying frequency of seropositivity each year [Figure 3]. Whereas only 19.4% of the cases were seropositive for NCC by ELISA of which 84.2% (64) were positive by both EITB and ELISA, whereas 15.8% (12) showed positive only by ELISA.

In our study, there were a total of 218 male cases, of them 34.4% (75) were found to be seropositive; similarly, in the total of 173 female cases, 30% (52) were seropositive. In addition, concerning age of the subjects, it was found that majority of the seropositive cases, among the total adult cases (284) 34.5% were positive, and from the total pediatrics cases (107) 27.1% were positive. The age distribution of seropositive cases was found higher in the age range of 15–40 years than that of 1–14 years and above 40 years of age [Figure 4]. The overall mean seropositive age group was found to be 30.9 years, and among children, the age group was found to be 9.5 and adult was 36.5 years. Although the pediatric rate of seropositivity was lesser than that of adults, the levels of seropositive are increasing with each year [Figure 5]. Overall impression presented from the study is that there is a statistically significant increase in the seropositive
cases among the adults and gender with each subsequent year, except pediatrics group.

**DISCUSSION**

According to the WHO, being a neglected tropical disease, cysticercosis lacks much attention among the health division. Monitoring the spread of the disease is becoming the major priority as the global impact of the disease remains unclear. The disease not only warrants for causing considerable mortality and life-long morbidity but also it leads to great socioeconomic loss to the country as well. This results in causing a significant impact on disability-adjusted life years (DALYs) of the nations endemic to it. Adding to which a study from Mexico and Cameroon estimates around DALYs of 0.25 and 9.0/1000 persons per year were lost due to clinical manifestations of NCC.

Our study shows an increasing trend line of seropositive cases over the study period considering the parameters of age and gender. We have carried out two different serological assays in our study, which employs two different diagnostic antigens. However, results obtained from EITB are considered as it is more specific even up to 100% and CDC recommends it as well. ELISA was performed as add-on to check for the seroreactivity with a different diagnostic antigen (CFA) of *C. cellulosae*. Moreover, it was found that it was not as equally sensitive as WCA in EITB.

The NCC seropositivity documented in the current study is considerably higher than the few earlier studies carried

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**Table 1: Year-wise prevalence estimate of neurocysticercosis seropositive in the study group**

| Parameters (P) | 2011 | 2012 | 2013 | 2014 | 2015 |
|---------------|------|------|------|------|------|
|               | n (S) | Percentage | n (S) | Percentage | n (S) | Percentage | n (S) | Percentage | n (S) | Percentage |
| Total samples (0.02) | 99 (10) | 10.1 | 68 (19) | 27.9 | 49 (4) | 29.1 | 71 (27) | 39.1 | 104 (57) | 54.8 |
| Male (0.03) | 54 (8) | 14.8 | 40 (11) | 27.5 | 26 (6) | 23.1 | 31 (13) | 41.9 | 67 (37) | 55.2 |
| Female (0.02) | 45 (2) | 4.4 | 28 (8) | 28.6 | 23 (8) | 34.8 | 40 (14) | 35 | 37 (20) | 54.1 |
| Adult (0.007) | 57 (5) | 8.8 | 51 (16) | 31.4 | 38 (11) | 28.9 | 54 (21) | 38.9 | 84 (45) | 53.6 |
| Children (NS) | 42 (5) | 11.9 | 17 (3) | 17.6 | 11 (3) | 27.3 | 17 (6) | 35.3 | 20 (12) | 60 |

Data analyzed using unpaired t-test, *P*<0.05 considered as significant. *n*: Total number of patients, *S*: Seropositivity, NS: Nonsignificant.
out on NCC from our institute. A similar study carried out for 5 years (during November 2005–March 2010) reported NCC serological response to be 16.2% by EITB, which was carried out on epileptic cases.[15] Likewise, other earlier reports on NCC seropositivity were found to be 6.48% and 5% in studies conducted in healthy blood donors and HIV patients, respectively.[17,21] Since the studies have different groups of study population they cannot be generalized yet the number of seropositives has increased with the period. As in our study, we observed a steady increase in the percentage of the seropositives with the consecutive years; the possible reason could be the study population chosen were clinically susceptible for NCC, unlike the other studies. The seasonal changes do not have any role in the incidence of seropositives as we can observe them almost all the months around the year.

Likewise, the study reports from other regions of Tamil Nadu; an epidemiological study carried out on twenty clusters from Kanyambadi block in Vellore district suggests a seropositivity of 19.2%,[22] In a community-based study of the tribal population from Kancheepuram reported 9% of NCC seropositive by antigen ELISA detection.[23] This makes it apparent from the studies that the prevalence of NCC is widespread in the districts of Tamil Nadu. In addition, with respect to other states along India a few to mention, 56% seropositively ELISA among patients presenting with symptoms for NCC was reported from a tertiary care hospital in Chinakakani district, Andhra Pradesh.[16] Another study from Odisha, on the prevalence of NCC among the epileptic cases, showed 28.1%.[24]

The gender-wise seroprevalence from our study showed males (34.4%) with higher rates than that of females (30%), which is in concordance with many other studies carried out on the prevalence of NCC. The study results based on age, we found that the overall pediatric seropositive by was 27.1% during the study period. A hospital-based study from Andhra Pradesh province on pediatric NCC[25] shows a higher prevalence of 37.7%, which is found to be slightly higher than our study. Although the ‘rise in’ pediatrics seropositives seems statistically nonsignificant compared with adults, we have seen a constant increase year-wise along the study period. Hence, it’s alarming that higher incidence of the disease will lead to a life-long morbidity in the upcoming generations of the country.

The study limitations are the lack of the demographic data and also the radiodiagnosis results for all the study population. This study presents only the serologic evaluation of the clinically suspected individuals, and this does not serve solely as a diagnostic criterion for NCC. As it is necessary to determine the patient has definitive NCC according to the Del Brutto’s criteria of classification. A more detailed patient history with a correlation of imaging studies would present much clear picture about the disease scenario. In addition, though there are patients from different regions of the country, our study setting being a single-centered study rather than a multicentric study a larger estimate or a fixed number of populations from different regions need to be analyzed to extrapolate the results into a general consideration.

**CONCLUSIONS**

This study shows a higher increase in seropositives of NCC among the study population, which majorly contributes to the Southeastern population of India. This underlines the unnoticed spread of the disease, which requires greater prioritization. This would create a need to explore the less investigated parasitic disease and also it creates awareness for the role of universal precautions among the general public sector. This warrants the need for support of the health professionals to promote the joint action of the community toward suppression of the spread of disease and its consequences. Moreover, early and effective diagnosis of the disease has to be formulated. The disease incidence can be kept in control as per the DRG6 report stating that WHO has included cysticercosis in Global Plan to combat NTDs in 2008–2009 with newer initiatives.[28] Which suggests administration of a large-scale combined preventive chemotherapy regime for humans as well as pigs where there is none so far for humans, it has to be formulated and provided starting from the rural and peripheral population to urban locations. Also, maintenance of community-wide total sanitation measures and awareness among the general public sector can pave a way for elimination of cysticercosis/teniasis in the endemic communities.

**Financial support and sponsorship**

JIPMER intramural research funds and the Science and Engineering Research Board, Department of Science and Technology (DST - SERB) funded the study.

**Conflicts of interest**

There are no conflicts of interest.

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