DIAGNOSIS OF HUMAN FASCIOLIASIS BY ELISA COPROANTIGEN DETECTION

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Abstract

This study was performed on 147 faecal samples collected from 17 patients with fascioliasis, 21 patients with clinically suspected fascioliasis, 12 patients with other intestinal parasitic infections and 15 healthy negative controls. Purified polyclonal hyperimmune serum from rabbits immunized with crude Fasciola gigantica antigen, was used in ELISA for Fasciola coproantigen detection in saline extracts of faecal samples. Results showed 100% sensitivity and 98% diagnostic accuracy of ELISA coproantigen detection versus 64.7% sensitivity and 88.3% diagnostic accuracy of egg finding with statistically significant differences (P < 0.001, P < 0.01 respectively). So, ELISA coproantigen detection is a reliable technique for early and sensitive diagnosis of human fascioliasis {Zag. Med. Ass. J., Vol. 8, No 4, October, 1995}.

Introduction

Human fascioliasis is a world wide problem. In Egypt, it is becoming a public health problem (Osman, 1991) as it is one of the causes of hepatobiliary disorders (Makled et al., 1988). The disease passes through 2 phases: an acute or invasive (prepatent) fascioliasis which coincides with parasite migration and hepatic invasion, and a chronic or patent fascioliasis which coincides with the persis-
tance of adult flukes in bile ducts (Barrett - Conner, 1986). Although diagnosis is based on finding eggs in stool of suspected cases (Knoblock et al., 1985), this method is often unreliable, since eggs never found in the stool during acute phase (De Weil et al., 1984) and are not easily recovered during the chronic phase due to either low intensity or irregular egg shedding (Levine et al., 1980). Moreover, the sensitivity of finding eggs was as low as 55% (Fawzy et al., 1992), 52% (Farag et al., 1993), 40% (Hammouda et al., 1995) and 47% (Hassan et al., 1995). Serological testes for detecting antibodies are mostly of limited value because of a false positivity with normal human sera (Hillyer and Capron, 1976) and extensive cross reactivity with other helminthic infections specially Schistosoma (Osman and Helmy, 1994). Moreover the positive titres persist after cure (Espino et al., 1990). In addition false negativity has been reported in recent infection (Khalil et al., 1990). Some workers tried to diagnose human fascioliasis by ultrasonography, but no gall bladder changes could be detected during the incubation period and the changes found in the chronic stage are not specific but suggestive for diagnosis (Fawzy et al., 1992).

Therefore, the need exists for an alternative technique for early and specific diagnosis and consequently proper management of human fascioliasis.

So, the present study was designed to assess the efficacy of ELISA coproantigen detection for diagnosis of human fascioliasis using purified polyclonal antibodies raised in rabbits hyperimmunized with F. gigantica crude antigen.

Patients and Methods

The present study was conducted on 147 stool specimens collected from 65 patients and subjects presented to the outpatient clinics of Zagazig University Hospital and categorized into 4 groups: Group I: 17 patients with confirmed fascioliasis in whom Fasciola eggs were detected in the stool. Group II: 21 clinically suspected cases of fascioliasis (suffering from fever of unknown origin, abdominal pain, hepatomegally, sple-
nomegaly, pallor and/or eosinophilia) in whom Fasciola eggs were not detected in stool. Group III: 12 patients with some intestinal parasites (S. mansoni: 5. A. lumbricoides: 3 and G. lamblia: 4) in whom Fasciola eggs were absent. Group IV: 15 apparently healthy persons proved to be free from any intestinal parasitic infection and considered as negative control group. Stool examination was done after the merthiolate - iodine - formaldehydeether concentration technique (Blagg et al., 1955).

Faecal supernatant fraction was prepared by mixing equal volumes of faecal material and 0.15 M phosphate buffered saline (PBS) containing 0.3% Tween 20 (Sigma), shaken vigorously and centrifuged at 2000g for 30 min. at room temperature. An equal volume of heat inactivated faetal calf serum was added to the supernatant before adding it to each ELISA well (Allan et al., 1992).

Fasciola gigantica crude antigen was prepared from adult worms obtained from condemned livers from slaughter house. Worms were washed in saline, homogenized in tissue grinder in 0.01M PBS (PH 7.0) and centrifuged at 500g, for 10 min. The supernatant was ultracentrifuged at 100,000 g for 1 hour at 4°C (Hillyer et al., 1979). Its protein content was estimated by Lowry method (Lowry et al., 1951).

Hyperimmune anti - Fasciola serum was prepared in New Zealand rabbits. They were hyperimmunized by a priming dose of 2mg protein/ml of Fasciola antigen in 1 ml complete Freund's adjuvant at several sites and boosted with 2 booster doses of the antigen in incomplete Freund's adjuvant one and two weeks later and bled one week after the last dose (Youssef et al., 1991). Gamma globulin fraction of the antisera was prepared by passage and low pH elution from protein A sepharose CL4B (Pharmacia). A portion of gamma globulin fraction was conjugated to horse - radish peroxidase type VI (Sigma) according to Allan and Craig (1989).

ELISA technique was performed according to Nageswaran et al., (1994) with some modifications. Briefly, wells of
ELISA plate were coated with 150 ml of purified gamma globulin fraction of hyperimmune anti-Fasciola serum, then rinsed the next morning and 150 ml faecal supernatent diluted in heat inactivated 50% foetal calf serum were added for 1 hour at room temperature. Gamma globulin fraction of hyperimmune anti-Fasciola serum conjugated to horse raddish peroxidase (150 ml of 1/200 in PBS) was added and incubated for 1 hour at 37°C. After wash, 150 ml of OPD was added for 1 hour at room temperature. The optical density readings were at 492 nm.

Statistical analysis of the results was done using Chi square and Student's t tests (Kirkwood, 1989).

Results

Results are presented in table 1, 2 & 3.

Table (1): Results of both microscopic examination for eggs and ELISA coproantigen detection for diagnosis of fascioliasis in stool specimens from different groups.

<table>
<thead>
<tr>
<th>Clinical cases</th>
<th>No. of persons</th>
<th>No. of specimens</th>
<th>No.+ ve for Fascioal eggs</th>
<th>No.+ ve by ELISA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fascioliasis</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>(group I)</td>
<td>5</td>
<td>10</td>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>18</td>
<td>10</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>17</td>
<td>34</td>
<td>22 (64.7%)</td>
<td>34 (100%)</td>
</tr>
<tr>
<td>Suspected fascioliasis</td>
<td>5</td>
<td>5</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>(group II)</td>
<td>9</td>
<td>18</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>21</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>44</td>
<td>0</td>
<td>20 (45.5%)</td>
</tr>
<tr>
<td>Other parasitic infections (group III)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Schistosomiasis</td>
<td>5</td>
<td>10</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Ascariasis</td>
<td>3</td>
<td>9</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Giardiasis</td>
<td>4</td>
<td>12</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>31</td>
<td>0</td>
<td>2 (16.6%)</td>
</tr>
<tr>
<td>Apparently healthy controls (group IV)</td>
<td>7</td>
<td>14</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>24</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
Diagnosis of human Fascioliasis

Table (2): Stool examination for eggs versus ELISA coproantigen detection for diagnosis of fascioliasis in stool specimens from all studied groups.

<table>
<thead>
<tr>
<th>Stool examination</th>
<th>ELISA for coproantigen detection</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Test + ve</td>
<td>Test - ve</td>
</tr>
<tr>
<td>Test + ve</td>
<td>22</td>
<td>0</td>
</tr>
<tr>
<td>Test - ve</td>
<td>34</td>
<td>91</td>
</tr>
<tr>
<td>Total</td>
<td>56</td>
<td>91</td>
</tr>
</tbody>
</table>

Probability $P < 0.001^*$

* Significant.

Table (3): Statistical analysis of both microscopic examination for eggs and ELISA coproantigen detection for diagnosis of fascioliasis in all studied group.

<table>
<thead>
<tr>
<th></th>
<th>Microscopic examination</th>
<th>ELISA</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>64.7%</td>
<td>100%</td>
<td>$P &lt; 0.001^*$</td>
</tr>
<tr>
<td>Specificity</td>
<td>100%</td>
<td>95%</td>
<td>$P &lt; 0.05^{**}$</td>
</tr>
<tr>
<td>Positive predictive value</td>
<td>100%</td>
<td>94.4%</td>
<td>$P &lt; 0.05^{**}$</td>
</tr>
<tr>
<td>Negative predictive value</td>
<td>85%</td>
<td>100%</td>
<td>$P &lt; 0.001^*$</td>
</tr>
<tr>
<td>Diagnostic accuracy</td>
<td>88.3%</td>
<td>98%</td>
<td>$P &lt; 0.01^{*}$</td>
</tr>
</tbody>
</table>

$^*$ Significant  $^{**}$ Nonsignificant
Discussion

Detection of parasite specific antigen in host's faeces by ELISA technique has been successfully developed for the diagnosis of giardiasis (Goldin et al., 1993), taeniasis (Allan et al., 1990), amaebiasis (Grundy et al., 1987) and strongyloidiasis (Nageswaran et al., 1994). Application of such technique may solve the problem encountered with early fascioliasis diagnosis and consequently proper management and saving from possible complications. Employing such technique in the present study using purified hyperimmune serum against crude Fasciola gigantica antigen, was proved to be an accurate method for diagnosis in both confirmed and clinically suspected fascioliasis. To the best of our knowledge, this is the first trial to develop and evaluate such technique for diagnosis of human fascioliasis. Results of the present study showed that out of 34 stool specimens obtained from 17 fascioliasis patients, only 22 were positive for eggs by microscopic examination (64.7% sensitivity). This indicates that detection of eggs is not a constant finding and consequently it is unreliable method for diagnosis. This is either due to recent infection since eggs are found in the stool only 6 - 8 weeks after infection (Espino et al., 1990) or due to intermittent egg shedding (Hillyer et al., 1984). This is in agreement with many authors (Fawzy et al., 1992, Farag et al., 1993 and Hassan et al., 1995) who reported lower sensitivity of egg finding in the stool.

On the other hand, the present study showed a 100% sensitivity of ELISA coproantigen detection technique. All the 34 stool specimens from fascioliasis patients showed O.D. readings (0.615 to 1.310) which were above the cut-off value (0.120) calculated as the mean O.D. readings of negative controls + 3 S. D. Out of such 34 specimens, 22 were positive for eggs and the ELISA positivity here was most probably due to egg-associated antigen. Twelve specimens were negative for eggs and the ELISA positivity here was most probably due to the presence of excretory / secretory products of adult worms rather than on the presence of egg-associated antigen. This indicates the ability of ELISA to diagnose chronic fascioliasis in cases of absence of
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eggs in the stool due to irregular shedding. Patients with clinically suspected fascioliasis with negative stool examination for eggs (group II) showed positive ELISA in 20 out of 44 stool specimens. Follow up of such patients by stool examination showed appearance of eggs 2-4 weeks later which confirm the positivity of ELISA and indicates its ability to diagnose infection at early stages. The ELISA positivity here was most probably due to excretory / secretory products of immature worms. Positive results obtained at different clinical cases suggests the presence of shared antigens in different developmental stages of Fasciola gigantica. Results of the present work are in agreement with Youssef et al., (1991) who detected Fasciola coproantigens early in infection using the counterimmunoelectrophoresis. Among patients with other parasitic infections (group III), all specimens (31) showed negative ELISA except for 2 specimens from one patient with schistosomiasis which showed positive ELISA at O.D values slightly above the cut off value and this may be due to either the presence of cross - reacting antigens or due to early and light fascioliasis with inapparent clinical manifestations.

Statistical Analysis

Statistical analysis of the results of the present study showed that the ELISA coproantigen detection technique had 100% sensitivity with a highly significant difference (P < 0.001) in relation to microscopic examination for eggs, 95% specificity, 94.4% positive predictive value, 100% negative predictive value and 98% diagnostic accuracy with significant difference (P < 0.01) in relation to egg detection.

So, in conclusion, this study emphasizes the application of ELISA coproantigen detection technique as a sensitive, specific and accurate method for diagnosis of acute and chronic fascioliasis.

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