VALUE OF PURIFIED SCHISTOSOMA SNAILS ANTIGENS IN DIAGNOSIS OF SCHISTOSOMIASIS

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ABSTRACT

Objectives: This study was done to assess the value of purified Schistosoma snails antigens in diagnosis of schistosomiasis.

Materials and Methods: Five antigens were used, S.mansoni adult worm crude antigen, snails antigens (foot and visceral hump of B.alexandrina and B.truncatus). Specific hyperimmune mice sera versus each antigen were prepared. Known positive and negative human sera and uninfected mice sera were used as control. Two ELISA techniques (conventional and sandwich) were performed.

Results: There was high similarity between S.mansoni crude antigen and B.alexandrina foot antigen in detecting S.mansoni antibodies (100% and 80% respectively) at serum dilution 1:50. B.alexandrina visceral hump antigen detected only 33.3%. Both B.truncatus antigens gave negative results.

Conclusion: Sandwich ELISA technique proved to be more species specific than conventional ELISA. B.alexandrina foot antigen was found to be the best antigen among the tested antigens that can replace S.mansoni adult worm crude antigen in diagnosis of schistosomiasis. antigens into circulation of the host. These antigens are classified according to the stage of development of the parasite into cercarial antigens, adult worms associated antigens (e.g. tegument or gut associated), and egg antigens²). Serodiagnosis is considered as one of important tools which lead to accurate diagnosis. Accuracy of serodiagnosis is affected by types and degree of purification done on the used antigen ³.

Common antigen fractions had been demonstrated to be shared between schistosome larval stages (miracidia, sporocysts & cercariae), adult schistosomes and their intermediate hosts ⁴. These antigenic fractions proved to be active in inducing immunity against schistosomiasis. Studies ⁵ had shown that antismail antibodies had been demonstrated in sera of patients infected with S.mansoni and S.haematobium using hepatopancreas of infected and uninfected B.glabrata snails. The authors mentioned that the surface coat or “glycocalyx” of Schistosoma cercariae contain antigens contracted from snail tissues and the antibodies against snail ( Biomphalaria glabrata) intermediate host antigens had been demonstrated in the sera of infected animals.

INTRODUCTION

Schistosomiasis remains a serious public health problem in Egypt and other subtropical and tropical countries despite extensive efforts of disease control⁶. Schistosoma parasites excrete and secrete a number of different antigens into circulation of the host. These antigens are classified according to the stage of development of the parasite into cercarial antigens, adult worms associated antigens (e.g. tegument or gut associated), and egg antigens²). Serodiagnosis is considered as one of important tools which lead to accurate diagnosis. Accuracy of serodiagnosis is affected by types and degree of purification done on the used antigen ³.

Common antigen fractions had been demonstrated to be shared between schistosome larval stages (miracidia, sporocysts & cercariae), adult schistosomes and their intermediate hosts ⁴. These antigenic fractions proved to be active in inducing immunity against schistosomiasis. Studies ⁵ had shown that antismail antibodies had been demonstrated in sera of patients infected with S.mansoni and S.haematobium using hepatopancreas of infected and uninfected B.glabrata snails. The authors mentioned that the surface coat or “glycocalyx” of Schistosoma cercariae contain antigens contracted from snail tissues and the antibodies against snail ( Biomphalaria glabrata) intermediate host antigens had been demonstrated in the sera of infected animals.

The aim of this study is identification of snails antigens able to diagnose the antibodies of its corresponding Schistosoma species.

MATERIALS AND METHODS

1- Preparation of S.mansoni adult worm crude antigen:
Cercariae of S.mansoni were obtained from experimentally infected B.alexandrina snails. These were provided by Schistosome Biological Supply Program Unit, Theodor Bilharz Research Institute, Giza, Egypt. Swiss albino mice (15-20gm) were infected with 20-30 cercariae by intra-peritoneal injection and 8 weeks later, adult worms were collected from liver and presmesenteric veins⁶. They were homogenized, sonicated then centrifuged at 20000 rpm for 1 hour at 4°C. The adult worm antigen was prepared ⁷ ⁸ and distributed into 1ml aliquots in plastic vials.

2- Preparation of snails antigens: Biomphalaria alexandrina and Bulinus truncatus snails were collected from Abu-Rawash Giza ⁹. Medium to large sized snails were dissected, foot and visceral hump were separated, homogenized, sonicated and left overnight for extraction ¹⁰. The protein content of the prepared antigens was measured ¹¹, divided into aliquots and stored at -20°C until used.

3- Specific hyper immune mice sera versus each selected antigen were prepared ¹². Known positive human sera obtained from patients admitted in Tropical Medicine Department in Kasr El-Ainy Hospital. Known
negative human sera and sera from non-infected mice were used as control.

4- Serological tests:

A-Indirect enzyme linked immunosorbent assay (ELISA) (13): to study the antigenic relationship between the five selected antigens versus their specific hyper-immune mice sera as well as positive and negative human sera.

B-Sandwich ELISA (14); for capturing the tested antigens between two antibodies. The first one was hyper-immune mice sera used in coating ELISA plates while the other was either known positive or negative human sera. The absorbance was measured at 490 nm using a micro-plate ELISA reader. The presented optical density (OD) values are mean values of three examined samples. The cut-off value was adjusted to be twice the mean OD value of the negative control. A positive reaction was defined as having an OD value greater than the cut-off value.

5- Statistical analysis:
The data were processed using statistical package SPSS Ver.10 on an IBM compatible computer. Differences between antigens were assessed using the ANOVA test (Two – Factor without Replication) and F-Test (Two-Sample for Variances).

RESULTS

Serological results of the tested antigen versus the different prepared hyperimmune mice sera by ELISA are presented in table (1). Sensitivity of B.alexandrina snails antigens in diagnosing S. mansoni infection in infected patients are presented in table (2). Results of sandwich ELISA are presented in table (3).

Table 1: ELISA OD values of the tested antigens versus different prepared hyperimmune mice sera (HIS) at dilution 1:50.

<table>
<thead>
<tr>
<th>Tested antigens</th>
<th>Anti- S. mansoni HIS</th>
<th>Anti-B. alexandrina foot HIS</th>
<th>Anti B. alex. vis. Hump HIS</th>
<th>Anti B. truncatus foot HIS</th>
<th>Anti B. trunc. vis. Hump HIS</th>
<th>Control (-ve mice sera)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. mansoni crude Ag</td>
<td>0.97</td>
<td>0.79</td>
<td>0.45</td>
<td>0.20 (-ve)</td>
<td>0.18 (-ve)</td>
<td>0.10</td>
</tr>
<tr>
<td>B. alexandrina foot Ag</td>
<td>0.83*</td>
<td>0.87</td>
<td>0.78</td>
<td>0.66</td>
<td>0.60</td>
<td>0.11</td>
</tr>
<tr>
<td>B. alex. vis. Hump Ag</td>
<td>0.66*</td>
<td>0.90</td>
<td>0.87</td>
<td>0.61</td>
<td>0.55</td>
<td>0.12</td>
</tr>
<tr>
<td>B. truncatus foot Ag</td>
<td>0.42**</td>
<td>0.50</td>
<td>0.40</td>
<td>0.82</td>
<td>0.70</td>
<td>0.12</td>
</tr>
<tr>
<td>B. trunc. vis. Hump Ag</td>
<td>0.42**</td>
<td>0.65</td>
<td>0.61</td>
<td>0.71</td>
<td>0.85</td>
<td>0.11</td>
</tr>
</tbody>
</table>

*P>0.05 (non significant difference)  **P<0.001 (highly significant difference)

Table 2: Sensitivity of B. alexandrina snails antigens in diagnosis of S. mansoni infection in naturally infected patients at 1:50 and 1:100 serum dilutions.

<table>
<thead>
<tr>
<th>Tested antigens</th>
<th>Human sera at dilution 1:50</th>
<th>Human sera at dilution 1:100</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ELISA OD value</td>
<td>Mean -ve control</td>
</tr>
<tr>
<td>S. mansoni crude Ag</td>
<td>0.34</td>
<td>0.09</td>
</tr>
<tr>
<td>B. alexandrina foot Ag</td>
<td>0.24*</td>
<td>0.09</td>
</tr>
<tr>
<td>B. alex. vis. Hump Ag</td>
<td>0.18**</td>
<td>0.08</td>
</tr>
</tbody>
</table>

*P>0.05  **P<0.05
Table 3: Results of sandwich ELISA in capturing different tested antigens using S. mansoni HIS and B. alexandrina foot HIS at dilution 1:50.

<table>
<thead>
<tr>
<th>Tested antigens</th>
<th>Coating Ab S. mansoni HIS at dilution 1:50</th>
<th>Mean sandwich ELISA OD values</th>
<th>Coating Ab B. alexandrina foot HIS at dilution 1:50</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2nd Ab Human sera (+ve and –ve) at dilution 1:50</td>
<td></td>
<td>2nd Ab Human sera (+ve and –ve) at dilution 1:50</td>
</tr>
<tr>
<td>S. mansoni crude Ag</td>
<td>+ve 0.28, -ve 0.09</td>
<td>+ve 0.28, -ve 0.12</td>
<td>+ve 0.28, -ve 0.12</td>
</tr>
<tr>
<td>B. alexandrina foot Ag</td>
<td>+ve 0.24**, -ve 0.09</td>
<td>+ve 0.44, -ve 0.12</td>
<td>+ve 0.44, -ve 0.12</td>
</tr>
<tr>
<td>B. alexandrina vis. Hump Ag</td>
<td>+ve 0.21**, -ve 0.07</td>
<td>+ve 0.39, -ve 0.11</td>
<td>+ve 0.39, -ve 0.11</td>
</tr>
<tr>
<td>B. truncatus foot Ag</td>
<td>+ve 0.14**, -ve 0.08</td>
<td>+ve 0.20, -ve 0.11</td>
<td>+ve 0.20, -ve 0.11</td>
</tr>
<tr>
<td>B. truncatus vis. Hump Ag</td>
<td>+ve 0.12**, -ve 0.07</td>
<td>+ve 0.11, -ve 0.10</td>
<td>+ve 0.11, -ve 0.10</td>
</tr>
</tbody>
</table>

*P<0.05  **P<0.001

DISCUSSION

Schistosomiasis is a major health problem in tropical and subtropical areas. Its importance lies in the fact that it affects people not only in their overall health status and fitness but also in their productivity (15). Many researches had been performed to develop sensitive and specific antigen for diagnosis (2). Shalaby et al. (16) used snail antigens for immunodiagnosis of Tremarode infections. The enzyme linked immunosorbent assay has been the technique receiving most attention for detection of antibodies to Schistosoma (17). It is the most widely used diagnostic method, being more sensitive and specific than indirect haemagglutination and immunofluorescence techniques (18). ELISA test sensitivity depends on the type of antigen used (19). Adult worm antigen had the advantages of being easily obtained, more reactive with patient’s sera and yield higher levels of diagnostic sensitivity and specificity than other types of antigens. The use of crude adult worm extract in ELISA provided a serological method with high sensitivity and specificity for diagnosis of acute and chronic schistosomiasis (20). Several investigators demonstrated the antigenic community between snails and their trematode parasites 

In this study the anti-S. mansoni antibody reacted specifically with its own antigen revealing higher mean ELISA OD values than those recorded versus B. alexandrina antigens. Previously Abdel-Rahman and Abdel-Megeed (23) reported that ELISA OD values obtained in homologous assays were higher than those in heterologous ones. It was found that sera from outbred mice immunized with a soluble B. glabrata antigen (SBgA) of non-infected snails recognized molecules of SBgA itself and S. mansoni AWA (24). While, Stein and Basch (25) found that anti-sera from mice given antigens derived from B. glabrata haemolymph did not form immunoprecipitates with soluble antigen from adult worms. As regards B. truncatus snails, were used for comparison. Antisera from mice given antigens (foot and visceral hump) derived from B. truncatus snails gave negative reactions with S. mansoni worm crude antigen. While the high ELISA OD values detected between anti-B. truncatius antibodies and both B. alexandrina (foot and visceral hump) antigens may be due to the presence of common antigens between these cold blood invertebrates.

In this study B. alexandrina foot antigen appeared more sensitive than its visceral hump antigen. It diagnosed 80% of S. mansoni infected patients with considerable positive mean ELISA OD value (0.24). B. alexandrina visceral hump antigen was less sensitive as it detected 33.3% only of S. mansoni positive patients, accompanied by weak positive mean ELISA OD value (0.18). It was excluded when using higher serum dilution (1:100). The results obtained by Shalaby et al. (16) revealed higher specificity of snail feet in antibody detection than hepatopancreas antigens.

In the present study, ELISA plates were coated with 4µg protein of antigen per well after checkerboard titration. Espino et al. (26) made several trials to eliminate the cross reaction by several antigen concentrations and different serum dilutions. They concluded that the best results were obtained when the ELISA plate was coated with 4µg protein per well. In this study, serum dilution 1:50 was found to be the optimum dilution after checkerboard titration as well as in the study of Sulahian et al. (27) all serum samples were tested at dilution 1:50, as no differences in sensitivity or background readings were detected when dilution 1:50, 1:100, 1:160 were used. Other authors (28, 29) observed that ELISA technique failed to differentiate the specific antibodies against target parasite at dilutions 1:100, 1:200, 1:400.

As regards the sandwich ELISA test in this study, the results showed that it was more
species specific than indirect ELISA as both B. truncatus antigens reacted negatively versus the two sandwich anti-sera. Ramzy and Hilyer (30) stated that the ELISA test had low species specificity (23 to 28%). Sandwich ELISA appeared sensitive and specific for immunodetection of active schistosomiasis and useful for monitoring its chemotherapy (31). Double sandwich ELISA was used to measure circulating antigen levels in S.mansoni infected children. Its sensitivity was 91.8% and specificity was more than 99% with no evidence of cross reactivity with other parasites (32).

Accordingly it may be concluded that B. alexandrina foot antigen was the best antigen among the tested ones that can replace S. mansoni adult worm crude antigen in diagnosis of schistosomiasis patients (P=0.05).

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29. Mousa WMA. Studies on the cross reactivity among some helminthes of veterinary and medical
المختصر العربي

قيمة مولدات الديم المنقاه والمحضر من مواقع البلهارسيا في تشخيص مرض البلهارسيا

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تعتبر البلهارسيا الأوسع انتشارًا فيما بين الأمراض الطفيلية. وبرغم من اتخاذ إجراءات العلاج الشامل، فضلت البلهارسيا مؤنَّادة وسريعة الانتشار نسبيًا. ويعتبر التشخيص السريري من أهم الوسائل التي تؤدي إلى دقة التشخيص وتساهم هذه الدقة بنوع ودرجة النتائج الطبية على الأنتيجين المستخدم. وقد تثبت وجود جزيئات للأنتيجين مشتركة بين دودة البلهارسيا والعائلات الواسعة. هذه الجزيئات تحت على المناعة ضد البلهارسيا. يهدف هذا البحث إلى دراسة قيمة مولدات الديم المعجمة والمحضر من مواقع البلهارسيا في تشخيص مرض البلهارسيا. تم تحضير مولدات الديم (الأنتيجينات) المستخلصة من دودة البلهارسيا المعجمة ومن مواقع البلهارسيا التقدم والأهشاء من الليموفولاريا والبوليس، وكذلك الأجسام المعجمة لكل أنتيجين في دم الفرد وتم استخدامهم في تشخيص مرض البلهارسيا المعجمة باستخدام نوعان من اختبارات الألزاج. اختبار الألزاج المباشر واختبار الساندوتون البيضاء.

أظهرت النتائج أن الأجسام المعجمة لدودة البلهارسيا المعجمة تتفاعل مع الأنتيجين الخاص بها في أعلى من التي سجلت مع أنتيجينات الليموفولاريا (القدم والأهشاء). كما أظهرت النتائج مقيدة الأجسام المعجمة للليموفولاريا على التفاعل مع أنتيجين دودة البلهارسيا المعجمة ولكن في قليل من التي تم الوصول عليها بالتفاعل مع الأنتيجينات الخاصة بها. وقد أوضح النتائج وجود تشابه كبير بين أنتيجين دودة البلهارسيا المعجمة والأنتيجينات الليموفولاريا في اكتشاف الأجسام المعجمة للبلهارسيا، وان أنتيجين القدم للليموفولاريا أكثر حساسية (80%) من أنتيجين الأهشاء (37%). وقد تم استخدام مواقع الليموفولاريا والمغارة وأظهرت النتائج أن الأجسام المعجمة للليموفولاريا كانت أقل أنتيجين القدم والأهشاء للليموفولاريا، والخلاصة أن أنتيجين القدم للليموفولاريا كان أكثر الأنتيجينات قدرة على أن يحل محل أنتيجين دودة البلهارسيا المعجمة في تشخيص المرضى المصابين بالبلهارسيا.