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Epidemiological and Immunological Study for Acute Amoebiasis Patients in Thi-Qar Governorate

Nadia Ahmed Al-Ubaydi[1*], Zahraa Sadoon Hadi[2] and Zainab Ali Alkaniny[3]

1. University of Thi-Qar / College of Education for Pure Sciences/ Biology Department. 2. University of Thi-Qar / College of Education for Women/ Biology Department. 3. University of Thi-Qar / College of Education for Pure Sciences/ Biology Department.

[2]zahra@utq.edu.iq and [3]zainabali.bio@utq.edu.iq

Correspondence author: Nadia Ahmed Al-Ubaydi, e-mail: Nadyaahmed.bio@utq.edu.iq

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Abstract

The present study aimed to find the relationship between Acute Amoebiasis and (Sex, Age and Location) in infected patients, also to estimate the role of interleukine-12 (IL-12) and Tumor necrosis factor –alfa (TNF-α) in immune response to Acute Amoebiasis patients by count the concentration of each one in study groups. Therefore, detected Acute infection with *Entamoeba histolytica* caused Acute Amoebiasis and studying some of epidemiological (Sex, Age, Location) and immunological (Cytokines IL12, TNF-α and Phagocytosis) parameters in infected patients. A total of (250) blood sample were collected from patients have diarrhea from Thi-Qar hospitals and private laboratories, divided into (100) infected samples with Acute Amoebiasis and (24) control from May 2018 to April 2019. The present study showed a relationship between infection and age groups, for example, the maximum percentage of infection in the age group (1-10) years which recorded 28% and the minimum percentage of infection in the age group (51-60) years which recorded 6%. The present results were not significant between female and male. The infection according to location found significant increased of percentage infection in rural area (68%) while decrease in urban area (32%).The present study showed significant increasing in Interlukine-12 and Tummor necrosis factor-alfa (TNF-α) in infected group compared with control group. Phagocytosis, the results showed increased significantly in the coefficient of phagocytosis in infected group compared with control group.

Keywords: Entamoeba Histolytica, Amoebiasis, IL-12, TNF-α, ELISA.

Introduction

Amoebiasis is currently defined as infection with the protozoan parasite *Entamoeba histolytica*. *E. histolytica* is an anaerobic parasitic amoebozoan. Normally resident in the large bowel, amoebae occasionally penetrate the intestinal mucosa and may disseminate to other organs (Hegazi *et al*., 2013). It has long been known that many people apparently infected with *E. histolytica* never develop symptoms and spontaneously clear the infection (Alvarado-Esquivel *et al*., 2015). Diarrhea is a major health problem worldwide, especially in developing countries. The causes of diarrhea vary from bacterial to bactericidal agents (*Escherishi coli, Salmonella* sp.), viral agents (*Astra virus, Corona*), Fungal agents (*Candida albicans*) and Parasitic agents (*Entamoeba histolytica, Giardia lamblia and Cryptospordium paravum*) (Kucik *et al*., 2004).

*Entamoeba histolytic*a contains lactin, which binds to galactose and N-acetylgalactosamine sugar on the surface of epithelial cells. Lactin is normally used to bind the parasite during swallowing (Ghosh *et al.*, 2019). The tissue amoeba parasite possesses a number of enzymes such as pore forming proteins, lipases and cysteine proteases that lead to the decomposition of epithelial cells causing cell necrosis and apoptosis, especially when the trophozoite of the parasite is then bound by lactin, These enzymes allow perforation of the intestinal wall and blood vessels and cross into the liver and other organs (James *et al.*, 2010). This leads to the formation of ulcers in the tissue and this effect may reach the blood vessels causing bloody diarrhea and amoebic dysentery (Shirley *et al*., 2018; Hung *et al*., 2008).

The trophozoite of the parasite reaches the bloodstream and is transmitted to the liver through the portal system leading to amoebic liver abscess. The parasite can reach the rest of the body causing the same pathological effect as the liver (Ralston *et al*., 2014; Cordel *et al*., 2013).

The body has different immune mechanisms in defense against amoebiasis. Interferon gamma (IFN-γ) contributes to protection against *Entamoeba histolytic*a, as in children, the rate of interferon (IFN-γ) is high, so the incidence of *E. histolytic*a diarrhea

is low (Moonah *et al*., 2013). Acquired immunity to protect against infection is involved through antibodies against parasite antigens (Ngobeni *et al.*, 2017).

As well as the production of a factors that is related to the tissue amoeba parasite and the production of cytokines during the infection, such as interleukin-8 (IL-8) protects the neutrophil and helps them to confront the parasite, when responding to interleukin (IL-8), it helps neutrophil cells pass through the intestinal cells and protect them from parasite infection (Sierra-Puente *et al*., 2009; Dickson-Gonzalez *et al*., 2009).

Material and Methods

1. **Collection of Data:** The current study performed on 250 patients suffer from diarrhea in Thi-Qar hospitals and special laboratories in Thi-Qar governorate. We collected 100 sample blood from infected patient with Acute Amoebiasis (infected group) and 24 sample blood from healthy persons(control group),also collected from two groups information about sex , age ,location ,family history , form period May 2018 to Aprile 2019.
2. **Diagnosis *Entamoeba histolytica*** by direct smear method and sedimentation technique according Stanley (2003).
3. **Using Enzyme-linked Immuno Sorbent Assay (ELISA) (Elabscience kit)** to counte concentration Cytokines (IL-12 and TNF-α).

Analyze technique

Improve 100 µL typical or sample to each well. Protect for 90 min at 37℃, Eliminate the liquid, Add 100 µL HRP Conjugate. Incubate for 30 min at 37℃. Remove and wash 5 times, Add 90 µL of Substrate Reagent. Incubate for 15 min at 37℃, improve 50 µL Stop Solution and Read at 450 nm immediately. Calculation of result.

4. **The phagocytosis:** studied the ability of phagocytic cells in peripheral blood to phagocyte Yeast Cells Killed by Somlata, (2014).

Statistical analysis

The current results were analyzed using T- test (unpaired, two tailed) to determine the significant differences between the infected study groups compared to the control group, Compare two independent proportions(two tailed) was also used to determine the relationship infection with sex and location. Correlation between the infection percentage and the age groups was used in this study.

Results

A. Epidemiological study

1. The Acute amoebiasis infection according to age groups

The current study showed a relationship between infection and age groups, for example, the highest percentage of infection in the age group (1-10) years which recorded 28% and the lowest percentage of infection in the age group (51-60) years which recorded 6%. The analysis used : correlation between the infection percentage and the age groups. The value of R is -0.993. This is a strong negative correlation, which means that high infection was seen with young groups and the less infection with the oldest groups. As in figure (1).

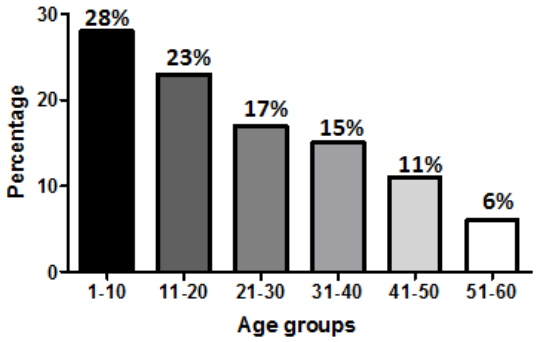

Figure-1: Showed correlation between the acute amoebiasis infection percentage and the age groups

2. The Acute amoebiasis infection according to sex

The present study found the highest infection percentage in females which recorded 58% compared to males that recorded 42% with non-significant differences. As in figure (2).

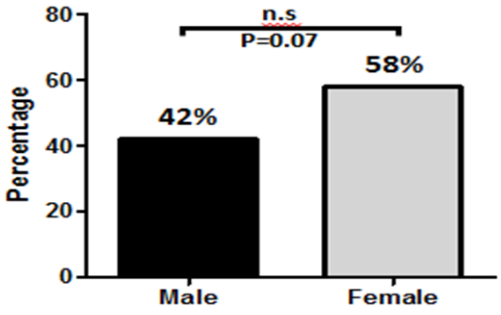

Figure-2: Relationship between the acute amoebiasis infection and sex

3. The Acute amoebiasis infection according to location

The results of this study found highest infection percentage in rural area which recorded 68%, while

lowerst infection rate was in urban area which recorded 32%. With significant differences. As in figure (3).

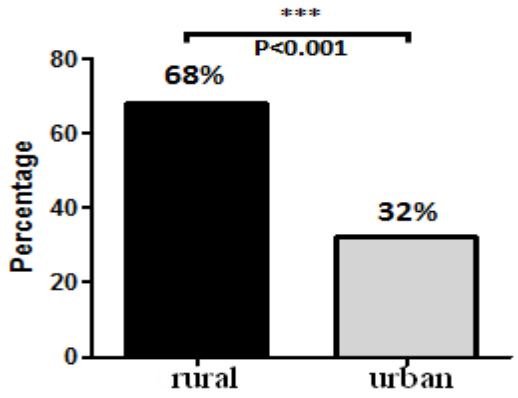

Figure-3: Relationship between Acute amoebiasis infection and location

B. Immunological study

1. Interleukine -12 results

The present results showed that IL-12 was elevated significant in the infected group which recorded 630.2 pg/ml concentration compared to the control which was 212.4 pg/ml. As in table (1) and figure (4).

Table-1: Interleukine -12 concentration in study groups

Groups	Control	Infected
Number of values	24	100
Mean	212.4	630.2
Std. Deviation	105.9	177.4
Std. Error	21.62	22.17

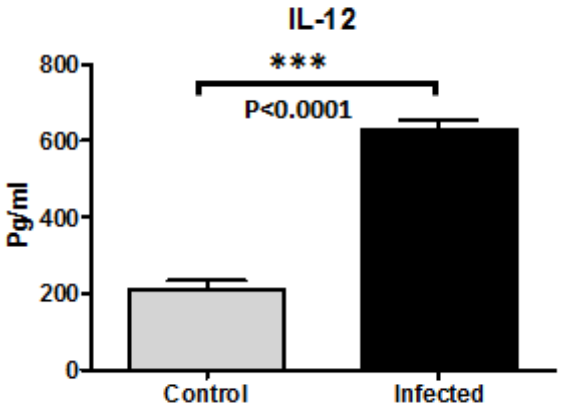

Figure-4: Effect of Acute amoebiasis infection on the concentration of interleukin-12

2. Tumor Necrosis Factor –alfa (TNF-α) results

The results of TNF-α showed significant increasing in infected group which recorded 365 pg/ml concentration compared with control group which recorded 232pg/ml .As in table (2) and figure (5).

Table-2: TNF-α concentration in study groups

Groups	Control	Infected
Number of values	24	100
Mean	232.0	365.0
Std. Deviation	65.00	70.05
Std. Error	13.27	8.756

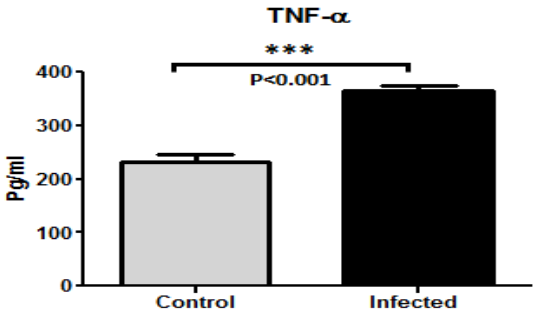

Figure-5: Effect of Acute amoebiasis infection on the concentration of TNF-α

3. Phagocytosis Results

The results of this study showed significant increasing in phagocytosis index, infected group recorded 57.53% compared with control which recorded 46.63%. As in table (3) and figure (6).

Table-3: Phagocytosis in infected group compared to control

Groups	Control	Infected
Number of values	24	100
Mean	46.63	57.53
Std. Deviation	6.628	6.850
Std. Error	1.353	0.8562

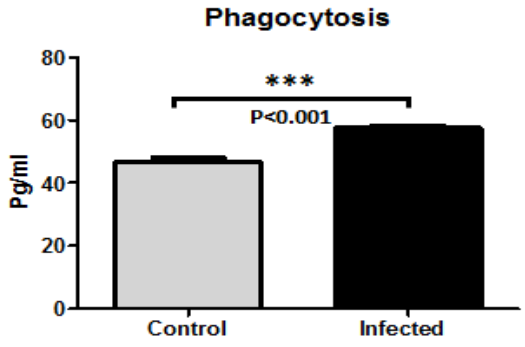

Figure-6: Effect of Acute amoebiasis infection on Phagocytosis

Discussion

The current study showed the infection of Acute amoebiasis in patients who attending the hospitals of Thi-Qar Governorate and the private laboratories for

the period from May 2018 to April 2019, the highest incidence of Acute amoebiasis in the age group 1-10 years (28%) and the lowest in 51-60 years (6%) with the highest percentage among children. This result was similar to that of Hadi (2011) with highest percentage in 6-10years (54.8%) and the lowest in > 21 years (27%) in the district of Shatrah and approach to results Ejaz *et al*,(2011),Al-Azawi, (2009), Raddam & Hasson (2008), Obadiah (2012), Al-Janabi and Tikriti (2014) due to the high proportion of children infected Amoebiasis because they are very active and they can pick up a lot of things and put them in the mouth be contaminated as well as unhealthy aids in children, such as putting the fingers in the mouth all these factors make more susceptible to parasitic infection, including *Entamoeba histolytica*, and the evolution of the immune system of children who are more sensitive to injury and general diseases especially parasites efficiency (Tasawar *et al*., 2010; Wegarshu *et al*., 2013).

The current study recorded a high rate of infection among females for males without significant differences. The results of this study were similar to those of Al-Khalidi (2016), Hadi (2011), Al-Masoudi (2009), Taswar *et al.* (2010). The present results differed with the results of Dakel *et al*. (2010) and Ejaz *et al.* (2011). These results may be due to similar conditions and habits leading to the spread of infection between both sexes, the convergence of the cultural level and the practice of the same behaviors and the nature of the studied areas In terms of the nature of the inhabitants and their treatment with contaminants, exposing both sexes to close rates (AL-Tourfy, 2014).

The study found that there were significant differences between the percentage of infection by location and the highest percentage of infection in the rural 68% and the lowest in city 32% differed with Al-Khalidi (2016), Al-Samraai (2008), Hamad & Ramzy (2012) And Al-Ebrahimi, (2013). Due to the poor sanitary conditions in the rural area as well as the lack of clean or non-availability of drinking water, the low level of education of most mothers, the lack of health services in the educational role in addition to the lack of environmental control that made the environment expose of biological pollutants (Ozer *et al.*, 2011).

The present study showed elevated of serum levels of cytokines (IL-12, TNF-α) and phagocytosis in the group that infected with acute Amoebiasis compared with control. The first line of Immune defense against E. histolytica is stomach acid which can killed the trophozoites while amebic cysts are very resistance (Mondal *et al*., 2012). Cysts excyst in leumen of intestine then, Trophozoites attach intestine tissues leading to distrupt the muscle layer to facilitate invasion of tissues (Lidell *et al*., 2006). This stage leading to discharge powerful cytokines to employee immune cells to the location of invasion (Bansal *et al*.,2009).The cytokines includes IL-12 , TNF-α and INF-ɤ which activate macrophages to discharge ROS and NO that destroy the parasite . IL-12 play acritical role in inflammatory immune response by activate the induction of TNF-α and IFN-ɤ from natural killer cells , IL-12 enhance the activity of $CD8^+$ cytotoxic T Lymphocytes and differentiates the T cells into T helper1.(Yu and Chadee ,1997).

Advanced TNF-α creation was lately shown to associate with *E. histolytica* diarrhea (Peterson *et al*., 2010). TNF-α trigger the cytotoxic activity by stimulates neutrophils and macrophages to release ROS and NO to fight the parasite and stimulates phagocytosis .TNF-α also, one of pro-inflammatory cytokine which mediate inflammation and stimulates acute phase protiens. (Lin *et al*., 1994).

Phagocytosis one of the most important defense against pathogens that invading host defense lines, neutrophils and macrophage play acritical role in this mechanism. neutrophils one of the first cells activated by TNF-α ,INF-ɤ and Lipopolysaccharides respond to amoebic invasion leading to activate amebicidal activity by phagocytosis and releasing reactive oxygen ROS (Nakada-Tsukui and Nozak, 2016).

This study agree with Peterson *et al*.(2010) study which establish an association between higher TNF-α creation and *Entamoeba histolytica* diarriah and an over –aggressive immune response from TNF-α cause improved inflammation and therfore diseases and the study of Rafiei et al. (2009) on 31patients suffering from amoebic colitis and 31 patients as ahealthy control ,serum levels of IL-12,IFN-ɤ,IL-13,IL-5 were higher in patients with amoebic colitis compared with control, the study of Helk *et al* .(2013) and study of Lotter *et al*.(2009) found that phagocytic activation increased and production of neutrophils and phagocytic cells also increased to defect against *E. histolytica.*

Conclusion

E. histolytica infection caused Acute amoebiasis continues to effect epidemiological and immunological in patients infected.

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