Diagnostic study of infection with the parasite E. histolytica and its relationship to some immunological and physiological indicators in infected patients in Najaf Governorate

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Abstract

The current study was conducted for the period from November 2021 until May 2022, which aims to study an epidemiological and diagnostic study of the parasite Entamoeba histolytica and to measure some immunological parameters of those infected with the parasite. The results of the microscopic examination of 1933 samples infested with 327 samples of tissue-forming amoeba parasites, with an infection rate of 17%. In the month of October, it was 8%, and the results showed that the highest percentage of infection with the tissue-dystrophic amoeba parasite was in the liquid stool samples, which amounted to 68.6%, respectively, and the lowest percentage was in the solid stool samples, which amounted to 2.4%. Males are higher than females, reaching 53.5% and 46.5%, respectively. The results also showed that the highest percentage of amoeba parasites was for the age group 21-30 years, which amounted to 23.9%, and the lowest percentage for group >50 reached 11.6%. The ELISA technique increased the concentration of interleukin-2 in serum for those infected with the histiocytic amoeba parasite, as the ratio reached 61.4 ng/ml compared with the serum group of healthy people, which amounted to 4.1 ng/ml. The results showed high concentrations of TNF-a and anti-IgE in serum for those infected with E. Histolytica parasite, it reached (28.7 and 29.3) ng/ml, respectively, compared to the healthy serum, where the concentrations of TNF-a and IgE antibody reached 25.3 ng/ml.

Keywords: E. histolytica, IL-2, TNF-a, IgE

Introduction

Intestinal parasite infection is an important health problem worldwide. According to the World Health Organization, it is estimated that more than 24% of people worldwide are infected with intestinal parasites, with a high prevalence in developing countries. (W. H. O, 2015) Amoebiasis is a disease Intestinal origin is responsible for a parasitic protozoan called mytolytic amoeba that infects humans and some animals (Fadile et al, 2013). Amoebic dysentery is classified as the third most common cause of death among diseases after malaria and schistosomiasis (Moustafa, 2013). Infections with this parasite are mostly without Symptoms But about 20% of cases show clinical symptoms such as dysentery, diarrhea accompanied by mucus when the parasite attacks the mucous layer of the colon and destroys tissue (Haque, 2009). The enzyme-linked immunosorbent assay (ELISA) is one of the modern methods in Diagnosing intestinal parasites by detecting antigens and using an antibody to the parasite to detect the antigen, and this technique is highly sensitive compared to other methods (Ryanet al. ., 2017; Saidinet al., 2019). Antigen-based ELISA has several important characteristics compared to other methods currently used for the diagnosis of parasitism and has excellent sensitivity and specificity, and this enhances its being a large-scale screening tool in epidemiological studies (Al-Hindi et al., 2021).

Sample collection

1933 samples were examined for patients coming to Najaf hospitals, including (Al-Hakim General Hospital and Al-Furat Al-Awsat Hospital) starting from November 2021 until May 2022. Sterile plastic containers were used to collect feces, and the personal information of each patient was recorded on them, including (gender, age, and sample type).

Stool sample Examination

Stool examination

Stool samples were examined by eye, prior to microscopic examination, to observe stool consistency (liquid, semi-liquid, solid and semi-solid) and stool color, as well as noting that the sample contained mucous, fat or blood droplets (Turgeon & Fritsche, 2001; Calderaro et al., 2006). Stool microscopic examination The samples were examined microscopically in two ways:

A. Direct Mount Method

A clean glass bottle was prepared and a drop of normal saline was placed on it, then it was mixed well with a small amount of stool using a wooden stick, then the slide cover was placed at an angle to avoid air bubbles and examined under the microscope under the influence of small and large forces (10 X, 40X). This method enables the observation of the active phase and the accumulating phase of the parasite (Markell et al., 1999).

B. Floatation Method

In this method, a saturated solution of saturated NaCl was used, which was prepared by dissolving 36.0 g of table salt NaCl in 100 ml of distilled water gradually, making sure of good mixing for the purpose of dissolving the food salt completely. As per the following steps:

1) Approximately 1 gm of stool sample is placed in a clean tube for the test
2) Then some drops of a saturated solution of table salt were added to the tube
3) Then mix the ingredients in the tube using a glass straw
4) The tube is filled to the end by adding another amount of saturated saline solution
5) Seal the tube nozzle with a glass slide for half an hour
6) Removing the glass slide and turning the tube slowly and smoothly, then placing the slide cover and then examining it under a microscope to detect parasites under study (Forbes et al., 2007).

Blood Samples

Withdrawing 5 ml of the blood of the infected patients who were confirmed to be infected with the parasite, after the area of withdrawal was sterilized with methyl alcohol (70%) by a sterile medical syringe. The serum was withdrawn using a sterile Pasteur pipette, and placed in Apendroff tubes for the purpose of carrying out diagnostic tests using the enzyme-linked immunosorbent adsorption method (ELISA). It is according to what was mentioned in (Dacie, 1984, Lewis).

Measurement of the concentrations of IL-2, IgE antibody, and TNF-σA in serum

Measurement of IL-2 concentration in serum

The test was done by the Enzyme-Linked Immunosorbent Assay (ELIA) method, using an assay kit produced by the American company Elabscience. The method of work was completed according to the instructions attached to the examination kit, with a map of the samples and the standard concentrations of the wells on the examination panel, which are as follows:

1 - All contents of the test kit have been placed at room temperature for 30 minutes before use
2- 100 micro-liters of the concentrations of the standard solutions and samples were added to the holes in the test plate according to the prepared map, and the plate was incubated for two hours at 37 °C.
3- After that, the fluid in the holes was removed by pouring it out and then 100 µl of Detection Reagent A was added to each hole. The plate was incubated for an hour at 37°C.
4 - Then it was washed three times with the washing machine, and in this step, the washing solution of the examination kit was used.
5 Then add 100 µl of Detection Reagent B to each hole. The plate was incubated for 30 minutes at 37°C.
6- Then it was washed with the washing machine and the washing solution of the test kit for five times.
7- Then 90 µl of the substrate solution was added to each well and the plate was incubated for 25 minutes at 37°C.

8- Add 50 micro-liters of Stop Solution. The results were read at a wavelength of 450 nm directly.

Statistical Analysis

The results were statistically analyzed using the SPSS program and using the chi-square test (X2) and LSD to find out the significant differences between those infected with the parasite, and in the serological immunological study to find out the significant differences between the treatments at the level of probability $P < 0.05$ (Walker and Shostak,. 2010).

Results

Total infection with the parasite E. Histolytica The results, upon microscopic examination of 1933 samples, showed that 327 samples were infected with amoeba histolytica as shown in Table (1).

Table (1) The total number, number and percentage of people infected with the parasite E. histolytica

Parasite name	*E. histolytica*	
The number of people examined	The number	percentage (%)
1933	**327**	**17**

Distribution of infection rates with E. histolytica parasite according to the months of the study

The results of the current study showed that the highest infection rate was in May, reaching 25.7%, followed by 19% in April. In March, February, and December, similar rates were recorded: 16.7%, 14.6%, and 14.1%, respectively, while they were lower. Ratio in the month of October for the first 8%. The results of the statistical analysis showed that there were significant differences at the level of probability ($P \leq 0.05$) as in Table (2).

Table (2) The number and percentage of people infected with the parasite E. histolytica, distributed by months

The months	Examined number	Infected number	Percentage (%)
October	113	9	8
November	275	25	9.1
December	94	10	10.6
January	199	28	14.1
February	206	30	14.6
March	240	40	16.7
April	327	62	19
May	479	123	25.7
chi-square arithmetic	5.3		
tabular chi-square p< 0.05	2.17		

Distribution of infection rates with E. histolytica parasites by type of stool sample

The results of the current study showed that there was a difference in the rates of infection with the histolytica amoeba parasite, according to the difference in the sample strength, where the highest rate of infection was recorded in liquid stools 68.6%, followed by semi-liquid stools with 18.3%,

while the infection rate of semi-solid and hard stools was 10.4% and 2.4% The results of the statistical analysis showed, respectively, that there were significant differences at the level of probability (P≤0.05) as in Table (3).

Table (3) Number and percentage of people infected with E. histolytica by type of stool

Stool type	*E. histolytica*	
	The number	Percentage (%)
Liquid	225	68.6
Semi-liquid	60	18.3
Solid	8	2.4
Semi-solid	34	10.4
The total	327	
statistical analysis	chi-square arithmetic	tabular chi-square p< 0.05
	6.8	0.71

Distribution of E. histolytica infection rates by sex

The results showed that there was a difference in the incidence rates between males and females, and there were significant differences (P≥0.05). in the percentage of infection between the sexes, and the percentage of infection for males reached (53.5%), while the percentage of infection for females reached (46.5%), as shown in Table (4).

Table (4) Number and percentage of people infected with the parasite E. histolytica, distributed by sex

The sex	Infected number	Percentage (%)
Male	175	53.5
Female	152	46.5
The total	327	100
chi-square arithmetic	2.3	
tabular chi-square p< 0.05	0.102	

The percentage of infection with the parasite E.histolytica, distributed by age groups

The results of the study showed significant differences in parasite infection rates with different age groups, as it was found that the highest infection rate was recorded in the age group that ranged between 21-30 years and was 23.9%, and the age group between 1-10 years showed a percentage of infection 20.2, while the lowest percentages were recorded in the age group older than 50 years, at 11.6%, as shown in Table (5).

Table (5) The number and percentage of people infected with the parasite E. histolytica, distributed by age groups.

Age categories	Infected number	Percentage (%)
1 – 10	66	20.2
11 – 20	60	18.3
21 – 30	78	23.9

31 – 40	42	12.8
41 – 50	43	13.2
> 50	38	11.6
The total	327	100
chi-square arithmetic	6.3	
tabular chi-square p< 0.05	1.15	

Effect of parasite infection. histolytica in the IL-2 concentration.

The results of the current study showed that the concentration of interleukin-2 concentrations of those infected with the parasite E. Histolytica, which amounted to 61.4 ng/ml, were high compared to the healthy ones, which were 4.1 ng/ml as shown in Table (6).

Table (6) Comparison between control and infected with parasites based on IL-2 . concentrations

Infected type	M±SD
The control	4.1 ± 3
E. histolytica	61.4 ±36.3
LSD P < 0.05	11.4

The effect of infection with the parasite E. histolytica concentrations of cytokinetic TNF-a and IgE antibody.

The results of the current study showed that the concentrations of TNF-a and IgE antibody in the serum of infected E. histolytica were high, respectively, reaching (28.7 and 29.3) ng/ml compared to the serum of healthy controls, where the concentrations of TNF-a and IgE antibody reached 25.3 ng/ml as in Table (7).

Table (7) Comparison between control and infected with E. histolytica parasite based on TNF-a cytokinetic and IgE antibody

Infected type	M±SD	Arithmetic T	Tabular T
The control	25.3 ± 3		
TNF-a	28.7±3.6	2.8	1.96
IgE	29.3 ± 4.2	4.3	1.96
LSD P < 0.05	2.2		

Discussion

Total infection with the parasite E. Histolytica The results of the study of the tissue-state amoeba parasite were identical to what was found by Al-Nasser in (2010) in the city of Tikrit, where the rate of infection with the tissue-state amoeba parasite was 17.03%, and to the percentage recorded by (Jasim & AL-Mugdadi 2011) when it was 17.3% in the city of Baghdad. The results of the study were close to what Lazar found in the year (2012) in the city of Kirkuk, where he found the infection rate of the parasite is 21.67%. The infection rates reached 2.33% and 7.52%, respectively, and AL-Moussawi (2006), where the parasite infection rate reached 10.1% in the city of Babylon, and the results of the current study were less than those recorded by Muhammad and his group

(2011) in the city of Dhi Qar, and Jawad (2019) in the province of Najaf, and Ibrahim (2021) in the city of Kirkuk, where they recorded the infection rates of the parasite were 68.9%, 46%, 26.9%, respectively. It is also lower than that recorded by Hamad & Ramzy (2012) in Erbil Governorate.

Distribution of infection rates with E. histolytica parasite according to the months of the study

The results of the study showed that the highest rate of infection was in the month of May, and this percentage is close to what Al-Hashemi (2019) found, where the infection rates were recorded in May 21.36%, as well as the percentage recorded in February, which amounted to 15.18%, and it was similar to some extent with the results). Which Ibrahim (2021) reached, where the infection rate in May was 35.4%, in addition to its congruence with the percentage recorded in February, March and April, where the infection rates were 14.6%, 15.3% and 17.6%, respectively. Al-Samarrai (2008) in the city of Samarra and for the months of May and Nesian, as the percentages were 23.3% and 21.2, respectively. The results of the current study differed with (Al-Mayahi, 2009) conducted in Al-Diwaniyah governorate on children less than eight years old. The highest percentage was recorded in the month of March, reaching 66.3 %.

Distribution of infection rates with E. histolytica parasites by type of stool sample

The results of the study showed that the tissue-forming amoebae parasite had the highest percentage recorded in liquid stool, and this percentage is consistent with Ibrahim (2021), who recorded the highest percentage of stool samples with liquid consistency, which amounted to 39.5%, followed by semi-liquid samples with 37.7%, and the lowest percentage of solid samples. It reached 12.7%. The study also agreed with (Al-Sumaida'i, 2013), when the results of the study recorded the highest percentage of liquid stool samples, which amounted to 15%. The results of the study differed with the results of the previous study (Zubaida, 2014), where the highest percentage of semi-solid stool samples was recorded, followed by solid stool samples.

Distribution of E. histolytica infection rates by sex

The results of the study showed that the infection rate of the histolytic amoebae parasite in males is higher than the infection rate in females. The results of the current study coincide with Abdul (2021) where the infection rate for males was 55%, which is higher than in females, as the percentage was 30%. Elijah Abdul-Zuhairi (2013) in Diyala governorate, where the infection rate for females was 49.13%. The results of the study also agreed with the construction study (2006) in Baghdad governorate, where the infection rate for males was higher than for females. And with Al-Bayati (2007) in Al-Diwaniyah Governorate, where the infection rate was recorded in males higher than in females. The current study did not agree with the study conducted by (AL-Bayati, 2009), as the infection rate in males was recorded at 23.46%, which is less than the infection rate in females who It reached 28.57%. Also with Al-Daoudi in (2019) in Kirkuk governorate, where he recorded close ratios between males and females, when they reached 20.48% and 20.05%, respectively.

The percentage of infection with the parasite E.histolytica, distributed by age groups

The results of the current study showed that the highest infection rate was for the group whose ages ranged between 21-30 years, reaching 23.9%. The results of the study coincided with Ibrahim (2021) in Kirkuk governorate, as the infection rate for the group 21-30 reached 20%, and the results also converged with that of Its record for the age group 31-40 years was 16%. The results of the study agreed with Al-Hashimi (2019) in the holy governorate of Karbala, as the infection rate was recorded for the group ranging from 10-5 years, reaching 19.18%. The results of the current study differed with Lazar (2012) in Kirkuk governorate, where the highest percentage was for the age group of 10- 1 year, and it was also different with Al-Sumaidaie in the year (2013), as it also recorded the highest rate for the category of 1-10 years. There is a difference in infection between age groups from what is expected to happen due to the different behavior of individuals and the

activities they practice as well as exposure to the infective phase of the parasite from the surrounding environment (AL- Bayati 2013).

Effect of parasite infection. histolytica in IL-2 concentrations

The results of the study using the ELISA technique to examine the serum of people showed a high concentration of interleukin-2 for people infected with the histolytic amoebae parasite compared with people who were not infected with the parasite, as there was a significant difference between them. Host immunity against parasites by a Th2 helper cell. These cells are characterized by the production of high levels of interleukins in response to infection, such as IL-2, IL-5, and others. This immune response has the role of controlling the parasite population by killing parasites within tissues or expelling them from the intestinal hollow. The host response against infection with intestinal parasites is linked With what is called hypersensitivity, as it leads to the expulsion of the parasite or an attempt to kill it (Maizels, 2016; Cruz et al., 2017). IL-2 plays a key role in stimulating the immune response (Muhsin et al., 2013). The results of the current study agreed with that of Mohamed et al. (2017) in Baghdad Governorate, also with the study conducted by Abdelmoneim et al. (2010) in Egypt.

The effect of infection with the parasite E. Histolytica in IgE antibody concentrations and TNF-a cytokinesis

The results of the current study using ELISA technique showed an increase in the concentrations of TNF-a and anti-IgE in the serum of infected E. Histolytica parasite in comparison with the healthy ones. The reason behind this may be due to the result of the immune response against infection with E. Histolytica parasite, these differ The response is different from the parasite that causes infection (Mukai et al., 2016). Infection with parasites leads to the production of IgE antibody, in addition to activating the role of eosinophils (Amâncio et al., 2012). High concentration of IgE antibody often leads to parasite expulsion and elimination Among its toxins, another mechanism that contributes to the elimination of parasites is antibody-mediated cytotoxicity (ADCC) through receptors, including IgE receptor (Mukai et al., 2016). TNF-α is an important factor in the regulation of immunity. And resistance to infection with different parasites (Deroguich-Guerour et al., 2001). It is produced by macrophages and in large quantities, can lead to inflammation in tissues by activating macrophages (Kristina et al., 2010). In the rate of TNF-σ in m The contact of infected persons is associated with a long duration of infection, which helps to eliminate parasites naturally (Long et al., 2010). Tumor necrosis factor-alpha (TNF-α) is a proto-inflammatory cytokine that is released during parasite infection (Grit et al., 2014). It has a significant effect on getting rid of parasites (Zhou et al., 2007).

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