Comparison of Casoni’s intradermal test with enzyme linked immunosorbent assay in the diagnosis of human hydatid disease

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Abstract

Casoni’s intradermal test and Enzyme-Linked Immunosorbent Assay (ELISA) was performed for the diagnosis of human hydatid disease. Casoni’s intradermal test was done by injecting commercially available antigen intradermally at a suitable site on the vein-free volar surface of the right fore-arm using a sterile tuberculin syringe fitted with a 26 gauge needle. An ELISA was performed for the detection of immunoglobulin G, E and M (IgG, IgE and IgM) antibodies to Echinococcus granulosus in surgically proven cases of hydatidosis, by use of sheep cyst fluid antigen. The diagnostic sensitivity and specificity of Casoni’s intradermal test was 59.3% and 60% respectively, whereas the diagnostic sensitivity of ELISA for the detection of IgG, IgE and IgM were 84.37%, 68.75% and 65.62% respectively. The specificity of ELISA for IgG, IgE and IgM were 90%, 80%, 85% respectively. The detection of IgG antibody was found more sensitive and specific than IgE and IgM antibody in the diagnosis of human hydatidosis. The ELISA was found more sensitive and specific than the Casoni’s intradermal test in diagnosing cases of hydatidosis, especially hydatid disease of liver.

Keywords: hydatid disease, casoni test, ELISA, IgG, IgM

Introduction

Cystic Echinococcosis (CE), caused by infection with larval stage of dog tapeworm Echinococcus granulosus has public health importance not only in areas of endemicity but also in countries or regions where migration of infected people and exchanges of livestock occur (Craig et al., 1996). Hydatid serological tests have a very long history, and almost all serological tests that have been developed were used in the diagnosis of human cases. There are considerable differences among the various tests in both sensitivity and specificity.Insensitive and non-specific tests, including the Casoni’s intradermal test, the complement fixation test, the indirect haemagglutination test and the latex agglutination test, have been replaced by the enzyme-linked immunosorbent assay (ELISA), the Immunoelectrophoresis (IEP) and Immunoblotting (IB) in routine laboratory application (Lightowlers and Gottstein, 1995). ELISA has been the technique which received most attention as an immunodiagnostic method for various parasitic infections. It is cheap and can be performed in poorly equipped laboratories (Zarzosa et al., 1999; Khosravi et al., 2012). The purpose of this study was to evaluate the efficacy of Casoni’s intradermal test and ELISA for the diagnosis of human hydatid disease.

Materials and Methods

Sample collection

Blood samples collected from 20 healthy individuals and 32 surgically confirmed hydatid patients were
centrifuged at 2000×g for 10 mins to obtain serum. Haemolysed and lipaemic serum was excluded from this study. All serum samples were stored at -70°C until antibody determination.

Antigen for Casoni test

Commercially prepared antigen marketed by Span Diagnostics® India was used. The antigen was standardised by immunoprecipitation test and was preserved in 0.01% thiomersal, and refrigerated at 2-8°C for the study period.

Casoni Intradermal test

Casoni antigen (0.1 ml) was injected intradermally at a suitable site on the vein-free volar surface of the right forearm using a sterile tuberculin syringe fitted with a 26 gauge needle; 0.1 mL of sterile normal saline was injected into the left forearm as control. Readings were taken after 15 mins, as well as after 30 mins for immediate reaction, and if both were negative, these were read again at the end of 72 hrs (for delayed response). A wheal measuring >24 mm in one direction or >22 mm in both the directions within 15-30 mins of the test with or without formation of pseudopodia was considered to be positive. The test was considered as borderline if the wheal measured 22 or 23 mm in the long axis and 21 mm or less in the other axis. Any wheal measuring < 22 mm in both axes was interpreted as negative (Lambria, 1975; Bhatia et al., 1990).

Antigen preparation for ELISA

Fertile hydatid cysts were obtained from sheep slaughtered at local abattoir. Hydatid fluid aseptically aspirated from the cyst and centrifuged at 2000xg for 20 mins and then the supernatant was passed through a Whatman membrane filter (WCN type cellulose nitrate, 47 mm diameter and 0.45 µm pore size). The fluid was then dialyzed against distilled water overnight at 4°C using dialysis tubing (Sigma Aldrich, USA) with molecular weight cut off 2000. Protein estimation of antigen was done as per the method of Lowry et al. (1951) with bovine serum albumin (BSA-Sigma Aldrich) as reference standard.

Enzyme Linked Immunosorbent Assay (ELISA)

The antibody detection was done by indirect ELISA technique as per the standard protocol described earlier with some modifications (Wattal et al., 1986). Briefly, microtitration plate was coated with 2 µg/100 µl of crude hydatid sheep antigen diluted in 0·1 M carbonate bicarbonate buffer (pH 9·6). Sera were diluted 1:200 for each immunoglobulin (IgG, IgM and IgE), in phosphate buffer saline (PBS). Goat anti-human IgG, IgM and IgE conjugated to horseradish peroxidase (Sigma Aldrich, USA), diluted 1: 4000, 1:2000 and 1: 2000, respectively, were used as the second antibody. Tetramethylbenzidine (TMB) and H₂O₂ were used to visualize the antigen-antibody reaction. Optical density (OD) was recorded at 450 nm after the addition of stop solution (2·5 N, H₂SO₄). Mean OD±3 standard deviations (SD) of the OD values obtained for the healthy sera was used to establish a cut-off value. Values greater than the cut-off value were considered positive for anti-hydatid antibodies.

Results

Out of 32 surgically confirmed hydatid patients 18 had hepatic cysts, 12 had cysts in the lung while 2 had cysts in multiple organs. The Casoni’s i.d test was positive in 19 (59.3%) patients, 12 in patients with liver hydatidosis, 6 in lung and 1 in multiple cysts. The Enzyme Linked Immunosorbent Assay (ELISA) for IgG was
Table 1. Immunological profile of 32 surgically proven cases of hydatid disease

<table>
<thead>
<tr>
<th>Cyst Localization</th>
<th>No. of cases tested</th>
<th>No. (%) of cases positive in Casoni’s i.d test</th>
<th>No. (%) of cases positive in IgG ELISA</th>
<th>No. (%) of cases positive in IgE ELISA</th>
<th>No. (%) of cases positive in IgM ELISA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung</td>
<td>12</td>
<td>6 (50)</td>
<td>9 (75)</td>
<td>4 (33.3)</td>
<td>4 (33.3)</td>
</tr>
<tr>
<td>Liver</td>
<td>18</td>
<td>12 (66.6)</td>
<td>16 (88.8)</td>
<td>16 (88.8)</td>
<td>15 (83.3)</td>
</tr>
<tr>
<td>Multiple organ</td>
<td>02</td>
<td>1 (50)</td>
<td>2 (100)</td>
<td>2 (100)</td>
<td>2 (100)</td>
</tr>
<tr>
<td>Total</td>
<td>32</td>
<td>19 (59.3)</td>
<td>27 (84.37)</td>
<td>22 (68.75)</td>
<td>21 (65.62)</td>
</tr>
</tbody>
</table>

Table 2. Immunological profile of non hydatid / Healthy cases

<table>
<thead>
<tr>
<th>Immunological Technique</th>
<th>No. of cases tested</th>
<th>No. of cases true negative</th>
<th>No. of cases false positive</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casoni’s i.d test</td>
<td>20</td>
<td>12</td>
<td>8</td>
<td>60 %</td>
</tr>
<tr>
<td>IgG ELISA</td>
<td>20</td>
<td>18</td>
<td>2</td>
<td>90 %</td>
</tr>
<tr>
<td>IgE ELISA</td>
<td>20</td>
<td>16</td>
<td>4</td>
<td>80 %</td>
</tr>
<tr>
<td>IgM ELISA</td>
<td>20</td>
<td>17</td>
<td>3</td>
<td>85 %</td>
</tr>
</tbody>
</table>

positive in 27 of 32 samples (84.37%), while ELISA for IgE and IgM were positive in 68.75% and 65.62% of patients respectively (Table 1). The Casoni’s i.d test and ELISA were found positive in large number of patients having cysts in the liver followed by lung. Lung hydatid cases were low reactors as compared with liver echinococcosis cases in both Casoni’s i.d test and ELISA. The sensitivity and specificity of the technique was determined by taking surgically confirmed cases as standard for sensitivity and healthy controls as standards for specificity. The ELISA was found more sensitive and specific than Casoni’s i.d test. The diagnostic sensitivity of ELISA for IgG, IgE and IgM were 84.37%, 68.75% and 65.62% respectively. The sensitivity of Casoni’s i.d test was only 59.3%. The anti-echinococcus antibodies of IgG class were found more sensitive and specific as compared to IgE and IgM class in the diagnosis of human hydatidosis. The specificity of Casoni’s i.d test was found only 60% were as the specificity of ELISA for IgG. IgE and IgM was found 90, 80%, 85% respectively (Table 2).

Discussion

Our results indicate that the IgG ELISA is highly sensitive and specific in detecting anti-echinococcus antibodies. We found ELISA useful in the diagnosis of human hydatidosis especially the liver hydatidosis. This is in contrast to the lower sensitivity and specificity of the Casoni’s i.d test in diagnosing human hydatidosis (Kagan, 1968; Kagan, 1976; Voller and Desavingny, 1981; Pini et al., 1983; Ray et al., 2002). Mathur and Bhave, (1985) also observed lower diagnostic sensitivity (58.33%) of Casoni’s test. However, Todorov et al. (1979) compared Casoni’s i.d test with other immunodiagnostic tests like
indirect haemagglutination (IHA) test, Complement fixation test and found Casoni’s i.d test positive in 83.3% of lung hydatid cases, we found only 59.3% of such cases to be positive. These findings for the Casoni’s i.d test are also corroborated by the result of other investigators (Mahajan et al., 1973; Todorov et al., 1976). The sensitivity of Casoni’s test varied from 60-80% in previous studies (Schantz, 1975). False negative reactions are found in calcified lung cysts which may be due to absence of precipitating antibodies in the infected host.

The measurement of hydatid specific IgG by the ELISA seems superior and better than even the detection of hydatid specific IgE and IgM antibodies. Similar observations were reported by Doiz et al. (2002). They also found higher diagnostic sensitivity of IgG ELISA. The higher diagnostic sensitivity of IgG was also reported by Hanillo et al. (2005) against crude hydatid cyst fluid antigen of sheep in ELISA. The moderate IgM antibody response is in accordance with previous investigations (Candolfi et al., 1985; Mantossian et al., 1991), but was not in agreement with some studies (Tassi et al., 1984; Orduna et al., 1986). Sbihi et al. (2001) observed low diagnostic sensitivity of IgE ELISA and suggested, the low sensitivity of this test was partly due to the low reactivity detected in the sera of patients with lung hydatidosis. The non-specific reactions in normal human sera may be caused by interaction with some blood group antigens, or with non-specific host proteins (serum albumin, host IgG) in hydatid fluid (Kanwar et al., 1992; Sunita et al., 2007). Regarding cyst location and specific antibody levels, it has been reported that the intensity of the immunological response to hydatid cysts may differ depending on the anatomical location of the parasite. In general, it has been postulated that the location of cysts in the liver will elicit higher antibody levels than those in the lung (Babba et al., 1994), and this will affect the sensitivity of a given serological test. The IgG ELISA is sensitive enough to detect even a low level of antibodies produced in human hydatidosis. Among the possible causes of negative serological response in some surgically confirmed patients are the number, site, integrity and morphology of hydatid cyst, high concentration of circulating immune complexes in hydatid disease, has been documented by previous work (Pavlov et al., 2006). Thus rendering antibodies unavailable for detection, also the possibility of antigen induced specific immunological tolerance has also been raised. Such complexes in the serum of hydatid cyst patients may cause false negative reactions in serological tests with clinically and surgically confirmed disease. This result may be due to the fact, that the immune response in large cyst is weak or completely absent because it has a thick fibrous capsule, which may prevent the release of antigens (Petrov et al., 2001).

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References


Tenguria et al., 2013


