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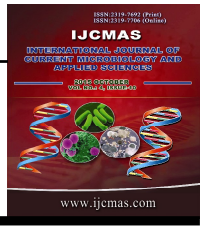

Original Research Article

Prevalence of Neurocysticercosis in and around Chinakakani, Andhra Pradesh, South India

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Keywords

ELISA,
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A B S T R A C T

Objectives of the work are to study the prevalence of neurocysticercosis [NCC] among patients attending our rural Hospital, Chinakakani, South India. Patient presenting with neurological manifestations suggestive of NCC were enrolled in the study group over a period of one year. All these patients were subjected to neuroimaging (CT or MRI). Serum from all suspected cases and CSF from those who have shown evidence of NCC on neuroimaging were subjected to enzyme linked immunosorbent assay (ELISA) for the presence of anti-cysticercus antibodies using Cysti-cheX commercial kit. A thorough general and systemic physical examination with emphasis on the nervous system was carried out along with hematological parameters after obtaining a detailed history. Out of 66 patients presented with manifestations of NCC, radiological evidence was found in 43 (65%) patients of which, 27 (62.8%) of them showed single lesions and 16 (37.2%) showed multiple lesions. Out of 43 patients, majority of them were positive 40(92%) for CSF ELISA than Serum 28 (65.1%) ELISA while 19 (44.1%) were positive both for serum as well as CSF. Patients in the age group 20–30 years were most affected. Majority of them presented with seizures 54 (81.82%), others had headache associated with vomiting 47 (71.21%), behavioral abnormalities 18 (27.27%), visual disturbances 12(18.18%) and localizing neurological manifestations. Significantly a large number of patients were vegetarians 31(47%), only 18 (27.3%) were pork eaters, while the remaining 17 (25.7%) never had pork in their meals. Both sexes were found to be equally affected. The sensitivity and specificity of Serum ELISA is 86.49% and 79.31% and that of CSF is 100% and 88.46%. The prevalence rate is 0.42% (<1%). High prevalence of anti-cysticercus antibodies in this region needs further evaluation. ELISA for CSF was found to be more sensitive and specific than serum using purified excretory and secretory antigens of cysticercus cellulosae. For hospitals lacking advanced neuroimaging techniques ELISA serves as the convenient modality.

Introduction

NCC is a disease caused by the metacestode or larval form of the tapeworm Taenia solium when it lodges in the central nervous system [CNS]. The disease is a major public health problem in the developing countries of Asia, Africa and Latin America (Fisser *et al.,* 1982) It is regarded as the most common parasitic disease of the CNS and in India, as a major cause of neurological morbidity accounting for 47% of cases of epilepsy in South India (Chowdary *et al.,* 2004). In North India, upto 50% of the children with partial seizures have a recognizable underlying cause and NCC was found to be the most common cause of the focal seizures in children (Singhi and Singhi, 1997).

It is a disease difficult to diagnose on the clinical picture alone as the manifestations of NCC are polymorphic; no symptom or sign is specific presenting with seizures, (most common) headache, hydrocephalus, chronic meningitis, focal neurological deficits, psychological disorders, dementia, occular and spinal cysts (Kashi Nath Prasad *et al.,* 2008). More over the symptoms differ according to the number, location of the cysts in the brain (parenchymal or ventricular) and the state of the parasites (vesicular, degenerating or calcified). The definitive diagnosis is made using a combination of methods including images of the cysts in the brain (by CT or MRI) and immunological methods (Detection of specific antibodies or antigens). Neuroimaging modalities (CT and MRI) have greatly improved the accuracy in the diagnosis of NCC (Kashi Nath Prasad *et al.,* 2008; Sharda *et al.,* 2002; Gracida and Del Brutto, 2003). MRI is considered the best neuroimaging tool for the detection of degenerating and viable cysticerci, while CT is the best for calcified lesions. More over MRI with gradient echo sequence phase imaging has overcome this also (Kashi Nath Prasad *et al.,* 2008; Sharda *et al.,* 2002).

A wide range of serological tests have been used in the diagnosis of NCC. Among which ELISA and electro immuno transfer blot (EITB) assays are the commonest, each exhibiting varying degrees of sensitivity and specificity based on the type of antigen and specimen used for the test (Kashi Nath Prasad *et al.,* 2008). A definitive and probable diagnosis of NCC can be made on the basis of proposed clinical, radiological and epidemiological criteria (Del Brutoo *et al.,* 2001; Garcia *et al.,* 2005).

Aims and objectives of the work: 1. To study the prevalence of neurocysticercosis (NCC) in patients attending our tertiary care hospital from in and around Chinakakani, a rural area in Guntur district. 2. To compare the sensitivity and specificity of enzyme linked immunosorbent assay (ELISA) by detecting anti-cysticercus antibodies in the serum and CSF of clinically suspected and radiologically proven cases using purified excretory and secretory antigens of cysticercus cellulosae.

Materials and Methods

Study Design: Descriptive study

Source of date: A total of 109 samples (66 serum, 43 CSF) were collected from patients attending the Outpatient Departments of General Medicine, Paediatrics and Neurology.

Study period: One year

Participants/patient selection: Patients included in the study group were those reporting to the hospital with neurological manifestations such as seizures, convulsions, fainting attacks, recurrent headaches, focal neurological deficits, signs of meningitis and dementia. They also included patients with

history of trauma following a fainting attack or seizure but clinically not suggestive of a cerebro vascular accident (CVA).

Patients excluded in the study group were previously diagnosed cases of NCC undergoing treatment or reporting for follow up in the hospital. Other exclusions were clinically diagnosed cases of CVA. Patients with history of alcoholism or trauma following alcohol intake and previously diagnosed cases of chronic renal failure, hepatic failure or electrolyte abnormalities were also excluded.

All these patients presenting with symptoms and signs suggestive of NCC were subjected to neuroimaging studies (CT scan or brain MR). Serum samples were collected from all of them where as CSF samples only from those who have shown lesions on CT scan. All patients were subjected to detailed history taking and an examination session following a planned protocol. History of seizures, fainting attacks, headache, recent development of subcutaneous or muscular nodules, food habits, occupation and previous medication were elicited in detail. A thorough general and systemic physical examination was carried out with emphasis on the nervous system.

Routine investigations such as hematological parameters (hemoglobin, TLC, DLC, ESR, absolute eosinophil count) were carried out on each patient. Taking aseptic precautions, blood samples from all (66) the clinically suspected NCC cases and CSF samples only from those who have shown evidence of NCC on neuro imaging (46) were collected.

Antibody assay

Serum was separated and both serum and CSF samples were stored at -20^{0}C until further use. Cysticercus IgG antibodies were estimated in sera and CSF samples by ELISA using Cysti-cheX a commercial kit which uses purified excretory secretory antigens of cysticercus cellulosae maintained in vitro. Tests were performed with diluted serum and CSF samples as per manufacturer's instructions and optical density (OD) was read at 450 nm using ELISA reader. Test samples having OD more than low positive control were taken as positive.

Statistical analysis

Descriptive statistics like tables, graphs and percentages were used to present the data.

Results and Discussion

A total of 66 cases presenting with manifestations of NCC were included in the study group. The greatest number of patients were found to be in the 20–30 years age group (30.3%), followed by 30–40 years ago group (15.15%), in both males and females. The male to female ratio of the patients among the study group was 1.2:1 (Table 1).

The common spectrum of clinical features manifested by these cases was given in figure 1. Majority of patients presented with seizures or seizures with headache followed by headache and vomiting. Minority of them were with visual disturbances and behavioral abnormalities.

In dietary terms, significantly large number of patient were vegetarians 31 (47%), only 18 (27.3%) were pork eaters while the remaining 17 (25.7%) never had pork in their meals.

Of the 66 cases, radiological evidence was found to be present by CT scan in 43 patients. A single lesion was found in 27 (62.8%) patients and multiple lesions in 16 (37.2%) patients (Table 2).

Out of 66 serum samples subjected to ELISA, 37 (56%) had anticysticercus antibodies of which 21 (56.75%) were males and 16 (43.25%) were females with M:F = 1.2:1.

In the group of 43 patients where both serum (collecting before subjecting the patients to CT) and CSF were collected, sera alone were positive in 28 (65.1%), CSF alone in 40 (93%) while in 19 (44.1%) cases both serum and CSF were positive. The sensitivity, specificity, Positive predictive value and Negative predictive value of serum is 86.49%, 79.31%, 84.1%, 82.14% and that of CSF is 100%, 88.46%, 93.02%, 100%. The prevalence rate is 0.42% (<1%) (Fig. 2).

To our knowledge, there is no information of NCC available in our region. So this study has been taken up to know the prevalence of the disease in and around chinakakani. Many rural households in this region rear pigs in small numbers providing the family not only with a means of income but also meat. In the absence of sanitary infrastructure, people use open areas and fields for defecation. Free raring pigs thus have access to human faeces which perpetuates transmission of the parasite from human to pig. The pork being sold to customers is not thoroughly inspected because of threat of condemnation and taxation.

Table.1 Demographic analysis of patients with NCC

Age	**Males [%]**	**Females [%]**	**Total [%]**
10 o 20	8 [12.1]	7 [10.6]	15 [22.7]
20 to 30	12 [18.1]	8 [12.1]	20 [30.3]
30 to 40	5 [7.5]	6 [9]	11 [16.6]
40 to 50	4 [6]	2 [3]	6 [9]
50 to 60	4 [6]	3 [4.5]	7 [10.6]
60 to 70	1 [1.5]	3 [4.5]	4 [6]
70 to 80	2 [3]	1 [1.5]	3 [4.5]
Total	36 [54.5]	30 [45.5]	66 [100]

Table.2 Distribution of cases according to the type of lesion

Type of cyst	No.of cases	%
Single	27	62.79
Multiple	16	37.21
Total	43	100

Fig.1 Distribution of cases according to symptomatology

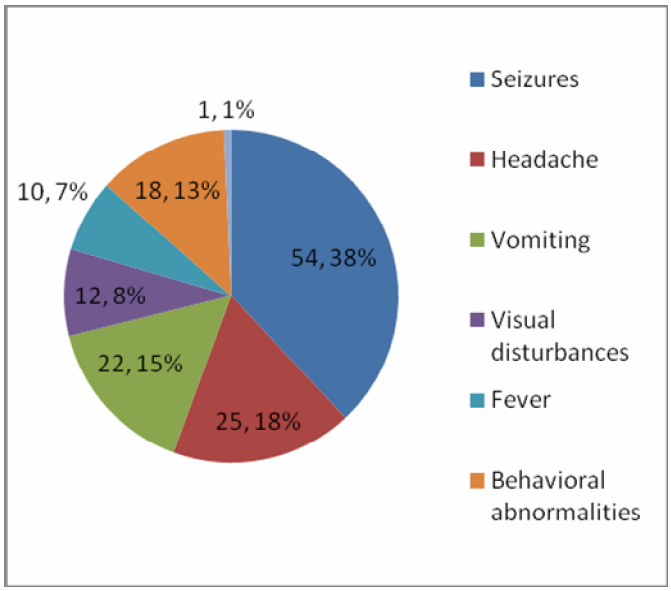
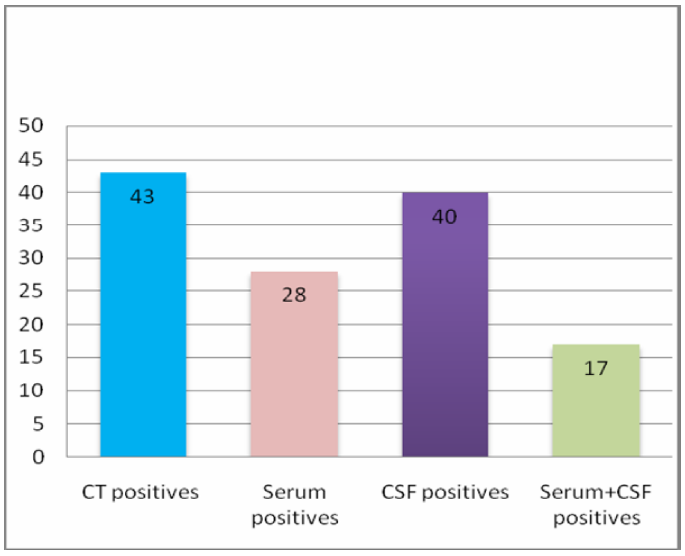


Fig.2 Distribution of CT positive cases in serum and CSF



In our study, a good number of cases were noted in the age group 10–40 years with maximum number between 20–30 years in both males and females. This is in accordance with most other studies (Janak kishore *et al.*, 2004; Palacio *et al.*, 1998; Del Brutto and Scotelo, 1998).

Both sexes were found to be almost equally affected (1.2:1). A large percentage of patients were found to be vegetarians 31 (47%). This may be related to the practice of consuming raw or unwashed vegetables/ fruits contaminated with the parasite eggs. Similar studies conducted by others have also shown that a large proportion of patients population belong to the vegetarian group (Ashish kumar Suhial *et al.*, 2006; Varma and Gour, 2002) while others have shown equal preponderance among

vegetarians and non-vegetarians (both pork eaters and non- pork eaters) (Ashish kumar Suhial *et al.,* 2006; Varma and Gour, 2002; Prasad *et al.,* 2002). Majority of cases presented with seizures (generalized or focal) followed by headache associated with vomiting and localizing neurological manifestations. Cysticercosis was the causative agent of seizures in 5.9% of 103 epilepsy cases in Changigarh (Janak Kishore *et al.,* 2004)

Many studies have reported a higher prevalence of epilepsy in developing countries than in the developed world (Athanase Millogo *et al.,* 2012; Roman *et al.,* 2000; Preux *et al.,* 2000). In a recent study in South India, a third of active epilepsy was related to neurocysticercosis (Dupak Goel *et al.,* 2011; Rajasekhar *et al.,* 2006). One hospital- based study in north India had also suggested that a good proportion of recurrent unprovoked seizures were due to NCC (Mohanty *et al.,* 2008). Another meta- analysis study reported a prevalence rate of 5.33 per 1000 population. (Sridharan and Murthy, 1999; Athanase Millogo *et al.,* 2012).

As far as lesions are concerned patients with single ring enhancing lesions in CT scan were more in number (62.8%) than multiple lesions (37.2%). In endemic countries of Latin America, Africa and most of Asia the disease is mainly multi-lesional. In India solitary cysticercus granulomas (SCG) contribute to almost 70% of the disease while multi-lesional NCC (MNCC) is seen in 30% of cases (Oommen *et al.,* 2004; Wadia *et al.,* 1987; Rajashekar and Chandy, 2000).

In the present study, ELISA was performed to detect anti-cysticercus antibodies both in the serum and CSF of patients using purified excretory secretory antigens of cysticercus cellulosae and an attempt was made to compare the sensitivity and specificity of ELISA for the same. Even though current data indicates that the most effective assay for the detection of anti-cysticercus antibodies is EITB (Proano – Narvaez *et al.,* 2002), because of cost effectiveness, ELISA was chosen as the test of choice.

Out of 66 clinically suspected cases of NCC subjected to CT, only 43 of them were showing evidence of single or multiple lesions, out of which majority of them were positive for CSF ELISA 40 (93%) than serum ELISA 28 (65.1%).

In the present study we found that the sensitivity and specificity of ELISA for serum is 86.49%, 79.31% and that of CSF is 100%, 88.46%. This is in accordance with other studies which have also shown that ELISA is more sensitive for the detection of antibodies in CSF than serum (Proano – Narvaez *et al.,* 2002; Wilson *et al.,* 1991). More over the sensitivity and specificity of ELISA depends upon the nature of antigen used, method of purification of antigen, number, location and stage of lesion, patient selected for the study, steroid intake, antibody responses etc (Rosas *et al.,* 1986; Flisser *et al.,* 1990; Del Brutto and Sotelo, 1998; Sotelo *et al.,* 2002).

Even though EITB assay is more sensitive than the ELISA especially when serum is being tested, (Proano – Narvaez *et al.,* 2002; Wilson *et al.,* 1991) a low sensitivity was observed with either technique in cases with single cysts or calcified lesions (Wilson *et al.,* 1991; Espinoza *et al.,* 1986). The complexity of the technique, time of execution, sophisticated equipment, need for availability of highly skilled personnel and cost makes EITB a difficult tool for routine use in developing countries like India. Thus, since ELISA being relatively cheaper, easy to perform, less time consuming, easily

available and convenient for quality assurance procedures can be used as an alternative tool.

In conclusion, the prevalence of anti-cysticercus antibodies in clinically suspected cases of NCC was significantly high. We believe that this is only a rough indicator. Small number of pork eaters and a significant number of vegetarians suggest other modes of transmission and need for further evaluation. CSF ELISA was found to be more sensitive and specific than serum ELISA. Since positive response with neuroimaging or serology depends on the stage of the disease, both tests should be done together to rule out NCC. But hospitals which can't afford and lack advanced techniques, ELISA remains the most convenient modality.

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