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PREVALENCE OF NEUROCYSTICERCOSIS IN AND AROUND OF RURAL KANCHIPURAM.

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ABSTRACT Cysticercosis has been reported as the most common illness affecting the central nervous system by cystic stage of Taenia. solium pork tapeworm. It is also referred as food borne zoonotic infection with T.solium larva or cysticercus stage in humans. Cysticercosis which affects central nervous system is called neurocysticercosis (NCC). The clinical manifestation of NCC largely depends on the number, type, size of the cysts, stage of development of cyst and degree of host immune response against the parasite. **Objectives:** The present study aimed to assess the burden of neurocysticercosis infection by CT imaging and antigen detection by ELISA in the community as well as hospital based patients. **Methods:** Blood samples were collected and antigen detection of cysticercosis by ELISA were done and CT Brain was done to confirm the diagnosis of neurocysticercosis among the participants. **Results:** The overall clinical presentation among 640 subjects showed seizure as the major presentation 446 (69.69%). The overall results of the commercial-Ag-ELISA revealed 61 of 446 sera to be positive for Cysticercus antigen, and thus confirming 13.68% total prevalence of neurocysticercosis in the study region. **Conclusion:** High index of exposure to T. solium cysticercosis in the study region of south Indian. An early diagnosis and appropriate treatment management can improve the quality of life of a patient.

KEYWORDS : *Cysticercosis, Taenia solium, neurocysticercosis,* ELISA, CT image and cyst.

INTRODUCTION:

Taenia solium cysticercosis has been reported as most common illness affecting the central nervous system in the World by cystic stage of *T.solium* pork tapeworm; these referred as food borne zoonotic infection with *T.solium* larva or cysticercus stage infect humans[1&2].

Humans acquire infection of cysticercosis by ingestion of infected under cooked pork, vegetables, contaminated food and water[3]. The larval stage of *T.solium* tapeworm is a major public health problem in endemic regions of the world, common cause was seizure disorder[4&5].

The infections of cysticercosis is found worldwide and most commonly seen in developing countries this may be due to poor hygiene habits, lack of routine meat inspection, poor pig husbandry and sanitary control. In add-on these infections are reemerging as significantly public health problem commonly seen in rural but also seen in urban areas where many infected pigs roam, transported and consumed.

Clinical manifestation will differ based on the site of cyst located like brain, spinal cord, muscles and eye and also depends on the number, size, location and stage of cyst, as well on the host immune response to the parasite[6&7], common symptoms like seizures, headache, neurological deficits, hydrocephalus[8]. The infection with cysticercus cellulosae larva in central nervous system with symptoms is noted as neurocysticercosis. In India Cysticercosis is prevalent in virtually in all the states. It is normally assume that the disease is more commonly seen in north than south India. Unfortunately, population-based epidemiological data on Cysticercosis are lacking in south India. Worldwide *T.solium* infected people noted as 50 million approximately and deaths per day of 50,000 were estimated [11].In 30 to 50% cases of neurocysticercosis associated with seizures to be common in the regions where *T.solium* cysticeri is endemic[9&10].

In India, the estimation of cysticercosis and neurocysticercosis was high this may be due to precipitation of unhygienic habits, contamination of food and water, poor sanitation and poor pig husbandry [11]. Diagnosis of NCC is made by imaging techniques like CT, MRI and less supplemented by serological investigations like antibody detection by serum [12]. A study from Vellore, south part of India the prevalence of NCC associated with 50% cases with seizures.[13]

Hence the present study aimed to assess burden of neurocysticercosis infection by CT imaging and antigen detection ELISA in the community as well as hospital based patients attending with seizures and to know the usefulness of serological Antigen ELISA with brain imaging for diagnosis of neurocysticercosis in rural Kanchipuram of Tamilnadu.

METHODS:

Study design, duration, and selection of study subjects:

The present cross-sectional study was conducted over a period of 3 years at Shri Sathya Sai Medical College and Research Institute (SSSMCRI). In the community screening for detection of prevalence of neurocysticercosis, a total of 322

subjects from Kanchipuram district Thirukazukundrem and Chengalpet Taluk (Nellikuppam, Ammapettai and kottamedu villages) were included following informed written consent to participate in the study by providing information and specimens required for laboratory investigations.

A detailed physical examination and preliminary clinical assessments of the subjects were made with the help of clinicians under supervision of a neurologist. Of 322 total subjects 128 had symptoms of seizures.

In the hospital based screening for estimating the prevalence of neurocysticercosis among patients with seizures, a total of 318 cases attending hospital of Kanchipuram District were included following informed written consent obtained in the study by providing information and specimens required for laboratory investigations. Routine physical examination and preliminary clinical classification of cases were made under supervision of a neurologist.

Both the community as well as hospital based screening studies were done upon the Institutional Ethical Committee (IEC) approval of the protocol.

Study subject inclusion in community screening, and patient selection was made as per the following inclusion and exclusion criteria. A detailed history of exposure, presenting signs and symptoms related to neurological health were recorded.

Inclusion criteria:
Subjects in the communities as well as patients within age range 6 – 65 years with seizures, chronic headache, and history of possible exposure irrespective of detection of *T.solium* taeniasis in stool were included.

Exclusion criteria:
Patients < 6 years and > 65 years age, history of accidents or trauma in brain, individuals with diagnosis of other chronic infections, neurodegenerative disorders, pregnancy and patients not willing to participate were excluded.

Brain Imaging:
A total of 446 subjects (128 form communities and 318 cases from hospital) were subjected to brain imaging by computed tomography (CT) in order to detect presence of lesions if any.

Specimen:
Up to 3 ml of venous whole blood sample was collected under aseptic precautions from each of the study subjects. Serum was separated from each sample and stored in aliquots using cryoprotective screw capped vials labeled with appropriate information for identification, and then stored at -80° C.

Cysticercus antigen detection in Serum by ELISA
ELISA for Cysticercus antigen detection was performed using a commercially available Cysticercosis Antigen (Ag) ELISA kit (REF 650501; (*apDia, Belgium*) [14].

Statistical analysis used: SPSS 22 statistical software and windows Excel-2007 and analyzed by using chi-square test.

RESULTS:
The overall clinical presentation among 640 subjects (recruited from communities and the hospital) showed seizure as the major presentation (69.69%). In the hospital based recruitment of cases, all patients had presentation with seizures (n=318) and thus considered for inclusion for further investigations. The seizure occurrence was only in 128 of 322 (39.57%) subjects form communities **(Table-1)**.

Table-1: Occurrences of seizures among the study subjects

Study subjects	No. (%) subjects presenting with seizures		χ2(p-value)
	Present	Absent	
Community (n=322)	128 (39.75)	194 (60.24)	274.93(0.0000)
Hospital (n=318)*	318 (100)	0 (0)	
Total (n=640)	446 (69.69)	194 (30.31)	

* In the hospital based recruitment of cases, all patients had presentation with seizures (n=318).

Seizures was higher in Hospital (100%) than Community (39.75%) and there was a statistical difference in the presence of seizures between Hospital and Community 2=274.93, p=0.0000.

Therefore those from communities which did not have any symptoms of seizures were excluded from the study for further investigations and analysis.

Besides seizures, other major neurological symptoms recorded in the finally recruited population (n=446) were seizures, neurological deficit, chronic headache, dementia, fever, neck pain, and vomiting.

A total of 446 subjects were subjected to imaging of brain by CT scanning, and their sera were tested for presence of Cysticercus antigen by ELISA. The overall results of the commercial-Ag-ELISA revealed 61 of 446 sera to be positive for Cysticercus antigen, and thus confirming 13.68% total prevalence of neurocysticercosis in the study region. The prevalence was 15.09% form hospital screening, whereas the same was 10.15% in case of study subjects from the community. The overall prevalence results of detection of Cysticercus antigens in serum are shown in **Table-2**.

Table-2: Overall prevalence of NCC in community and hospital screening by Ag-ELISA

Origin of subjects presenting with seizures	Commercial Ag-ELISA in serum for NCC showing no. (%) subjects		χ2(p-value)
	Positive	Negative	
Community (n=128)	13 (10.15)	115 (89.84)	1.885(0.1698)
Hospital (n=318)	48 (15.09)	270 (84.90)	
Total (n=446)	61 (13.68)	385 (86.32)	

The cut-off OD and antigen index were calculated according to the protocol as provided in the commercial kit (*apDia, Belgium*) used in the present study. A total 61 positive for Cysticercus antigen, 41 (9.19%) were male and 20 (4.48%) were female.

In the present study, the overall data on brain imaging findings by CT scanning revealed 64 of 446 (14.34%) subjects to have at least one lesion in brain having appearance in accordance with features as in NCC [17]. The prevalence was 16.03% from the hospital and 10.15% form community screening **(Table-3)**.

Table-3: Detection of lesions on brain by imaging (CT) in the study subjects

Origin of subjects presenting with seizures	CT imaging showing no. (%) subjects		χ2(p-value)
	Positive	Negative	
Community (n=128)	13 (10.15)	115 (89.84)	2.569(0.1090)
Hospital (n=318)	51 (16.03)	267 (83.96)	
Total (n=446)	64 (14.35)	382 (85.65)	

Among the 64 positive subjects with at least one lesion in brain, the major neurological symptoms were seizures (generalized and focal), other symptoms (*viz.*, neurological deficit, chronic headache, dementia, fever, neck pain, and vomiting).

Brain CT findings from 64 subjects with a remarkable finding reveled presence of suspected parasitic lesion(s) predominantly in only parietal region (46.47%) followed by other brain parts such as only frontal lobe (15.49%), fronto-parietal (14.08%), parieto-occipital (11.26%), only temporal lobe (8.45%), and only occipital lobe (4.22%) **(Figure-1).**

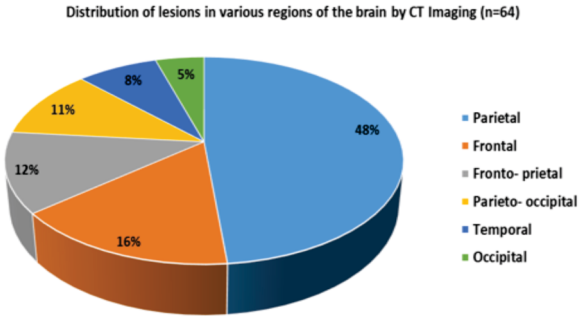

Figure-1-: Distribution of lesions in various regions of the brain by CT Imaging

Gender wise distribution of subjects with a remarkable finding on CT, of 64 subjects with lesion(s) in brain, there were 43 (9.64%) males and 21 (4.7%) females**.** Of 43 male subjects with a positive CT finding, 11 were from community and 32 from hospital. Similarly, of 21 female subjects 5 were from community and 16 from hospital.

Age group wise distribution of subjects with findings on CT, of 64 subjects with lesion(s) in brain, there were 24 (5.38%) children and 40 (8.96%) adults**.** Of 24 children with a positive CT finding, 4 children were from community and 20 from hospital. Similarly, of 40 adults 13 were from community and 27 from hospital. Of 64 subjects with a remarkable finding on CT, 45 had single lesion and 19 had multiple lesions. The commercial-Ag-ELISA results showed only marginally differences when detection rates compared between presences of single lesion vs multiple lesions in brain among the study subjects. Of 45 subjects having a single lesion in brain, 42 were ELISA positive. Similarly, of 19 subjects having multiple lesions, 17 were positive for ELISA. Surprisingly, two subjects having no lesions in brain also were observed to be ELISA positive **(Table-4).**

Table-4: Results of the Commercial-Ag-ELISA in serum with respect to numbers and types of lesions in brain on CT scanning

Number of lesions on CT	Lesion types	No of subjects	No of subjects positive for Ag-ELISA
Single lesion	Vesicular	26	26
	Granular-nodular	11	10
	Calcified cyst	8	6
	Sub-total (A)	45	42
Multiple lesions	Granular-nodular	3	3
	Calcified	16	14
	Sub-total (B)	19	17
	No lesion (C)	0	2
Total (A+B+C)		64	61

Representative Images of CT scanning of brain among study subjects are depicted in **(Figure-2).**

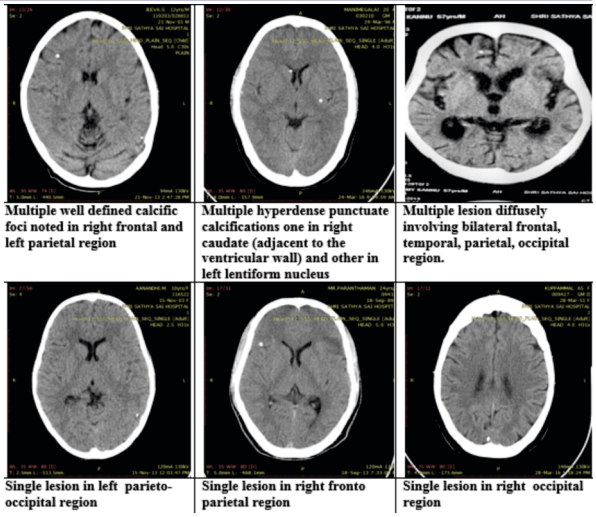

Figure-2: CT-Brain plain images (Multiple & Single lesions at various regions)

In this study, a confirmatory diagnosis of NCC was given if one subject if found to be either positive for serum Ag-ELISA or presence of imaging features of brain lesion(s) consistent with NCC. Therefore, a total 64 of 446 (14.34%) subjects with symptoms of seizures were confirmed to be due to NCC (**Table-5**)

Table-5: Comparison of results of the Commercial-Ag-ELISA with positive CT findings

Commercial Ag-ELISA test result	No (%) of subjects with at least one lesion	No (%) of subjects with no lesion	Total (%)	$\chi 2$(p-value)
Positive	59 (13.22)	2 (0.44)	61 (13.68)	390.108(0.0000)
Negative	5 (1.12)	380 (85.20)	385 (86.32)	
Total	64 (14.34)	382 (85.65)	446 (100)	

DISCUSSION:

Our study is the first to report the prevalence of NCC in Kanchipuram district of South India in both community and hospital patients by CT imaging and antigen ELISA.

In the present study, CT imaging confirmed NCC were 14.34%, on serology antigen ELISA were 13.68%. Globally NCC is endemic in most of the countries and is considered to be a major health problem in developing countries [15]. Good diagnostic test accessibility is necessary not only for diagnosis and treatment of NCC but also for epidemiological studies. Diagnosis of NCC by CT and MRI are costly, which may not accessible in many endemic areas[16]. Therefore there is urgent need to the serological test for diagnosis in poor resource settings.

There are difficulties in estimating the correct prevalence of NCC due to the miscellany of clinical manifestations. The symptoms attribute mainly on the host immune response, stage of cyst, location, size and there number[17]. In the present study NCC associated with seizures as a major presentation of 69.69% besides seizures; other major neurological symptoms were neurological deficit, chronic headache, dementia, fever, neck pain and vomiting[18].

In relation to the gender distribution of the patients, the higher prevalence of NCC by Commercial ELISA was (9.19%) in males and (4.48%) in females. Prevalence of NCC by CT was 9.64% in males and in females 4.7% there was no statistical difference seen. Similar studies obtained in different parts of world Buitrago et al., 1995 who found that 60% of the infected cases were man and also by Li et al., 2006 reported that the prevalence of cysticercosis was higher in men (6.6%) than

women (1.8%). Fleury et al. reported the infection of cysticercosis gender wise there was no significance[19].

The influence of age in prevalence of NCC by CT findings among children below 6-15 years the positivity of NCC were (5.38%) and in adults 15-65 years (8.96%). There were predominantly adult cases than children NCC and there was a statistical difference in the presence of lesions on brain imaging between adult and children in our study. In other Indian study also Maximum incidence of NCC was in adults[20]. Similar studies were obtained by Sarti et al., who found that the positivity increase with age and reach the peak at 46-55 years, our study supports the same results [21]. Some other studies mentioned that the frequency of infection significantly increases with age and the highest age group was above 46 years. Furthermore, Lkejima et al., 2005 found that the peak of the infection with cysticercosis was in the adulthood. On the other hand Adjide et al., found no statistical significance according to the age of the patients[22]. The positive influence of the patient's age on the prevalence of cysticercosis may be due to daily activities and contacts to the eggs of *T. solium*. The young adults, who roam from place to place, which is required for their job, may be exposed to the food that is available there with or without any hygiene precautions; they can contact the infection from infected food handlers with *T.solium*.

The results obtained by this study, the prevalence of NCC in males were quite high when compared with other studies, at the same time the infection seen in adults was high when compared with children.

Since 1989, the diagnosis of cysticercosis by antigen detection ELISA (monoclonal) has been reported [23]. Brandt et al. thrive ELISA (monoclonal) for diagnosis of NCC against glycoprotein and excretory secretory of *T. saginata* showed specificity 97% and sensitivity of 85% with one or more viable lesions in brain[24].

The present study showed the seroprevalence of cysticercosis (NCC) was high 13.68%, when compared with some studies like Assiut and Sohage Governorates, the seroprevalence of *T.solium* / cysticercosis in humans was 6.5% by using ELISA test [25]. Similar studies recorded by Parija and Priyadarshi sahu 2003 (6.1%), DeGiorgio et al., 2005 [26] and Rodriguez-Hidalgo et al., 2006 (2.25%) [27]. On the other hand our finding was closer to that are obtained by Oliverira et al., 2006 (11.3%), other study by Carrique-Mas et al., (22%) high [28]. Sarah Gabriel et al. evolve ELISA (monoclonal) showed 100%sensitivity and specificity of 84 % [29]. In present chapter the diagnosis of NCC by Antigen detection ELISA (monoclonal) by serum found to be 13.68%, which was high when compared with other studies [32, 25-27]. These results adjoin the worth and importance of antigen ELISA for diagnosis of NCC significant in limited equipped laboratories with inadequate or absence of neuroimaging techniques.

Out of CT 64 positive subjects, 45 showed single lesions and 19 showed multiple lesions which was also reported in another studies of NCC, small single CT calcified lesion and single CT enhancing lesion and together accounted for 40% of etiological factors of seizure (Murthy JM et al., 1998). The present study showed there was a significant association was observed between lesion in brain and the ELISA-positivity as proven by our study.

Prevalence of NCC in rural Kanchipuram district without seizures, was found to be high (4.63%) by CT and ELISA. In our study, most of the patients were from rural community, the living habits of this community were highly clustering cases, pork eaters, low socioeconomic habits and immigrant people from different parts of India where cysticercosis can be endemic. They may not be visiting the referral hospitals for the diagnosis of NCC, they move closer to the hospital for diagnosis. Therefore the number of case and prevalence of NCC was high in our study when compared with other studies.

In the present study, seizures (69.68%) followed by chronic headache (57.96%) as a most common clinical manifestation presented by NCC subjects in both community and Hospital based studies. This conclusion supports the other available studies. For example, a study on current review found that seizures of symptomatic patients or headache were the commonest clinical manifestation of NCC (70-90%) [30&31].

Limitations of the present study were detection of antigens in CSF could not be performed due to limitations of the commercial-Ag-ELISA kit which was recommended to be used for serum specimen from human and porcine sample.

CONCLUSION:

Overall >14% prevalence of neurocysticercosis among subjects with symptoms of seizures is of concern. Therefore a high index of exposure to *T. solium* cysticercosis in the study region of south India calls for an urgent attention for an improved animal husbandry, sanitation and meat inspection, health education, and awareness campaigns in order to prevent further transmission of this neglected parasitic disease.

It is recommended that in endemic regions, physicians should pay attention on the possibility of infection with both adult and larval stage of the parasite in respective of presence or absence of corresponding symptoms of the other if either one infection is detected.

An early diagnosis and appropriate treatment management can improve the quality of life of a patient. The currently used commercial-Ag-ELISA meant for diagnosis of early stage infection was found to be useful in the diagnosis of NCC. It could be used as an adjunct to neuroimaging where the combination of both diagnostic modalities could probably provide a confirmatory diagnosis; however there is need of developing *in-house* detection materials in endemic developing regions. Indigenous diagnostic material development and validation might also be more appropriate to be used for screening in large scale studies for a better economic reason.

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