A Comparative Study of Single Versus Multiple Cysts of Neurocysticercosis Based on their Clinico-Serological Profile

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ABSTRACT

Introduction: Neurocysticercosis is a common disease in developing countries. Its diagnosis is based on clinical, epidemiological, imaging findings and laboratory tests.

Aim: To study and compare the clinical profile and diagnostic utility of NCC IgG ELISA in patients with single and multiple neurocysticercosis.

Materials and Methods: This cross-sectional study was conducted in the Department of General Medicine, Himalayan Institute of Medical Science (HIMS), Swami Ram Nagar, Dehradun, over a period of 12 months from August 2014 to July 2015. Patients were grouped into those with single NCC and multiple NCC based on their radio imaging findings. A total number of 60 patients, 30 in each group were taken in the study and their clinical profiles were studied. Serum Immunoglobulin G(IgG) Enzyme-Linked Immunosorbent Assay (ELISA) test was applied on both the groups and serological profile was thus compared.

Results: Most commonly affected age group was 18 to 35 years of age (60%). Most common symptom at presentation was seizure (53%). Out of 60 patients, 39 suffered from generalized tonic-clonic seizures. Status epilepticus was found exclusively in patients with multiple NCC (26.6%). Sensitivity of serum IgG ELISA was 53.3% amongst single NCC and 76.7% amongst multiple NCC.

Conclusion: NCC may present with varied clinical features. No sign or symptom is pathognomic of the disease entity. Diagnostic tests are the only method to clinch the diagnosis. Detection of specific antibodies in patients with NCC is a useful tool for diagnosis, especially when radio imaging facilities are not available or inconclusive. Serological tests are useful if they are positive. Negative tests, however, do not exclude the diagnosis of NCC.

Keywords: Taenia solium, Tonic-clonic seizures, Idiopathic epilepsy, Status epilepticus

INTRODUCTION

It is well known that cysticercosis is caused by larval stage of tapeworm *Taenia solium*. Cysticercosis poses a major public health problem, especially in the developing countries like India. A study has shown that Neurocysticercosis (NCC) is the most common parasite infestation of the central nervous system and the single most common cause of epilepsy in the developing world [1].

Neurocysticercosis being the most common aetiology of community acquired active epilepsy has been demonstrated in 26.3% to 53.8% active epilepsy cases in the developing countries including India and Latin America [2]. In a study conducted in Uttarakhand, India the prevalence of acute symptomatic seizures related to NCC was found to be 2.5 per 1,000 population [3].

Humans are usually infested with *T. solium* eggs by the fecal-oral route. The eggs develop into larvae, which penetrate the intestinal wall and are then transported by the bloodstream mainly to the brain, muscle, and subcutaneous tissue [4].

Amongst the various diagnostic tools for NCC, serology holds an established reputation. Enzyme-Linked Immunoelectrotransfer Blot (EITB) that detects specific antibodies against *T. solium* cysticerci in serum is considered to be the gold standard serologic test. This test offers a sensitivity of 98% and specificity of 100%. However, the test poses technical difficulties and is expensive, hence not feasible in most resource poor laboratories in endemic and remote areas. The ELISA, however, is a more feasible test and detects specific antibodies and antigens in the serum [5].

The definite diagnosis of NCC requires neuroimaging studies which are sparsely available in resource poor countries and need mobilization of the patient to the health care facility. So, in countries like India where there are poor transport facilities and expenses are a big issue, immunodiagnostic tests represent an important tool for diagnosis of cysticercosis. Furthermore, new low-cost diagnostic methods offering good sensitivity and specificity are needed [6].

Uttarakhand is a newly formed state of India and a large portion is covered by hills of Himalayas. Medical infrastructure is poor and economic status of people is poor. One pilot study from Uttarakhand state showed a high prevalence rate of NCC. The high frequency of NCC in the Uttarakhand state may be due to poor sanitation and non-vegetarians in the large segment of the population [7]. The present study was aimed to study and compare the clinico-serological profile of patients with single and multiple NCC. Also, to study the diagnostic utility of NCC IgG ELISA in these patients.

MATERIALS AND METHODS

This cross-sectional study was conducted at the Department of General Medicine, Himalayan Institute of Medical Science (HIMS), Swami Ram Nagar, Dehradun, over a period of 12 months from August 2014 to July 2015. Subjects were recruited from patients presenting in the Department of General Medicine with a primary diagnosis of NCC after obtaining written informed consent and taking ethical clearance from the Institute.

Patients were classified to be suffering from NCC on the basis of Del Brutto O. H. diagnostic criteria for NCC and their baseline characteristics were noted which included their clinical profile,

personal history, radiological findings and serological findings [Table /Fig-1].

Patients were then grouped into those with single NCC and

Categories of criteria	Criteria
Absolute	Histologic demonstration of the parasite from biopsy of a brain or spinal cord lesion
	Evidence of cystic lesions showing the scolex on neuroimaging studies
	Direct visualisation of subretinal parasites by fundoscopic examination
Major	Evidence of lesions highly suggestive of neurocysticercosis on neuroimaging studies
	Positive serum immunoblot for the detection of anticysticercal antibodies
	Resolution of intracranial cystic lesions after therapy with albendazole or praziquantel
	Spontaneous resolution of small single enhancing lesions
Minor	Evidence of lesions suggestive of neurocysticercosis on neuroimaging studies
	Presence of clinical manifestations suggestive of neurocysticercosis
	Positive CSF ELISA for detection of anticysticercal antibodies or cysticercal antigens
	Evidence of cysticercosis outside the central nervous system
Epidemiologic	Individuals coming from or living in an area where cysticercosis is endemic
	History of frequent travel to disease-endemic areas
	Evidence of household a contact with *T. solium* infection

[Table/Fig-1]: Diagnostic criteria for neurocysticercosis.
Note: Definitive diagnosis: 1absolute criterion, or 2 major + 1 minor and 1 epidemiological criterion; Probable diagnosis: 1 major +2 minor criteria, or 1major + 1 minor and 1 epidemiological criteria or 3 minor + 1 epidemiological criteria

multiple NCC based on their radio imaging findings. A total number of 60 patients, 30 in each group were taken in the study on the basis of convenient sampling technique. Also 30 controls were taken who were proven negative cases of NCC.

Inclusion Criteria: A minimum of 60 patients above the age of 18 years who were diagnosed cases of neurocysticercosis as per Del Brutto O. H. diagnostic criteria were taken for the study [1].

Exclusion Criteria: Patients of seizures due to lesions other than NCC and patients with idiopathic epilepsy were not included in the study.

ELISA test for NCC

A blood sample of 3–5 ml was drawn from each subject using a sterile Vacutainer (Becton-Dickinson Vacutainer System) for routine medical purposes, and the serum was separated via centrifugation (3000 rpm for 15 mins). The serum specimens were then screened for the NCC IgG antibodies using commercially available kit for serum IgG ELISA which was procured from DRG International Inc™ and standard procedure as described by manufacturer was done. Based on the manufacturer's instructions, positive results were recorded when the absorbance reading of the serum specimen was ≥0.3 OD units, and negative results were recorded for a absorbance reading of <0.3 OD units.

STATISTICAL ANALYSIS

Data was collected and entered in MS excel 2007. Statistical analysis was performed using SPSS software 22. Categorical data was analyzed using Chi-square test. For comparison between two groups Z-test was applied. The statistical comparison in terms of sensitivity, specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV) in both the groups were calculated. Level of significance for this study was $p<0.05$ was considered significant.

RESULTS

In the present study, 60 patients diagnosed with NCC were enrolled. Patients were then grouped into those with single NCC and with multiple NCC. Thirty patients in each group were included and their clinical profiles, symptomatology, risk factors, personal habits and serological findings were studied.

Most commonly affected age group was 18 to 35 years i.e. 36 patients (60%). Male to female ratio was 1.6: 1 [Table/Fig-2]. Most common symptom at presentation was seizures [Table/Fig-3]. Out of 60 patients, 39 suffered from tonic-clonic seizures. Status epilepticus was found exclusively in patients with multiple NCC [Table/Fig-4]. There were no cases presenting as meningo-encephalitic syndrome.

The role of IgG ELISA was studied in diagnosis of single versus multiple NCC. Sensitivity of serum IgG ELISA was 53.3% (16 out of 30) amongst single NCC and 76.7% (23 out of 30) amongst multiple NCC and overall specificity was found to be 100% in both the groups. Overall the sensitivity was found to be 65% [Table/Fig-5].

Age (years)	Single NCC Number (%)	Multiple NCC Number (%)	Total Number (%)
18-35	21 (70)	15 (50)	36 (60)
36-50	7 (23.3)	9 (30)	16 (26.7)
51-65	2 (6.7)	6 (20)	8 (13.3)
Sex			
Male	18 (60)	19 (63.3)	37 (61.7)
Female	12 (40)	11 (36.7)	23 (38.3)

[Table/Fig-2]: Demographic profile of patients with single and multiple NCC.

Symptom	Single NCC Present Frequency (%)	Multiple NCC Present Frequency (%)	Total Present Frequency (%)	Statistics Chi-square test	Odds ratio
Headache	19 (63.3)	25 (83.3)	44 (73.3)		0.35
Seizure	26 (86.7)	27 (90)	53 (88.3)	2.38 p-value: 0.497	0.72
FND	3 (10)	1 (3.3)	4 (6.7)		3.23
PS	1 (3.3)	3 (10)	4 (6.7)		0.31

[Table/Fig-3]: Symptom wise distribution of patients in single & multiple NCC group.
FND= Focal neurological deficit
PS= Psychiatric symptoms
Level of significance: p < 0.05

Type of seizure	Single NCC Frequency (%)	Multiple NCC Frequency (%)	Total Frequency (%)	Statistics Chi-square test	Odds ratio
GTCS	19 (73.1)	20 (74.1)	39 (73.6)	3.49 p-value: 0.321	0.86
Focal	7 (26.9)	7 (25.9)	14 (26.4)		1
Status epilepticus	0	8 (26.7)	8 (13.3)	-	-

[Table/Fig-4]: Pattern of seizure in patients with history of seizures and comparison between single & multiple NCC group.
GTCS: Generalized tonic clonic seizures

Serum IgG ELISA	Single NCC	Multiple NCC	Z-value	p-value
Sensitivity	53.3%	76.6%	3.5	0.0006
Specificity	100%	100%	0	1
Positive predictive value (PPV)	100%	100%	0	1
Negative predictive value (NPV)	68.2%	81.1%	2.1	0.036
Accuracy	75%	86.6%	2.0	0.049

[Table/Fig-5]: A comparison of serum IgG ELISA sensitivity between two groups. Level of significance p < 0.05

DISCUSSION

NCC is one of the most serious problems especially in the developing nations. It has varied clinical presentations. CT scan has proved helpful in diagnosis of NCC but it does not differentiate NCC from other sinister entities. The advent of ELISA has provided a lot of promise in the diagnosis of NCC [7].

In the present study, maximum number of patients i.e. 36 patients (60%) were in the age group of 18-35 years. In another study conducted by Varma A et al., in year 2002 in Uttarakhand, majority of cases were in the age group of 21 to 30 years [8]. Kuruvilla in 2001, reported an age range of 24 to 62 years and a mean age of 35.2 years for NCC in their study at a tertiary care centre in Kerala [9]. In another study by Kokotey et al, maximum number of patients (43.13%) in the age group of 21 to 30 years [7]. Our study has shown similar results as described in previous studies.

In the present study, seizure was the presenting complaint in 88.3% of cases. In a major study conducted by Del Brutto OH et al., they reported seizure as the primary or sole manifestation of NCC in almost 70% of patients [10]. In other studies from India, conducted by Kokotey RK et al., and Kuruvilla A et al., they reported 100% incidence of seizure in patients with NCC in their respective populations studied [7,9].

In the present study, 73.6% patients presenting with seizure had generalized tonic-clonic seizure while 23.4% had focal seizures [Table/Fig-3]. In another study from Uttarakhand by Varma A et al., they also reported generalized seizures to be more commonly affecting 62.75% of the studied population [8]. However, other studies from India by Kokotey RK et al., [7] and Kuruvilla A et al., show that majority of cases with NCC suffered from focal seizures affecting 21.6% and 36% of studied population, respectively [9].

In the present study, headache was presenting symptom in 73.3% patients. In study by Stepien L et al., headache occurred in 97.7% of cases [11]. While Varma A et al., reported 37.5% and Kuruvilla A et al., reported 63.6% incidence of headache [8,9].

In the present study, 6.7% patients presented with focal neurological deficit mimicking stroke syndrome. In a study by Del Brutto OH et al., they reported a 3% incidence of stroke syndrome amongst patients with NCC [12]. In the present study, 6.7% patients had psychiatric manifestations at presentation. Similar findings have been described in literature by Forlenza OV et al., [13]. In the present study, status epilepticus was found in 8% of the case suffering from NCC, of who all had multiple NCC. While in a study by Kokotey RK et al., in 2006, they demonstrated a 5.9% incidence of status epilepticus in the population studied [7]. In the present study varied clinical presentations of NCC were seen, ranging from simple headache to status epilepticus. Therefore, the clinical features seen in our study were comparable with those described in literature.

In the present study, serum IgG ELISA was found to be positive in 39 out of 60 cases taken, resulting in 65% sensitivity and 100% specificity. Among the two groups, sensitivity was more in the patients with multiple NCC (76.7%) as compared with single NCC (53.3%).

In the study conducted by Kokotey RK et al., sensitivity of the test came out to be 82.6% [7]. In the study by Shukla N et al., they found a sensitivity of 92% [14]. In the study conducted by Kirmani S et al., they found a sensitivity of 13.3% in the population studied [15]. In another study by Mittal V et al., they found a sensitivity of 10.4% [16].

The previous studies have shown results with both high and low sensitivity. The current study is comparable in this regard with above mentioned studies.

Although, a low sensitivity especially in those with single NCC, is a big hindrance for the test to be used in masses for making a diagnosis of NCC.

LIMITATION

The study took into account only 60 cases of NCC due to financial constraints. Further large scale studies and observations are required to understand the utility of ELISA in a better way. Moreover, comparison of EITB and ELISA in a large scale study will further help with our understanding.

CONCLUSION

Detection of specific antibodies in patients with NCC is a useful tool for diagnosis, especially when radio imaging facilities are not available or inconclusive. Serological tests are useful if they are positive as shown in our study by their high positive predictive value. Negative tests, however, do not exclude the diagnosis of NCC. Serological tests using serum may serve as a logical tool to aid in confirming the diagnosis of NCC.

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