

Original Research Article

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Seroprevalence of Human Cysticercosis in a Tertiary Care Center in South India

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ABSTRACT

Cysticercosis is a major public health problem in the tropical and subtropical areas. In this, neurocysticercosis remains the most common cause of epilepsy in these regions with considerable morbidity and mortality. Wide variations were seen in its prevalence due to various factors which influence it. This study was carried out in a tertiary care center located in South India to ascertain the seroprevalence of human cysticercosis for a period of 2 years. All patients with clinically suspected cases of cysticercosis were enrolled for the study. Serum samples were tested by enzyme-linked immunoelectro transfer blot or Enzyme-linked immunosorbent assay to detect IgG antibodies against *Taenia solium* larval stage. Of the total 92 patients enrolled and their serum tested, fifty six (58.33%) samples were tested positive; 46 of 77 by enzyme-linked immunoelectro transfer blot and 10 of 19 by ELISA. Maximum cases were in the age range of 10-19 years (26.98%) followed by 0-9 years. Male preponderance was seen over the female patients. It was also seen that asymptomatic patients were also seropositive. Also, high number of suspected cases was found to be seronegative. Being a hospital based one, the study observed a high seroprevalence of human cysticercosis compared to other regions. Still, the study reflects higher rate in the patients of south India and its prevalence which can be supplemented with community based studies and its risk factors.

Keywords

Cysticercosis;
Enzyme-linked
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electrotransfer blot,
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Introduction

Cysticercosis in man is caused by *Cysticercus cellulose*, the larval stage of *Taenia solium* and is labeled as a major neglected disease by World Health Organisation (1,2). It is a major

public health burden due to its high prevalence rate, especially in developing countries of Asia, Africa and Latin America. Neurocysticercosis (NCC) remains the most common cause of epilepsy in these regions with considerable morbidity and mortality (2).

Cysticercosis is acquired by humans by ingesting tapeworm eggs shed in the feces of someone infected with an adult intestinal tapeworm.

In India, wide variations is seen in the prevalence of human cysticercosis owing to difference in geography, ethnicity, religious beliefs, socioeconomic and hygiene standards, food habits, educational and awareness status etc (3). Lack of appropriate diagnostic modalities in rural setups and lack of adequate surveillance systems may also affect the prevalence rates. Hence, this study was carried out to estimate the seroprevalence of cysticercosis among clinically suspected patients attending a tertiary care centre in south India.

Materials and Methods

Study design

A descriptive study was carried out in a tertiary care center located in South India catering to the needs of healthcare of the neighbouring regions too to ascertain the seroprevalence of human cysticercosis. The study was conducted for a period of 2 years from July 2017 to June 2019 and a total of 96 patients were enrolled during this period.

Study participants

Inclusion criteria

Patients from different age groups, and both genders presenting as clinically suspected cases of cysticercosis with following signs such as seizures, persistent headache, focal neurologic signs, intracranial space occupying lesions, mobile subcutaneous nodules, retinal edema, and uveitis were included. All such patients irrespective of outpatient or inpatient were included.

Exclusion criteria

Individuals having past history of accident and trauma in brain, current history of any other chronic diseases, symptoms of febrile seizures (particularly in children) were excluded from the study. Short term visitors or tourists, and out of state individuals living in the study area for less than 1 year period were also excluded (4).

Sample collection

Three milliliters of venous blood were collected aseptically without anticoagulant from 96 cases. Serum was separated and stored at -80°C until assay. Serum samples were tested by enzyme-linked immunoelectrotransfer blot (EITB) or Enzyme-linked immunosorbent assay (ELISA) to detect IgG antibodies against *Taenia solium* larval stage (5).

***Taenia solium* IgG ELISA in serum**

A commercially procured ELISA Kit (NovaTec Diagnostics TM, Germany) was used for detection of anti-Cysticercus IgG antibodies in all the collected sera as per the manufacturer's instructions. Antigen coated wells (as supplied by the kit manufacturer) were incubated with 1: 100 diluted patient serum (diluted with the serum diluent fluid provided in the kit) for one hour at 37°C . This is followed by addition of the enzyme conjugate containing Protein A, horseradish peroxidase and the chromogen (tetramethylbenzidine) with subsequent washes after each incubation steps.

A negative control serum, a positive control serum, and a cut off control serum were used for testing validity of the test. Absorbance at 450nm (OD450) was measured using microplate reader (6).

Enzyme-linked immunoelectro transfer blot

T. solium infected pork meat was obtained from the abattoir. The cysts (*C. cellulosae*) were dissected out and washed thoroughly to remove the tissue fibers using phosphate-buffered saline (PBS) pH 7.2. To prepare the native whole cyst somatic antigen (WCA), the cysts are homogenized using a glass tissue homogenizer with PBS (pH 7.2) containing PMSF, phenylmethylsulfonyl fluoride (0.1mM) under cooling conditions 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 4°C for 30 mins at 14000 rpm. The clear supernatant is collected and stored in aliquots at -20°C until further use.

For testing by Enzyme-linked immunoelectro transfer blot (EITB), lentil lectin-specific *T.solium* metacestode glycoproteins were subjected to, a 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis gel was ran using mini-PROTEAN (Bio-Rad Laboratories, USA) . Antigen strips were 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 using Ponceau S (Hi-Media, India)/ coomassie protein stain. The strips were then blocked using 3% BSA for an hour followed by incubation with primary antibody, 1: 100 diluted patient sample and then with secondary antibody, 1: 1000 diluted anti-human IgG horseradish peroxidase conjugate (GeNei, India) with washing with PBS (pH 7.2) containing 0.1% Tween 20 (PBS-T), after each incubation. Finally, diaminobenzidine (Sigma-Aldrich Corp., USA) is used as the chromogen along with

hydrogen peroxide to visualize the reactive protein bands. The major immune-reactive 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 was considered to be of positive serology.

Statistical analysis

The data were analyzed using chi square test using EpiData Analysis software (version 2.2.2.186) and $P < 0.05$ was considered to be statistically significant.

Ethical considerations

Ethical approval for the study was obtained from the Institute Ethics Committee.

Results and Discussion

A total of 96 cases were investigated for cysticercosis over a period of two years from July 2017 to June 2019. The bulk of the clinically suspected cases of human cysticercosis were from Medicine and Pediatric departments and least was seen from the patients of cardiology department. (Fig 1) No positive cases were seen from the patients in Endocrinology, Gynaecology and Surgery through suspected cases were there. Among the analyzed serum samples, fifty six (58.33%) samples were tested positive; 46 of 77 by EITB and 10 of 19 by ELISA.

The age wise distribution of seropositive cases is depicted in figure 2. Maximum cases were in the age range of 10-19 years (26.98%) followed by 0-9 years. In our study, there were a total of 52 male cases, of them 30 were found to be seropositive. Similarly, in the total of 44 female cases, 26 were seropositive as depicted in fig 3.

The seasonal variation of the positive cases with more than 80% of seropositive cases occurred during December, August,

November, September, February, January and as observed through the study period all round the year.

The seropositivity of 58.33% documented in the current study was considerably higher than a former study carried out from January 2011 to December 2015 in our institute, which reported seroprevalence of 32.5% tested by EITB only (5). Likewise, the study reports from other regions of Tamil Nadu which is an epidemiological study carried out using EITB on twenty clusters from Kanyambadi block in Vellore district suggests a seropositivity of 15.9 % (7). This makes it apparent from the studies that the prevalence of cysticercosis is widespread in the districts of Tamil Nadu. In addition, in other states of

India a few to mention, similar findings were observed by Vora *et al.*, in a cross sectional study from Goa, which reported a seroprevalence of cysticercosis to be 22.4% (8). Another study from Odisha, carried out among the epileptic cases, showed 28.1% seroprevalence (6). The above studies used ELISA as the method of testing serum sample. Comparison of seroprevalence of cysticercosis in some studies in India and the world are shown in tables 1 and 2 respectively. It is seen that seroprevalence is quite high in south India compared to other parts of India. The higher seroprevalence rate in the present study maybe due to a hospital based study and the study population included presented with clinical symptoms of cysticercosis.

Table.1

Burden of Human Cysticercosis: Indian Scenario					
Author	Year of publication	States	Seroprevalence (%)	Gender predominance	Age group predominance
<i>Vora et al.,</i>	2008	Goa	22.4	-	Adults
<i>Shukla et al.,</i>	2010	Lucknow	3.48	Male (52.85%)	< 18 yrs (52.87%)
<i>Cherian et al.,</i>	2014	Kerala	14.12	-	Adults (mean: 34 yrs)
<i>Sahu et al.,</i>	2015	Odisha	28.12	Male (83.33%)	Adults (median: 25yrs)
<i>Thamilselvan et al.,</i>	2016	Puducherry	32.5	Male (59.05%)	15-40 years (mean: 30.9 yrs)

Table.2

Burden of Human Cysticercosis: World Scenario			
Author	Year of publication	Countries	Seroprevalence (%)
<i>DeGiorgio et al.,</i> ¹³	2005	California, USA	1.1
<i>Xu et al.,</i> ¹⁵	2010	Philippines	24.6
<i>Mestrovic et al.,</i> ¹⁶	2011	Croatia	1.5
<i>Mwape et al.,</i> ¹⁷	2013	Zambia	36.0
<i>Coral et al.,</i> ¹⁸	2014	Ecuador	31.22
<i>Moyano et al.,</i> ¹¹	2016	Peru	36.9

Figure.1 Department wise distribution of cases

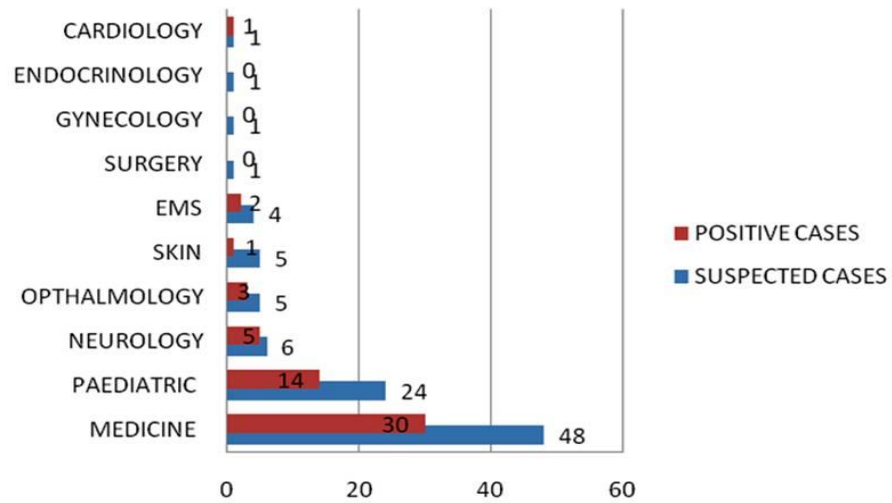


Figure.2 Age wise distribution of seropositive cases

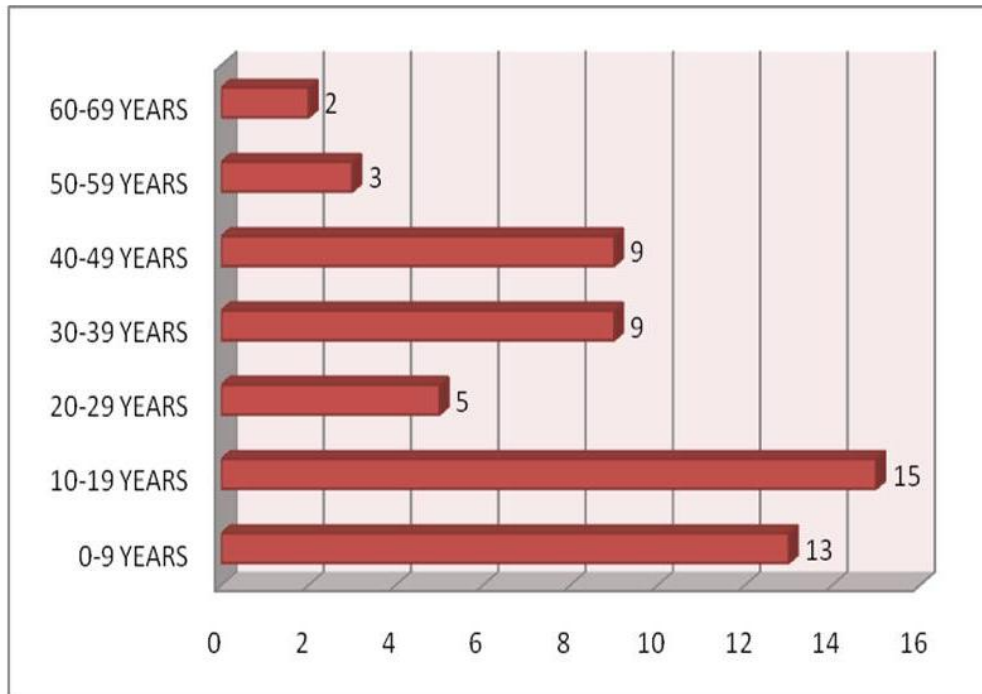
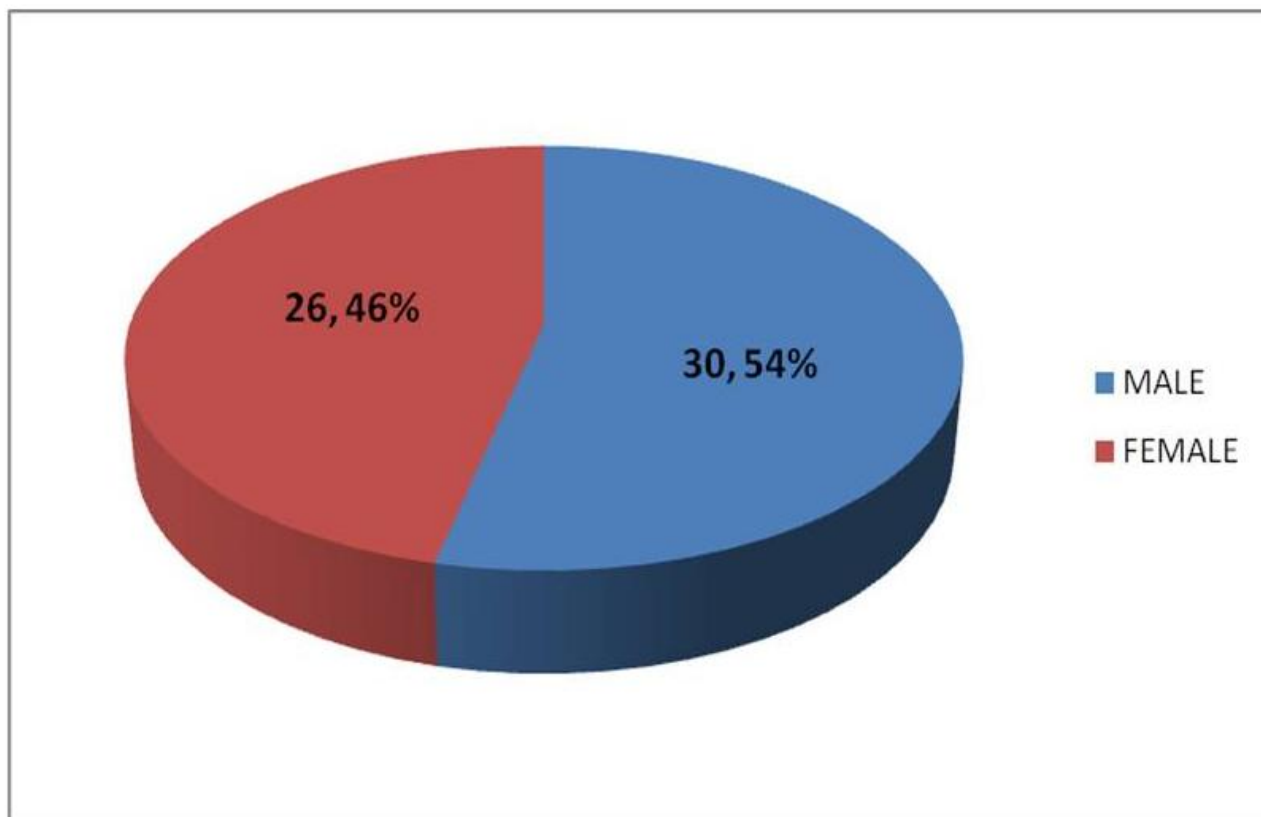


Figure.3 Gender wise distribution of seropositive case



Our study is in accordance to an institutional based study carried out in Chinakakani district, Andhra Pradesh (4) which showed a seroprevalence of 56%. In contrast to our study, a cross sectional study carried out in Lucknow in both urban and rural settings showed an overall seroprevalence of cysticercosis to be 3.48% only (9).

Among the 96 analyzed serum samples, fifty six (58.33%) samples were tested positive. It was also seen that 46 of 77 by EITB and 10 of 19 by ELISA. In another study conducted for neurocysticercosis where 165 serum samples were analysed, 18 were confirmed to be NCC positive by EITB (10). In another study conducted in Peru, 142 (36.9%) serum were positive for antibodies against cysticercosis among a total of 385 patients enrolled (11). Surprisingly, majority of the enrolled patients

were clinically asymptomatic (83%, 333/403) (11).

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. Similar finding was reported by a previous study conducted in the same institute (5). The gender-wise seroprevalence from our study showed males (34.4%) with higher rates than that of females (30%). The difference in seropositivity between males and females is not statistically significant (P value= 0.89). Similar findings are observed in studies carried out by Cherian *et al.*, (12).

Prabhakaran *et al.*, (7) reported in their study that the prevalence of cysticercus antibodies was significantly higher in rural women than in rural men but did not differ between urban

men and women. Though the tertiary care center is located in an urban area, it caters to most of the rural areas of the neighbouring states and hence many reflect the higher seroprevalence in our findings.

In our study, the age wise distribution of seropositive cases was found to be highest in the age range of 10-19 years (26.78%) but the difference in seropositivity across age groups is not statistically significant (P value = 0.78) which is similar to studies carried out by Prudhivi *et al.*, (4) which showed a maximum seroprevalence of 30.3% in the age group of 20-30 years followed by 22.7% in the age group of 10-20 years. In contrast to our study, another study reported higher prevalence in older age groups (8). In a study conducted in California, United States, *Taenia solium* cysticercosis and *T. solium* taeniasis antibodies were not detected in children (13). Similar findings were seen in studies conducted in various parts of the world (14-18). Comparative burden of the cysticercosis in India and the world had been depicted in tables 1 and 2 respectively.

Limitations

Being a single centric hospital based study; the results of our study may not be generalisable to other regions and hence cannot be taken as a baseline epidemiological data with respect to the whole community, thereby limiting its usefulness towards formulating control strategies for prevention of cysticercosis. Still the study reflects higher rate in the patients of south India and its prevalence which can be supplemented with community based studies.

For the definitive diagnosis of NCC, the most common presentation of cysticercosis in man, revised diagnostic criteria for neurocysticercosis by Del Brutto need to be followed that consist of a more detailed

patient history, imaging studies and histopathological aspects (14). This study presents only the serologic evaluation of the clinically suspected individuals which needs to be supplemented with other diagnostic modalities.

In conclusion, despite a hospital based study, it showed that cysticercosis is much prevalent among the patients of south India. Further studies can be carried out taking a more detailed patient history, radioimaging and histopathological aspects to evaluate associated risk factors of cysticercosis. To conclude, it confirms that a large proportion of apparently asymptomatic patients can be seropositive but can have complications in future which may necessitate a long term follow up. This also calls for a community based study along with effective control interventions in *T. solium* endemic regions around the world to reduce the incidence of the disease.

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