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BRIEF COMMUNICATION

Cysticercus ANTIBODIES AND ANTIGENS IN SERUM FROM BLOOD DONORS FROM PONDICHERRY, INDIA

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SUMMARY

The aim of the present study was to screen the serum of blood donors, which are apparently healthy and residing in Pondicherry or its neighboring districts of Tamil Nadu State, for specific detection of *Cysticercus* antigens and antibodies. A total of 216 blood samples were collected from blood donors at the Central Blood Bank, JIPMER Hospital, Pondicherry, India during January and February 2004. Enzyme-linked immunosorbent assay (ELISA) was used to demonstrate anti-*Cysticercus* antibodies and the Co-agglutination (CoA) was used to detect antigen in sera. 14 (6.48 %) males were positive for either anti-*Cysticercus* antibodies or antigens. Of these eight sera were positive for anti-*Cysticercus* antibodies and six were positive for antigens. Results of the present study show that serum *Cysticercus* antigen detection may be a useful adjunct to antibody testing for seroprevalence studies of cysticercosis in the community. The present study is the first kind of study, carried out to determine both cysticercal antibodies as well as antigens in the serum samples collected from the healthy blood donors.

KEYWORDS: Cysticercosis; Blood donors; ELISA; Co-Agglutination test; Seroprevalence.

Taeniasis and cysticercosis caused by the cestode *Taenia solium* are widely prevalent in human and porcine hosts in many developing countries of Latin America, Africa and Asia. It has been estimated that *T. solium* infections affect 50 million people and approximately 50,000 succumb to this disease worldwide every year[8]. Human cysticercosis caused by *Cysticercus cellulosae*, the larval cysts of *T. solium*, results from the ingestion of food, water and vegetables contaminated with *T. solium* eggs or even by autoinfection in case of persons with intestinal taeniasis. For each person with intestinal taeniasis, there are thought to be 10 or more persons infected with the cystic stage of the parasite[13]. The central nervous system (CNS) is the most common site of severe symptomatic infection where the condition is referred to as neurocysticercosis (NCC).

In India, the cases of cysticercosis including the NCC has been documented from various parts of the country and is increasingly related to the poor hygienic conditions especially in the slum dwellers and in the certain pockets of the rural area. Most of the data are based on the hospital-based studies, related to the clinical cases of NCC being admitted in the hospitals for treatment. Diagnosis of these cases, especially at the tertiary hospitals being made by imaging methods such as CT and MRI; and frequently supplemented by serological tests. Hospital based studies on autopsy of the brain documenting the cases of NCC have also been documented from different parts of India including Pondicherry[18,20]. However, population prevalence study on human cysticercosis is less frequently reported from the country.

The aim of the present study was to screen the serum of blood donors who are apparently healthy and residing in Pondicherry or its neighboring districts of Tamil Nadu state for specific *Cysticercus* antigens and antibodies using immunological techniques developed and evaluated in this laboratory.

A total of 216 blood samples were collected from blood donors randomly at the Central Blood Bank, JIPMER Hospital, Pondicherry during January and February 2004 (The total number of donors we have received at our hospital blood bank was 446 in the study period). The median age of donors was 37 years (ranging from 18 years to 55 years) and number of male donors (95.37%) were predominant over females (4.62%). The serum was separated and stored at –20 ^{0}C until used.

ELISA was performed as per the procedure described by standard method of CROWTHER with few modification[4]. Briefly, 1 µG protein per 100 µL concentration of the antigen was prepared in antigen coating buffer (PBS pH 7.2) as per the method described earlier[17] and used for

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coating plates (NUNC) followed by blocking non-specific binding sites by 2% BSA in PBS (pH 7.2). Optimum dilution (1:50) of test serum sample and known *Cysticercus* antibody-positive serum samples were prepared in sample dilution buffer were added in duplicate in the wells and goat anti-human-IgG-HRP conjugated secondary antibody (Bangalore Genei, India) was added to each well for detection. Substrate solution prepared freshly by adding 6 mG of OPD in 10 mL of PBS pH 7.2 and 10 µL of H_2O_2 (30%) was added just before to use. The reaction was stopped by adding 50 µL of 2N H_2SO_4 in each well to avoid over reaction and development of optimum colour. The absorbance was taken at 492 nm in an ELISA reader (TEKAN).

Various controls were used for validity of the assay such as antigen blank, antibody blank, negative control serum, and known positive control serum. Serum sample which gave OD_{492} value of more than *cut-off* (mean of 50 negative serum samples + 2 SD) was considered as positive. The cut-off used in the present study was 0.157.

The Co-agglutination (Co-A) test to detect *Cysticercus* antigen in the serum was carried out as per the method described by us before[9]. In this test Cowan's strain of *Staphylococcus aureus* rich in Protein A (SAPA) cells, sensitized with polyclonal antibodies (hyperimmune serum against *C. cellulosae*) were used to detect specific *Cysticercus* antigen in the serum. The hyperimmune serum was used at the concentration of 100 µL/ 10 mL of SAPA cells suspension (1%) as the optimal sensitizing dose (OSD).

The test was performed on a glass slide, which was marked by a pencil into two equal halves. 25 µL of the test serum was placed on each half of the slide. An equal volume (25 µL) of sensitized SAPA cell suspension (2%) was added to the test serum on one half. The same volume (25 µL) of a suspension of unsensitized SAPA cells 2% was added to the serum on another half of the glass slide as a cell control. The slide was then rotated manually for two min and then observed. In positive Co-A tests, the presence of *Cysticercus* antigen was detected by the formation of large visible clumps of bacterial cells within two min and clearing of the suspension in the test specimen. In a negative reaction, no visible clumps were observed. Agglutination with the sensitized cells and not with the unsensitized cells was considered to be a positive reaction (Fig. 1). It is a qualitative test, so there is no such cut-off used. Appropriate controls were put with each test. With each batch, known positive and negative serum controls were tested.

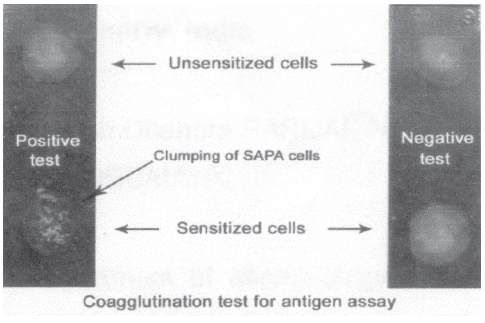

Fig. 1 - Antigen detection by Co-A: (a) test serum positive for antigen showing clumping with cells sensitized with anti-*Cysticercus* antibody but no clumping with unsensitized cells; and (b) test serum negative for antigen showing no clumping with either sensitized cells or unsensitized cells.

From the 216 studied subjects, screened for the *Cysticercus* antigens and antibodies, 206 (95.3%) were male and 10 (4.62%) female. Of these 14 males were positive for either *Cysticercus* antibodies or antigens, which accounts 6.48% (14 out of 216) of the total number of donors. The median age of 14 seropositive blood donors was 28.35 years (range from 19 to 36 years). No serum sample from female donors was positive. ELISA demonstrated reactive *Cysticercus* antibody titre in eight serum samples collected from blood donors. The Co-A detected *Cysticercus* antigen in six (2.77 %) serum samples out of total 216 blood donors.

The seroprevalence of anti-*Cysticercus* antibodies in general population has been found to vary from 1.2% to 21% as seen in several endemic countries[5,7,11,12,13,19]. In a study carried out in Brazil, IgG antibodies against total saline extract of *C. cellulosae* was detected in blood donors where the 4.7%, 4.8%, 5.0% and 13.5% serum samples were found to be positive for anti-*Cysticercus* antibodies in four different cities. But in this study the serum samples were not screened for *Cysticercus* antigens[16]. Similarly in another serological study in Portugal a total of 489 individuals including the blood donors and patients receiving treatment in the departments of orthopedics, neurology and psychiatry, were tested for anti-*Cysticercosis* antibodies; the overall seropositivity rate for *Cysticercus* antibodies among these individuals was 12.1%. *Cysticercus* antibodies was detected in 14.9% of the blood donors and patients from the department of orthopedics, in 11.5% of the patients from the department of neurology, and 7.6% of the patients from the department of psychiatry[21].

In the present study 6.48% (14 out of 216) serum samples were positive for either *Cysticercus* antibodies or antigens. Donors showing a positive test, either by antigen or antibody assay were from Villupuram (8.51%), Cuddalore (13.33%) and Thiruvanamalai (18.75%) districts, that are immediately neighboring to Pondicherry (4.87%), indicating the prevalence to be clustered in and around the studied place than the districts located far away. However, further study with larger population may be able to provide accurate information.

The presence of diagnostic anti-*Cysticercus* antibodies in eight blood donors indicates that these donors either have suffered from cysticercosis in past or having an infection with no overt clinical manifestation. The mean titer of the negative subjects (*i.e.*, 0.118) are close to that of healthy normal controls (*i.e.*, 0.117) where the mean titer of positive subjects (*i.e.*, 0.533) is higher than that of negative subjects and close to that of positive control subjects (*i.e.*, 0.355). The antigen positive subjects showing a negative titre for antibody showed the value a mean titer of 0.15, which is close to the cut-off OD (*i.e.*, 0.157).

Earlier studies including those in Brazil[1,2] have detected the prevalence of *Cysticercus* antibodies in sera of blood donors and other patients but studies for detection of antigens in these individuals are lacking. In the present study the *Cysticercus* antigen was detected in sera of six (2.77%) blood donors by the Co-A test; the presence of *Cysticercus* antigen in these persons may be due to sub-clinical infections *C. cellulosae.* Because none of these donors gave any history

of having any overt clinical manifestations of NCC or other form of cysticercosis. An interesting finding is that none of these *Cysticercus* antigen-positive sera were positive for anti-*Cysticercus* antibodies. This may be due to low or no response of the host to certain *Cysticercus* antigens, which are recognized by polyclonal antibodies raised in rabbit. Also could be those immunologically less reactive antigens usually persisting longer in the circulation without a detectable antibody response. Further characterization of the circulating antigens may be necessary in support of this hypothesis, which was beyond the scope of the present study.

The problem of a low sensitivity in the antibody detection test may be due to no or low immune response in patients with cysticercosis, as the cystic stages of the parasite may not evoke a strong detectable antibody response while they remain alive and intact. Hence serological study for *Cysticercus* antibodies alone may fail to detect all the cases of cysticercosis and thus may not provide an accurate data on seroprevalence of cysticercosis in the general population. Results of the present study show that detection of *Cysticercus* antigen in serum may be a useful adjunct to antibody testing for seroprevalence studies of cysticercosis in the community. Also an antigen detection assay provides useful evidence of recent infection as it is observed in case of other parasitic diseases[10]. Combined antigen and antibody detection is found to be always useful[3].

The low sensitivity and specificity of the CoA test for antigen detection, as observed in the present study may be due to the use of polyclonal antibody that is raised against crude antigens of the parasite. However, in an epidemiological study in endemic areas, an antigen positive test along with the history of exposure to the parasite may help in initial screening of the infected but asymptomatic subjects. But for a confirmatory diagnosis there is need of developing a more sensitive, specific as well as economically affordable serological tests suitable for studies in developing countries.

RESUMO

Antígenos e anticorpos de *Cysticercus* em soro de doadores de sangue de Pondicherry, Índia

O objetivo do presente estudo foi examinar o soro de doadores de sangue que são aparentemente saudáveis e residentes em Pondicherry ou seus distritos vizinhos do estado de Tamil Nadu, para a detecção específica dos antígenos e anticorpos de *Cysticercus.* Um total de 216 amostras de sangue foram coletadas de doadores de sangue do "Central Blood Bank, JIPMER Hospital", Pondicherry, Índia, durante janeiro e fevereiro de 2004. ELISA foi usado para demonstrar anticorpos anti-*Cysticercus* e co-aglutinação (CoA) foi utilizada para detectar antígenos no soro. 14 (6.48%) homens foram positivos para antígenos e anticorpos anti-*Cysticercus.* Destes, oito soros foram positivos para anticorpos anti-*Cysticercus* e seis positivos para antígenos. Os resultados do presente estudo mostram que a detecção de antígenos de *Cysticercus* no soro pode ser um adjunto útil para o teste de anticorpos para estudos de soroprevalência de cisticercose na comunidade. O presente estudo é o primeiro do tipo, realizado para determinar tanto anticorpos de *Cysticercus* como antígenos nas amostras de soro coletadas de doadores de sangue aparentemente saudáveis.

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