EVALUATION OF IMMUNOGLOBULIN M (IGM) AND INTERLEUKIN 2 (IL-2) ASSAY IN TRUE INFECTION OF *ENTAMOEBA HISTOLYTICA*

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ABSTRACT

Eighty six sera samples were collected from patients suffering from gastrointestinal symptoms (less than one year-67 years old) had been established from the beginning of May to the end of November 2010 who attends to the Maternity and Childhood Teaching Hospital and Al-Diwania Education Hospital in Al-Diwania Governorate. Nested polymerase reaction and restriction endonuclease were done previously and classified the specimen into positive for *Entamoeba histolytica* mixed with *E. dispar*, positive for *E. dispar* only and negative for *Entamoeba* sp. and usually control groups. IL-2 was detected by using ELISA in microtiter plate and designed the concentration of it in the serum. Data were translated into a computerized database structure. An expert statistical advice was sought for. Statistical analyses were computer assisted using SPSS version 13. The result showed there was an elevation in the median concentration of IgM and IL-2 in positive group for *E. histolytica* mixed with *E. dispar* in comparing with control groups, and those which was negative for *Entamoeba* and positive for *E. dispers* only. In conclusion the levels of the serum. IgM and IL-2 which are a mediator of inflammation gave a high sensitivity and specificity in relationship with invasive amoebasis due to the high titter of IgM and IL-2, so it can be used in early diagnosis of *E. histolytica* infection.

Keywords: Interleukin 2; Igm; *Entamoeba Histolytica*. 
تقييم مستوي الضد والمدور المناعي الثاني في الإصابة الحقيقية للزحار الأمبي

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الخلاصة

تم تصميم هذه الدراسة الجزئية المناعية باختيار 86 عينة مصل والتي جمعت من المرضى (ذكور وإناث) يعانون من أعراض معوية (أقل من سنة واحدة - 67 سنة) وللفترة الزمنية من بداية أيار حتى نهاية تشرين الثاني 2010 والذين راجعوا مستشفى النساء والأطفال التعليمي والمستشفى العام التعليمي في محافظة القادسية، بالإضافة إلى ذلك جمعت عشرون عينة مصل من أشخاص أصحاء كمجموعة سيطرة. أجري فحص البلمرة المتسلسل نوع (Nest.PCR) ويلي تقنيه استعمال الالزيمات القاطعة والداخلية للحامض (Restriction Endonuclase) سابقاً. تم تصنيف العينات إلى أربعة مجموعات هي المجموعة موجبة لأمييا الزحار مخلوطة مع أميا الدسبار، والمجموعة الموجبة لأمييا الدسبار فقط، المجموعة السالبة لجنس الأمبيا وأخيراً مجموعة السيطرة. تم تقييم دور الضد في التشخيص المبكر لطفيلي (IgM) immunoglobulin وتم تكليف المضادات من المصل بتطبيق الألزيمات القاطعة والداخلية (Single Radial immunodiffusion Assay) أظهر وجود قيمة إحصائية معنوية (P<0.001) بين كل من السالبة لطفيلي الأمبيا ومجموعة السيطرة وبين السالبة والموجبة لجنس طفيلي الأمبيا وبين الموجبة للأمييا الحالة للنساء المرضى والموجبة لأمييا الدسبار غير المرضية. تم قياس تركيز المدور الثاني بالمصل بإستخدام الألياف بأطواق خاصة. إحصائياً تم نقل النتائج إلى الحاسوب وفق برنامج خاص للتحليل الإحصائي. أظهرت النتائج إرتفاع في متوسط التركيز لمستوى المدور الثاني ومستوى الضد في مصل العينات الموجبة لأمييا الزحار الممزوجة مع أميا الدسبار بالإضافة إلى تلك النتائج لأمييا الدسبار فقط وتلك السلبية لجنس الأمبيا.
INTRODUCTION

Entamoeba histolytica is a unicellular protozoan parasite that infects about 45-50 million people each year, causing 40 thousand to 100 thousand deaths annually and may cause potentially life-threatening diseases such as hemorrhagic colitis and/or extraintestinal abscesses (1). Most infections are asymptomatic, but E.histolytica can invade the gut wall, causing severe ulceration and amoebic dysentery characterized by bloody stools (2). If the parasites gain access to damaged blood vessels, they may be carried to extraintestinal sites anywhere in the body, the most important of which is the liver, where the amoebae cause hepatic amoebiasis (liver abscess)(3). The two morphologically identical but genetically distinct species E.histolytica, which is potentially invasive, and E. dispar, which is not, together, E. histolytica and E. dispar infect about 10% of the world’s population (4). Trophozoites can infect the human liver for several months or years before abscesses are diagnosed. Expectedly, a host immune response is triggered, leading to the production of circulating immunoglobulin. (5). The IgM levels become negative in a short period of time after infection, with more than half of the subjects having negative results at 6 months or 100% becoming negative by 46 weeks after treatment (6).

MATERIALS AND METHODS

Patient groups and Healthy groups as control for comparing

Patient groups were suffering from differential diagnosis of digestive tract symptoms (abdominal pain, diarrhea, loss of appetite, dryness) of unknown etiology, no patient was treated prior to specimen collection. An eighty-six serum samples were collected from patients their age was less than one year-67 years old and had been established from the beginning of May to the end of November 2010. All patients were positive for Entamoeba spp using microscopic examination (Wet mount, concentration method) were tested at the laboratories of (Maternity and Childhood Teaching Hospital and Al-Dewania Education Hospital) in Al-Dewania Governorate. The collection done by drawing a 5-10 milliliter venous blood samples using a sterile syringe storing at room temperature for 30 minutes, and were centrifuged (2000xg, 5 minutes), serum was separated and placed in other vials and kept frozen at −20°C until used to serological test. Twenty serum were collected from healthy individual (had neither digestive symptoms nor diarrhea a previously history) using as control groups. All specimen were classified into four groups (1st positive for Entamoeba histolytica mixed with E. dispar, 2nd positive for E. dispar only, 3rd negative for Entamoeba sp, 4th control groups) according to the molecular methods which done previously.

Immunoglobulin M (IgM) assay in serum

Single Radial Immunodiffusion (SRID) is a well established technique, based on the binding of antigen and antibody to produce a visible precipitin ring in a gel. Measurement of the ring diameter enables quantification of specific proteins present in a test sample.
Interleukin 2 (IL-2) assay in serum

The steps were done according to the ELISA kit instruction of supplied company (US. Biological USA).

Statistical analysis

Statistical analyses were computer assisted using SPSS version 13 (Statistical Package for Social Sciences). Frequency distribution for selected variables was done first. The outcome quantitative variables (IL2 and IgM) were non-normally distributed. Such variables are described by median and interquartile range. The association between 2 categorical variables was assessed by Chi-square test. P value less than the 0.05 level of significance was considered statistically significant (7).

RESULTS AND DISCUSSION

Antibody detection test results

As shown in Table 1 and Figure 1 the median conc. of serum IgM was highest among those with mixed (*E. histolytica* and *E. dispar*) infection (413.1 mg/dl) and lowest in control group (22 mg/dl). The difference in median conc. between the 4 groups was statistically significant. The median IgM conc. in control group was significantly lower than the remaining 3 groups. The median IgM conc. in the mixed (*E. histolytica* and *E. dispar*) group was significantly higher than the remaining 3 groups. No important or statistically significant difference in median IgM conc. was observed between those with *E. dispar* only (89.2 mg/dl) and those cases negative for *Entamoeba* spp. (107.8 mg/dl).
Table (1): The differences in median concentration of serum IgM between 4 groups

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Negative for Entamoeba spp.</th>
<th>E. dispar</th>
<th>Mixed (E. histolytica+E. dispar)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum IgM concentration mg/dl</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>(19.9 - 23.1)</td>
<td>(24.2 - 180.6)</td>
<td>(24.2 - 310.9)</td>
<td>(53 - 781)</td>
</tr>
<tr>
<td>Median</td>
<td>22</td>
<td>107.8</td>
<td>89.2</td>
<td>413.1</td>
</tr>
<tr>
<td>Interquartile range</td>
<td>(21 - 22.6)</td>
<td>(54.1 - 142.5)</td>
<td>(46.5 - 138)</td>
<td>(338.9 - 491.1)</td>
</tr>
<tr>
<td>No.</td>
<td>20</td>
<td>24</td>
<td>46</td>
<td>16</td>
</tr>
<tr>
<td>Mean rank</td>
<td>3.5</td>
<td>43.81</td>
<td>41.47</td>
<td>81.13</td>
</tr>
</tbody>
</table>

P (Kruskal-Wallis) for difference between 4 groups < 0.001

P (Mann-Whitney) for difference between:

Control X Negative for Entamoeba spp. <0.001

Control X E. dispar <0.001

Control X Mixed (E. histolytica+E. dispar) <0.001

Negative for Entamoeba spp. X E. dispar =0.65[NS]

Negative for Entamoeba spp X Mixed (E. histolytica+E. dispar) <0.001

E. dispar X Mixed (E. histolytica+E. dispar) <0.001

NS=no significant
Interleukin IL-2 concentration in true infection with *E. histolytica*

As shown in Table 2 and Figure 2 the median conc. of serum IL-2 was highest among those with mixed (*E. histolytica* and *E. dispar*) infection (117.7 pg/ml) and lowest in control group (1.7 pg/ml). The difference in median conc. between the 4 groups was statistically significant. The median IL-2 conc. in control group was significantly lower (P<0.001) than the remaining 3 groups. The IL-2 conc. in the mixed (*E. histolytica* and *E. dispar*) group was significantly higher (P<0.001) than the remaining 3 groups. No important or statistically significant (P>0.001) difference in median IL-2 conc. was observed between those with *E. dispar* only (6.7 pg/ml) and those cases negative for *Entamoeba* spp. (6.7 pg/ml).
The production of antibodies is the main immunological manifestation of invasive amoebiasis in humans, although its presence is not associated with resistance to infection or with protective immunity (8). Serology can be seriously examined for the diagnosis amoebiasis and the absence of reactive IgG, IgA and IgM antibodies during convalescence is strong facts against invasive infection with *E. histolytica* (2,3). In the present study there was a statistically significant (P <0.001) in the concentration of IgM between control and negative groups, control and mixed (*E.histolytica* and *E.dispar*) groups and, negative and mixed groups. This may be due to the presence of invasive *E.histolytica* trophozoites that stimulate B-lymphocyte which have an important role in disease protection The P value was not significant between those of negative groups and infected with *E.dispar* only (0.65) that proved that *E.dispar* did not pathogen and did not stimulate the production of IgM like those infected with *E. histolytica*.
These results agree with a previous study which demonstrated the IgM in the experimental infection with *E. histolytica* showed the conc. of IgM 465mg/dl while there was no elevation in the conc. of those infected with *E.dispar* (9). (10) recommended the measuring of IgM was very useful in the diagnosis of amoebic dysentery. In the past study of (11), designate IgM is quite sensitive (->94%) and specific (->95%) when combined with serum, antigen detection while in the present study the sensitivity (87.5%), specificity (100%), this difference in the result may be due to the SRLD measuring the total IgM while other study using ELISA the specific IgM for *E.histolytica*, whereas (3) mentioned that the detection of antigen in stool was only ~50% positive while the estimation of IgM in the serum was 88%. The result of present study was conformity with a previous study done by (12) who revealed that in acute symptomatic infection the production of *E. histolytica* specific antibodies IgM and in non-endemic areas, serum antibodies to *E.histolytica* are useful for diagnosis (~70-80% positive in acute stage) but can be negative for the first 7-10 days of illness. Also(13) determined the incidence of probable invasive amebiasis in 15 symptomatic patients by several immunological tests, 13 out of 15 patients showed significant antibody titers and were probably infected with tissue-invading strains of *E. histolytica* (despite negative stool examination in two of them), and should be considered for radical treatment with appropriate amebicidal drugs.

Serological techniques have been developed to help establish the diagnosis of amebiasis. In the present study there was elevation in the cytokines which had been tested, and this may be due to the activation of neutrophiles results in amebicial activity mediated by nitric oxide production and that this activation may be essential for innate immunity against the parasite (14). In previous study, it had been observed that several mediators of inflammation tend to become elevated during *E. histolytica* infections. The concentrations of some pro-inflammatory cytokines was nearly similar to the present result, especially IL-2 in systemic circulation were reported to increase in acute stage and reach 154pg/ml (15). The present study showed that the median concentration of IgM, IL-2 were elevated in pathogenic *Entamoeba* than those infected with *E.dispar*, negative for *Entamoeba* and control group, and there was significant difference between them (p<0.001) and this result was similar to that conducted by(15), who concluded that the level of some pro-inflammatory cytokines were higher in amoebic desentary with other causes of diarrhea. In previously study(16) showed the analysis of the inflammatory response during intestinal amebiasis in human and animal models of the disease has revealed an important regulatory role for chemokines and cytokines. Recruitment and activation of inflammatory cells can also be modulated by secreted amebic factors, such as amebapores and monocot locomotion inhibitory factor. Several cytokines, such as and IL-2, have been shown to be associated with the development of amebiasis and was increasing during acute disease; the concentration of this cytokines were 232pg/ml.
A previous study (17) measured the supernatant concentration of a panel of cytokines and found that pro-inflammatory cytokines (IL-1β, IL-6, IL-8, IFN-γ, and TNF-α) were significantly and specifically secreted in the presence of *E. histolytica* but not in the presence of *E. dispar* this was a sign of the *E. histolytica* which activate the innate immune response while *E. dispar* was not, this finding was agreement with present study. Other cytokines (such as IL-2, IL-4, and IL-10) were not detected in the supernatant of the colonic explants cultured in the presence or absence of parasites (18). These findings are also in agreement with the earlier reports showing low IL-10 production during *E. histolytica* infection in both susceptible mice and a human epithelial cell line, while (19) successfully produced *E. histolytica* infections in IL-10, IL-2 high in mice. The high levels of pro-inflammatory cytokines induced here by *E. histolytica* trophozoites demonstrated that the ex vivo human model can be exploited to study the initiation of inflammatory responses at early stages in amoebiasis and that the explants' responses were specifically induced by amoebic virulence factors.

**REFERENCES**


