IMMUNOGLOBULIN PROFILE OF E. HISTOLYTICA AND E. DISPAR IN HUMAN SERA

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ABSTRACT

Determine the profiles of S- IgA , and IgA, IgG, IgM.In Human Sera (40) stool sample from suspected patients with E. histolytica /E. dispar and (50) sample of healthy individuals as a control ,were examined by direct smear method and wet preparation method to differentiated for infection with E. histolytica and E. dispar by CP5 and ED-1 primers. All blood samples were subjected to determine the S- IgA , by ELIZA technique and IgA, IgG, IgM by immune diffusion method.ELISA titer of secretory- IgA was (8.582 ± 0.351) µg /dLfor E. histolyticahigher than (5.213 ± 0.421) µg /dL for E. dispar with significant differences, and the concentrations of immunoglobulin IgA , IgG, IgM, (387.9 , 707.4, 294.9 mg/dl) respectively, were higher in E. histolytica patients group compared to E. Dispar patients and control group , with a significant differences.**s**erum anti-E. histolyticaIgG, IgA and IgM compared with serum anti-E. dispar was generally high , also the S- IgA was for E. histolyticahigher than for E. disparThese results emphasize the relevance of secretory IgA antibodies in the phenomenon of E. histolytica adherence to epithelial cells.

No: of Tables: 2 **No: of Figures**: 5 **No: of References**: 22

INTRODUCTION

Serological tests may be useful in the diagnosis of amebiasis in developed countries since E. histolytica infection is uncommon. Whereas in developing countries, infection due to E. histolytica remains endemic (Ohnishi et al., 2004).This makes definite diagnosis of amebiasis by antibody detection difficult because of the difficulty to corroborate the present from past infection (Parijaet al., 2014).

Several members of the genus Entamoebainfect humans. Among these only E. histolytica is considered pathogenic and the disease it causes is called amebiasis or amebic dysentery. Humans are the only host of E. histolytica and there are no zoonotic reservoirs. E. dispar is morphologically identical to E. histolytica and the two were previously considered to be the same species(Wiser, 2010).

This parasite has a very simple life cycle in which the infective form is the cyst that is considered a resistant form of the parasite. The asymptomatic cyst passers and the intestinal amoebiasis patients are the natural transmitters; they excrete cysts in their feces, which can contaminate food and water sources. Cysts are round structures around 10–16 μm in diameter (Ximénezet al.,2011).

IgG antibodies remains detectable for years after infection, whereas IgM antibodies clears from the circulation within a short period and can only be detected during current infection,ELISA remains an important diagnostic tool in patients with invasiveamoebiasis and has no cross-reactions with other nonpathogenic Entamoeba species contributing to high specificity. Indirect immunofluorescence assays have been shown as a rapid, reliable and reproducible methodology of antibody detection to differentiate amoebiasis from nonamebic diseases, and also past from current infection (Parijaet al., 2014).The disadvantages of antibody detection is its low sensitivity in developing countries where infections are endemic (Haqueet al., 2003; Fotedaret al., 2007).

In the study of Al-Yaquob, (2010) used an Enzyme Linked Immunosorbent Assay (ELISA) for detection of E. histolytica \ E. dispar and comparing it with microscopic examination .Sixty five samples were examined by ioden staining was positive in 35 (53.6 %) then these iodine positive samples examined by ELISA a lot of which 33 (94.2 %) were positive in the meantime 4 (13.33 %) were positive in ELISA although that they were negative in microscopic exam. It can be concluded that microscopical examination has low sensitivity and specificity in comparison with ELISA.

MATERIALS AND METHOD

1- Fecal samples collection.

All samples(40) were collected from hospitals in Babylon province ,all patients undergo full history and full information were obtained from the patients .Stool samples were taken from each patient

and collected in sterile containers for microscopic examination (wet mount). positive sample were frozen for DNA detection by conventional PCR.

wet mount preparation method, with an applicator stick picked up a small amount of specimen butting on clean sterilized slide and mixed with a drop of saline and use cover slip to get a clear vision and examine in 40x, 100x Identification of the parasite by its motile and size.

2-Blood samples collection.

From the same patients a three ml venous blood samples were drawn by disposable syringe 5ml and putting in a plain tube and centrifuge at 3000 rpm for 5 minute to separate the serum and then placed in another sterile tubes and kept in deep freeze at -20 C° until used in immunological tests.

Assay for measurement the level of immunoglobulins A in sera of control and patients with amoebiasis

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 , and measurement of ring diameter enables quantification of specific proteins present in a test sample.

Test procedure according to LiofilchemS.r.l. (Italy)

1- Remove easy rid from the envelope, open the plate and leave to stand for 5 min. at room temp. so that any condensed water in the wells can evaporate.

2- Fill the well with 5 µl of undiluted samples .

3. Close the plate with the lid , after the samples have diffused into the gel for about 20 leave to stand , overturned into the envelope at room temp. for 48 h.

Assay Procedures for Secretory IgA (S-IgA) ELISA for stool samples.The procedure deducted according to Cal biotech (USA-California).

Statistical analysis:

Chi-Square was used for detect statistical differences of data prevalence of disease and effect of other factors at significant differences p≥0.05 by use the application of statistical package social science (SPSS) (VERSION 17)(Al-Rawi, 2000).

Results :

Immunoglobulin S- IgA levels for E. histolytica , E. Dispar patients and control group byEnzyme Linked Immunosorbent Assay (ELISA) .

The level of secretory IgA showed significant differences in patients with E. histolytica compared with control group, The level of secretory- IgA was (8.582 ± 0.351) µg /dLfor E. histolytica and (5.213 ± 0.421) µg /dL for E. disparand (1.935±0.03) for control group, as showedintable (1).

Table (1): The level of S-IgA (µg /dL) in stool for E.histolytica, E. Dispar patients and control group

parameter	*E. histolytica* n=29	*E. dispar* n=11	Control group n=50
S-IgA	*8.582 ± 0.351	5.213 ± 0.421	1.935±0.03
P value 0.05 = 0.0214			

* significant differences at p ≤ 0.05.

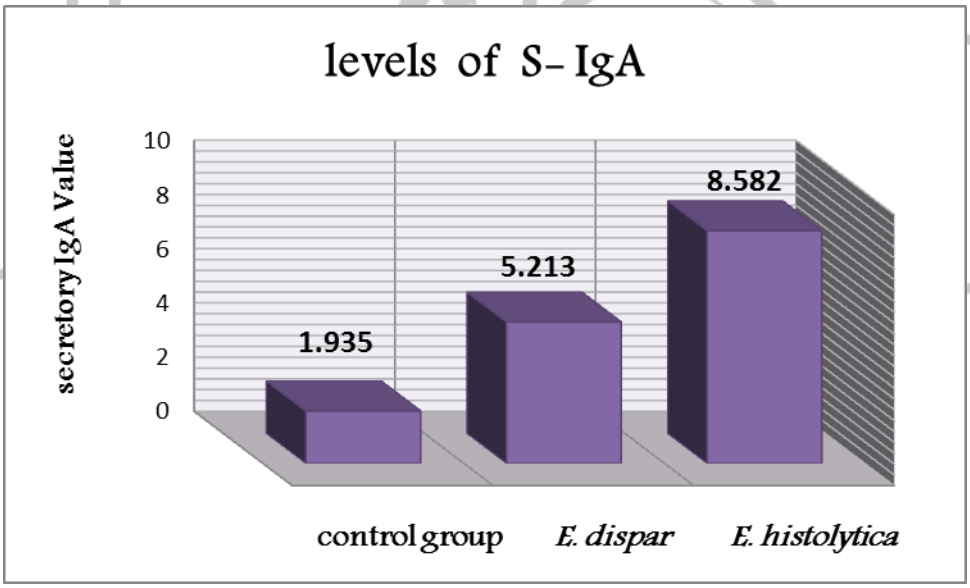

Figure (1): level of S-IgA (µg /dL) in stool for E.histolytica, E. Dispar patients and control group

Immunoglobulin levels IgA , IgG, IgM for E. histolytica and E. Dispar patients and control group byimmunodiffusion assay.

The result of the present study as shown in table (2) ,revealed the concentrations of immunoglobulin IgA , IgG, IgM, (387.9 , 707.4, 294.9 mg/dl) respectively, were higher in E. histolytica patients group compared to E. Dispar patients and control group , with a significant differences at p ≤ 0.05 for E. histolytica group .

Table (2): The level of Immunoglobulin IgA , IgG, IgM for E. histolytica and E. Dispar patients and control group byimmunodiffusion assay.

parameter	*E. histolytica* n=29	*E. dispar* n=11	Control group n=50
IgA	387.9±1.95	102.3± 2.5	*85.6± 3.6
IgG	*707.4± 22.5	293.2± 2.81	172.8± 2.8
IgM	294.9± 32.6	90.4± 1.7	*76.5± 5.4
P value 0.05 = 0.0322			

* significant differences at p ≤ 0.05.

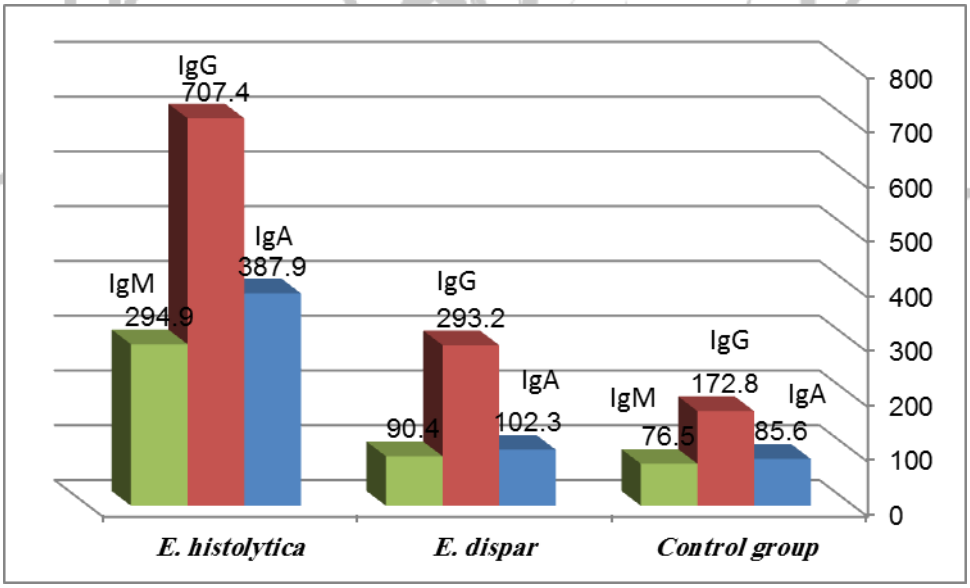

Figure (2): levels of Immunoglobulin IgA , IgG, IgM for E. histolytica and E. Dispar patients and control group byimmunodiffusion assay.

Figure (3): single radial immune diffusion for IgM for E. histolytica patients.

Figure (4): single radial immune diffusion for IgG for E. histolytica patients.

Figure (5): Single Radial Immune diffusion for IgA for E. histolytica patients.

DISCUSSION

Relation of Immunoglobulin S- IgA levels for E. histolytica and E. Dispar infections .

In the current study the level of secretory IgA showed significant differences in patients with E. histolytica compared with control group, the high level of secretory-IgA were agree with other studies as in the study of AL-Mahdawyetal.(2016) inAL-Yarmouk teaching hospital and AL-Tifil central hospital , which found that Secretory IgA increased significantly (5.46 ± 0.03) µg /mL for mail patients and (5.42 ± 0.03) µg /mL for female ,also agree with a studyin Indiarecorded the highest level of specific secretory anti-amoebic IgA were observed in the patients infected with intestinal amoebiasis and liver amoebic abscess (Sehgalet al ., 2010).

From our result It was concluded that a mucosal IgA antibody response is associated with immune protection against E. histolytica. the infection with E.histolyticaantigen specific secretoryimmunoglobulin A (IgA) antibodies have been found to mediate protection against intestinal infection by E.histolytica. it serves as lines of defense against microorganisms(Blaise, 2007).

Secretory IgA (SIgA) serves as the first line of defense in protecting the intestinal epithelium from enteric toxins and pathogenic microorganisms. Through a process known as immune exclusion, SIgA promotes the clearance of antigens and pathogenic microorganisms from the intestinal lumen by blocking their access to epithelial receptors, entrapping them in mucus, and facilitating their removal by peristaltic and mucociliary activities. In addition, SIgA functions in mucosal immunity and intestinal homeostasis(Mantis et al., 2013).

ELISA titer for sera results showed that both diarrheic and non-diarrheic individuals possess high levels of E. histolytica-specific

IgG compared to IgA and IgM ,specific IgA assay showed that 10 diarrheic samples and all 30 non-diarrheic were non-reactive to the parasitic (Rivera et al., 2012).

The assay for Diagnosis of intestinal amoebiasis using salivary IgA antibody detection was less satisfactory because of the overall sensitivity and specificity of the IgA ELISA was 36% and 72% respectively. The false positive rate was 28% and the false negative rate was 64%. The predictive value of a positive ELISA was 47% and the predictive value of a negative ELISA was 63%. The diagnostic accuracy for the presence of salivary IgA antibodies to E. histolytica was 58%. Therefore, the membrane antigens were more appropriate than those of soluble antigens for the diagnosis of salivary IgA antibodies in intestinal amebiasis(Punthuprapasaet al.,2001).

The sIgA response developed against some antigens of E. histolyticatrophozoites during the intestinal infection in humans and experimental rodents confers temporal protection against the re-infection, probably by inhibiting the adherence of the parasite to the colonic wall. Until now, the most feasible strategy for the development of a vaccine against intestinal amoebiasis is the use of one of the three antigens recognized by human S-IgA and involved in the adherence of the parasite to colonic cells, Gal-lectin, CREHP and SREHP, or their combination as the constituents of a preparation that will be delivered by oral or nasal routes(Carreroet al., 2007).

Human and parasite genetics along with environmental factors might have a role as it has been found that not all children are equally susceptible to infection. Malnutrition extensively increases disease susceptibility. Gut microbiome also influence Eh. infection susceptibility .Both host innate and adaptive immune response take part in the elimination of invasive Eh. The host immune system builds up a rapid inflammatory response by the secretion of cytokines/chemokines, recruitment of immune cells(neutrophils, macrophages),and the activation of inflammasome to control invasive parasites. This parasite alsodevelops multiple strategies to subvert host immune responses and to promote its own survival. Eh induces host cell killing primarily by apoptosis, which is a non-inflammatory cell death mechanism. Induction of apoptotic cell death is an active and step wise process. After inducing cell death, the parasite also clears the corpse by phagocytosis to inhibit further inflammatory responses. Another mechanisms amebic trogocytosis where Eh bites live cells very rapidly and induces cell death (Begum et al.,2015 ;Ralston et al.,2014).

Relation of Immunoglobulin levels IgA , IgG, IgM for E. histolytica and E. Dispar patients byimmunodiffusion assay.

The result of the present study revealed the concentrations of immunoglobulin IgA , IgG, IgM, (387.9 , 707.4, 294.9 mg/dl) respectively, were higher in E. histolytica patients group compared to E. Dispar patients and control group , with a

significant differences at p ≤ 0.05 for E. histolytica group.

Our study agree with other studies as in the study ofAl-Oumashiet al,(2012)in Al-Diwania Governorate ,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. dispar 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 amoebiasis due to the high titter of IgM and IL-2, so it can be used in early diagnosis of E. histolytica infection.

A study in Iran it was found out that there was significant increase in IgG level in amoebiasis group while, no significant increase was observed in IgA level. IgM level was increased only in amoebic liver abscess cases compared to negative controls (Ardalanet al., 2011).

The serum IgG antibodies persist for years after E. histolytica infection, whereas the presence of IgM antibodies is short-lived and can be detected during the present or current infection. An ELISA for detection of serum IgM antibodies to amebic adherence lectin was successfully used with patients suffering from acute colitis for less than 1 week, as 45% had detectable antilectinIgM antibodies(Haqueet al. , 2003).

A study in Theqar, showed that the mean concentration of IgG and IgA in patients withintestinal amoebiasis was more than those of control group with significant differences (p<0.05) , while there was no significant differences in concentration of IgM between two studied groups(Hussien and Shani, 2006).

SRID results also showed that the patients had a high concentration of IgA when compared with control group and these results identical with the results of Al – Kubassi (2002) and this may be attributed to the fact that the IgA had an important activity in humoral immunity against parasite which participating in reducing the infection and preventing the repeated infection (Haqueet al. , 2003) . Generally the elevated levels of IgG and IgA which documented in patients of the recent study may be due to the systemic sensitization of B – cells (Valenzuela et al. , 2011) . the latest worker showed that the serum IgM increases in patients with hepatic liver abscess but not in patients with intestinal amoebiasis , the negative results of IgM may be due to the fact that the patients of present study is in a different stage of infection (acute or chronic) (Valenzuela et al. , 2011).

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