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ORIGINAL RESEARCH ARTICLE

CYSTICERCI SEROPREVALENCE AND RISK FACTORS FOR NEUROCYSTICERCOSIS: AN OBSERVATIONAL HOSPITAL BASED STUDY

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ABSTRACT

The serological test, enzyme linked immunosorbent assay for circulating antigen of Taenia Solium (Ag-ELISA) , was carried out in a series of 90 cases of seizure disorders, aged more than 14 years, presented to B. P. Koirala Institute of Health Sciences, Dharan, Nepal between March 2008 to May 2009. Taenia solium antigen were detected in 13 (15%) of the patients. The diagnosis of neurocysticercosis was made in 35% cases (26 out of 75) based on clinical and neuroimaging findings. Neuroimaging was not done in 15 cases. Seropositivity was associated with neuroimaging studies consistent with NCC (OR=13.295% CI 1.43- 305.79, P= 0.014). It is significant for multiple ring enhancing lesions (P= 0.00935 for CT Head and 0.00274 for MRI Head) and not found to be statistically significant for single ring enhancing lesion (P= 0.637 for CT Head and 1.00 for MRI Head). Living in KACHCHA house, family members >5 and age > 60 years have higher odds ratio for positive serology of cysticercosis although there was no statistical significance. The serology for cysticercosis had sensitivity of 41.5% and specificity of 98.4%.

Key Words: *Neurocysticercosis, Risk factor, Seroprevalence*

INTRODUCTION

Epilepsy is a major problem in tropical developing countries.[1] The incidence and prevalence of the disorder in these countries are very high because of high rate of infectious and parasitic diseases.[2] Neurocysticercosis (NCC), caused by infestation of the human central nervous system (CNS) with the tissue cyst of *Taenia solium*, is the most common parasitic disease of the human nervous system.[3]

T. Solium exists worldwide but is most prevalent in Latin America, Sub-Saharan Africa, China, Southern and Southeast Asia and Eastern Europe. Cysticercosis occurs in industrialized nations largely as a result of the immigration of infected persons from the endemic areas.[4] Hospital based studies in Nepal and elsewhere suggests that NCC of the human CNS by *T. solium* is the main cause of late onset epilepsy in most developing countries. This has been confirmed by field studies and a close relationship between human and porcine infection.[5, 6]

Given the fact that *T. solium* is endemic in Eastern part of Nepal where cohabitation with the pigs is quite common in some of the ethnic communities. Though neuroimaging studies are used for the diagnosis of NCC, they are costly, not available in many part of the world where the disease is prevalent and does not always provide definitive result. Serological tests would help to solve some of these problems. Hence it is decided to study the frequency of cysticercosis in epileptic patients attending B. P. Koirala Institute of Health Sciences using the enzyme linked immunosorbent assay for antigen (Ag-ELISA).

MATERIAL AND METHODS

The observational study was conducted from March 2008 to May 2009 at B. P. Koirala Institute of Health Sciences, Dharan, Nepal. Patients aged above 14 years and having history of at least one episode of seizure episode within a month were eligible. Seizures occurring owing to traumatic brain injury were excluded. Cases were recruited from the medical ward, emergency department, medical OPD & Psychiatry OPD of BPKIHS. All cases were evaluated, investigated and treated as per standard guidelines and the clinician's judgment. The investigator enrolled the cases after verbal consent obtained from the patient or guardian in cases of minors.

Special investigations like CT scan and MRI head were done as per requirement and feasibility.

The serum samples were collected by the investigator in a glass vial using universal precaution and stored at -20°C temperature in refrigerator of the institution after centrifugation. The collected samples were transported to National Zoonoses and Food Hygiene Research Centre,

Kathmandu, Nepal for the serological test. The test was done free of cost to the patients.

It is a monoclonal based sandwich ELISA using monoclonal antibodies against excretory –secretory products (ES products) of living *T. saginata* cysticerci. This immunoassay can be used to monitor the evolution of circulating ES-products during the course of infection. It is used in diagnosis of the presence of living metacestodes of *Taenia* sp in human, porcine or bovine serum samples.

The serology for the circulation cysticercosis antigen (excretory–secretory product) was performed using sandwich ELISA technique. It has a maximum sensitivity of 85% in cases of multiple lesions and 65% in cases of single viable cyst or only enhancing lesions. The manufacturer indicated the possibility of cross reactivity with the serum from patients with other taeniids only.

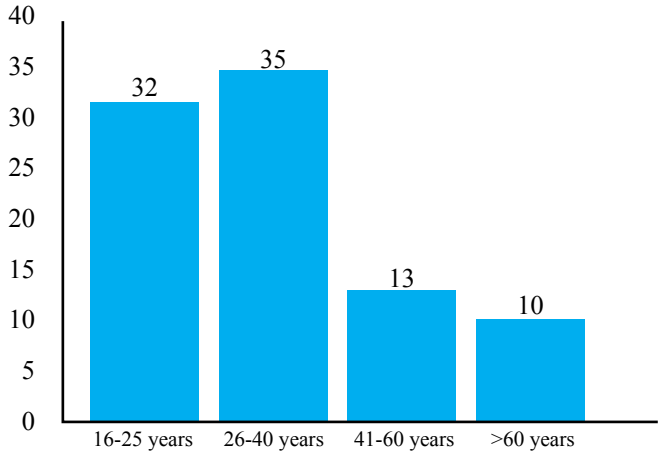
STATISTICAL ANALYSIS

All clinical and laboratory parameters were recorded in the proforma. The data were entered into Microsoft Excel 2003 spread sheet. Data were analyzed using SPSS (PC+) version 13.0. Test of significance for continuous variables was assessed by linear by linear association and for categorical test by Chi-square or Fisher's Exact Test. Test for association for risk factors for seropositivity for cysticercosis was done.

RESULTS

Out of 90 cases, 51 were recruited from medical ward or emergency department and the rest (39) were recruited from medical or psychiatry OPD. The Male: Female ratio was 1.19:1. The age ranged from 16 to 78 years with a mean of 34.60 years ±16.96.

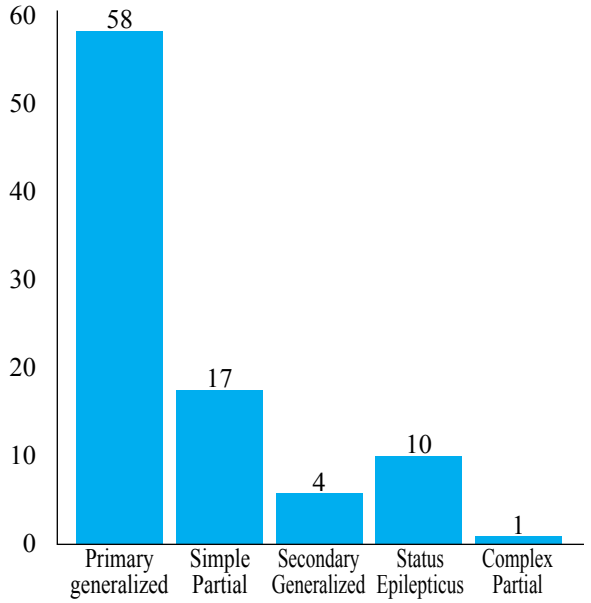
Fig 1: Age distribution of the 90 seizure cases



The cases came from 13 different districts of Nepal and one case came from of India (**Table 1**). As expected the majority of the cases were from Sunsari 37.7% (33/90) followed by Jhapa 20% (18/90) and Morang 17.8% (16/90). However there were cases also from far and remote districts such as Taplejung, Tehrathum and Bhojpur.

The mean family size was 6.36 (SD 2.64) with 7 families having more than 10 members. Sixty one cases (67.8%) were married. The type of houses was as follows: 44.4% KACHCHA house, one third (33.3%) corrugated sheet roof and 21% in PAKKA house made up with pillars. The source of water in 63.3% (57/90) households was deep tube-well, 28.9% (26/90) use piped water provided by the government and rest 7.8% (7/90) had to depend upon surface water for their source of water. Only 57 (63.3%) households had toilet facilities in their homes. The majority (92.2%) were non-vegetarians and 41 (45.6%) consumed pork. Pig rearing was seen in 34 (37.8%) households. The pattern of seizure is shown in fig 2.

Fig 2: Specific types of seizure in 90 seizure cases



The majority of cases (81.1%) had normal physical examination. Baseline investigations are shown in the **table 1.**

Table 1: laboratory investigations in 90 seizure cases

Tests	Mean	SD	Minimum	Maximum
Hb gm/dl	12.303	2.3	5.9	20.9
TLC/ cu m m	13266.67	6734.55	2500	43500
Neutrophil %	74.27	11.38	43	93
Lymphocyte %	23.09	11.46	5	55
Monocyte %	2.64	4.14	0	15
RBS mg/dl	116.97	51.12	40	338
Urea mg/dl	35.56	40.69	10	271
Creatinine mg/dl	1.305	2.094	0.1	18.5
Na+ mEq/L	138.82	7.03	114	162
K+ mEq/L	4.172	4.1	2.5	7.6
Albumin gm/dl	5.356	5	2.7	8
SGOT IU/dl	109.17	29	18	1323
SGPT IU/dl	132.87	28	18	1629

Radio imaging was done in 75 (83.3%) cases: CT scan (Head) in 51(56.67%) and MRI (Head) in 24 (26.67%). Findings of CT scan and MRI are shown in Table 2. Commonest CT abnormality was multiple ring enhancing lesions (25.49 %) and in MRI single ring enhancing and multiple ring enhancing lesion were seen in equal frequency. The other diagnosis included tuberculoma, CNS tumours and infarcts. CT/MRI was normal in only 41.33 % (31/75) of cases.

Table 2: CT Scan/ MRI (Head) findings in 51 seizure cases

CT/MRI findings	No of cases			
	CT Scan (N= 51)		MRI (N=24)	
Normal	21	41.17%	10	41.67%
Single ring enhancing lesion	7	13.73%	3	12.5%
Multiple ring enhancing lesion	13	25.49%	3	12.5%
Calcified granuloma	6	11.76%	0	0%
Other diagnosis	4	7.84%	8	33.33%

The cysticercosis antigen- ELISA test was done on all 90 serum samples and it was positive in 13 samples (15%). In one of the seropositive case, neuroimaging was not done. Seropositivity was assessed under various categorical variables and appropriate test of significance was applied (Table 3). Significance was seen only those with abnormality in CT scan.

In the 13 (15%) of cases of neurocysticercosis defined by the serology, association of risk factors was analyzed by univariate analysis, (Table 4). None of them were seen to be significant although odds ratio (OR) was higher for age > 60years, family size > 5 members and consuming pork.

Table 3: Variables and seropositivity in 90 seizure cases

S.N.	Variable	Categories	Serological test		P value	Remarks
			Positive	Negative		
1	Data Source	Admitted	6	25	0.88	Non-significant
		OPD	7	32		
2	Sex	Male	7	42	0.962	Non-significant
		Female	6	35		
3	Marital Status	Married	8	53	0.603	Non-significant
		Unmarried	5	24		
4	Dietary habit	Non-veg	12	71	0.99	Non-significant
		Vegetarian	1	6		
5	Toilet facility	No	30	3	0.359	Non-significant
		Yes	47	10		
6	Rears pig	Yes	8	48	0.956	Non-significant
		No	5	29		
7	Consumes pork	Yes	9	32	0.120	Non-significant
		No	4	45		
8	Status epilepticus	Yes	4	13	0.258	Non-significant
		No	9	64		
9	Old or newly diagnosed	Old	8	40	0.521	Non-significant
		New	5	37		
10	Treatment	No	10	49	0.530	Non-significant
		Yes	3	28		
11	Compliance	No	1	8	0.428	Non-significant
		Yes	2	20		

S.N.	Variable	Categories	Serological test		P value	Remarks
			Positive	Negative		
12	Family history of seizure	Yes	1	11	1.000	Non-significant
		No	12	66		
13	Neuro-cutaneous marker	No	0	1	1.0	Non-significant
		Yes	13	76		
14	Urinary abnormality	Yes	5	29	0.956	Non-significant
		No	8	48		
15	CT Scan	Normal	0	21	0.015	Significant
		Abnormal	8	22		
		Total Done	8	43		
16	MRI Scan	Normal	1	9	0.615	Non-significant
		Abnormal	3	11		
		Total Done	4	20		

Table 4: Risk factors for seropositivity univariate analysis

Variable	Sero-positive (n=13)	Sero-negative (n=77)	Total (n=90)	OR (95%CI)	P value
Female	6	35	41	1.03 (0.27-3.83)	0.962
Male	7	42	49	Referent	
Age>60 yrs	**3**	**7**	**10**	**3 (0.51-16.36)**	**0.313**
<=60 yrs	10	70	80	Referent	
Family members >5	**9**	**41**	**50**	**1.98 (0.5-8.43)**	**0.283**
<=5 members	4	36	40	Referent	
Living in kaccha house	2	17	19	0.64 (0.09-3.57)	0.857
Not living in kachcha	11	60	71	Referent	
Drinking surface water	**1**	**6**	**7**	**0.99***	**0.584**
Drinking tubewell or piped water	12	71	83	Referent	
Eats Non-veg diet	**12**	**83**	**7**	**1.01***	**0.734**
Veg diet	1	7	83	Referent	
Not toilet facility	**3**	**30**	**33**	**0.47 (0.09-2.08)**	**0.43**
Have toilet	10	47	57	Referent	
Rears pig	**8**	**48**	**56**	**0.97 (0.25-3.81)**	**0.959**
Doesn't rear pig	5	29	34	Referent	
Consumes pig	**9**	**32**	**41**	**3.16 (0.79-13.55)**	**0.12**
Doesn't take pork	4	45	49	Referent	

The imaging studies and seropositivity was analyzed. Neuroimaging abnormalities suggestive of NCC were significantly associated with seropositivity. (Table 5).

Table 5: Neuroimaging findings in seizure cases who have gone for imaging

Variable	Seropositive (n=12)	Seronegative (n=33)	Total (n=45)	OR (95% CI)	P value
CT/MRI with single or multiple lesion	11	15	26	13.2 (1.43-305.79)	0.014
CT/MRI normal or other diagnosis	1	18	19	Referent	

*Confidence limit invalid to calculate

Association was looked for neuroimaging studies for single and multiple lesions separately for CT scan and MRI. (Table 6). Those with multiple lesions in neuroimaging (both for CT scan and MRI) were statistically significant with seropositivity.

Table 6: Specific finding in neuroimaging and seropositivity

S.N.	Neuroimaging	Sero-positivity		OR (95% CI)	P value	Remark
		+	-			
CT scan	Single lesion	1	6	0.38 (0.01- 4.55)	0.637	Not significant
	Multiple lesion	7	6	18.67 (1.59- 502.18)	0.00935	Significant
	Calcified granuloma	0	6	*	0.155	Not significant
	Other diagnosis	0	4	*	0.550	Not significant
MRI scan	Single lesion	0	3	*	1.000	Not significant
	Multiple lesion	3	0	*	0.00274	Significant
	Calcified granuloma	0	0	*		
	Other diagnosis	0	8	*	0.055	Not significant

* Confidence limit invalid to calculate

Taking the clinical and radiological findings for making a diagnosis of NCC, we looked at the validity of the serological test. The Ag-ELISA test had a sensitivity of 41.5 % and specificity of 98.4 %. (Table 7).

Table 7. Sensitivity and specificity of the serological test:

			NCC (according to clinical and radiological diagnosis)		Total
			+	-	
Serology	+		12	1	13
	-		14	63	77
	Total		26	64	90
PPV	NPV	LR+	LR-	Sensitivity	Specificity
92.3%	81.8%	25.93	0.59	41.5%	98.4%

DISCUSSION:

The present study was carried out to study the seroprevalence of cysticercosis in adults presenting with seizure to B. P. Koirala Institute of Health Sciences, Dharan, Nepal. The serological test for the E-S product of cysticercosis using Ag- ELISA was performed in all 90 consecutive cases. The various socio-demographic and clinical profiles were studied and risk factors associated with seropositivity was evaluated.

In the cysticercosis, the diagnosis can be difficult. A consensus conference has proposed absolute, major, minor and epidemiological criteria for the diagnosis.[4] Diagnostic certainty is possible only with definitive demonstration of the parasite (absolute criteria). This task can be accomplished by histological observation of the parasite in excised tissue, by the fundoscopic visualization of the parasite in the eye (in anterior chamber, vitreous, or subretinal space) or by neuroimaging studies demonstrating cystic lesion containing the characteristic scolex. In most cases, diagnostic certainty is not possible. Instead a clinical diagnosis is made on the basis of a combination of clinical presentation, radiographic studies, serologic tests and exposure studies.[4]

Various assays have been developed for the detection of *T. solium* antigen in serum or cerebrospinal fluid with variable result.[7] The present study was based on the monoclonal antibody based enzyme linked immunosorbent assay designed to detect the presence of excretory-secretory antigen from viable parasite. There are many studies done in various countries based on serology either for antigen or for antibody in community surveys or hospital setting with variable results. In a serological study undertaken in 3 rural Venezuelan communities (Conoabo, Sanare and Rio Tocuyo) using trapped ELISA to detect a secreted product of viable parasite showed the circulating parasite antigen of 9.1 % in Canoabo, 6.1 % in Sanare and 5.7% in Rio Tocuyo.[8] In a study from 3 rural localities in the West and Northwest provinces in Cameroon, *T. solium* antigen was detected in the sera of 1.2% of the patients.[9] In another study in Viet Nam done in 1999 had seropositivity of 5.7% of the community including all 5 persons with epilepsy for detecting circulating antigen.[10]

The study which detected antibodies to *T. solium* cyst antigen showed higher prevalence of cysticercosis. For instance, seroprevalence to detect antibodies was reported 36.5% in Canoabo, 36.5% in Sanare and 4% in Rio Tocuyo in Venezuela.[8] Specific antibodies against the parasite were detected in 44.6% of the patients in Cameroon.[9] Antibodies against a specific form of antigen were detected in 15.2% of patients in the study done in Brazil.[11]

Similarly the seroprevalence of human cysticercosis antibody was reported 8% in Peru, 10.8% in Mexico and ranged 10% to 17% in Guatemala.[12-14]

The differences between Ag-ELISA and Ab-ELISA could be because Ag-ELISA detects only living cysts whereas Ab-ELISA detects dead cysts as well. However, antibody detection has 2 important limitations. Firstly it may indicate only exposure to infection and not necessarily the presence of established, viable infection and, secondly, antibody may persist long after the parasite has been eliminated through the immune mechanism and/or drug therapy.[15, 16] In the former case, the presence of antibodies to *T. solium* in a patient with neurological symptoms living in an endemic zone may result in false diagnosis of neurocysticercosis and delay the search for other pathological condition. In the latter, antiparasitic therapy

may be unnecessarily indicated since the parasite may not be viable.

The studies done in hospital setting with those presented with seizure disorders also vary in their seropositivity rate. In a study in Brazil, 6% of the patients who attended an ambulating clinic in the district of Mulungu do Morro, had the circulating parasite product as tested by capture ELISA.[11] In a case control study for epilepsy in Burundi, Ag- ELISA revealed 38.3% positivity in epilepsy cases.[17]

Study of 1038 randomly selected cases of epilepsy in Chandigarh showed the cysticercosis hemaagglutination test to a useful adjunct in the diagnosis of cysticercosis as an etiological factor. It was positive in 25.7% epileptic cases but in only 2% of healthy controls.[18] The higher rate of seropositivity compared to the present study is because they used hemaaglutination test to detect antibody against cysticercosis.

In a hospital based study done in Nepal during the period of 2002- 2006 the occurrence of neurocysticercosis was 13.34% of epileptic admission episodes in 6 hospitals by using EITB.[19] The present study showed seroprevalence of 15% using antigen-ELISA in the adults presenting with seizures. The seropositivity rate is higher in our study even though we have used Ag-ELISA which is a better indicator of active infection than antibody based assay. Serological test using antibody may only indicate exposure to infection and antibody may persist long after the parasite has been eliminated. It appears that cysticercosis prevalence is much higher in Eastern Nepal. Further community based larger studies are needed to establish true seroprevalence. These variability's in seroprevalence rates are contributed by various factors depending on presence or absence of various risk factors in different communities including poverty, pork consumption, poor pig husbandry practices, hygiene standard and customs like consumption of raw pork in some communities. For example, it is almost non-existing in Japan and Singapore due to increasing economic prosperity and accompanying infrastructure; while in others such as the Islamic countries of the Middle East and West Asia, religious proscription of the consumption of pork had made a similar result.[20]

The present study revealed that 11 of 26 (42.3%) of patients with seizure and active lesions in neuroimaging (CT or MRI) were seropositive. This is less than seropositivity specified in the literature which revealed 85% with multiple lesions and 65% in single lesion. This could be because almost a third (30%) patient had single ring enhancing lesion which may have low antigen detection rate. The seropositivity was relatively high (62.5%) in patients with multiple lesions.

No significant difference was observed between the prevalence in males and females in the present study. It showed no statistical significance of the risk factors including dietary habit, source of water supply, presence of toilet facility, type of house and co-habitation with pigs although higher odds ratio was observed for family size of more than 5 members, age > 60 years and those consuming pork. This may have been due to smaller sample which was a limitation in our study. It might also be because neurocysticercosis is transmitted by feco-oral route and infective stage may have been harboring in the community.

On the basis of clinical and imaging findings, the serological test was found to have sensitivity of 41.5% and specificity of 98.4% with the positive predictive value of 92.3% and negative predictive value of 81.8%. So, in the settings where imaging facilities are not available a positive serological test could qualify to start the treatment for NCC. In conditions of negative serological test but if NCC is suspected on clinical grounds then neuroimaging is advocated and could be the reason for referral for neuroimaging. Caution has to be taken while starting antiparasitic therapy without neuroimaging as there may be extraparenchymal lesion(s) though the percentage for so is low. These can be associated with intense inflammatory reactions and complications.

Thus the antigen ELISA test may have stronger implication in clinical decision making in cases of clinically compatible NCC with neuroimaging unavailable or inconclusive. Further large scale studies are required to establish the true seroprevalence and verifying the strength of the test.

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