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Original Article

Microbiology Section

Seroprevalence of Neurocysticercosis among Epilepsy Patients in Chennai, Southern India- A Cross-sectional Study

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ABSTRACT

Introduction: Neurocysticercosis (NCC) is the leading cause of epilepsy in the developing world and is also the most common parasitic infection of the Central Nervous System (CNS). The disease is one of the "major neglected diseases" as per World Health Organisation (WHO). There is an immense burden of epilepsy in the developing countries like India, but little is known about its cause. Data regarding the prevalence of epilepsy due to NCC is unavailable in many regions in India though in some areas it is reported more frequently.

Aim: To estimate the seroprevalence of NCC among epilepsy patients in Southern India.

Materials and Methods: This prospective cross-sectional study was conducted at Stanley Medical College, Chennai, Tamil Nadu, India, among epilepsy patients attending neurology clinic for a period of one year (August 2011-September 2012). All the participants were evaluated for NCC by Magnetic Resonance Imaging (MRI) and detection of *Taenia solium (T. solium)* anti-cysticercal antibody by Enzyme Immuno Transfer Blot (EITB) and Enzyme Linked Immuno Sorbent Assay (ELISA). The statistical analysis was done by Chi-square test using Statistical Package for the Social Sciences (SPSS) software (version 19.0).

Results: In the present study, of the 100 epilepsy cases studied, about 20% of the participants had MRI findings suggestive of NCC and colloidal vesicular stage was the most common stage (11%). Anti-cysticercal antibodies were detected in 27% by EITB and 16% by ELISA. However, there was no statistical significant association between seropositivity type of epilepsy and MRI findings. In comparison to EITB, the sensitivity and specificity of ELISA were 59.3% and 90.4%, respectively.

Conclusion: The findings of this study suggests that there has been an exposure of NCC in this population and hence this disease should be considered in all epilepsy cases for appropriate treatment.

Keywords: Enzyme immuno transfer blot, Seizures, *Taenia solium*

INTRODUCTION

The NCC is the most common parasitic infection of CNS and is the leading cause of epilepsy in the developing world [1,2]. About 20 million people are infected with cysticerci and nearly 2.5 million people worldwide are tapeworm carriers. Of late Cysticercosis/ Taeniasis is emerging as a serious public health problem in many developing areas of the world especially Latin America, India, Africa and China [3,4]. There has been rising trends of the disease even in the developed countries due to ease of international travel and increasing immigrants from the endemic areas [2,5].

Though NCC is known to present with varied clinical manifestation, epileptic seizure is the most common presentation seen in 70-90% of the cases [6,7]. A recent meta-analysis of the published studies has estimated that about 29.6% of epilepsy cases in the endemic countries are associated with NCC lesions in brain identified by neuroimaging [8,9]. There is an immense burden of epilepsy in the developing countries like India, but little is known about its causes [10,11]. Data regarding the prevalence of epilepsy due to NCC is unavailable in many regions in India though in some it is reported more frequently [12]. Although theoretically easy to control and declared eradicable, cysticercosis remains a neglected disease due to the lack of information and lack of awareness of the burden of the disease in the endemic areas [13]. WHO has now declared cysticercosis as the "major neglected disease" [14].

India being a developing country, with large populations below the poverty line, NCC is highly prevalent here. But there is little epidemiological data on the prevalence of taeniasis, human or porcine cysticercosis [12,15,16]. Hence, in view of the burden of epilepsy patients in our country and the significant contribution of NCC to this, the present study was undertaken to estimate the seroprevalence of NCC among epilepsy patients in Chennai, Southern India.

MATERIALS AND METHODS

This prospective cross-sectional study was conducted from August 2011-September 2012 at Stanley Medical College a tertiary care hospital in Chennai, Tamil Nadu, India among patients attending the neurology clinic with complaints of seizure or epilepsy. The study was approved by the Institution Ethics Committee (IEC). All participants were informed about the study and written consent was obtained.

Inclusion criteria: The study group included adult population aged >12 years, who came with complaints of first episode of seizure (new onset seizure cases) and known epileptic patients who came for follow-up (chronic epilepsy).

Exclusion criteria: Cases of epilepsy secondary to encephalitis or meningitis, non infectious aetiology like metabolic disorder, alcohol withdrawal seizures or post-traumatic epilepsy.

Study Procedure

Each participant was interviewed with a questionnaire to collect data regarding risk factors like toilet facilities and pork eating. Clinical information regarding the type of epilepsy and MRI findings were obtained from the medical records.

Neuroimaging studies: Brain imaging by MRI was performed in all cases for the presence of ring enhancing lesions suggestive of NCC. The NCC lesions were further classified depending on the stage as vesicular, colloidal vesicular and nodular calcified.

Sample collection: Three mL of blood was collected from each patient by venipuncture and was centrifuged. The serum separated was stored at -20°C until serological tests were done.

***T.solium* anti-cysticercal antibodies by ELISA:** A commercial ELISA kit (IVD research, SCIMEDX Corporation, USA) was used for the detection of IgG anti-cysticercal antibodies in the serum of

the patients. The test was performed following the manufacturer's instructions. The sensitivity and the specificity of the test kit was 42.9% and 90.6%, respectively [17].

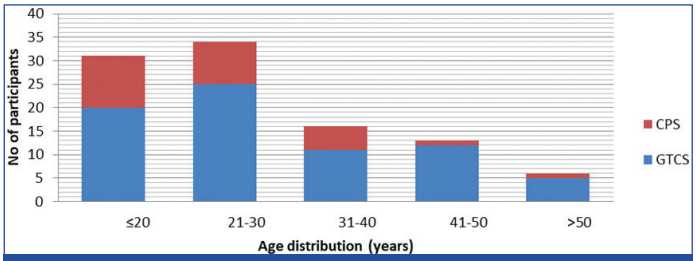
Enzyme Immuno Transfer Blot (EITB): EITB was performed to detect specific antibodies to *T. solium* using *T.solium* metacestode somatic antigens, the methods adopted being as per the previous studies [18]. Briefly, first the somatic antigens were separated based on the molecular weight by Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) followed by EITB. Antigen antibody reactivity at 40 kDa, 24 kda, 32 kDa, 18 kDa were taken to be reactive for anti-cysticercal antibodies.

STATISTICAL ANALYSIS

The results were analysed using Chi-square test in SSPS software (version 19.0). The p<0.05 was considered significant.

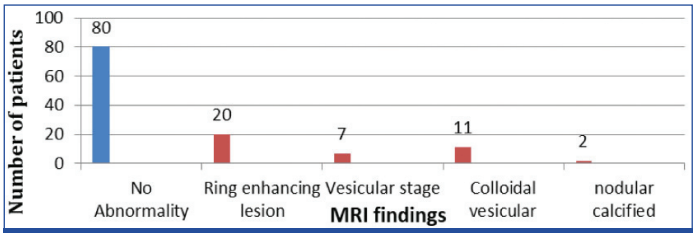
RESULTS

Based on the inclusion criteria about 100 patients presenting with epilepsy were recruited. About 32% of the participants in the study population were 21-30 years [Table/Fig-1]. The mean age of study population was 28.42±12.76 years. The most common type of epilepsy was Generalised Tonic Clonic Seizure (GTCS) (71%).



[Table/Fig-1]: Clinical characteristics of study population.
CPS: Complex partial seizures; GTCS: Generalised tonic clonic seizures

Imaging results of the study population: Based on the MRI findings of the study population, 20% of the cases were diagnosed/suspected to have NCC [Table/Fig-2]. Majority of the epileptic patients with ring enhancing lesion suggestive of NCC were found in the age group <20 and 21-30 years age groups and predominantly in males. About 41% presented with generalised seizure of less than one year duration. Colloidal vesicular stage of NCC was the common finding seen in 11% of the cases. Nodular calcified stage was seen in 2% in the age group of 41-50 years [Table/Fig-3].



[Table/Fig-2]: MRI findings among patients with epilepsy.
*Distribution of the different stages of ring enhancing lesion of parasite in MRI (20=7+11+2)

Age group (years)	No abnormality	MRI findings			
		Ring enhancing lesion	Vesicular stage	Colloidal vesicular	Nodular calcified
≤20	24	6	3	3	0
21-30	31	6	2	4	0
31-40	13	1	0	1	0
41-50	8	4	0	2	2
>50	4	3	2	1	0
Total	80	20	7	11	2
Chi-square	13.4				
df	12				
p-value	0.4				

[Table/Fig-3]: Age wise distribution of MRI findings among patients with epilepsy.

Detection of anti-cysticercal antibodies by EITB: About 100 samples from the study population were tested by EITB for detecting antibodies to cysticercus. Antibodies were detected in 27% of the samples. Maximum seropositivity of 44.4 % (12/27) was detected in the age group of <20-30 years. With respect to the type of seizures among the seropositives, 66.7% (18/27) presented with generalised seizures and 33.3% (9/27) presented with partial seizures. The association of detection of anti-cysticercal antibody to the type of seizure was found to be not statistically significant (p-0.36).

As shown in [Table/Fig-4], among the seropositives, only 8 (29.6%) had Solitary Cystic Granuloma (SCG) suggestive of NCC in MRI. Antibodies were also detected in about 19 (70.3%) of those with no MRI abnormalities. On comparing the seropositivity with the various stages of the lesion in MRI, slightly higher positivity was found in the colloidal stage i.e., 18.5%, but there was no statistical significance.

Type of epilepsy	No. of cases tested	Result of *T. solium* IgG antibody detection By EITB		
		No. (%) of sera tested positive	No. (%) of sera tested negative	p-value
Seropositivity	100	27 (27)	73 (73)	-
Age (years)				0.99
≤20	29	7 (26.0)	22 (30.1)	
21-30	33	12 (44.4)	21 (28.8)	
31-40	18	3 (11.1)	15 (20.6)	
41-50	12	3 (11.1)	9 (12.3)	
>50	8	2 (7.4)	6 (8.2)	
Gender				0.59
Male	64	12 (44.4)	52 (71.2)	
Female	36	15 (55.6)	21 (28.8)	
Risk factors				0.07
Pork consumption	18	13 (72.2)	5 (27.8)	
Lack of personal hygiene and toilet facilities	45	20 (44.4)	25 (55.6)	
Type of epilepsy				Chi-square 0.753 df-4 p-0.386
GTCS	73	18 (66.7)	55 (75.3)	
CPS	27	9 (33.3)	18 (24.7)	
MRI findings				0.143
No lesions	80	19 (70.3)	61 (83.6)	
Solitary cystic granuloma s/o NCC	20	8 (29.6)	12 (16.4)	
-Vesicular	7	3 (11.1)	4 (5.5)	
-Colloidal	11	5 (18.5)	6 (8.2)	
-Nodular calcified	2	0	2 (2.7)	

[Table/Fig-4]: Results of *T.solium* IgG antibody detection by EITB among the study population and its correlation with clinical findings.
Chi-square test

Comparison of Anti-cysticercal Antibodies Detection by ELISA and EITB

Though EITB is considered the standard diagnostic test for NCC, it is not widely available. Hence, serum samples were tested by widely available ELISA for detection of anti-cysticercal antibodies to *Taenia solium*. As can be seen from [Table/Fig-5], of the 27 which were

Result of EITB for detection of IgG antibodies	No. of cases	ELISA	
		Antibodies detected by ELISA	Antibodies not detected by ELISA
Reactive	27	16	11
Non reactive	73	7	66
Total	100	23	77

[Table/Fig-5]: Comparison of the results of EITB and ELISA for detection of *T.solium* Ig G antibodies.

confirmed by EITB, ELISA detected antibodies in only 16, giving the test a sensitivity of 59.3% and specificity of 90.4%. This translates into a PPV of 69.7%, NPV of 85.7% [Table/Fig-6].

Sensitivity	59.3%
Specificity	90.4%
Positive predictive value	69.7%
Negative predictive value	85.7%

[Table/Fig-6]: Sensitivity and specificity of ELISA.

DISCUSSION

Epilepsy due to NCC is a major public health problem especially in developing countries, where nearly one third of the active epilepsy is attributed to it [19,20]. Knowledge of the prevalence of the disease in that region and a high index of suspicion are essential for the diagnosis and treatment of NCC. Though similar studies done in other regions have shown the occurrence of seizures and cysticercosis, there has been no data from this region [16,21,22].

Seropositivity among the study population: The significant finding in the current study was the detection of anti-cysticercal antibodies to *T.solium* in 27% of the study population presenting with seizures by immunoblot. In a community-wide screening survey from rural Peru, the seroprevalence was found to be 24% and was associated with seizures with an odd's ratio of 2.1 [23]. This finding was also consistent with other similar studies done across various geographical locations in India and seropositivity ranged from 26-32.5% [Table/Fig-7] [15,16,24-27]. As all these studies lack uniformity in the method of detection, the trend in the seropositivity over the years cannot be commented. However a rising trend has been seen in consecutive years in a study done in a tertiary care centre in Puducherry from 2011-2015 and a higher seropositivity of 32.5% was seen [28]. The varied prevalence rates across different geographical areas in India is due to factors like literacy rate, religious reasons, pig rearing community as stated in a review [29].

Authors	Year of publication	Place of study	Methodology	Seropositivity detected
Rajshekhar V et al., [16]	2006	Vellore	EITB	13%
Devi KR et al., [15]	2021	Assam	ELISA	27.7%
Sahu PS et al., [24]	2015	Odisha	ELISA	28.12%
Thamilselvan P et al., [25]	2017	Puducherry	EITB	32.5%
Pappala BC et al., [26]	2016	Visakhapatnam	ELISA	27.5%
Anadure RK et al., [27]	2020	Mumbai and Bangalore	ELISA	26%
Present study	2021	Chennai	EITB	27%

[Table/Fig-7]: Prior studies on the prevalence of Neurocysticercosis (NCC) in epilepsy patients [15,16,24-27].

The present study showed predominance of seropositivity among males than females and higher in the age group of 21-30 years. Though other Indian studies have also showed similar male predominance, no significance can be derived from the gender distribution about its causal role in NCC [18,25]. The maximum incidence of neurocystcercosis in the age group of 20-30 years has been demonstrated in other studies and have shown solitary cysts to be usually seen in adults (majority under 20 years of age) presenting with newly developed seizures [24,25,30,31]. This was observed in present study and found vesicular stage was predominantly seen in MRI in less the age group of 21-30 years.

The most common type of epilepsy seen in present study among seropositive patients was generalised seizures. Though most of the earlier studies have demonstrated this, a similar study in Odisha has found partial seizures to be common [24]. The differences in the type of epilepsy in these patients seen across various geographical locations remain enigmatous and warrant further studies. The generation of epilepsy in NCC is due to the compression of the surrounding brain parenchyma and inflammatory reactions [32]. The type of epilepsy depends on the location of the cyst within the brain parenchyma, with frontal lobe cysts manifesting as generalised seizures and extra parenchymal and parietal cyst manifesting as partial seizures.

The SCG was detected in 20% of the study population by imaging. In terms of the type of parenchymal lesions, colloidal was the most common stage seen in 11%, vesicular in 7% and calcified in 2%. In contrast, Anadure RK et al., had found vesicular forms to be the most common seen in 30%, calcified in 28% and colloidal forms in 17.5% among NCC cases [27]. Anti-cysticercal antibodies were detected in only 40% (8/20) by EITB among the SCG cases. The low sensitivity of antibody detection assays among SCG was due the low cyst numbers resulting in poor immunological response [33]. But antibodies were detected in about 23.8% (19/80) of cases with no imaging findings. This could probably indicate the exposure to the antigen rather than active infection.

In the current study, the sensitivity of ELISA in comparison to EITB was found to be low i.e., 59.3%. Previous studies have shown varied sensitivity of ELISA depending on the antigen used for detecting antibody. But overall the sensitivity of ELISA in comparison to immunoblot was found to be low i.e., 19.2% and frequent cross reactions were also noted [28,34]. Though the presently employed commercial ELISA kit detects antibody against the parasite undefined antigen, usage of specific cysticercal antigens have been shown to enhance the sensitivity and specificity of the test for diagnostic purpose [35]. Sensitivity is also lowered in cases with solitary and calcified cyst which could be attributed to the low antibody titers seen in these cases [36]. Hence, EITB is recommended in cases with suspected NCC however the results should not be interpreted in isolation as all antibody-detecting techniques do not have the capacity to distinguish between exposure, inactive and active infection [37]. This study was the first study to our knowledge, done in this unexplored region in Tamil Nadu to assess the seroprevalence to *T.solium* in epileptic patients.

Limitation(s)

The limitations of this study were small sample size and the use of antibodies for detecting NCC. Though the results of this study cannot be extrapolated to the general population, this study could form the basis for future studies with larger study population. As antibody detection doesn't correlate with disease but rather indicates the exposure, antigen based assays are recommended for definitive diagnosis of NCC.

CONCLUSION(S)

The present study shows that there is evidence of NCC in this unexplored region. Hence, the potentiality of possible NCC should be considered in patients presenting with epilepsy. The study also shows that serological tests like ELISA and EITB aid the better identification of clinically and radiologically suspected active NCC cases. ELISA has low sensitivity and high specificity than EITB in detecting the antibodies. Based on the findings from this study it is evident that NCC may be a growing public ill health in our country and there is a need to take public health measures like by proper sanitation and hygiene to control this disease.

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