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Original Article

Microbiology Section

Seroprevalence of Cysticercus Antibodies in Japanese Encephalitis Patients in Upper Assam, India: A Hospital Based Study

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ABSTRACT

Introduction: Co-infection of Japanese Encephalitis (JE) and Cysticercosis is attributed mainly to the common epidemiological features between the two diseases. Not much is known about the clinical implications of one infection over the other.

Aim: The study aimed at establishing whether JE-Cysticercosis co-infection is prevalent in the Upper Assam districts and to explore additional details about such co-infections both clinically and epidemiologically.

Materials and Methods: The present study was a retrospective cross-sectional hospital based study conducted between July 2013 and June 2014 and included 272 Acute Encephalitis Syndrome (AES) patients. Out of this, 137 JE positive and 135 non-JE Acute encephalitis patients were taken as cases and controls respectively. The diagnosis of JE and Cysticercosis was established by ELISA.

Statistical Analysis: EpiInfo ver. 7 was used for statistical analysis. Chi-square was used and p-value < 0.05 was considered to be statistically significant.

Results: The association of Cysticercosis with JE was found to be statistically significant (14.6%, $p = 0.0019$) in the cases with reference to the controls (3.7%). Moreover, the co-infections were found to be more common in case of adults (19.32%, $p = 0.0360$); with males having a greater odds (5.25, $p = 0.0008$) of harbouring the parasite as compared to females.

Conclusion: The study proves that the association of Cysticercosis and JE holds true in this region.

Keywords: Acute encephalitis syndrome, Elisa, Taenia solium

INTRODUCTION

Japanese Encephalitis (JE) and Cysticercosis share quite a few epidemiological features and their co-infection has already been described in literature [1,2]. The state of Assam, especially the Upper Assam districts of Dibrugarh and Sibsagar have a high incidence of JE, especially during the monsoons [3].

Pig rearing is a common practice and a source of livelihood in these endemic areas of Assam. Pigs are an integral part of the natural life cycle of both JE and Cysticercosis. In JE, they act as the amplifying host for the virus and in case of the tapeworm *Taenia solium* which causes Neurocysticercosis, pigs harbour the larval forms known as *Cysticercus cellulosae*. With nearly 55.15% of the rural population in these areas involved in pig rearing [4], there is a high probability that these two organisms may co-exist in the indigenous population.

In 2013, as many as 1388 Acute Encephalitis Syndrome (AES) cases were reported from Assam out of which 495 were diagnosed as laboratory confirmed JE cases. The figures increased further in 2014 with 2194 AES cases out of which 761 were confirmed JE cases [5]. Only Uttar Pradesh and West Bengal have reported a higher number of AES cases in India. So far, JE has been detected in as many as 21 states in India with Uttar Pradesh, West Bengal, Assam and Bihar reporting the highest number of cases over the years. The virus is widely prevalent in most parts of south central, northern and northeast states of India. Still, a part of Maharashtra and states like Gujarat, Rajasthan and Madhya Pradesh are free from JE [6].

This study was undertaken with the sole objective of exploring whether this hypothesis holds true in the JE endemic zone of Upper Assam. This might throw light on the dynamics of this complex disease in other endemic areas such as most parts of south central, northern and northeast states of India [6] as well as abroad [7]. Knowledge of prior co-infection might also help in predicting the severity of disease, thus serving as a prognostic indicator.

MATERIALS AND METHODS

The present study was a retrospective cross-sectional hospital based study which included 272 Acute Encephalitis Syndrome (AES) patients admitted in our hospital. Out of these, 137 JE positive patients (including 50 male and 87 females) and 135 age and sex matched non-JE AES patients were taken as cases and controls respectively. Patients of all age groups and both the sexes admitted in our hospital during July 2013 to June 2014 were included in the study according to the clinical case definition of AES as per WHO guidelines [8]. Clinically, a case of AES is defined as a person of any age, at any time of year, with the acute onset of fever and a change in mental status (including symptoms such as confusion, disorientation, coma, or inability to talk) and/or new onset of seizures (excluding simple febrile seizures). Other early clinical findings can include an increase in irritability, somnolence or abnormal behaviour greater than that seen with usual febrile illness [8]. Cases presenting with similar clinical features but which did not satisfy the WHO case definition were excluded from the study. The study was carried out according to the guidelines of the Institutional Ethics Committee (Human) of the hospital. As the samples used in the study were archived, anonymised samples, written informed consent from the patients or their guardians was not deemed necessary. The samples (CSF and serum) had originally been collected according to standard protocol as part of the ongoing AES surveillance program under the National Vector Borne Disease Control Program (NVBDCP), Govt. of India. For this study, the samples were anonymised after removing all identifying features.

The diagnosis of JE was established using the JE specific IgM ELISA kits manufactured by NIV, Pune and supplied by the NVBDCP under the AES surveillance program. An Optical Density (OD) value ≥5 times the negative control (NC) was considered to be positive and an OD value that was ≤ 1 of the negative control was considered to be negative. Samples which had an OD value 1-5 times of the NC were considered to be equivocal and were not included in the study.

The presence of *Taenia solium* IgG antibodies was confirmed by the Ridascreen *Taenia solium* IgG ELISA kit manufactured by R-Biopharm, Germany. Samples whose OD value exceeded the OD value of the cut-off point (calculated by adding 0.15 to the average OD of the two negative controls) by a factor of 1.1 were considered positive and which were less than 0.9 times the cut-off were considered to be negative. Equivocal results were those in between and were excluded from the final analysis.

Data analysis was done using Epi-Info ver.7.0. Chi-square p-value < 0.05 was considered to be statistically significant.

RESULTS

In the study population of 272 AES patients, JE specific IgM ELISA revealed 137 positive cases and 135 JE negative controls. Out of the 137 JE positive cases, 20 cases (14.6%) were found to be positive for *Taenia solium* IgG antibodies, indicating that they had been previously exposed to the parasite. In the JE negative AES cases (n=135) which constituted the control population, the prevalence of *Taenia solium* IgG antibodies was found to be 3.7% (5 positive cases out of 135). The association of *Taenia solium* antibodies was found to be statistically significant (p=0.0019) in the JE cases as compared to the non-JE AES controls with the odds of developing JE being as much as 4.44 times higher amongst those who had a previous exposure to *Taenia solium* [Table/Fig-1].

	JE Positive (n=137)	JE Negative (n=135)	Odds Ratio	95% CI	p-value
With Cysticercosis	20	5	4.44	1.6166-12.2189	0.0019
Without Cysticercosis	117	130			

[Table/Fig-1]: Prevalence of Cysticercus antibodies in JE positive and JE negative cases.

Clinical/ Demographical features	With Cysticercosis (n=20)	Without Cysticercosis (n=117)	Odds Ratio	95 % CI	p-value
Age ≥ 15 y	17	71	3.6714	1.0185-13.2341	0.036
Age < 15 y	3	46			
Male	14	36	5.25	1.8671-14.7620	0.0008
Female	6	81			
Change in mental status	15	91	0.8571	0.2847-2.5801	0.7838
No change in mental status	5	26			
With Seizure	5	53	0.4025	0.1373-1.1801	0.0895
Without Seizure	15	64			

[Table/Fig-2]: Differences in clinical and demographical features in JE positive cases with and without Cysticercosis.

The peak incidence for JE during the study period was observed in the month of July'13. Considering the demographical features amongst the JE positive cases with and without prior exposure to the parasite, it was found that the adult age group had a higher rate (19.32%) of exposure as compared to the paediatric (6.1%) age group [Table/Fig-2] and the difference was statistically significant (p=0.036). Even amongst the sexes, males had a higher odds (5.25) of having been previously exposed to the parasite and subsequently developing JE as compared to the female population and this was also statistically significant (p=0.0008).

Two clinical features, viz. change in mental status and presence or absence of seizures were also examined amongst the exposed and non-exposed group in JE positive cases to look for any significant differences [Table/Fig-2]. But none of them proved to be statistically significant. Fever was not considered as it was universally present in all the cases and controls which were included as per the WHO AES guidelines.

DISCUSSION

The co-infection of Cysticercosis, especially Neurocysticercosis with JE has been shown in different autopsy based [1], serological [2] and imaging [9] studies by different researchers in both India and abroad. However, their findings are diverse and there is no uniformity in the rates of detection of the two conditions in different geographical regions. This may be due to variations in the local epidemiological and demographical characteristics in the respective study regions as well as variations in population and viral dynamics.

The present study demonstrates that the co-existence of Cysticercosis and JE in this endemic region is not a chance occurrence. However, there are a few differences as evident from studies carried out elsewhere. The overall rate of detection of IgG antibodies to *Taenia solium* in JE positive cases was found to be 14.6% which compares favourably with a study carried out by Handique et al., in Assam [9]. However, Desai et al., found this number to be substantially higher (37.42%), which may be explained by the fact that the study setting was different (Karnataka which is also endemic for JE, especially the southern regions) and they had used all three modalities of investigation (autopsy, serology and CT imaging) for detection of Cysticercosis [2].

The higher rate of prevalence of anti-cysticercal antibodies in the adult population (19.32% vs 6.1%) is in contrast to the findings of Handique et al., and Kalita et al., [9,10]. Historically however, Cysticercosis has been shown to primarily involve the adult population who have a greater chance of being exposed to the parasite. The incidence of JE in Assam has also shown a shift in the trend of the disease from the paediatric to the adult population [3] in recent times and hence the finding that co-existent infections are more common in the adult population may in fact be a valid one.

Neurocysticercosis has been shown to be a poor prognostic marker in cases of viral encephalitis [2]. The immunological response against *T. solium* by an infected host is also diverse and involves multiple immune effectors at the level of the central nervous system [11] which may influence the pathogenesis of encephalitis caused by neurotropic viruses. Intestinal parasitic infections, and especially neurotropic parasites have been shown to cause generalised immune-suppression and disruption of the blood-brain barrier, thereby facilitating easy entry of the JE virus leading to fulminant encephalitis and higher mortality rates [1,2,12]. However, since the cases in the current study were completely anonymised, it was not possible to evaluate the outcome in all the cases. Moreover, many other socio-economic, demographic and immunological parameters have to be taken into consideration in this region before arriving at a conclusion regarding the prognostic significance of Cysticercosis co-infection in JE cases. This is because poor socio-economic status, malnutrition and immunosuppression are risk factors for both the conditions. Now that the hypothesis regarding JE – Cysticercosis co-infection [1,2,9,13,14] in this region seems valid, the direction of future studies in this population group should be to explore the immunological response of the infected host in the setting of JE

– Cysticercosis co-infection with a view to understand how one pathogen influences another and if one can serve as a prognostic indicator for the other.

LIMITATIONS

The current study used only serology for diagnosis of Cysticercosis and this might have been a limiting factor. The use of additional modalities for diagnosis such as radiological imaging and autopsy findings (in case of deceased patients) will probably increase the sensitivity of detection of co-infection. Another drawback of the study was the inability to determine the long term outcome of the patients with JE – Cysticercosis co-infection. This might have thrown some light on the prognostic significance of these cases with co-infection. However, this can be addressed with the help of future studies carried out to assess the long term implications of JE – Cysticercosis co-infection.

CONCLUSION

Nevertheless, the current study shows that Cysticercosis co-infection in JE cases is prevalent in Upper Assam. It might therefore be prudent to include Cysticercosis, especially Neuro-Cysticercosis (NCC) in the differential diagnosis of AES in this region. The policy makers should make provisions to include *Taenia solium* IgG serology in the AES surveillance program carried out under the National Vector Borne Disease Control Program (NVBDCP). This will help in detecting hitherto undetected cases of NCC and also help in prediction of disease severity.

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