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Demonstration of Class Specific Antibody in the Diagnosis of Neurocysticercosis by Staphylococcus Aureus Protein-A Antibody Mediated Co-Haemagglutination Assay (SAPA-AMHA)

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Abstract

A simple and sensitive method has been described for the demonstration of class specific antibody to Cysticerous cellulosae by passive haemagglutination (PHA) reaction through intact cells of Staphylococcus aureus Protein - A (SAPA). SAPA binds rapidly to Fc portion of IgG with high affinity without affecting the immunological reactivity of bond immunoglobulin. Double aldehyde stabilized (DAS) sheep red blood cells sensitized with soluble antigens from porcine cyst sonicate were used in the test. This study was conducted on 142 cerebrospinal fluid (CSF) specimens. Of these, 5 cases were confirmed neurocysticercosis, 66 were clinically suspected and 71 controls. For comparative evaluation, all CSF specimens were examined by enzyme linked immunosorbent assay (ELISA).

The results indicate that PHA and SAPA-AMHA tests are sensitive methods in demonstration and quantitation of anticysticercal antibodies. The results also suggest that reaction was mediated predominantly by IgG and in some cases by both IgG and IgM which finally has importance in the diagnosis of the disease.

Key words -

Neurocysticercosis,
Staphylococcus aureus,
Haemagglutination assay,
Cerebrospinal fluid,
ELISA

Neurocysticercosis (NCC) is the most important parasitic disease of the central nervous system (CNS) in the Asian subcontinent [1]. The diagnosis of NCC has been made by clinical criteria, computed tomography (CT) and by serological procedures. A number of immunological methods have been described for the diagnosis of NCC such as complement fixation test (CFT) [2], indirect haemagglutination (IHA) [3], [4] and immunoenzymatic techniques [5], [6]. IHA was described in 1966 and found to be reliable in the diagnosis of NCC [3]. Later, IHA test was applied effectively

and evaluated using tanned cells and it was found that this test was superior to CFT [7]. In recent years, with the advent of newer techniques like enzymeimmunoassay, the detection of class specific immunoglobulin has been made possible. There are two different versions regarding the class specific antibody which mediates the reaction that has diagnostic importance. It was shown that IgG is the class of antibody which is of diagnostic value [5], [6]. Other studies have shown that IgM is the class of antibody, which would aid in the diagnosis of NCC [8]. Therefore, the present study aims at the demonstration of class specific antibody and its application in the diagnosis of NCC by indirect or passive haemagglutination (PHA) and modified PHA called, Staphylococcus aureus Protein-A antibody mediated co-haemagglutination assay (SAPA-AMHA). The efficacy of SAPA-AMHA was evaluated by comparing with that of enzyme linked immunosorbent assay (ELISA).

Material and Methods

Antigen

The soluble antigen of Cysticercus cellulosae was prepared from the cysts obtained by carefully dissecting them from infected pork, avoiding any inclusion of host tissue. All cysts were homogenized in 0.1 M PBS (0.1 M Na2 HPO4 / KH2 PO4, 0.15 M NaCl, pH 7.4) containing ampicillin, kanamycin and gentamycin along with protease inhibitors (phenylmethyl sulfonyl fluoride 0.006%, p-hydroxy mercuric benzoate 0.04%). The homogenate was sonicated at 20 kHz for 30 min at 4° C. The pocine cyst sonicate (PCS) was centrifuged at 17,000 Xg for 30 min at 4° C. The protein content of the supernatant was estimated by the method of Lowry et al [9] and stored in small aliquots at -20° C.

Hyperimmune serum

The hyperimmune serum was obtained by immunizing rabbits with the pellet of PCS, which was resuspended in small amount of the supernatant. 1.5 mg of protein of PCS was emulsified in equal amount of Freund's complete adjuvant, injected intradernally at multiple sites. The animals were bled 8-10 days after the last injection and sera were stored at -20° C with 0.1% azide.

CSF specimens

One hundred and forty two CSF samples were obtained from patients with various neurological disorders. These were categorised into the following four groups. group -I: confirmed neurocysticercosis (n=5), group - II: clinically suspected neurocysticercosis (n=66), group - III: infectious neurological disorders (n=49) and group - IV: non-infectious neurological disorders (n=22).

Staphylococcus Aureus Protein - A (SAPA)

Staph. aureus (Cowan I) was cultured on Mueller-Hinton agar. The formalin fixed heat killed SAPA cells were prepared by the method described by Kessler [10]. A 10% suspension of SAPA cells in PBS with 0.1% azide, was stored at 4° C. Similarly, a 5% suspension of Staph. aureus (Wood 46) in PBS with 0.1% azide were prepared and stored at 4° C.

PHA test procedure

This was essentially by the method described earlier [11]. Sheep red blood cells were stabilized successively with pyruvic aldehyde and glutaraldehyde - double aldehyde stabilized (DAS) cells. These were sensitized with predetermined optimal sensitizing dose of PCS antigen (200µ gm/ml).

Microtitration

Microtitration was carried out in haemagglutination plates (U-bottomed, NUNC plates) using calibrated microdroppers. Prior to titration, all CSF samples were absorbed with unsensitized 10% DAS cells and 5% Wood-46 cell suspension at 37° C for 1 hr. After absorpiton 25µ l of CSF was dispensed to 1st and 2nd wells and serially double diluted in adjacent wells from 2-8 which contained 25µ l of diluent (PBS with 1% BSA) 25µ l of unsensitized red cells were dispensed to 1st well and sensitized red cells to wells 2-8. The plate was gently rotated for 2 min on level surface and incubated at room temperature for 2 hr. Haemagglutination patterns were read according to Stavitsky [12]. The CSF antibody titre was taken as the highest dilution showing 2 + reaction or above. The 12th row was used as negative control, that is, diluent with sensitized cells. Thus the initial dilution of each CSF was 1:8.

SAPA-AMHA test for assessment of IgG class of antibody [13]

The procedure is essentially similar to that of PHA excepting the addition of SAPA cells prior to the addition of sensitized red cells. The hyperimmune serum and pooled CSF samples from confirmed neurocysticercosis cases were titrated with different dilutions of SAPA cells against sensitized red cells. The lowest concentration of SAPA cells showing maximum co-haemagglunitation (OCHA) dose valid for the batch of SAPA cells. For SAPA-AMHA test, CSF samples were diluted as for PHA. 25µ l of SAPA cells (predetermined OCHA) were dispensed to each well and the plate was gently agitated at room temperature for 30 min. Unsensitized and sensitized red cells were then dispensed as described earlier for microtitration. Agglutination patterns were scored as for PHA.

PHA test for assessment of IgM class of antibodies [14]

After completion of PHA test, all positive samples were treated with equal volumes of 0.2M 2-mercaptoethanol (2ME) and incubated at room temperature for 1 hr., after which they were retitrated against sensitized DAS cells, as described above.

ELISA

This was done as described earlier with slight modifications [6]. 100µ l of PCS antigen (10µ m/ml) was coated onto 96 well rigid polystyrene plates (NUNC, Denmark) using carbonate buffer (pH 9.6) for 16 hr at 4° C. Subsequently, the plates were washed thrice with PBS containing 0.05% Tween 20 (PBST) and quenched using 1% BSA in PBS for 16 hr at 4° C. After three washes with PBS-T, the plates were incubated with four fold dilutions of CSF at 37° C for 2 hr (the initial dilutionof being 1:4) A 1:1000 dilution of rabbit antihuman IgG peroxidase labelled conjugate (DAKOPATS, Denmark) was added. The bound immunoglobulins were detected by colour development with O-phenylene diamine substrate solution. The plates were read visually.

Results

Haemagglutintion titre of 1:8 or more was considered as suggestive of neurocysticercosis because, none of the 22 samples of group- IV had the reactivity in the titre mentioned above. The results obtained with PHA, SAPA-AMHA and ELISA in the four groups studied are shown in table I. IgG type of antibodies were demonstrated by quantitative enhancement of titres in SAPA-AMHA over PHA since Staphylococcal protein - A binds mainly to IgG class of antibody. In all the positive cases

by PHA, there in increase in titre, thus showing it is IgG mediated reaction. In order to detect IgM calls of antibody, all positive samples by PHA were treated with 0.2M 2-ME. Any fall in titre is suggestive of IgM mediated reaction. When titres of PHA before and after 2ME treatment are compared, in 7 of 15 cases the reaction was also mediated by IgM (table II).

Table I - Comparative evaluation of PHA, SAPA-AMHA and ELISA for the measurement of antibodies to PCS in groups I, II and III and IV

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Table II - PHA test for assessment of IgM class of antibodies in cases belonging to first 3 groups

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Discussion

Passive haemaggultination and Staphylococcus aureus. Protein - A antibody mediated co-haemaggultination tests have been used for the demonstration of class specific anticysticercal antibodies. In addition an evaluation has been made to compare the efficacy of SAPA-AMHA with that of ELISA. In this study, DAS cells were used in PHA as it offers several notable advantages such as

(a) agglutinable property of cells is increased;
(2) the cells become insusceptible to lysis;
(3) aledhdes not only stabilize the cells but also act as coupling agents by reacting with amino, sulphydı [11], [15].

It has been possible to demonstrate the class-specific antibody either IgG or IgM by modified PHA which is known for its simplicity and sensitivity. IgG mediated reactions were detected by SAPA-AMHA test, since Staph. aureus Protein -A is known to bind rapidly specifically to IgG with high affinity without affecting the immunological activity of bound IgG [13]. Similarly, all positive cases by PHA were treated with mercaptans like 2-mercaptoethanol since 2 ME selectively inactivates IgM [14]. This study shows that the class of antibody which mediates the reaction is predominantly IgG and in some cases both IgG and IgM (tables I and II).

For the diagnosis of neurocysticercosis by immunological methods, either in confirmed or in clinically suspected cases, there was no difference between haemagglutination and ELISA with respect to detection of anticysticercal antibodies. This is in agreement with earlier reports [16]. However, SAPA-AMHA yielded 2-8 fold higher titres as compared to PHA and these titres are comparable to that of ELISA (table I). Three out of 49 CSF samples in patients belonging to group - III showed anticysticercal antibodies. All these 3 were pyogenic meningitis cases. In none of the tuberculosis meningitis cases (both culture positive and clinical) the antibodies were detected. The possibility of cross reaction in the 3 pyogenic meningitis cases cannot be over emphasised since in other cases of pyogenic meningitis due to the same etiological agents the test have been negative. In view of this, the probability of co-existence of neurocysticercosis in these 3 cases should be considered.

The experience of other workers with malaria [15], echinococcosis [17] and leprosy [18] showed that the use of DAS cells greatly enhanced the sensitivity of PHA. The sensitized cells have long shelf-life (12-14 weeks), prolonged stability and retention of haemagglutinating potency, if they are stored at 4° C. In our experience, the PHA and modified PHA tests with DAS cells have proved to be simple and highly sensitive methods for the measurement of antibodies to C. cellulosae.

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