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THE ASSOCIATION BETWEEN HIGH TITER ANTI-DS-DNA ANTIBODY AND ANTI – *ENTAMOEBA HISTOLYTICA* ANTIBODY TITER

Dr. Ali Abdul Hussian Mahdi* and Rugia A. Haider

College of Health and Medical Technology.

***Corresponding Author: Dr. Ali Abdul Hussian Mahdi**
College of Health and Medical Technology.

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ABSTRACT
The study was designed to estimate the prevalence of ds-DNA Antibody antibody. (DS-DNA) in 350 patients infected with *Entamoeba histolytica*. Diagnosis of DS-DNA & *Entamoeba histolytica* was done by using enzyme linked immune sorbent assay (ELISA) technique, their age ranged 15-40 years. The results were compared to control group which constitute of fifty apparently healthy men, their age ranged (15-40) years which is compatible to study group age. It was observed that there is a positive correlation coefficient between DS-DNA & *Entamoeba histolytica* (r=0.35729). Also there is significantly difference (t-test, P< 0.05) between mean of DS-DNA and *Entamoeba histolytica* of study and control groups. It was concluded that ds-DNA Antibody antibody. associated with *Entamoeba histolytica* infection due to the damage in intestinal tissue.

KEYWORDS: DS-DNA & *Entamoeba histolytica.*

INTRODUCTION

Entamoeba histolytica is an anaerobic parasitic protozoan, part of the genus Entamoeba. Predominantly infecting humans and other primates, E. histolytica is estimated to infect about 50 million people worldwide. Previously, it was thought that 10% of the world population was infected, but these figures predate the recognition that at least 90% of these infections were due to a second species, E. dispar. Mammals such as dogs and cats can become infected transiently, but are not thought to contribute significantly to transmission.[1]

Only about 10% to 20% of people who are infected with *E. histolytica* become sick from the infection. The symptoms are often quite mild and can include loose feces (poop), stomach pain and stomach cramping. Amebic dysentery is a severe form of amebiasis associated with stomach pain, bloody stools and fever. Rarely, *E. histolytica* invades the liver and forms an abscess (a collection of pus). In a small number of instances, it has been shown to spread to other parts of the body, such as the lungs or brain, but this is very uncommon.[2]

Diagnosis of amebiasis can be very difficult. One problem is that other parasites and cells can look very similar to *E. histolytica* when seen under a microscope. Therefore, sometimes people are told that they are infected with *E. histolytica* even though they are not. *Entamoeba histolytica* and another ameba, *Entamoeba dispar*, which is about 10 times more common, look the same when seen under a microscope. Unlike infection with *E. histolytica*, which sometimes makes people sick, infection with *E. dispar* does not make people sick and therefore does not need to be treated.[3]

A blood test is also available but is only recommended when your health care provider thinks that your infection may have spread beyond the intestine (gut) to some other organ of your body, such as the liver. However, this blood test may not be helpful in diagnosing your current illness because the test can be positive if you had amebiasis in the past, even if you are no longer infected now.[4]

Cysts and trophozoites are passed in feces. Cysts are typically found in formed stool, whereas trophozoites are typically found in diarrheal stool. Infection by Entamoeba histolytica occurs by ingestion of mature cysts in fecally contaminated food, water, or hands. Excystation occurs in the small intestine and trophozoites are released, which migrate to the large intestine. The trophozoites multiply by binary fission and produce cysts and both stages are passed in the feces. Because of the protection conferred by their walls, the cysts can survive days to weeks in the external environment and are responsible for transmission. Trophozoites passed in the stool are rapidly destroyed once outside the body, and if ingested would not survive exposure to the gastric environment. In many cases, the trophozoites remain confined to the intestinal lumen (noninvasive infection)

of individuals who are asymptomatic carriers, passing cysts in their stool. In some patients the trophozoites invade the intestinal mucosa (intestinal disease), or, through the bloodstream, extraintestinal sites such as the liver, brain, and lungs (extraintestinal disease), with resultant pathologic manifestations. It has been established that the invasive and noninvasive forms represent two separate species, respectively E. histolytica and E. dispar. These two species are morphologically indistinguishable unless E. histolytica is observed with ingested red blood cells (erythrophagocystosis). Transmission can also occur through exposure to fecal matter during sexual contact (in which case not only cysts, but also trophozoites could prove infective).[5][6]

Anti-dsDNA antibodies

Anti-dsDNA antibodies are a group of anti-nuclear antibodies (ANA) and their target antigen is double stranded DNA. Blood tests such as enzyme-linked immunosorbent assay (ELISA) and immunofluorescence are routinely performed to detect anti-dsDNA antibodies in diagnostic laboratories. They are highly diagnostic of systemic lupus erythematosus (SLE) and are implicated in the pathogenesis of lupus nephritis.[1][2]

MATERIAL AND METHODS

- Entamoeba histolytica (.) ELISA Kit (Biochic) / USA.
- Ds-DNA Antibody (.) ELISA Kit (Biochic) / USA.
- Apparatus ELISA (Biochic) / USA.

Principle of the Entamoeba histolytica (.) ELISA Kit

Abcam's anti-Entamoeba histolytica IgG Human in vitro ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for the accurate qualitative measurement of IgG class antibodies against Entamoeba histolytica in Human serum and plasma.A 96-well plate has been precoated with Entamoeba histolytica antigens to bind cognate antibodies. Controls or test samples are added to the wells and incubated. Following washing, a ProteinA peroxidase labelled anti-Human IgG conjugate is added to the wells, which binds to the immobilized Entamoeba histolytica-specific antibodies. TMB is then catalyzed by the ProteinA to produce a blue color product that changes to yellow after adding an acidic stop solution. The density of yellow coloration is directly proportional to the amount of Entamoeba histolytica IgG sample captured in plate.[11]

Method

***Reagent preparation**

Allow all components to reach room temperature prior to use in the assay.

The microtitration plate is vacuum-sealed in a foil with desiccant. The plate consists of a frame and strips with breakable wells. Allow the sealed plate to reach room temperature before opening. Unused wells should be stored refrigerated and protected from moisture in the original cover carefully resealed. Prepare a sufficient amount of wash solution by diluting the concentrated wash buffer 10 times (1 + 9) with distilled or deionized water.

For Example

10 mL wash buffer concentrate (2) + 90 mL distilled water.

ASSAY PROCEDURE

1- Dilute samples with sample diluent (3) 1:6,
e.g. 200 mg or 200 µL faeces + 1.0 mL sample diluent (3)
2- Avoid any time shift during dispensing of reagents and samples.
3- Make sure the soak time of the wash buffer in the wells is at least 5 seconds per wash cycle and that residual fluid is completely drained in every single wash cycle.
4- Avoid light exposure of the TMB substrate solution.
5- Working Steps Warm all reagents to room temperature (RT) before use. Mix gently without causing foam.[11]

Principle of the DS-DNA test

Ds-DNA Antibody (DS-DNA) antibodies ELISA kit is based on binding of DS-DNA antibodies from serum samples to purified DS-DNA antigen immobilized on microtiter wells. After a washing step, goat anti-mouse IgG-HRP conjugate is added. After another washing step, to remove all the unbound enzymes conjugate, chromogenic substrate TMB is added and color developed. The enzymatic reaction (blue color) is directly proportional to the amount of DS-DNA antibodies present in the sample. The reaction is terminated by adding stopping solution (converts blue to yellow). Absorbance is then measured on a microtiter well ELISA reader at 450 nm.

Sample Preparation

- Collect blood specimens and separate the serum.
- Specimens may be refrigerated at 2-8°C for up to seven days or frozen for up to six months. Avoid repetitive freezing and thawing.

Assay Procedure

Bring all specimens and kit reagents to room temperature (18-26°C) and gently mix.

Place the desired number of coated strips into the holder. Negative control, positive control and calibrator are ready to use. Prepare 1:21 dilution of test samples, by adding 10 µl of the sample to 200 µl of sample diluent. Mix well. Dispense 100 µl of diluted sera, calibrator and controls into the appropriate wells. For the reagent blank, dispense 100 µl sample diluent in 1A well position. Tap the holder to remove air bubbles from the liquid and mix well. Incubate for 20 minutes at room temperature. Remove liquid from all wells. Wash wells three times with 300 µl of 1X wash buffer. Blot on absorbance paper or paper towel. Dispense 100 µl of enzyme conjugate to

each well and incubate for 20 minutes at room temperature. Remove enzyme conjugate from all wells. Wash wells three times with 300 µl of 1X wash buffer. Blot on absorbance paper or paper towel. Dispense 100 µl of TMB substrate and incubate for 10 minutes at room temperature. Add 100 µl of stop solution.

9. Read O.D. at 450 nm using ELISA reader within 15 min. A dual wavelength is recommended with reference filter of 600-650 nm.[12]

RESULTS

A cohort study carried on 350 patients infected with Entamoeba histolytica, all of them were Entamoeba histolytica (+ve) and DS-DNA (+ve and –ve) as a study group and 50 healthy men, Entamoeba histolytica (-ve) and DS-DNA (+ve & -ve) as a control group.

Table (1) shows that the (mean±S.D.) of age, DS-DNA and Entamoeba histolytica titer were (32.27±5.026), (24.11±13.850) and (14.95±5.609) for study groups respectively, while (32.96±4.898), (5.09±2.410) and (3.73±1.702) for control group respectively. The present work shows a positive correlation coefficient between DS-DNA & Entamoeba histolytica titer (r=0.34729). About the mean of age of studied groups were statistically insignificant (t-test, P>0.05), whereas the difference between mean of DS-DNA and Entamoeba histolytica titers of study and control groups were statistically significant (t-test, P< 0.05).

Table 1 – Mean of age, DS-DNA and Entamoeba histolytica titer of study and control groups.

	Age*	DS-DNA titer**	Entamoeba histolytica titer**
Range	15-40years	Cut-off (10 IU/ml)	Cut-off (7 IU/ml)
study groups (n=100)	32.27±5.026	24.11±13.850	14.95 ±5.609
control group (n=50)	32.96±4.898	5.09±2.410	3.73±1.702
correlation coefficient between DS-DNA & Entamoeba histolytica (r)	0.35729		

* Difference between mean of age of study and control groups were statistically insignificant (t-test, p > 0.05).
** Differences between mean of DS-DNA and Entamoeba histolytica titers of study and control groups were statistically significant (t-test, p < 0.05).

Result appeared in table (2) shows that the mean of age, DS-DNA and Entamoeba histolytica titer among study and control groups who were without history of acute infection. The result of patients from study group Entamoeba histolytica (+ve) versus DS-DNA (+ve & -ve) who had no history of acute infection and control group who had Entamoeba histolytica (-ve) versus DS-DNA (+ve & -ve) as a control groups shows that the difference between mean of age, DS-DNA and Entamoeba histolytica titer were statistically significant (t-test, P<0.05).

Table -2: Mean of DS-DNA and Entamoeba histolytica titer without history of Acute infection from study and control groups.

	Study group Entamoeba histolytica +ve (n=15)	Control Entamoeba histolytica -ve (n=50)	P value
Age	29.93±6.638	32.96±4.898	P > 0.05
DS-DNA titer	19.0±7.653	5.09±2.410	P < 0.05*
Entamoeba histolytica titer	10.4±3.80	3.73±1.70	P < 0.05*

* Differences between mean of age, DS-DNA and Entamoeba hitolytica titers of women without history of acute infection from study and control group
were statistically significant (t-test, p < 0.05).

DISCUSSION

Table (1) shows that there is a positive relation between DS-DNA titer and Entamoeba histolytica titer among studied groups (r=0.35729). This result agrees with researcher who reported that DS-DNA antibodies in infected men are found to be the important factor for recurrent infection especially in the Acute infection.[13] Also the table demonstrate that there is a statistically insignificant difference in mean of age for patients and control groups, that is mean the age may did not influence in the titer of DS-DNA and Entamoeba histolytica titer (P>0.05).

In the current study, there is a statistically significant difference between mean of DS-DNA and Entamoeba histolytica titer for study and control groups (P<0.05). This result agrees with a study done by Lockshin *et.al.* who seems that high level of DS-DNA antibodies. may

be found transiently in patients with Entamoeba histolytica.[11]

Results denoted in table (2) shows that there is statistically significant difference between mean of DS-DNA and Entamoeba histolytica titer of study and control groups who have no history of Acute infection. This data disagrees with researchers who clarified that this antibody (DS-DNA) is believed to cause thrombosis in the maternal circulation leading to events that lead to clot.[13-14]

Our explanation to demonstrated result in table (3) that the low concentration level of DS-DNA and Entamoeba histolytica titer may not effect to cause clot in patiens.

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