



RESEARCH ARTICLE

The Impact of Amoebic Dysentery on Immune Parameters in Age Groups 1-20 Years in Fallujah, Iraq: A Comparative Study Between Genders

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ARTICLE INFO	ABSTRACT
Received: May 21, 2024 Accepted: Jun 18, 2024	Fecal samples were collected from patients attending Fallujah Women's and Children's Hospital and some private laboratories in Fallujah, Anbar Governorate, for age groups ranging from (1-20) years, for both sexes. The collection period lasted from July 2023 until December 2023, and Entamoeba histolytica was identified through microscopic examination of fecal samples in 30 samples out of 120 samples, with a percentage of 25%. The highest infection rate was recorded in females at 27.69%, while in males the infection rate reached 21.81%. The highest infection rate was recorded in the age group (6-10) years, where the percentage reached 26.66% in female and 35.29% in males, and the lowest percentage was in the age group (16-20) years, where the percentage reached 12.5% in female and 14.28% in males. Highly significant differences appeared for INF Y for those infected with the parasite compared to healthy people, while for IgA and NK cells, a significant difference appeared for those infected compared with healthy people, and for Cysteine and TGF Beta, no significant differences appeared for those infected with the parasite compared with healthy people. The results of the current study showed that there were high significant differences for IGA for people infected with E.histolytica of both sexes compared to healthy people. As for INF Y and NK Cells, a significant difference was shown for those infected with E.histolytica, and the infection rate in females was higher than in males compared to healthy people. Cysteine and TGF Beta did not show any significant differences for those infected with E. histolytica of both sexes compared to healthy people. Highly significant differences appeared for IgA for those infected with E. histolytica, as an increase in its levels was observed for all age groups compared to healthy people, and for INF y, it showed a significant difference and an increase in its levels was observed for all age groups infected compared with healthy people, as for Cysteine, NK Cells, and TGF. Beta: There were no significant differences between the affected age groups compared to the healthy ones.
Keywords Amoebic dysentery Some immunological criteria Iraq	
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INTRODUCTION

Intestinal infections spread throughout the world, and their prevalence is increasing, especially in tropical and subtropical regions. Amoebiasis is considered one of the serious health problems in the world, and its infection rate is estimated at about 45 million people, or 12% of the world's population, annually (Kumar & Singh, 2016). Infection with amoebiasis is the third most common parasitic

infection in the world after malaria and schistosomiasis as a cause of death (Shirley et al., 2018). Amoebiasis may lead to 100,000 deaths annually, according to WHO, especially in tropical and subtropical regions (Wesel et al., 2021). The reason for the spread of infection and high rate of infections is due to the contamination of food and water (Al-Warid et al., 2013). Also, climatic conditions such as a humid climate, a low economic situation, unhealthy water, and poor drainage methods. These conditions are ideal for the presence of parasite cysts and retention, With its vitality (Kumar & Singh, 2016). *E. histolytica* is becoming increasingly prevalent in developing regions due to the resistance of the parasitic cysts to long-term survival in warm, humid conditions, as well as the lack of hygiene (Hofman, 2016). It may be transmitted to developed countries with travelers and immigrants to endemic areas (Gwairgi and Ghildyal, 2018). The cyst is considered the main source of infection, as well as for asymptomatic carriers of the parasite (Bogitsh et al., 2018).

Infection with the parasite stimulates the host's natural immune response, as mucous substances and lytic enzymes are secreted by the mucous membranes lining the digestive system and bowel movement, all of which represent natural defense methods that the body displays to eliminate the parasite (Shannon, 2013). As Pinnilla et al., (2008) mentioned, they indicated that the common hosts of the parasite are humans, dogs, monkeys, pigs, cats, rats, and mice. The infection spreads throughout the world and poses a major threat to health (Raza et al., 2013).

This parasite lives and settles in the large intestine and may cause symptomatic amoebiasis, such as bloody diarrhea, intestinal colic, and fever. It may not cause any symptomatic amoebiasis and is thus called a symptomatic carrier. Through the studies conducted, it was found that 10% of people infected with *E. histolytica* will show disease symptoms, while it was found that approximately 90% may not show asymptomatic symptoms, meaning that the disease does not develop in them, and they are called carriers of the disease (Ghasemi et al., 2015). The infection with this parasite is transmitted from one person to another person, either through contamination of water or food, or with the feces of infected people containing infectious cystic stage of parasite (Davhana et al., 2020). This parasite has a simple life cycle, as it consists of two stages, which are the feeding stage, the cystic stage, and the cystic stage. The feeder will cause damage to the host, which leads to cell damage, causing painful cup ulcers, thus causing amoebic dysentery because this stage lives in the lumen of the large intestine and feeds on the mucous membrane lining the intestine (Petri & Frederick, 2005). Upon infection with this parasite, the humoral immune response will be stimulated, as High levels of IgG, IgM, and IgA antibodies were observed.

E. histolytica was classified based on genetic, immunological and biochemical methods according to (Roberts et al., 2013) into the kingdom of protozoa, Class: Lobosea. Order Amoebida., Family Endamoebidae.

IgA antibody: It is the main type of antibody that is found in body fluids and blood serum. IgA is involved in the immune response against parasites, and IgA can also be secreted across the mucous membrane, which is considered one of the main antibodies in the intestinal lumen. IgA binds to microorganisms and causes them to lose their attachment to mucous surfaces. It is also characterized by its resistance to digestive enzymes, which makes it more resistant to intestinal bacteria. It also prevents the adhesion of microorganisms to mucous membranes, such as viruses and bacteria (Levinson, 2016). Studies have indicated that when infected with *E. histolytica*, the IgA type participates in the host's immunity, as it controls On the surfaces of the mucous layer of the intestine, it is the first immunoglobulin that contributes to the extermination of intestinal bacteria and protozoa (Ishii et al., 2008).

Cysten is the main virulence factor of *E. histolytica* and recent studies have demonstrated that it has a role in pathogenesis and leads to death of host immune cells, e.g., IgG, IgM, as well as mucus depletion and microbial, amoebiasis dysbiosis as the main virulence factors (Allain., 2019).

Interferon gamma INF-: It is mainly produced by lymphocytes located between epithelial cells when they are stimulated, which in turn activates natural killer cells, Interferon gamma has an important role in autoimmune diseases (Huang *et al.*, 2022). When the intestine is infected with *E.histolytica*, it is accompanied by the production of various forms of cellular motility as a result of the damage occurring in the intestinal lining, and (INF) is considered one of these motility. These motilities are produced by epithelial cells that make up the intestine or by the immune system adjacent to it. This action acts as a special chemoattractant for inflammatory cells, which include macrophages, mononuclear cells, neutrophils, and lymphocytes.

These cells are , NK cells are natural killer cells produced under the influence of the thymus considered an important type of white blood cells that respond quickly to non-specific foreign substances, which means that NK cells detect foreign substances that enter our bodies, so they will take immediate measures to kill them, and natural killer cells work to attack and kill tumor cells (Bain, 2017).

TGF Beta is an extracellular protein that is produced mostly by a group of T cells and is multifunctional as it regulates the growth and differentiation of different cell types and is involved in various processes such as normal development and immune function (Lucilia Campelo *et al.*, 2012).

The study aimed to investigate the extent of the spread of amoebic dysentery in some areas of Anbar Governorate, the extent of the impact of some prevalence criteria on the infection rate such as age groups, area of residence, standard of living, etc., while evaluating the extent of the impact of infection with the parasite on some immune parameters such as IGA, INF Y, TGF Beta. NK Cells Cysteine.

MATERIALS AND METHODS

1- Sample collection: 90 faecal samples were collected from patients attending Fallujah Women's and Children's Hospital and some private laboratories in Fallujah, Anbar Governorate. The collection period lasted from July 2023 until December 2023. The study included examining samples from both males and females, with ages ranging from one years to 20years. The collected faecal samples were stored in sterile, dry plastic bottles with airtight seals prepared for this purpose. Then record the patients' information in terms of (gender, age, residence, standard of living, presence and duration of diarrhea, and other symptoms, and odor of each sample.

2- Collect blood samples: 5 ml of blood was drawn from patients suffering from diarrhea and confirmed to be infected with *E. histolytica*, in addition to uninfected people within control groups. The blood is placed in test tubes free of anticoagulant and left for approximately 15 minutes at 37 degrees Celsius, after which the serum is obtained using a centrifuge at a speed of 3000 rpm for 5 minutes and kept at 20 degrees. -) °C in new, clean plastic tubes until physiological and immunological tests are performed.

3- Examination of stool samples: Stool samples were examined, where an amount of stool was taken as much as the head of a matchstick and from many places using a clean wooden stick, and the amount of stool taken was mixed with the physiological saline solution present on the slide, after which the cover of the slide was placed and then examined. Using an optical microscope with an oil lens 100 to confirm the presence of the parasite (Singh *et al.*, 2009).

Immunological tests:

1: Estimation of IgA concentration: The IGA concentration was estimated using the analysis kit (Kit) produced by the Chinese company Genrui and according to the steps attached to it.

2: Estimation of the concentration of interferon gamma INF Y: The concentration of INF Y was estimated using the analysis kit (Kit) produced by the Chinese company Sunlong.

3: Estimating the concentration of natural killer cells (NK Cells): The concentration of NK Cells was estimated using the analysis kit (Kit) produced by the Chinese company Sun long.

4: Cysteine concentration estimation: The concentration of Cysteine was estimated using the analysis kit (kit) produced by the Chinese company Sunlong.

5: Estimate concentration TGF Beta: The concentration of TGF Beta was estimated using the analysis kit (kit) produced by the Chinese company Sunlong, according to the steps attached to it.

RESULTS AND DISCUSSION

Faecal samples were collected from patients attending Fallujah Women's and Children's Hospital and some private laboratories in Fallujah, Anbar Governorate, for age groups ranging from 1-20 years of age, for both sexes. The collection period lasted from July 2023 until December 2023, and the *E. histolytica* parasite was identified through microscopic examination of fecal samples in 48 samples out of 90 samples, with a percentage of 53.3%.

The results of this study agreed with what was recorded by Ismail (2006), where he recorded an infection rate of 47.7% in Kirkuk, but did not agree with what was found by (AL-masoudi, 2009) in Babylon, where the infection rate reached 34.3%, and with the study ('Kadir *et al.*,2018/) in Tikrit, where the infection rate reached 9.3%.

The difference in the rate of infection with this parasite in this study and the previous and mentioned studies is due to the difference in population density, personal hygiene, geographical location, the range of age groups in which the study was conducted, the number of samples examined, as well as the level of sanitation, climatic conditions, and living conditions, while the similarity in the rate of infection is due to Similarity in terms of environmental conditions and cultural, social and health levels (Kurt *et al.* 2007).

The highest infection rate was recorded in females, reaching 54.34%, while in males, the infection rate reached 52.27%, as shown in Table (1).

The results of the current study, according to gender and not percentage, agree with what was found by (Jasim, 2011) in Baghdad, where the infection rate in males was 54.6%, and in females, the infection rate was 45.4%, and with what was found by (Ibraheem, 2008) in Kirkuk, where he recorded a percentage The incidence in males is 49.1% and in females 34.6%. The reason for these differences may be due to environmental and physiological differences, especially hormonal ones. Environmental factors include the difference in exposure to pathogens due to the different behavior between females and males (Zuk and Mckean, 2000), and the difference between the glands Deafness and immunity make a difference between males and females (Zahida *et al.*, 2010).

The results of the current study showed the highest infection rate in the age group (1-10) years, where the percentage reached 56.60%, but the lowest infection rate was recorded in the age group (11-20) years, where the percentage reached 48.64%. As in Table (2).

The results of the current study showed high significant differences for INF Y for those infected with *E.histolytica* compared to healthy people. As for IgA and NK cells, a significant difference was shown for those infected compared to healthy people. As for Cysteine and TGF Beta, no significant differences were shown for those infected with *E.histolytica* compared to With healthy people, as in Table (3).

The results of our current study in INF y value agreed with another study conducted on laboratory mice, which proved the close connection between interferon gamma and infection with the parasite, as interferon gamma works on the process of building the parasite's proteins by binding through special receptors present in the parasite's membranes through which it stops the process of Building proteins specific to the parasite, as they found that the parasite isolated from infected tissues of the

colon has a high affinity for binding to interferon gamma (Pulido_ortega *et al.* 2019). An increase in the concentration of interferon was observed. Gamma in infected people, as interferon gamma plays an important role during infection with *E. histolytica*, as the concentration of interferon gamma increases during infection with this parasite (Haque *et al.*, 2007). Interferon gamma is considered one of cytokines that regulate the immune response when infected with the parasite (Grodon and Martinez 2010). As for IgA, the results of the current study showed that there are significant differences in people infected with *E. histolytica*, and it agreed with many studies that showed An increase in IgA upon infection with *E.histolytica* , as IgA is able to neutralize toxins and bind with pathogenic microorganisms, making them lose their ability to It is able to bind to mucous surfaces through its ability to move across the mucous membranes, and when a defect occurs in the mucous membranes, there will be an increase in the level of immune antibodies in the mucous sites and a decrease in the serum (Macpherson *et al.*, 2008).

As for Cystatin, no significant differences were recorded, as it agreed with (Shahi *et al.* 2019 ; Ng *et al.*, 2018.) who also recorded a significant increase in the concentration of Cystatin between infected and healthy people, and that the reason for the significant increase in Cystatin for those infected with *E. histolytica* is due to the fact that cysteine is one of the main virulence factors of the parasite, as it plays an important role in invading tissues and disrupting the host's defenses by degrading the mucous layer lining the intestine, as well as stimulating the production of antibodies and produced by the parasite (Melendez - Lopez *et al.*, 2007).

As for NK cells, I agreed with (Fabricio *et al.*, 2012), where significant differences were also recorded, and the reason for the increase in level of natural killer cells is that NK cells contribute to resistance against *E. histolytica* infection and to controlling colitis resulting from the infection.

As for TGF β , the results of our current study agreed with (Lucilia *et al.* 2015) in France when they studied the effect of TGF β and INF γ on the functional activity of mononuclear cells in the presence of *E. histolytica*. These results indicated that cytokines play an important role for the host by activating mononuclear cells against *E. histolytica*, the increase in the death of amoebae during mononucleosis indicates that the cytokines TGF β and INF γ can modify the functional activity of mononuclear cells and that these cytokines may be important in controlling amoebic infections.

The results of the current study showed that there were high significant differences for IGA for people infected with *E.histolytica* of both sexes compared to healthy people. As for INF γ and NK Cells, a significant difference was shown for those infected with *E.histolytica*, and the infection rate in females was higher than in males compared to healthy people. Cysteine and TGF Beta did not show any significant differences for those infected with *E.histolytica* of both sexes compared to healthy people, as in Table (4).

Interferon gamma is one of the cytokines that regulates the immune response when infected with the parasite (Grodon and Martinez 2010). It has proven the important role of interferon gamma in controlling amoebic infections through its important role in modifying the functional activity of monocytes and activating them against *E.histolytica*.

As for Cysteine, I agreed with (Shahi *et al.*,2019) and (Ng *et al.*,2018), who recorded a significant increase in the concentration of cysteine for those infected with the parasite compared to healthy people. Perhaps the percentage of cysteine produced by the feeding phase of parasite will increase, as it plays an important role. Penetrating the intestines, which stimulates antibodies (IgA, IgG) (Que *et al.*, 2003). Many studies have indicated that infection with intestinal parasites leads to a mixed immune response represented by an increase in antibodies (IgA, IgG, IgM), (Buss *et al.*, 2008). As for NK Cells and TGF β , no significant differences were recorded, but the current results recorded a significant increase in levels in females more than in males. Infection with *E. histolytica* causes severe damage in males more than in females, although the infection rate is the same in both sexes. It may

be due to modification of the main mechanisms of the innate immune response by sex hormones. Complement mechanisms and estrogen-dependent natural killer cells lead to early elimination of the parasite in females, while the pathological immune axis is stimulated in males (2023, Marco Er-Lukowiak).

The results of the current study showed high significant differences in IgA for those infected with *E.histolytica*, as an increase in its levels was observed for all age groups compared to healthy people. As for INF, a significant difference was shown and an increase in its levels was observed for all age groups infected compared to healthy people. As for Cysteine, NK Cells, TGF Beta. No significant differences appeared between the affected age groups compared to healthy ones, as in Table (5).

Many studies have indicated a high level of IgA antibodies in the sera of people infected with *E. histolytica*, as the diseases caused by the parasite will lead to an immune response represented by the production of antibodies (Petri, 1999; Stewart, 2002). As for interferon gamma, our current results agreed with (Lucilia Campello *et al.*, 2015) where they also recorded significant differences, and an increase was observed in all age groups of infected people compared to healthy controls. Interferon plays a beneficial role for the host against the parasite, as it can control amoebic infections. It also works To stimulate and modify immune responses, interferon gamma is mainly produced by natural killer cells, as it plays a beneficial role in autoimmune diseases (Huaang *et al.*, 2022). The concentration of interferon gamma also increases during infection with the parasite, in addition to the role of interferon. Gamma helps limit and reduce parasite infection by activating macrophage cells that kill the parasite (Uddin *et al.*, 2022). As for cysteine, no significant differences were recorded between age groups, but the level of cysteine decreased for the age group (1-10), as cysteine It has an important role in the pathogenicity of the parasite, It has an important role in tissue invasion and in disrupting the epithelial system, as well as disrupting the host's defenses by degrading the mucous layer lining the intestine (Melendez-Lopez *et al.*, 2007). It is considered one of the most important virulence factors in the amoeba histolytica and plays an important role in tissue invasion and in disrupting the epithelial system as well as disrupting the host's defenses by decomposing the mucous layer lining the intestine. As for natural killer cells, which agreed with the findings of Ana Faria *et al.*, 2012, an increase in the levels of NK cells was observed for all age groups of infected people compared to healthy people, while natural killer cells contribute to resistance against parasite infection as well as in controlling amoebic infections. These cells play an important role in defending the host against parasitic infection and preventing infection.

An increase in the levels of NK cells was observed for all age groups of infected people compared to healthy people, while natural killer cells contribute to resistance against parasitic infection as well as in controlling amoebic infections. These cells have an important role in defending the host against parasitic infection and also prevent the development of amoebic liver abscess.

Table (1) Percentage of infection with *E. histolytica* by gender

Gender	, number of people tested	number of infected people	Percentage
FEMAL	46	25	%54.34
MAIL	44	23	%52.27
TOTAL	90	48	%53.3

Table (2) Percentage of infected people by age group for both genders, number of people tested, number of infected people, percentage

Age groups	, number of people tested	number of infected people	Percentage
1-10	53	30	%54.34
11-20	37	81	%52.27
TOTAL	90	48	%53.3

Table (3): Average immune titers for infected and healthy people according to the general analysis

group	TGF Beta Mean ± S.D	NK Cells Mean ± S.D	Cysteine Mean ± S.D	INF Y Mean ± S.D	IgA Mean ± S.D
The patients	309.0±29.9	101.2±60.7	3.70 ± 3.611	1696±86.4	6.96 ± 3.64
Healthy people	298.1±35.8	60.7±9.24	10.74±2.49	828 ± 25.9	3.64 ± 1.15
P-Value	0.862 ^{ns}	0.028*	0.302 ^{ns}	0.018**	0.021*

*Indicates that there are significant differences at the probability level of 0.05

**Indicates that there are highly significant differences at the probability level of 0.05

ns indicates that there are no significant differences at the probability level of 0.05

Table (4): Average immune parameters for infected and healthy people by gender

group	TGF Beta	NK Cells	Cysteine	INF Y	IGA
Male patients	298.9a ±32.8	63.8bc ±14.15	14.5a ±2.500	1354b±59.3	6.56a ±2.540
Female patients	317.1a ±41.3	104.7a ±10.20	13.35a ±3.260	1558a ±63.7	7.31a ±2.209
Healthy males	303.6a ±42.2	53.1c ±8.760	11.48a ±2.800	757d ±53.1	4.74b ±1.960
Healthy females	290.5a ±36.8	71.3b ±7.691	11.70a ±1.643	928c ±58.4	3.09b ±1.290
P-Value	0.997 ^{ns}	0.047*	0.898 ^{ns}	0.039*	0.007**

* Similar letters indicate that there are no significant differences at the 0.05 probability level

*Different letters indicate significant differences at the 0.05 probability level

Table (5): Average immune parameters for infected and healthy people by age

Age group	TGF Beta	NK Cells	Cysteine	INF Y	IGA
Patients 1-10	312.9a±31.2	68.22a±7.31	10.026a±3.51 7	1160.0a±68.8	6.958 a±2.3 23
Patients 11-20	280.2a±27.6	66.47a±9.72	9.838a±1.561	1527.0a±90.6	7.012a±2.2 30
Healthy 1-10	305.8a±39.8	64.82a±7.51	11.220a±2.01 2	842.4b±47.0	3.551a±1.4 40
Healthy 11-20	275.1a±24.9	63.30a±9.36	9.300a±2.630	786.1b±69.2	2.906b±0.8 66
P-Value	0.839 ^{ns}	0.915 ^{ns}	0.822 ^{ns}	0.028*	0.005**

* Similar letters indicate that there are no significant differences at the 0.05 probability level

*Different letters indicate significant differences at the 0.05 probability level

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