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TO STUDY THE ROLE OF IMMUNODIAGNOSIS IN NEUROCYSTICERCOSIS

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ABSTRACT: Neurocysticercosis is the most common parasitic infection of the central nervous system (CNS) worldwide. The study was conducted in the Department of Microbiology, Rama Medical College, Hospital and Research Centre, Kanpur over a period of 12 months. The study group comprised of 56 patients attending OPD/IPD who were radiologically (CT and/or MRI) diagnosed as a case of Neurocysticercosis.⁷ The investigations done were CT scan and/or MRI and Serum ELISA for IgG antibody to *T. solium*. The *T. solium* specific IgG antibodies in patient's sera were assessed using the commercially available ELISA kit, IVD Research, Inc, Carlsbad, CA 92008. Twenty two patients (39.28%) out of 56 patients were vegetarians while 34 (60.72%) were non-vegetarians. None of the patients was a pork-eater. Sensitivity of serum ELISA for IgG antibodies was found to be 48.22%. Serum ELISA was positive in 27 (48.21%) out of 56 CT/MRI proven cases of NCC showing a 100% positivity in cases of calcified lesions and 45.28% in cases of active lesions. More seropositivity (72.72%) was seen in multiple lesions as compared to single lesions (42.22%).

KEYWORDS: *Taeniasolium*, Neurocysticercosis (NCC), ELISA.

INTRODUCTION: *Taeniasolium*, commonly called the pork tapeworm, has been known from the time of Hippocrates. The name *Solium* has been derived from the latin word "Solus" meaning 'Solitary' because usually only a single worm is found in infected persons. The infestation by *T. solium* was differentiated from that caused by *Taeniasaginata* by Kuchenmeister and Leuckart.¹

Humans are the definitive hosts for *Taeniasolium* which harbours in the small intestine.² Faecal shedding of eggs usually leads to ingestion of eggs through contaminated water or food by an intermediate host, typically human or pig. Once inside the intestine, the eggs are released and produce primary larvae that enter the circulatory system. Hematogenous spread to muscular, ocular and neural tissue then occurs. Once inside the brain, the primary larvae develops into secondary larvae, the cysticerci. Clinical manifestations of Neurocysticercosis (NCC) are varied and non-specific, related to number, age, location of the cysts and the accompanying host immune response. The parenchymal cysts may remain dormant for many years, and symptoms usually coincide with larval death and subsequent intense inflammatory reaction induced by larval antigens. Subsequently, the cyst transforms into a granuloma. The cyst then shrinks and granuloma eventually calcify or more frequently disappear completely. The most common manifestations of NCC are seizures, headache, behavioural disturbances and signs and symptoms of increased intracranial pressure.³

An accurate diagnosis of NCC requires interpretation of clinical and epidemiological data plus neuroimaging studies and specific serological tests. Computerized Tomography (CT) and Magnetic Resonance Imaging (MRI) findings in parenchymal NCC depend on the stage of involution of cysticerci.

Immunodiagnosis has the great advantages of lower cost than CT or MRI and presence of specific anticysticercus antibodies may confirm the disease. Enzyme Linked Immuno Sorbent Assay

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(ELISA) has a high sensitivity between 75-90% with serum and cerebrospinal fluid (CSF) ELISA gives better results. The main disadvantage of ELISA is cross reactivity with other helminthic infections since the antigen employed is a crude homogenate of cysticerci or vesicular fluid. Enzyme Linked Immuno Electro Transfer Blot (EITB) overcomes cross reactivity because it employs an enriched fraction of glycoproteins. When two or more cysticerci are present, blotting is 100% sensitive and 100% specific with serum samples.⁴

NCC is the most common parasitic infection of the central nervous system (CNS) worldwide. An estimated 50 million people are infected and 50,000 people die annually as a result of this disease.⁵ It accounts for 11% of hospital admissions and 35-45% of all neurological procedures in certain endemic areas.⁶

MATERIALS AND METHODS: The study was conducted in the Department of Microbiology, Rama Medical College, Hospital and Research Centre, Kanpur over a period of 12 months. The study group comprised of 56 patients attending OPD/IPD who were radiologically (CT and/or MRI) diagnosed as a case of Neurocysticercosis.⁷

Detailed case history of every patient was taken with special emphasis on the type of diet (vegetarian/nonvegetarian), type of seizures, any focal neurological deficit etc. Twenty healthy people were included as a control group for ELISA.

The investigations done were:

1. CT scan and/or MRI.
2. Serum ELISA for IgG antibody to *T. solium*.

RADIOLOGICAL STUDY: CT scan was performed using a Siemens Somatom ARC machine taking 5 mm and 10 mm axial cuts as per requirement. Both plain and contrast enhanced images were obtained. MRI was done with a Siemens megatom vision machine of 1.5 Tesla as and when required. T1 and T2 weighted images were obtained.

SEROLOGICAL STUDY:

Blood in all these 56 Cases was taken as follows: Three to four ml of patient's blood was collected by venipuncture using aseptic techniques. Blood was allowed to clot at room temperature. The clot was removed and plasma was centrifuged to separate out serum. Serum was stored in a sterile vial at - 20° C in a freezer.

The *T. solium* specific IgG antibodies in patient's sera were assessed using the commercially available ELISA kit, IVD Research, Inc, Carlsbad, CA 92008. The test was performed strictly according to the instructions of the manufacturer.

Samples and controls (positive and negative) were set up. Long interruptions were avoided between each step of the assay procedure. The plate was immediately read with an ELISA reader set for bichromatic readings at 450/ 650-620 nm. ELISA reader used was Anthos HT-II. The assay was considered valid if: O.D of the positive control was greater than 0.35 O.D units at 450/650 - 620 nm. O.D of the negative control was less than 0.35 O.D units at 450/650 - 620 nm. The observations in our study were evaluated in comparison to radiologically proven cases of NCC.

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RESULTS:

Age Group (yrs)	Total no. of patients	No. of seropositive patients	%
≤ 10	17	07	41.18
11- 20	21	10	47.62
21-30	10	07	70.00
31-40	05	01	20.00
41-50	02	01	50.00
> 50	01	01	100.00
Total	56	27	

Table 1: Correlation of seropositivity on ELISA with age

It was noted that seropositivity was 50% (24/48) in age group less than 30 years with maximum positivity (70%) in age group from 21-30 years. Serological positivity was 37.50% (3/8) in age group above 30 years.

Religion	Number (%)	Male (%)	Female (%)
Hindu	48(85.72%)	31(64.58%)	17(35.42%)
Muslim	08(14.28%)	07(87.50%)	01(12.50%)
Total	56	38	18

Table 2: Religion wise distribution of patients

Out of 56 patients, 48 patients (85.72%) were Hindus while 08 patients (14.28%) were Muslims. Amongst 48 Hindu patients, 31 patients (64.58%) were males and 17 patients (35.42%) were females whereas out of 08 Muslims patients, 07 patients (87.50%) were males and only one patient (12.50%) was a female.

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Dietary Habit	Number	%
Vegetarian	22	39.28
Non-Vegetarian	34	60.72
Total	56	

Table 3: Dietary habits of patients

Twenty two patients (39.28%) out of 56 patients were vegetarians while 34 (60.72%) were non-vegetarians. None of the patients was a pork-eater.

Result of serum ELISA	No. of patients	%
Positive	27	48.22
Negative	29	51.78
Total	56	

Table 4: Results of Serum ELISA for IgG antibodies to T. Solium

Sensitivity of serum ELISA for IgG antibodies was found to be 48.22%.

Antibody levels (O. D Units)	Number	%
< 0.35	29	51.78
0.35 – 1.00	19	33.93
1.01 -2.00	05	8.93
2.01 -3.00	02	3.58
> 3.0	01	1.78

Table 5: IgG antibody levels to T.solium

Majority of the reactive samples showed an O.D between 0.35–1.00 (33.93%) and only 3 above 2.00 O.D.

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CT/ MRI Findings	Number	ELISA	
		Antibody +ve No. (%)	Antibody -ve No. (%)
Active lesions			
Single	44	18(40.90)	26 (59.09)
Multiple	09	06(66.67)	03 (33.33)
Total	53	24(45.28)	29 (54.71)
Calcified Lesions			
Single	01	01(100)	00
Multiple	02	02 (100)	00
Total	03	03 (100)	00
TOTAL	56	27(48.21)	29 (51.78)

Table 6: Correlation of CT Scan/MRI findings with serum ELISA

p> 0.05.

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Serum ELISA was positive in 27 (48.21%) out of 56 CT/MRI proven cases of NCC showing a 100% positivity in cases of calcified lesions and 45.28% in cases of active lesions. More seropositivity (72.72%) was seen in multiple lesions as compared to single lesions (42.22%).

DISCUSSION: NCC is difficult to diagnose; neuroimaging is a very important tool for the diagnosis of disease but it is expensive. The design of a simple and dependable immunodiagnostic test in serum has long been sought as it would help medical and epidemiological studies.

The most common age group affected in our series was between 11-20 years (37.5%) followed by patients less than 10 years of age (30.35%). Majority of patients were under 30 years of age (85.71%). Other Indian workers have also reported a higher incidence of 78%⁸ in the age group 11-20 years and 46% in the age group below 15 years.⁹ Seropositivity was also higher (47.62%) in the 11-20 years age group, same has been noted by Sharma P et al¹⁰ from Chandigarh.

Males (67.86%) were more commonly affected than females (32.14%). Other workers have also noted NCC more commonly in males.^{10,11}

Poor hygiene in children and open air defaecation in rural areas allows pig's access to human faeces thus putting people at risk of developing taeniasis/cysticercosis. Chances of autoinfection in children are also higher. A higher occurrence in males than females could be because females are usually less willing to seek medical advice than males.

Though sporadic cases have been reported from all over India, prevalence of NCC appears to be higher in the states of Haryana,¹⁰ Uttaranchal¹¹ Punjab,¹² Uttar Pradesh,¹³ Delhi,¹⁴ and Pondicherry.¹⁵ NCC has not been commonly reported from Mumbai (western) and Kolkata (eastern) regions.¹⁶

Serum ELISA for IgG was positive in 27 cases showing a sensitivity of 48.22% and a specificity of 100%.

Our study is more comparable to a study done by R.Biswas¹⁵ showing a sensitivity of 43.75% and specificity of 98%. G Singh et al¹² have also shown a sensitivity of 48% with a similar commercially available ELISA kit as was used in our study.

Variation in sensitivity and specificity in different studies may be due to different kits, using different types of antigen (Ref. Table 2) and also the type of antibody being detected.

Accuracy of serological tests relies on the highly variable humoral immune response of the host against cysticerci. False negative results in serum (As compared to radiologically confirmed cases) are related to the local production of antibodies in the CNS without a parallel increase of antibodies in peripheral blood or due to immune tolerance to the parasite without production of anticysticercal antibodies. However as also seen in our study, patient compliance for obtaining CSF samples is very poor especially since clinical condition in very few of the patients was serious enough to warrant lumbar puncture.

Importance of serum ELISA cannot be overemphasized as a diagnostic tool in areas of high endemicity.

Serum ELISA was positive in 27(48.22%) out of 56 CT/MRI proven cases of NCC showing a 100% positivity in cases of calcified lesions and 45.28% in active lesions. This difference was not found to be statistically significant ($p>0.05$).

Several studies have shown poor seropositivity in calcified lesions.^{17,18} An apparently higher seropositivity in calcified lesions in our study may be because these cases were very few and a larger

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study group needs to be evaluated to arrive at a proper conclusion. Secondly, there may have been other viable but asymptomatic or otherwise undetected cysticerci leading to higher seropositivity.

More positivity (66.67%) was seen in multiple active lesions as compared to single active lesions (40.90%). The same has been reported by Espinoza et al.¹⁹

CONCLUSION: The detection of 56 cases of NCC and its association with seizures during a short period of 12 months definitely indicates a need to reduce and eliminate this preventable and treatable infection in this state. Serum ELISA is rapid, simple and easily available in clinical laboratories. Detection of specific antibodies in patients with NCC is a useful tool for diagnosis, especially when CT/MRI are not available or inconclusive. Serological tests are useful if they are positive. Negative tests do not exclude the diagnosis of NCC. Serological tests can be used as an initial noninvasive test for diagnosis. Serological tests using serum may serve as a logical alternative not only for screening and diagnosis but also for carrying out epidemiological studies on a large scale.

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