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Evaluation Of Igg Elisa in Radiologically Positive and Negative Cases of Neurocysticercosis at A Tertiary Care Hospital in Western Uttar Pradesh: A Cross-Sectional Study

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A B S T R A C T

Introduction: One of the most prevalent infections of the human neurological system is neurocysticercosis. Most of the world is affected by this disease. The diagnosis of neurocysticercosis (NCC), where the survival of the parasite is a major determinant, and is challenging due to the variety of clinical manifestations and the course of the disease. This study evaluates ELISAs for the detection of anti-*Taenia solium* metacestode IgG antibodies in serum utilizing *T.solium* antigens.
Aim: To evaluate CT-positive cases against IgG ELISA to determine the utility of antibody ELISA and/or antigen ELISA in the diagnosis of neurocysticercosis cases at UPUMS, Saifai, Etawah, (U.P.) The primary goal is to diagnose NCC cases by using the antibody ELISA at UPUMS Saifai.
Materials and methods: 100 patients who had symptoms and radiological findings strongly suggestive of NCC made up the study group. *T. solium* IgG ELISA was used to identify anti-*T. solium* antibodies.
Results: The most frequent complaint among new patients was seizures. In many studies of IgG antibodies against cysticerci by ELISA, researchers have found 17% seropositivity and 83% of patients tested negative for clinical and radiological characteristics of NCC. This demonstrates the diagnostic sensitivity of ELISA for NCC. The sensitivity of the IgG-antigen-based ELISA is 30%. In contrast, the specificities of the ELISA were 96% for IgG antibody detection in serum, 60.57% for PPV, 87% for NPV, and 63% for accuracy when antigen was used.

Keywords: Neurocysticercosis, Seizures, *Taenia solium*, IgG, ELISA, Antigen

INTRODUCTION

Neurocysticercosis is the most prevalent parasitic illness of the central nervous system, which accounts up to 50% of cases of late-onset epilepsy in some countries where prevalence is more than 10%.[1]

This disease is prevalent in all states of India, and it is highly prevalent in some of the states such as Bihar, Orissa, Uttar Pradesh, and Punjab. Kerala has a very low incidence disease rate, which is attributed to the State's high literacy rate, predominately due to vegetarian population, and strict sanitation laws. Moreover, the frequency is low in Jammu and Kashmir. [2]

Untreated tropical illness neurocysticercosis is a major cause of epilepsy and neurological morbidity worldwide. *T.solium* metacestode larvae in the Central Nervous System (CNS) produce Neurocysticercosis (NCC), a serious health issue that

affects people of all ages and is one of the main causes of human epilepsy in several hyperendemic areas of South America, Asia, and sub-Saharan Africa. According to WHO, NCC affects 2.5 to 8.3 million people annually, resulting in a burden of 2.8 million Disability-Adjusted Life Years (DALYs) for people worldwide. [3]

A recent Indian study report on the seroprevalence investigation from a South Indian province found anti-*T. solium*metacestode antibodies in 15.9% of individuals.[4] Similar to this, a prior study from North India found that anti-*T. solium*metacestode antibodies were present in 17.3% of the population, with slum regions having the highest frequency, rural areas having the second-highest prevalence, and the organized urban sector having the lowest prevalence.[5]

Eggs containing the hexacanth larvae spread hematogenously to the brain, developing into metacestode larvae or cysts. Thiscyst develops by ingesting infected eggs passed by a person with taeniasis, either directly through contact with another tapeworm carrier, indirectly through contaminated food, water, or hands, or autoinfection.[6]

The clinical presentation of NCC is similar to a wide range of neurological conditions making clinical diagnosis difficult, especially in low-income country settings. The location, quantity, and survival of the cysts, as well as the immune response of an individual patient, are the key determinants of clinical symptoms.NCC presentation can vary from being asymptomatic to sudden death.[7]

NCC has been reported in both adults and children who are present with cysts that range from single to multiple and are parenchymal or intraventricular in location. NCC is not lethal on its own, but is associated with substantial morbidity that lowers the quality of life.[8]

The most frequent cause of community-acquired active epilepsy is identified as NCC. According to reports in India, seizures are the most prevalent symptom.Amajor portion of late-onset seizures in developing nations is caused by NCC.[9]

Aside from these symptoms, meningoencephalitis, aberrant behavior, transitory paresis, intermittent obstructive hydrocephalus, disorientation, and vision issues may also be present. Neuroimaging is the most helpful analytic review for parenchymal infection.[10]

The presence of distinct parasite developmental stages in the brain with diverse clinical and radiological signs complicates the diagnosis of NCC.Individuals with NCC may go months or years without showing any symptoms, and frequently, a diagnosis is established by accident when neuroimaging is done. [11]
A medical diagnosis of NCC is difficult on clinical data alone so the definitive diagnosis is made using a combination of methods including imaging techniques (by Computed Tomography or Magnetic Resonance Imaging) and immunological methods (detection of specific antibodies or antigens).[12]
The most common finding in radio imaging is ring-enhancing lesions. But imaging is inconclusive in many cases as it can miss small cysts or lesions close to the skull bone or in the posterior fossa and MRI is poorly sensitive to calcified cysts.[13]
Under these conditions, serological findings may provide some clues for diagnosis in clinically suspected cases of NCC. Several immunological methods have been evaluated for the demonstration of specific antigens/antibodies in the serum and cerebrospinal fluid (CSF) over the last many decades. Among different methods, enzyme-linked immunosorbent assay is more sensitive and easy method, which is widely used in the detection of anti-T. Antibodies against *T. solium* in various samples from NCC patients.[14]

The current study was conducted at a tertiary care center in Western Uttar Pradesh to know the prevalence of neurocysticercosis andto determine the utility of IgG antibody ELISA in the diagnosis of neurocysticercosis cases.
IgG ELISA is considered a good adjunctive test that helpsin confirming the diagnosis in clinical-radiologically suspected cases of NCC,and more so, the absence of antibodies in sera also play important role in ruling out the diagnosis of NCC in doubtful cases where single ring-enhancing lesion of the brain remain a diagnostic dilemma.

MATERIAL AND METHODS

Study Setting and Duration: Across-sectional study was conducted on patients attending OPD of Neuromedicine and were recruited if fulfilling the inclusion criteria. All samples were collected, processed and evaluated within the stipulated time period of study, i.e., from January 2021 to June 2022 were evaluated and their outcomes were noted. A total of 100 samples with recent epileptic episodes were enlisted against their written consent and after the study protocol had been approvedby the Institutional EthicsCommittee.

Inclusion criteria

1. The study included clinically and radiologically confirmed 50 cases suggestive of NCC and 50 controls, clinically suggestive of NCC based on the history of seizure but without any lesion in CT.
2. The study included all patients aged >14 years

3. Those who gave consent

Exclusion Criteria:

1) All cases of epilepsy with other proven aetiology like tubercular meningitis, brain tumours, strokes, viral encephalitis, metabolic or systemic causes.
2) All patients with epilepsy where a CT scan was not available
3) Those who did not give consent.

Brain imaging by computed tomography (CT)

CT imaging was performed inall patients. The study subjects were initially hypothesized as probable cases of NCC based onone major neuroimaging criteria plus any two clinical /exposure criteria as per the revised diagnostic criteria suggested by Del Brutto.[15]

Sample collection

Venous blood samples (5 ml) were collected and processed by allowing them to stand for 15-20 minutes at room temperature followedby centrifugation for 10 minutes and the serum was stored at -20 until further use.

Methodology- Serological testing for the presence of antibodies to *T. Solium* was done using the ELISA method with *a T. Solium* IgG diagnosis kit (**Demeditec Diagnostics GmbH – Germany**). The qualitative immune enzymatic determination of specific antibodies is based on the ELISA (Enzyme-Linked Immunosorbent Assay) technique.All the samples were diluted 1in 100 with IgG sample dilution buffer.Theabsorbanceof all the wells was measured at 450nm (OD450). The sensitivity and specificity of this anti-Cysticercus antibody ELISA in serum were 30% and 95% respectively.

Statistical analysis:

The collected data was analyzed with IBM SPSS Statistics for Windows, Version 23.0.(Armonk, NY: IBM Corp).To describe the data descriptive statistics frequency analysis, percentage analysis was used for categorical variables and the mean & SD were used for continuous variables. To find the significance of qualitative categorical data Chi-Square test was used. Similarly,if the expected cell frequency was found to be less than 5 in 2×2 tables then Fisher's Exact was used. In all the above statistical tools the probability value of 0.05 was considered significant.

RESULTS

The study showed positive anti-Cysticercus IgG-ELISA results in 15 out of the total 50 cases, whereas only two of the 50 controls were IgG ELISA positive, indicating an overall positivity of 17%.(Table-1)

The Fisher exact test was used in our study to analyze seizure rates among cases and controls, and the results showed that there was a significant statistical correlation of seizure in cases and controls. The most frequent form of seizure in symptomatic NCC patients is a Generalized Tonic-ClonicSeizure (GTCS), which accounts for 60% of all seizures. (Table -2)

In our study, calcified lesions accounted for18 cases (36%), whereas vascular lesions were found in 10% of the cases and active perilesional inflammation was observed in 9% of the cases. No radiological findings were seen in controls.(Table-3)

This study shows the relation between the anti-Cysticercus-IgG and CT findings in cases. CT scan revealed an equal percentage of single vs. multiple lesions in the various regions of the brain. Only 30% of the cases in this study tested Positive for anticysticercusIgG ELISA. (Table-4)

Seizure was the most prevalent symptom in both cases and controls (100%),it was found to be statistically significant by Pearson's Chi-Square test.Headachewas reported in 45%, nausea and vomiting as reported in 30% of the cases.Other symptoms observed were fever (8%), mono paresis (4%), confusion (3%), altered behavior, depression, drowsiness and coma in 1% of cases and controls which was not statically significant, no participants in our study exhibitedparaparesis.Among all the symptoms as quoted above controls had only feverupto 1% only.

Radiological findings and IgG ELISA were found to be statistically significant among cases, (figure-1)

The IgG ELISA showed sensitivity, specificity, PPV, NPV, and accuracy of 30%, 96%, 60.57%, 87%, and accuracy was 63, respectively.(Table-5)

Table-1:Comparison of IgG ELISA among cases and controls by Fisher's exact test

			Groups		Total	χ 2 - value	p-value
			Cases	Controls			
IgG ELISA	Negative	Count	35	48	83	11.977	0.0005 **
		%	70.0%	96.0%	83.0%		
	Positive	Count	15	2	17		
		%	30.0%	4.0%	17.0%		
Total		Count	50	50	100		
		%	100.0%	100.0%	100.0%		
** Highly Statistical Significance at p < 0.01 level.The above table 1 shows comparison of IgG ELISA among cases and controlsbyFisher's exact test were χ2=11.977, p=0.0005<0.01 which shows highly statistical significance.							

Table-2:Comparison of Seizure among cases and controls by Fisher's exact test

			Groups		Total	χ 2 - value	p-value
			Cases	Controls			
Seizure	Focal seizure	Count	34	6	40	32.667	0.0005 **
		%	68.0%	12.0%	40.0%		
	GTCS	Count	16	44	60		
		%	32.0%	88.0%	60.0%		
Total		Count	50	50	100		
		%	100.0%	100.0%	100.0%		
** Highly Statistical Significance at p < 0.01 level. The above table shows comparison of Seizure among cases and controlsbyFisher's exact test were χ2=32.667, p=0.0005<0.01 which shows highly statistical significance.							

Table- 3:Comparison of Radiological finding among cases and controls by Pearson's Chi-Square test

			Groups		Total	χ 2 - value	p-value
			Cases	Controls			
Radiological finding	Calcified lesion	Count	18	0	18	100.000	0.0005 **
		%	36.0%	0.0%	18.0%		
	Colloidal lesion	Count	7	0	7		
		%	14.0%	0.0%	7.0%		
	Granular nodular stage with inflammation	Count	9	0	9		
		%	18.0%	0.0%	9.0%		
	Granular-nodular with inflammation	Count	6	0	6		
		%	12.0%	0.0%	6.0%		
	No lesion	Count	0	50	50		
		%	0.0%	100.0%	50.0%		
	Vesicular lesion	Count	10	0	10		
		%	20.0%	0.0%	10.0%		
Total		Count	50	50	100		
		%	100.0%	100.0%	100.0%		
** Highly Statistical Significance at p < 0.01 level The above table shows comparison of Radiological finding among cases and controlsby Pearson's Chi-Square test were χ2=100.000, p=0.0005<0.01 which shows highly statistical significance.							

Table-4: Correlation between the anti-Cysticercus-IgG and CT findings in cases.

Number of lesions	Lesion type	**No. of cases shows lesion**	No. of sera tested positive by anti-CysticercusIgG-ELISA
Single lesion	Vesicular	**5**	**0**
	Calcified cyst	**09**	**4**
	Granular-nodular with inflammation	**11**	**5**
>1lesions	Vesicular	**5**	**1**
	Calcified cyst	**12**	**3**

	Granular-nodular with inflammation	**3**	**0**
	Colloidal	**5**	**2**
Total	**50**		**15 (30%)**
This table shows that 30% cases turned out to be positive using IgG ELISA whereas radiologically lesions were seen in all cases.			

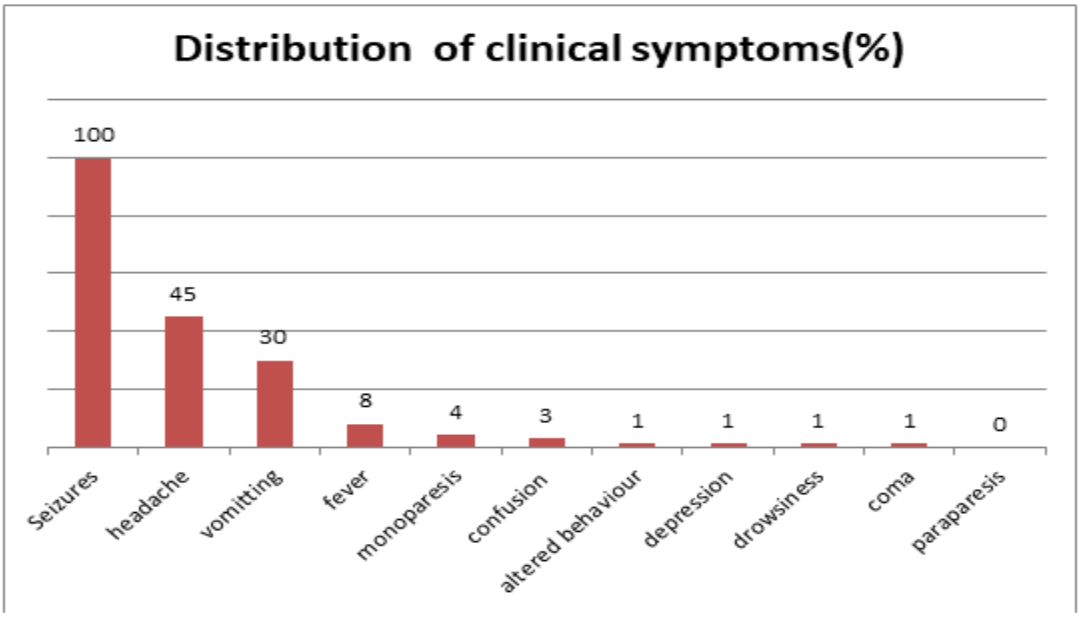

Figure-1 Distribution of clinical symptoms among cases and controls

Table –5: Diagnostic Efficacy of IgG ELISA

	Cases Symptomatic NCC	Healthy controls	Prevalence= 17%
Test results: Positive (NCC ELISA)	True positive 15 (TP)	False positive 2 (FP)	PPV= 60.57%
Negative	False negative 35 (FP)	True negative 48 (TN)	NPV=87%
	Sensitivity= 30%	Specificity=96%	Accuracy= 63%
This table shows the Diagnostic Efficacy of IgG ELISA			

DISCUSSION

NCC is one of the major causes of epilepsy in developing countries. It has varied clinical presentations. Therefore, rapid and reliable diagnostic techniques need to be evaluated for its accurate diagnosis. We studied the role of anticysticercusantibody ELISA (IgG) in diagnosis of NCC to determine its sensitivity and specificity among all suspected patients suggestive of NCC.

The present study contributes valuable insight into the diagnostic role of IgG ELISA in neurocysticercosis (NCC) among patients presenting with seizures in Western Uttar Pradesh. Our findings confirm that seizures, particularly generalized tonic-clonic seizures (GTCS), remain the most common clinical manifestation of NCC. Brain imaging, especially CT, demonstrated characteristic lesions in all cases, while the IgG ELISA test showed a modest sensitivity (30%) but high specificity (96%). This highlights the value of ELISA as a confirmatory tool in radiologically suspected cases of NCC and as a useful exclusion test in ambiguous clinical presentations.

Despite the low sensitivity, serological testing remains advantageous in resource-limited settings due to its non-invasive, cost-effective nature and ease of implementation. Integration of both imaging and serological testing can significantly

improve diagnostic confidence. Notably, calcified lesions were the most frequently observed radiological finding, aligning with emerging Indian data emphasizing post-inflammatory sequelae in endemic populations.

Seizure is the most common clinical manifestation of the disease as also noted in our study in our patients (100%), followed by headache in (45%). In a systematic review among NCC patients in neurology clinics, seizure was found to be the most common clinical feature seen in about 79% of patients followed by headache in 38% and this finding corroborates with our study.[15,16]

ELISA performed on all cases and controls (100) showed seropositivity &seronegativity rates 17% and 83%, respectively and among all the 17 positives 15 were from cases alone while 2 were from controls. A similar study conducted in a university teaching hospital in Odisha State, where patients were recruited from Neurology clinic as well as from Medicine Out Patient Department between September2012 to April 2013 revealed an overall anti-CysticercusIgGELISApositivity of 12% (18 of the total 64 cases).[17,18] And the findings are in accordance with our study. Intapan et.al., (2008) while studying IgG antibodies against cysticerci by ELISA observed 17% seropositivity and 83% of patients tested negative.[18] while Verasteguiet.al.,(2003) noted 20% of cases were seropositive.[19]

However, higher seropositivity is noted by many other authors. Ocanaet.al., (2009) noted a seropositivity of 37.2%.[20]In the present study, CT scans revealed an equal proportion of single and/or multiple lesions in all regions of brain in 50% in cases (25) which is contradictory when compared with many other single lesion-based studies reports from India.[21]

In our study, the calcified lesions were predominant with a percentage of 36%. In a recent study from Odisha, calcified lesions were predominant, accounting for 47.06% of the total patients suspected of NCC.[22]

In other studies, either mixed lesions, or immunologically active lesions were reported to be predominant. Hence, the results of our study were found to be in accordance with findings from other Indian studies.[23]

In our study, ELISA showed sensitivity and specificity of 30% and 96% respectively. A similar study conducted by Samian Kirmani et.al., (2014) revealed sensitivity and specificity of 13.3% and 100% respectively of ELISA.[24]

Shukla et.al., (2008) found a sensitivity of 92% and specificity of 84% of ELISA while studying immunodiagnosis of NCC in definitive cases andKotokeyet.al.,(2006) reported 78.43% sensitivity and specificity of 100%.Thedifference in high sensitivity and high specificity of ELISA as reported by different authors as compared to our study, may be explained by the gold standard used in the studies. The visibility of scolex in the CT scans is an absolute diagnostic criterion and which also denote active lesions, hence this may be the reason of high sensitivity and high specificity.[25]

Mittal et.al.,(2001) found a sensitivity of 10.4%. The low sensitivity observed in our study can also be attributed to false negative serology because of immune tolerance.[26]

Based on the different diagnostic tools for NCC such as neuro-imaging and serological tests, revealed that most of the NCC patients were positive by both methods.[27]However, there were cases where patients showed positive by imaging and negative by serology and vice versa. A possible reason for this discrepancy could be due to the presence of very low levels of antibody/antigen titer detectable by serological tests.[28] Or if the cyst is at the earlier stages of transition or has undergone calcification or cystic lesions due to other parasitic or microbial infections.[29]

Recent literature emphasizes the need for focused and targeted programs that also encourage for stronger public health approach based on equity for prevention, control, and management of epilepsy in India. Our study is the first hospital-based report of NCC cases from Etawahdistrict of Uttar Pradesh in India where the burden of seizures due to NCC was never explored before. We found NCC to be more common in males with low socioeconomic status living in rural areas with GTCS seizures.

Given the endemicity of Taenia solium in rural India, including Etawah district, and the diagnostic dilemmas posed by overlapping clinical symptoms, it is critical that NCC be considered in all cases of new-onset seizures. IgG ELISA testing should be employed in tandem with neuroimaging to guide prompt clinical decision-making, particularly in resource-constrained tertiary centers. Future studies with larger sample sizes, improved molecular diagnostics, and comparative analyses with antigen-based assays are warranted to refine the current diagnostic algorithm for NCC in the Indian context.

Detecting anti-cysticercus IgG antibodies in serum could reveal the potentiality of possible NCC as an underlying cause of recent onset seizures in the studied cases. Although, the CT/MRI scan is a potent diagnostic tool compared to antibody detection, the diagnostic decision can be better made if both modalities are considered together. However, it is recommended to suspect NCC as one of the major differentials and so it should be ruled out in every recent onset seizure

case with or without an imaging-based supportive diagnosis, especially in the regions which are endemic for *T. solium*taeniasis/cysticercosis.

The importance of combining neuroimaging with serological markers for improved diagnostic accuracy. Advanced antigen detection techniques and the inclusion of cerebrospinal fluid (CSF) analysis in future protocols could enhance sensitivity and minimize false negatives, especially in cases with single small lesions or low immune response [30]. Public health interventions, including improved sanitation, pork hygiene, and mass deworming, have also shown promise in reducing NCC incidence in endemic areas and should be actively promoted [31].

CONCLUSION

To conclude, the present study adds information on NCC ELISA test accuracy & its association with clinical-radiological profile of neurocysticercosis from a tertiary care center in North India. Generalized tonic-clonic seizures are the commonest clinical manifestation and single calcified ring enhancement in brain imaging is the commonest radiological feature of NCC. Non-invasive & feasible nature of ELISA-based kit helps in making an early diagnosis of NCC. More so its high specificity plays important role in differentiating these cases from other causes of similar neurological presentation unambiguously. Low sensitivity may be the weakness of this test, however, that can be overcome by testing CSF along with blood for NCC antibodies in future studies. It may prove to be an effective tool for diagnosis of NCC provided we use IgG ELISA along with brain imaging in clinically suspected cases.

DECLARATIONS:

Conflicts of interest: There is no any conflict of interest associated with this study

Consent to participate: There is consent to participate.

Consent for publication: There is consent for the publication of this paper.

Authors' contributions: Author equally contributed the work.

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