



## Original Research Article

# Detection of Neurocysticercosis by Comparing the Radiological Changes in CT Imaging and ELISA Test using ES Antigen

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## ABSTRACT

### Keywords

Neuro-cysticercosis, ELISA, Neuro-imaging, IgG antibodies

Neurocysticercosis (NCC) is a clinical condition in humans, which infects brain caused by a encysted larvae, cysticercoid of *Taenia solium*. NCC is common disease but its diagnosis remains problematic. Suspected cases of Neurocysticercosis were underwent CT/MRI neuroimaging and also blood samples were collected. Enzyme Linked Immunosorbent Assay (ELISA) used for detection of IgG antibodies in serum samples using Excretory-Secretory (ES) antigen. Positivity for IgG antibodies seen in 59 patients about 71.9%. ES antigen was positive in 4(44.4%) of 9 patients with live cysts, 18 of 61 patients (29.5%) with degenerative cysts and only 1 of 6 patients ( 16.6%) with calcified cysts. Maximum positive results on immunological tests were obtained when the number of lesions were high, when the lesions were in contact with CSF and when there were live cysts. Comparative study of Radioimaging and ELISA gives better results and helpful in early diagnosis and accurate treatment.

## Introduction

Neurocysticercosis (NCC) is a clinical condition in humans, which infects brain caused by a encysted larvae, cysticercoid of *Taenia solium*. *Taenia solium* is a cestode which is commonly called as Pork tapeworm. Cysticercosis is one of the most important of the parasitic disease of the nervous system, because of its worldwide distribution, high incidence and variety of symptoms and signs it produces.

The tapeworm that causes Cysticercosis is endemic to many parts of the world including China, Southeast Asia, India, Saharan Africa, and Latin America<sup>1</sup>. Some

studies suggest that the prevalence of cysticercosis in Mexico is between 3.1 and 3.9 percent. Other studies have found the Seroprevalence in areas of Guatemala, Bolivia and Peru as high as 20 percent in humans and 37 percent in pigs in Ethiopia, Kenya and Democratic Republic of Congo around 10 percent of the population is infected, in Madagascar 16 percent. The frequency has decreased in developed countries owing to strict meat inspection, better hygiene and better sanitary facilities.<sup>2</sup>

Both adults and cysts are pathogenic. The cyst, with cellulosae are more pathogenic,

which cause Cysticercosis. Most cysts produced in skin, skeletal muscles, eye and CNS. In the brain, the cysts survive by overcoming host defenses. Cysticercosis generally affects adults and rarely children. NCC is the leading cause of adult-onset epilepsy and is probably one of the most frequent causes of childhood epilepsy in the world.

NCC is common disease but its diagnosis remains problematic. Neuroimaging studies are usually abnormal but in most cases, not pathognomonic, immunological tests have been developed to support the diagnosis. During recent meeting on NCC held in August 2000 in Lima, Peru, a panel of experts agreed upon more accurate and stringent revised criteria for the diagnosis of NCC<sup>3</sup>.

Its prevalence is still underestimated because of lack of diagnostic facilities in rural areas where the reported cases represent only the tip of the iceberg. Hence this study has undertaken to know the prevalence of Neurocysticercosis and also to compare the radioimaging and ELISA diagnostic modalities which are routinely used to detect Neurocysticercosis.

### **Materials and Method**

This is a randomized study conducted for one year as a prospective analysis in 2011 at NRI medical college. Informed consent has been taken from the patients before doing the diagnostic procedures and the ethical committee has approved to do this study.

All the suspected cases of Neurocysticercosis as per diagnostic criteria<sup>3</sup> from medicine, surgery, paediatric departments were subjected to CT/MRI Neuroimaging at the radiology department.

Blood samples were collected from all Neurocysticercosis patients by a sterile needle in labeled test tubes under aseptic precautions and then centrifuged to obtain serum, and stored at -4 degree centigrade until reuse<sup>4</sup>. All samples were collected before administering the therapy. All samples were considered as potential biohazards and were handled with care using universal precautions. By using excretory secretory (ES) antigen, ELISA was done on serum samples of all patients and IgG antibodies were estimated (Cysticercosis test).

Patients' complaints were noted and were carefully examined for subcutaneous nodules, muscle hypertrophy, levels of sensation and focal neurological deficits. In all patients complete hemogram with absolute eosinophil count, CSF analysis, chest X-ray, Mantoux tests were done.

The results of all diagnostic works were evaluated and tabulated.

### **Results and Discussion**

A total of 82 blood samples were collected from patients suspected of Neurocysticercosis (NCC). All the samples were subjected to ELISA test.

Among 82 patients, males were shown higher preponderance affected with Neurocysticercosis about 62.1% than females. Most commonly affected age group was young adults (15-25 years) about 43.9% followed by 26-40 years (25.6%).

Symptomatology assessment was done among NCC patients (Table No:1). Based on these proposed criteria (Del Brutto et al)<sup>3</sup>, diagnosis of NCC in our study group was definitive in 48 patients (58.5%) and probable in the remaining 34 patients (41.4%).

Out of 82 patients, 9 patients (10.9%) had subcutaneous nodules (multiple in seven and single in two). 16 patients (19.5%) had one or other focal neurological defects, 7 (8.5%) had bilateral papilloedema, 5 (6%) had reduced visual acuity, 3 (3.6%) had hemiplegic and one had unilateral third nerve paralysis.

Hemogram study shown that 40 patients (48.7%) had hemoglobin level less than 12 g/dl, of these in 11 (13.4%) patients hemoglobin level was less than 10 g/dl. 23 patients (28%) showed leukocytosis (>11,000). Absolute eosinophilic count was > 500/c.mm in 42 patients (51.2%). In 49 patients (59.7%) ESR was > 20 mm / 1<sup>st</sup> hour and in 13 patients (15.8%) ESR was more than 40mm/1<sup>st</sup> hour.

CSF study shown that most of the patients with absent or mild pleocytosis presented with seizures and those who had significant pleocytosis had chronic meningitis like presentation. In 38 patients (46.3%), there was CSF pleocytosis (>5 cells/c.mm). CSF protein was 41-60 mg% in 27 (32.9%) patients and more than 60 mg % in 8 (9.7%) patients. Maximum CSF protein observed was 190 mg%. CSF sugar was low, < 40 mg% in 3 (3.6%) patients. In 43 (52.4%) patients CSF sugar was between 40-60 mg%. In 26 (42.6%) patients CSF sugar was > 60 mg%. The lowest CSF sugar observed was 10 mg%.

ELISA has performed to detect IgG antibodies using Excretory Secretory antigen on serum samples. Positivity for IgG antibodies seen in 59 patients about 71.9% (Table No.2).

Cysts in brain after diagnosed by CT or MRI imaging in total of 82 patients, categorized as degenerating cysts, live cysts and calcified cysts. Their positivity has depicted in Fig.No.1

Parenchymal changes were noted in Suspected cases of Neurocysticercosis. Edematous changes in brain noted as a significant factor on CT scan (Fig.No:2).

On repeat imaging of Neurocysticercosis cases among 45 patients about 54.8%, most of the cysts in brain were disappeared, few were in same size or calcified (Fig.No.3).

Out of 82 patients, 20 patients (24.3%) cysts were in frontal region, in 46 patients (56%) in parietal region and in 22 patients (26.8%) lesions were widespread.

Out of 82 patients, 28 patients (34.1%) cysts were located closer to CSF (10 near cortical surface, 5 near ventricular surface and 7 near both cortical and ventricular surface), 55 (67%) patients cysts were located away from CSF flow.

Neurocysticercosis (NCC) is a pleomorphic disease, although it sometimes produces no clinical manifestation. The pleomorphism is due to parasitic factor like number of vesicles, their size and multiplicity of location, their toxic qualities and the possibility of immunological reaction on penetration of oncospheres. In terms of host there may be difference in the severity of the immune response to the parasite. Neurocysticercosis is an extremely rare entity. Control of disease in human depends on the control of the disease in animals.

NCC is more common in the age group of 15-25 years and more preponderance in males (62.1%) in this study. Cysticerci generally affects adults and rarely children. However Lefever-et al<sup>5</sup> reported a predominance of patients below the age of 20 years and some even below the age of 10 years. Escalante<sup>6</sup> found that more than 50 percent patients were males, where as Stepien<sup>7</sup> found a higher incidence among

women. These variations may be differences in food habits and hygienic practices in different communities.

In this study, majority of the patients (79.2%) presented with seizures. Focal motor with secondary generalization was the commonest seizure type. Seizures were also the most common symptoms in the series reported by Del Brutto et al<sup>3</sup> Rajasekhar V<sup>8</sup> and Chowdary. G.V.S<sup>9</sup> being seen in 70%-90% of the patients. Headache and associated vomiting was the second commonest symptoms seen in 45% of the patients in our study. Gracia HH<sup>10</sup> et al found that in 52% of this patients with NCC had headache.

In general disseminated cysticercosis was more common in Asian patients with NCC compared to others<sup>11,12</sup>

In the present study serum was selected for ELISA testing using ES antigen to detect IgG antibodies against NCC. Because measuring IgG antibodies in Serum was more sensitive and specific than using CSF as per different studies<sup>2,13,14</sup>.

As per this study, among 57 patients who had single CT lesions, ELISA was positive for serum in 38 patients (66.6%) with excretory – secretory antigen. Subbarao V Atluri<sup>15</sup>, P.Singhi<sup>16</sup>, Kandelwal N<sup>17</sup>, Malla N et al<sup>18</sup> found positive anticysticercal antibody in serum against ES Antigen among single cysts diagnosed patients. Similar low immunological positivity in patients with single cysticercal cyst is seen in other studies as well. Sotelo et al<sup>19</sup> found that immunological positivity depends on site and number of the cysticercal lesions, Khandelwal N<sup>17</sup> found that antibody titre correlated with number of CT lesions. Positivity was maximum when there were

live cysts and minimum when there were only calcified cysts. Espindola NM et al<sup>20</sup> found that 94% of patients harbouring live cysticercal cysts had IgG antibody in contrast to 44% of patients harbouring calcified cysticerci.

In the present study the positivity of ELISA is compared with Stages of larvae by CT imaging. Positivity was maximum when there were live cysts and minimum when there were only calcified cysts. ES antigen was positive in 4(44.4%) of 9 patients with live cysts, 18 of 61 patients (29.5%) with degenerative cysts and only 1 of 6 patients (16.6%) with calcified cysts. However this was not statistically significant.

Of the 4 parameters (number of CT lesions, stage of larva, proximity to CSF lesion and clinical syndrome) only the relation between proximity of lesion to CSF and positivity of ELISA was statically significant (p value < 0.05).

Thus immunological tests are of only limited value in establishing the diagnosis of NCC in general, but important in selected group of patients. In cysticercal meningitis, imaging is normal in most of the cases and diagnosis usually depends on exclusion of other common conditions. In such cases ELISA is very useful in making a diagnosis. The yield of positive result is high in patients with multiple lesions. Hence ELISA is useful in patients with multiple cystic lesions without definite scolex where the possibility of tuberculoma, microabscess and metastatic lesions cannot be excluded by imaging. Since the positivity is high with lesion closer to CSF surface, ELISA is useful in determining the nature of such lesion even when the number of lesions are few.

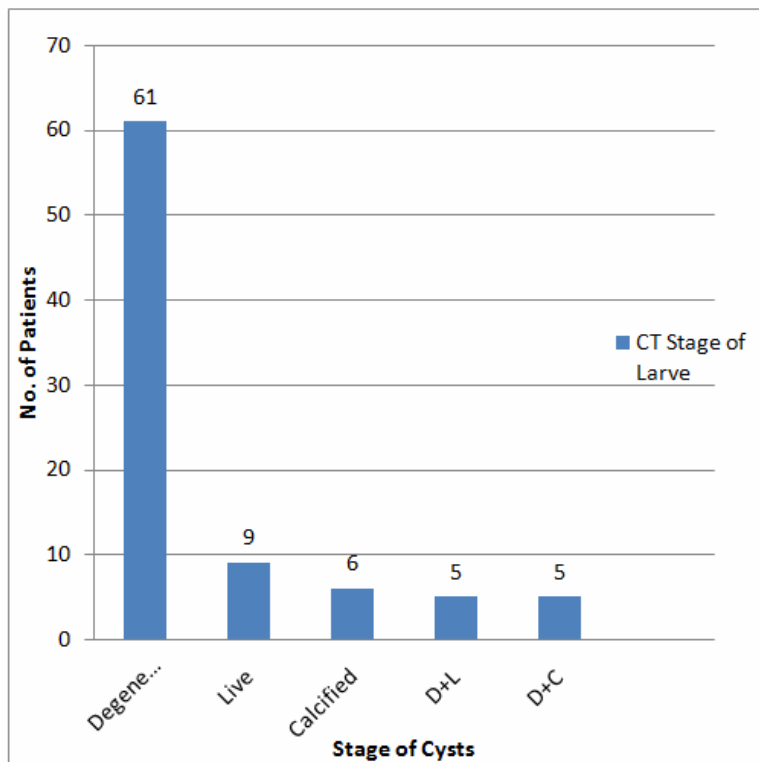
**Table.1** Showing Percentage of Various Symptoms among NCC Patients

Symptoms	No. of patients	Percentage
Seizures	65	79.2%
Headache	39	47.5%
Vomiting	33	40.2%
Visual disturbance	23	28%
Fever	22	26.8%
Hemiplegic	3	3.6%
Behavioral abnormalities	3	3.6%

**Table.2** Distribution of Serum Positive Cases in Both Single and Multiple Cysts

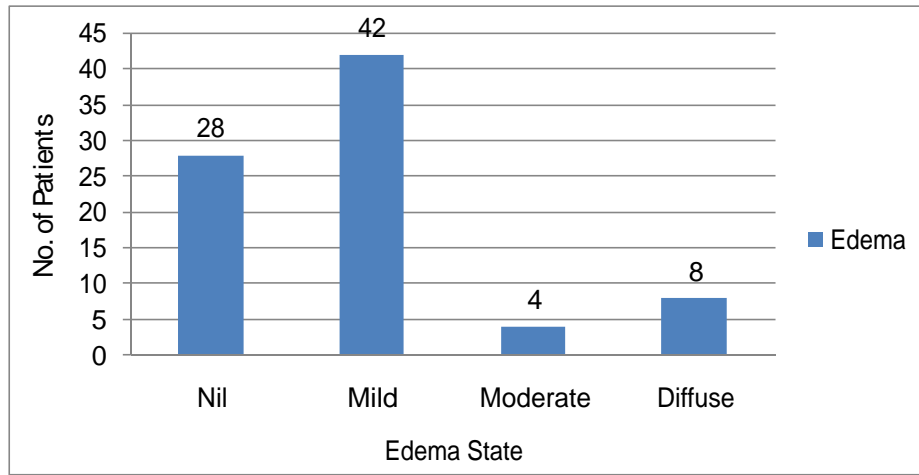
		Serum	Percentage
Single	Positive	38	66.6%
	Negative	19	33.3%
	Total	57	69.5%
Multiple	Positive	22	88%
	Negative	3	12%
	Total	25	30.4%

**Fig.1** Distribution of Data According to Stage of Larva

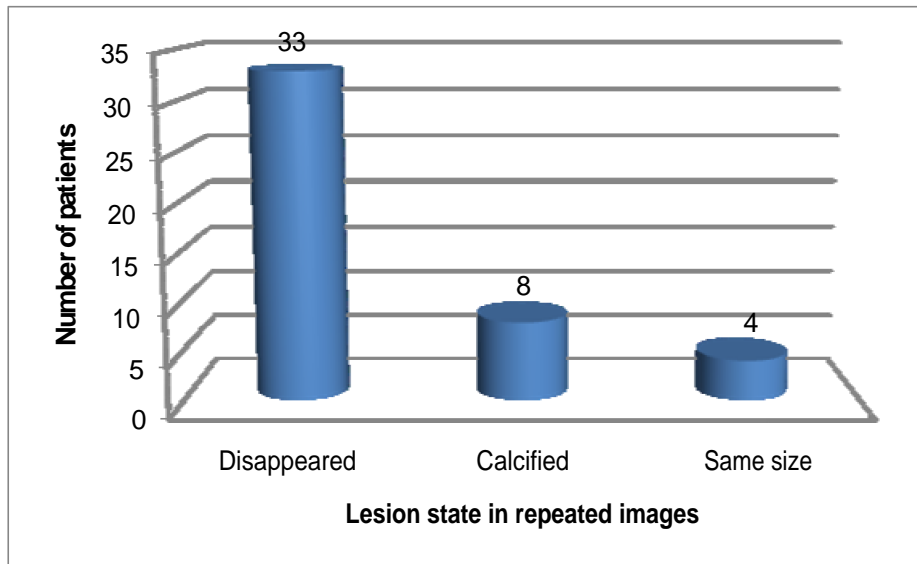


D-Degenerating; L-Live; C-Calcified

**Fig.2** Distribution of Data According to Edema State



**Fig.3** Distribution of Data According to State of Lesions in Repeated Images



Maximum positive results on immunological tests were obtained when the number of lesions were high, when the lesions were in contact with CSF and when there were live cysts. High levels of personal hygiene and prevention of faecal contamination with foods also play a major role in preventing the disease. Even though CT / MRI, EITB are important modalities for the diagnosis of NCC cases, they may not be always

available in small equipped laboratories and small hospitals in rural areas. In such situations ELISA can be preferred to CT /MRI and EITB for the detection of cysticercal antibody in serum samples. ELISA has become increasingly popular as field test also. Comparative study of Radioimaging and ELISA gives better results.

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## Reference

- 1.Harrison, U., Correa, D., Sandoval, MA et al.1989. Human neurocysticercosis: Comparison of enzyme immunoassay capture techniques based on monoclonal, polyclonal antibodies for the detection of parasite products in cerebrospinal fluid. *Transr Soc Tropo Med Hyg.* 22:471-7.
- 2.Bucardo, F et al. 2005. The seroprevalence of *Taenia solium* cysticercosis among epileptic patients in Leon, Nicaragua, as evaluated by ELISA and western blotting. *Annals of Tropical Medicine and Parasitology.* 99(1):41-45.
- 3.Del Brutto, OH and Sotelo, J. 1998. Neurocysticercosis: an updates. *Reviews of Infectious Diseases.* 10(6):1075-87.
4. Parija, SC.2004. Textbook of medical Parasitology. 2nd edition. Madras, AIPD., 205-235.
- 5.Del Brutto, OH., Noboa, CA., Lefever. 1991. late onset epilepsy in Ecuador: aetiology and clinical features in 225 patients. *J Trop Geogr Neurol.* 1:31-34.
- 6.Escalante, S. 1973. Cisticercosis. I. Epidemiología y clínica Consideraciones anatómicas. II. Cisticercosis Porcina. Tesis Doctoral, Universidad Peruana Cayetano Heredia, Lima.
- 7.Stepien, L. 1962. Cerebral cysticercosis in Poland. *J Neurosurg.* 19:505-13.
- 8.Rajasekhar, V., Chandy, MJ. 1997. Validation of diagnostic criteria for solitary cerebral cysticercus granuloma in patients presenting with seizures. *Acta Nourol Scand.* 96:76-81.
- 9.Chowdary, GVS., Murthy, JMK., Vijay, S et al. 2004. Prevalence of seizure disorders associated with neurocysticercosis: a community based study comprehensive rural epilepsy study – Oceanic congress of Neurology, Singapore.
- 10.Gracia, HH., Martinez, RH., Gilman, T., Herrera, G., Tsang, VCW., Pilcher, JB., Diaz, F., et al.1991. The Cysticercosis working Group in Peru-Diagnosis of cysticercosis in endemic regions. *Lancet.* 338:549-551.
- 11.Del Brutto, H., Sotelo, J., and Roman, G.1998. Neurocysticercosis: A Clinical Handbook, Swets & Zeitlinger, Lisse, The Netherlands. 23-45.
- 12.Wadia, NH., Singh, G.2002. *Taenia Solium: A historical Note-Taenia Solium Cysticercosis: From Basic to Clinical Science*, CABI Publishing. 157-168.
- 13.Nussenzweig., Rosas, N., Sotelo, J., and Nieto. 1986. ELISA in the diagnosis of neurocysticercosis. *Arch Neurol.* 43:353 – 356.
- 14.Diwan, JF., Coker-Vann, P., Brown, D., Subianto, B., Yolken, R., Desowitz, Ret al. 1982. Enzymelinked immunosorbent assay (ELISA) for the detection of antibody to cysticerci of *Taenia solium*. *Am J.Trop.Med Hyg.*31: 364-369.
- 15.Atluri Subba Rao, V et al. 2009. NCC immunodiagnosis using *T.solium* cysticerci crude soluble extract, excretory, secretory and lower molecular mass antigens in serum and urine samples of Indian children.

- Acta Tropica. 110(1):22-27.
- 16.Singhi, PD., Jain, V., Khandelwal, N. 2004. Corticosteroids Versus Albendazole for Treatment of Single small Enhancing Computed Tomographic Lesions in Children with Neurocysticercosis. *J. Child Neurol.*19: 323 – 27.
  - 17.Khandelwal, N., Mandal, J., Singhi, PD and Malla N. 2006. Evaluation of ELISA and dot bolts for the serodiagnosis of neurocysticercosis, in children found to have single or multiple enhancing lesions in computerized tomographic scans of the brain. *Ann Trop Med Parasitol.* 100: 39-48.
  - 18.Malla, N., Kaur, R., Ganguly, NK., Sawhney, IM and Mahajan, RC. 2005. Utility of specific IgG4 response in saliva and serum samples for the diagnosis and follow up of human neurocysticercosis. *Nepal Med College J .* 7: 1-9.
  - 19.Sotelo, J., Del Brutto, OH and Roman, G. 2002. *Neurocysticercosis: A Clinical Handbook*, Swets & Zeitlinger, Lisse. The Netherlands. 457.
  - 20.Espindola, NM., Fernandes, I., Takyanagui, OM., Machado Ldos, R., Livramento, JA., Mendes Mala, AA. et al. 2005. Cysticercosis immunodiagnosis using 18- and 14 kilodalton proteins from *Taeniacrassiceps cysticercus* antigen obtained by immunoaffinity chromatography. *J.Clin. Microbiol.* 43:3178 -3184.