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Prevalence of Amoebiasis and Estimation of Certain Cytokines (IL-17, IFN-γ and TNF-α) in Children with Amoebic Infection in Sulaimani Province/Iraq

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Abstract
Objectives: We aimed to determine the prevalence of amoebic infection in Sulaymaniyah province and measuring of some immunological parameters among amoebic infected children.
Methods: The current study was carried out in the pediatric teaching hospital in Sulaymaniyah governorate from September to December 2021. A total of 560 stool samples were collected from diarrheal children for direct microscopical examination. Also, in a case-control study serum was taken from 80 infected children and 80 parasite-free children for estimation of IL-17, IFN- γ and TNF-α levels using the ELISA technique. Statistical analysis was performed by using the SPSS program, using Chi-square and ANOVA test. $P \leq 0.05$ consider a significant difference.
Results: The total prevalence of amoebic infection was (16.1%), males recorded a higher infection rate was (17.7%), while the female was (14.3%), ($P > 0.05$). The age group between (1–6) years had a significantly higher prevalence (21.5%), while those under than (1 year) old was recorded the lowest lower infection rate (7.54%), ($P < 0.05$). Prevalene in rural (20.3%), in urban (13.8%), ($P < 0.05$). The patients who used general tap water for drinking recorded the highest rate of infection (19%). The highest prevalence documented in September (19.5%), followed by October (16%), then November (11.2%), without significant difference, ($P > 0.05$). The highest amoebic infection rate was recorded in those children whose mother was illiterate (19.8%). Serum levels of IL-17 were not significantly different between infected children and control groups, ($P > 0.05$); however, IFN γ level was reported to be significantly different ($P < 0.05$) while, TNF-α serum level recorded a highly significant difference, ($P < 0.001$).
Conclusion: We concluded that the prevalence of amoebic infection was (16.1%) in Sulaymaniyah province among symptomatic children based on the microscopic diagnosis. The immunological assessment of IL-17 showed that there was no significant difference between infected and control individuals, while the rest of IFN- γ and TNF-α documented significant and highly significant differences respectively.
Keywords: Amoebic infection, *E. histolytica*, microscopy, cytokines

Introduction

Amoebiasis is a parasitic infection of the human gastrointestinal tract caused by *E. histolytica,* a primary parasite that leads to widespread morbidity and mortality. It affects 40–50 million people worldwide through diarrheal disease.[1] The National Institute of Health in the United States has categorized this infection as a category B priority biodefense pathogen.[2] According to reports, *E. histolytica* infection affects one-tenth of the world's population, and a large number of infected people (100,000 deaths worldwide) die each year.[3,4] After malaria, African trypanosomiasis, and leishmaniasis, this parasite is the fourth leading cause of death and the third leading cause of morbidity after malaria and trichomoniasis.[5]

E. histolytica can infect the intestinal mucosa and cause abscesses in organs like the liver and lung. Amoebic colitis and a potentially fatal liver abscess are among the symptoms of invasive amoebiasis. *E. dispar* and *E. moshkovskii,* for instance, may cause infection of humans.[1]

Transmission occurs through ingestion of food and water contaminated with amoebic cysts.[6-8] One of the main reasons that this disease is widely spread in developing countries is the lack hygienic measurements and contamination of water, in addition, the disregarding of parasitic infections in such countries increases the number of these infections.[9,4]

The main laboratory diagnosis of *Entamoeba* spp. in humans is the microscopic examination of stool samples but it is not possible to distinguish between these types through it.[10,11]

Because there is currently no vaccine for this lethal disease, an understanding of the human immune response toward the parasite would greatly enhance the ability to develop effective immunotherapies. As the parasite invades the colon, the host deploys a sequence of immune defences. The amoeba, on the other hand, has evolved sophisticated strategies for evading host defences and promoting its survival.[12]

Parasite-specific immune responses are regulated by cytokines and chemokines that lead to the development of immunity,[13] Among those cytokines are Interleukin-17 (IL-17), a potent proinflammatory cytokine, produced by activated T-cells, γ δ T-cells, natural killer (NK) cells as well as innate lymphoid cells.[14] The IL-17 receptor is found in a variety of tissues and cell types, such as lymphocytes, monocytes/macrophages, and epithelial cells. These cells produce diverse proinflammatory cytokines and chemokines in response to IL-17 stimulation. The effector role of IL-17 is primarily for accumulation of neutrophils and IL-17, while its effector role is primarily to accumulate neutrophils and increase the production of proinflammatory cytokines and antimicrobial peptides.[15,16]

Another important cytokine that contributes to protection against *Entamoeba histolytica* is Interferon-gamma (IFN-*γ*), as in children, the rate of interferon (IFN-*γ*) is high, so the incidence of *E. histolytica* diarrhea is low.[12] Acquired immunity to protect against infection is involved through antibodies against parasite antigens.[17] Moreover, Tumor necrosis factor-α (TNF-*α*) induces neutrophils and macrophages to secrete reactive oxygen species (ROS) and nitric oxide (NO) to fight the parasite, but an extra amount of TNF-*α* can cause indirect damage to host tissue.[18] Therefore, the initiation of effective immunity against *Entamoeba* requires the activation of antigen-specific T-cells, which cause either Th1 or Th2 phenotype depending on the secretion of appropriate cytokines.[19]

Aim of the Study

Since *Entamoeba* spp. is important enteric protozoa causing invasive and non-invasive disease, this study is designed to detect the pathogenic *Entamoeba histolytica* based on routine laboratory microscopically method for identification, this will lead to the determining of the Epidemiology and assessing some immunological parameters.

The objectives of the present work were:

1. The diagnosis of *Entamoeba* spp. from human stool samples using the microscopical method.
2. To determine the Epidemiology of *Entamoeba* species infection among symptomatic children in the pediatric teaching hospital of Sulaimani province based on microscopic examination.
3. To evaluate the serum levels of (IL-17, INF gamma and TNF-*α*) in infected patients with *E. histolytica.*

Materials and Methods

Sample Collection

Sampling Setting

In the present study a total of 560 children, suffering from diarrhea including 288 males and 272 females, ages 6 months - 15 years, mean of age (6.4 ± 3.93) year, were attended to the pediatric teaching hospital (Dr. Jamal Ahmed Rashed Hospital) in Sulaimani/Kurdistan region-Iraq, from the beginning of September to the end of November 2021. The children and their parents were interviewed, then an informative questionnaire form was organized for each patient including data such as age, gender, residence (urban, rural), maternal educational state, source of water drinking and period of infection history.

Collection of the Stool Sample

The stool samples were collected in a sterile container labelled with the names of the patients and brought to the laboratory of the Pediatric teaching hospital for macroscopic and microscopic examinations.

Collection of Blood Sample

A total (160) blood samples were achieved, (80) of the patients suffering from diarrhea visited the pediatric teaching hospitals as a result of infection with *E. histolytica* (patients' group) and (80) children were not infected with the same parasite (control group). From the venous of both infected and control groups (5 ml) of blood was collected. The collected sample was transferred immediately in a plain plastic tube (without EDTA) and left to clot at room temperature, then the samples were centrifugated for 5 minutes at 3000 rpm to separate the serum and put in other sterile tubes, each sample of serum was divided into two parts. The serum was collected in 3 Eppendorf tubes and each tube was labelled by a special identified number (ID) for all patients as well as the control group and stored at refrigerator (–70˚C).

Microscopical Examination

The collected fresh stool samples of symptomatic individuals were directly examined macroscopically by the naked eye for appearance, colour and the presence of blood then examined microscopically for trophozoite and cyst stages of *Entamoeba histolytica* using saline and iodine wet mount techniques.

Measurement of Immunological Parameters

The present case-control study involved serum sample from (80) symptomatic patients which compared to serum obtained from (80) healthy individuals without parasitic infections (negative control), for estimation of IL_17, INF- γ, and TNF-*α*, by using sandwich enzyme-linked immunosorbent assays (ELISA) technique and absorbance was measured at 470 nm according to the manufacturer's instructions (ElabScience®, USA).

Measurement of IL-17

Procedure:

This ELISA kit uses the Sandwich-ELISA principle. The micro ELISA plate provided in this kit has been pre-coated with an antibody specific to Human IL-17A. Standards or samples are added to the micro ELISA plate wells and combined with the specific antibody. After that, each microplate well is incubated with a biotinylated detection antibody specific for Human IL-17A and an Avidin-Horseradish Peroxidase (HRP) conjugate free components are washed away. The substrate solution is added to each well. Only those wells that contain Human IL-17A, biotinylated detection antibody and Avidin-HRP conjugate will appear blue. The enzyme-substrate reaction is terminated by the addition of stop solution and the colour turns yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The OD value is proportional to the concentration of Human IL-17A. Possible to calculate the concentration of Human IL-17A in the samples by comparing the OD of the samples to the standard curve.

Measurement of IFN-γ and TNF-α

Since the brand and the manufacturing company of the kits used for detection of serum IFN-*γ* and TNF-*α* levels are the same as that of IL-17, therefore, the principle of the reaction and the applied procedure is similar. Taking into consideration the differences in the type of the antibody pre-coated on the microplate for each respective immune marker. In addition to the differences in the serial dilutions and their standard curves.

Statistical Analysis

All recorded questionary was coded and given an identify the number (ID). The data was set into a Microsoft Excel

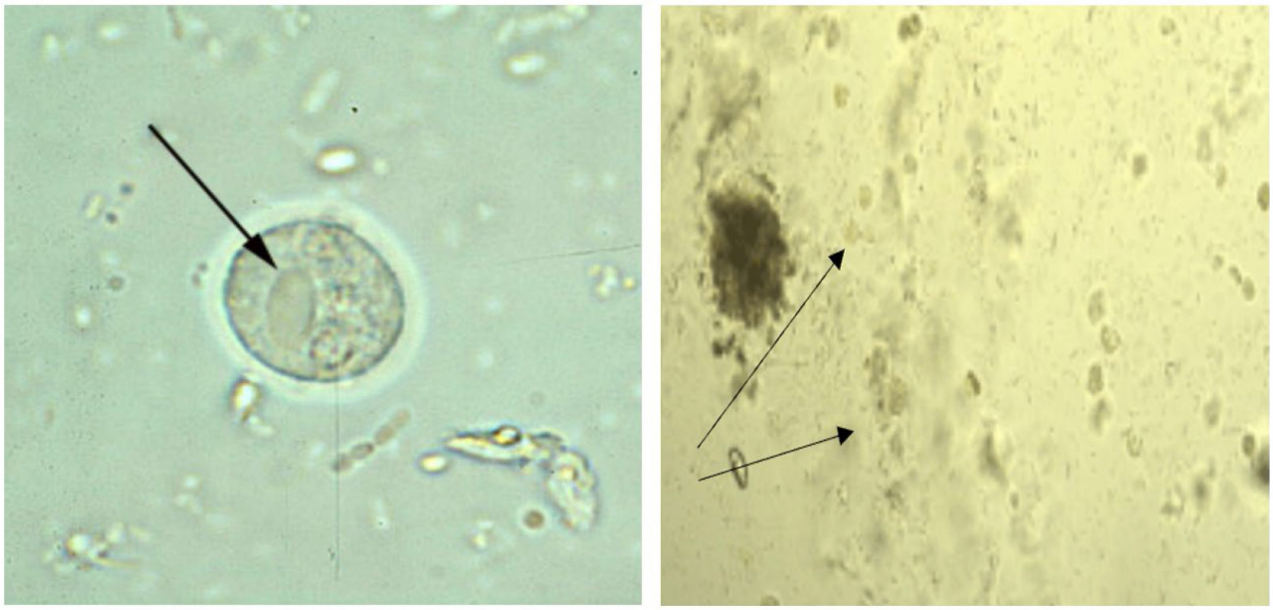

Fig. 1 **Cystic stage of *E. histolytica* using Saline (Left) and Iodine (Right) wet mount of stool sample under magnification power of (40X) of a light microscope.**

Spreadsheet, after data sorting, the data was transported into Statistical Package for the Social Science (SPSS) (version 23) software program for statistical analysis.

Descriptive statistics (number and percentage) were calculated for each variable; as well as analytical statistics were done to find the association between the variables. The relation between variables was calculated by using the applicable statistical test by Person's Chi-square (χ^2). *P*-value ≤ 0.05 was considered significant. The quantitative variables were presented as Mean ± Standard Deviation (S.D) and analyses used one-way analysis of difference (ANOVA). Also using a *t*-test to determine the significant differences between the infected study groups compared to the control group. $P \leq 0.05$ consider a significant difference.

Ethical Consideration

Ethical approval for the current study was sought from the Ethical committee of the college of medicine at the university of Sulaimani.

Results

Microscopical Examination of E. histolytica

During microscopical diagnosis out of 560 stool sample, 90 sample were positive for *E. histolytica* infection, direct wet tested either by saline or iodine. The cystic stage appeared as a small spherical body (10–15 µm) which contains granules and (2–4) nuclei mature cyst contains (2–4 nuclei) as shown in (Figure 1).

The Trophozoite stage of *E. histolytica* has indefinite boundaries, amoeboid shape and measured about 15–60 µm, it revealed fixed directional motility during saline wet mount examination (Figure 2). Trophozoites have a single spherical nucleus. The nucleus has a single centrally located karyosome, with chromatin which is delicate and evenly distributed, however, this feature was not observed. RBCs were seen in most trophozoites.

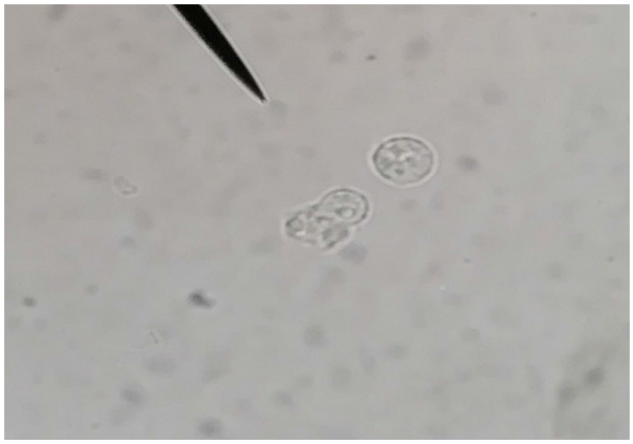

Fig. 2 **The trophozoite stage of *E. histolytica* in stool specimen using Saline wet mount under a light microscope (40X).**

Prevalence of Amoebic Infection

In the current study, from the microscopic examination of 560 stool samples, the total prevalence of *Entamoeba* infection (positive) was found to be 90 (16.1%) of diarrheal child individuals (Figure 3).

Prevalence of Amoebic Infection According to Gender

In the present study out of (560) diarrheal children (288) were males and (272) were females, (56.7%) of the infected child were males and (43.3%) were females, therefore the prevalence of amoebic infection was (17.7%) in males and (14.3%) in females without significant difference between the gender (*P*-value = 0.278) (Table 1).

Prevalence of Amoebic Infection According to Age

From the results of the relationship between age groups and the prevalence of infection with amoebic infection (Table 2), there was a significant difference observed in the rate of infection and age group ($P = 0.033$), the highest prevalence rate was

found in children aged (1–6) years old (21.5%), then followed by those children who aged between (6–12) years old (13.9%), then the children who older than (12) years old (11.5%), while the lowest infection rate (7.5%) was recorded in the age group of lower than (1 year) old.

Prevalence of Amoebic Infection Among Residency

From the study out of 560 examined stool samples of the children, 362 cases were from urban and 198 from rural areas, only 90 cases were positive for *Entamoeba histolytica* (50 in urban and 40 in rural habitats), the infection rate by this parasite was (13.8%) in the urban area, while in the rural area it was (20.3%), with a significant difference ($P = 0.031$) which is (<0.005) between both areas infections as shown in (Table 3).

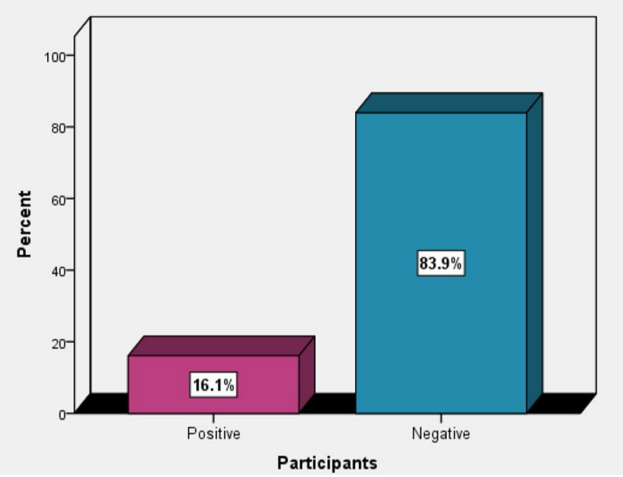

Fig. 3 **Prevalence of amoebic infection.**

Prevalence of Amoebic Infection According to the Source of Water Drinking

From our results, it was obvious that children who drink general water (chlorinated water) had more infections 60 children out of 316, which recorded the highest prevalence (19%), While 28 children out of 207 children who used underground well water were infected with the prevalence of (13.5%). On the other hand 2 out of 37 children who drink another source of water like (bottled water) were infected with the same parasite, and recorded the lowest prevalence rate (5.4%), with a significant difference (P-value = 0.047), as shown in (Table 4).

Prevalence of Amoebic Infection Among the Months of the Study

The results showed that the prevalence of Amoebic infection was different between the three months of sample collection. It was (19.5%, 16% and 11.2%) during the months of September, October and November respectively, without a significant difference in the percentage of infection ($P = 0.97$) (Table 5).

Prevalence of Amoebic Infection According Maternal Educational Status

Regarding maternal educational level, it was demonstrated that the highest infection rates were among illiterate level

Table 1. **Prevalence of amoebic infection according to gender**

Variables			Examined sample		Total	*P*-value
			Positive	Negative		
Gender	Male	Count	51	237	288	
		% within Examined Sample	17.7%	82.3%	100%	0.278
	Female	Count	39	233	272	
		% within Examined Sample	14.3%	85.7%	100%	
Total		Count	90	470	560	
		% within Examined Sample	16.1%	83.9%	100.0%	

Table 2. **Prevalence of amoebic infection according to age**

Variables			Examined sample		Total	*P*-value
			Positive	Negative		
Age Class	< 1 Year	Count	4	49	53	
		% within Examined Sample	7.5%	92.5%	100%	
	1–6 Year	Count	45	164	209	
		% within Examined Sample	21.5%	78.5%	100%	
	6–12 Year	Count	38	234	272	
		% within Examined Sample	13.9%	86.1%	100%	0.033*
	> 12 Year	Count	3	23	26	
		% within Examined Sample	11.5%	88.5%	100%	
Total		Count	90	470	560	
		% within Examined Sample	16.1%	83.9%	100.0%	

Table 3. **Prevalence of amoebic infection among residency of the individuals**

Variables			Individuals		Total	*P*-value
			Positive	Negative		
Residence	Urban	Count	50	313	363	0.031*
		% within Examined Sample	13.8%	86.2%	100%	
	Rural	Count	40	157	197	
		% within Examined Sample	20.3%	79.7%	100%	
Total		Count	90	470	560	
		% within Examined Sample	16.07%	83.9%	100.0%	

*Significant difference.

Table 4. **Prevalence of amoebic infection according to the source of water drinking**

Variables			Individuals		Total	*P*-value
			Positive	Negative		
Source of water	General	Count	60	256	316	0.047*
		% within Source of water	19%	81.0%	100.0%	
	Well	Count	28	179	207	
		% within Source of water	13.5%	86.5%	100.0%	
	Other	Count	2	35	37	
		% within Source of water	5.4%	94.6%	100.0%	
Total		Count	90	470	560	
		% within Source of water	16.1%	83.9%	100.0%	

*Significant difference.

Table 5. **Prevalence of amoebic infection among the months of the study**

Variables			Individuals		Total	*P*-value
			Positive	Negative		
Month	September	Count	43	177	220	0.097
		% within Month	19.5%	80.5%	100.0%	
	October	Count	30	158	188	
		% within Month	16%	84.0%	100.0%	
	November	Count	17	135	152	
		% within Month	11.2%	88.8%	100.0%	
Total		Count	90	470	560	
		% within Month	16.1%	83.9%	100.0%	

(19.8%) followed by school education (13.3%), in comparison to university level (9%) with a statistically significant difference with maternal educational status ($P = 0.037$) (Table 6).

Estimation of the Cytokines

Serum levels of, IL-17, IFN γ and TNF-α were measured using the ELISA technique; the serum levels of (80) patients infected with *E. histolytica* were compared to the control negative group (80) without any parasitic infections. The results of IL-17 showed increased level without significant differences between the two studied groups with *P*-value = 0.282, in the patient group the mean ± St. Dev = 15.24 ± 2.60, minimum serum level was 10 pg/ml and maximum = 21 pg/ml, while in control individual mean ± St. Dev = 14.78 ± 2.84, minimum was 10.8 pg/ml and maximum= 21 pg/ml.

Regarding the serum level of IFN-γ, records showed higher level in infected children with *E. histolytica* compared to control individuals with significant difference ($P = 0.04$), mean ± St. Dev = 18.94 ± 15.64, minimum serum level was 6 pg/ml and maximum = 75 pg/ml for patient individuals and mean ± St. Dev = 13.06 ± 8.70, the minimum level was 7.4 pg/ml and maximum = 52 pg /ml for the control group.

Finally, there was a highly significant difference regarding to TNF-α, ($P < 0.001$), mean ± St. Dev = 12.03 ± 5.09, minimum serum concentration was 5.4 pg/ml, and maximum = 33 pg/ml for children patients and mean ± St. Dev = 9.54 ± 1.88,

minimum serum level was 7.4 pg/ml and maximum = 99 pg /ml for control negative individuals, as showed in (Table 7).

The statistical results of the present study showed that those children who were infected between 1–3 days ago before taking their serum, were recorded with maximum mean of serum concentration of IL-17, IFN-γ and TNF-α, as follow (15.53 ± 2.42), (21.17 ± 17.68) and (12.74 ± 5.63) respectively. (Table 8 and Figure 4), but statistically, there was no significant difference between their serum level and the period of infection ($P > 0.05$).

Discussion

Parasitic infection of *E. histolytica* is a major health problem particularly in developing countries of the world, and the factors that control the pathogenesis of *E. histolytica* are poorly understood. Also, prominent features are the ability of the organisms to lyse host cells and cause tissue destruction, with induced immune responses occurring in invasive diseases.[20] Direct examination confirmed *E. histolytica* existence in both trophozoite and cysts stages, the bloody appearance of the samples is highest in number among our samples, this might be explained by *E. histolytica* infection that we found in our study because most studies pointed that *E. histolytica* normally caused bloody diarrhea as one of their well-known symptoms. Additionally, clinical diagnosis of the sample revealed mucoid stool in most of the samples caused by increased secretion of mucus in the intestine during the infection, as it is known that the intestinal mucosa is the first line of defence against intestinal infection, this also as suggested by others researchers *E. histolytica* have the ability to secret protease, glycosidase to destroy mucin resulting in mucous diarrhea.[21]

Many studies on the prevalence of intestinal parasites were done in many parts of the world and they have shown difference in the prevalence of infections depending on localities and geographical regions, hygienic habits and sanitary environment of people living.[22]

In the present study as shown in (Figure 3) the microscopic diagnosis of *E. histolytica* showed that the total prevalence of symptomatic child amoebic infection in Sulaymaniyah

Table 6. **Prevalence of amoebic infection according to Maternal educational status**

Variables			Individuals		Total	*P*-value
			Positive	Negative		
Maternal Educational Level	Illiterate	Count	56	227	283	0.037*
		% within Maternal Educational Level	19.8%	80.2%	100.0%	
	School	Count	28	182	210	
		% within Maternal Educational Level	13.3%	86.7%	100.0%	
	University	Count	6	61	67	
		% within Maternal Educational Level	9.0%	91.0%	100.0%	
Total		Count	90	470	560	
		% within Maternal Educational Level	16.1%	83.9%	100.0%	

*Significant difference.

Table 7. **Statistical analysis of mean serum IL-17, IFN- γ and TNF-α, between the control group and infection with *E. histolytica***

Variables		Individuals			*P*-value
		Patients	Controls	Total	
IL17 (pg/ml)	Mean ± St. Deviation	15.24 ± 2.60	14.78 ± 2.84	15.01 ± 2.72	0.282
	Maximum	21.00	21.00	21.00	
	Minimum	10.00	10.80	10.00	
	N	80	80	160	
IFN-γ (pg/ml)	Mean ± St. Deviation	18.94 ± 15.64	13.06 ± 8.70	16 ± 12.96	0.04*
	Maximum	75.00	52.00	75.00	
	Minimum	6.00	7.40	6.00	
	N	80	80	160	
TNF-α (pg/ml)	Mean ± St. Deviation	12.03 ± 5.09	9.54 ± 1.88	10.79 ± 4.02	<0.001**
	Maximum	33.00	19.00	33.00	
	Minimum	5.40	7.40	5.40	
	N	80	80	160	

*Significant difference; **Highly significant difference.

Table 8. **Mean serum concentration of (IL-17, IFN- γ and TNF-α) according to infection periods**

Variables		Infection Period Class					*P*-value
		< 24 Hour	1–3 Day	3–6 Day	> 6 Day	Total	
IL-17 (pg/ml)	Mean ± Std. Dev	13.71 ± 2.08	15.54 ± 2.43	14.77 ± 3.46	14.39 ± 1.00	15.24 ± 2.60	
	Std. Error	1.04	0.32	0.89	0.50	0.29	0.394
	N	4	57	15	4	80	
IFN-γ (pg/ml)	Mean ± Std. Dev	11.42 ± 5.21	21.17 ± 17.69	14.91 ± 6.76	9.82 ± 2.14	18.94 ± 15.65	
	Std. Error	2.60	2.34	1.75	1.07	1.75	0.220
	N	4	57	15	4	80	
TNF-α (pg/ml)	Mean ± Std. Dev	10.62 ± 3.06	12.74 ± 5.63	10.23 ± 3.13	10.15 ± 1.04	12.03 ± 5.09	
	Std. Error	1.53	0.75	0.80	0.52	0.57	0.283
	N	4	57	15	4	80	

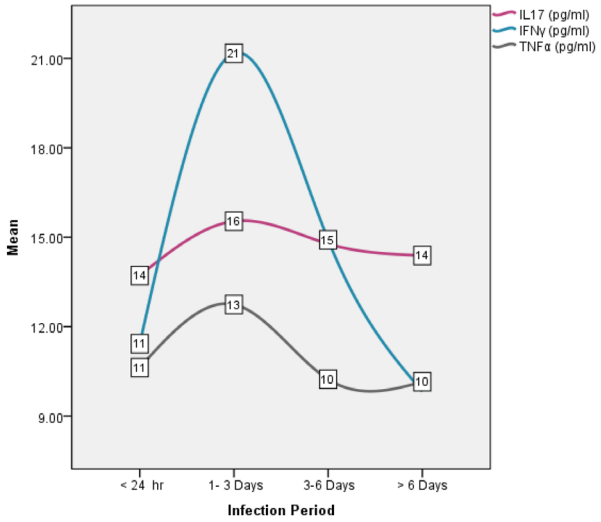

Fig. 4 **Mean serum concentration of (IL-17, IFN- γ and TNF-α) according to infection periods.**

province was (16.1%). This indicates that Sulaimani province is endemic with this parasite; the endemicity may be related to ingestion of contaminated food with the cystic stage of this parasite, socioeconomic condition, and untreated water supplies as a source of drinking and poor hygiene.

Results have been reported by other researchers in Kurdistan Provinces. In a study in Erbil, the infection rate was 30% of 200 examined samples,[23] but the infection rate of 15% was reported in a study in Duhok city.[24] In Kirkuk, the infection rate of *E. histolytica* among 221 diarrheal samples of children was 19.91%.[25] While in Sulaymaniyah city 10.3% were recorded from 300 diarrheal samples from children[26] also, 12.93% was recorded.[27] However, the variance between the prevalence of this parasitic infection from the present study and others may be consequent to different factors such as environmental, alimentation, socio-economic, geographical circumstance, demographic and health-related conduct as well as the number of participants in screening study and sampling, duration of the study, months of collection and age groups in a given study may affect the outcoming results.[28-30]

The current study recorded a higher prevalence of infection (17.7%) in males than in females (14.3%) without significant difference Table 1, similar results were documented in Al-Qadisiya province; 58.3% of *E. histolytica* infections were males and 41.6% were females.[31] Furthermore similar results were recorded in Anbar,[32,33] also in Sulaymaniyah province reported higher prevalence in males than females.[27] This may be attributed to the behavioural activities of males at this age and more exposure to sources of *E. histolytica* infections than females, such as more frequent consumption of contaminated outdoor food and water and contact with infected individuals.[34]

Table 2 showed that a statistically significant difference regarding age as a risk factor for *E. histolytica* infection (P = 0.033). The minimum rate of infection was in children lower than 1 year of old (7.5%), and it is consistent with the study conducted in Saudi Arabia[35] as well as in Erbil Province.[23] This finding is perhaps because parents are responsible for their hygiene.[36] The infection rate was highest in the illiterate age group (1–6 years) were (21.5%). The study among Najaf children were also supported our study,[37] as well as among Erbil children.[23] This is may be due to children at this age having high physical activity with continuous exposure to pathogens and small children engage more frequent hand to mouth habits which can be contaminated, as well as other unhealthy aids in children, such as putting the fingers in the mouth. All these factors increase the chance of expose 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.[38] After the two previous age groups the prevalence infection of the age group of (6–12) years old was (13.9%) then followed by those children who are older than (12) years old (11.5%).

The findings of this study, revealed a significant difference between the prevalence rate of *E. histolytica* infection by location (P = 0.031) and the higher infection rate of *E. histolytica* among children in the rural areas in comparison with that of urban regions 20.3% and 13.8% as documented in Table 3. Several studies were in agreement with this result and they recorded a similar relation of the infection with residency, reported that the rural children had a higher *E. histolytica* infection rate than urban children.[39-42] This result could be due to the problem of food and drinking water contamination by faecal rodents, dogs, cats and sheep that act as reservoirs for these parasites and water contamination is of great importance in this respect, because chlorinated tap water is not available in most of the areas and infected people using contaminated

water supplies, together with suitable environmental factors such as moisture and temperature that facilitate spreading and completion of the life cycle of these parasites.[22] Additionally, due to low sanitary services, low educational level of mothers, absence of regular hygiene toilets, lack of health services in the educational role and malnutrition which significantly increases susceptibility to *Entamoeba histolytica* in children;[43,44] finally environmental, social and economic factors are also playing a role in this aspect.[35]

Our result as recorded in the Table 4 showed significantly higher ($P < 0.05$) rates of infections with *E. histolytica* that were observed in individuals using general water (19%) than those who drank well water (13.5%). While the lowest rate was recorded in other water sources such as bottled water (5.4%). The result is due to the fact that general water is more prone for contamination which contains the infective stage of these parasites (cysts).[45] Additionally, general water can be contaminated through releasing of wastewater into the rivers or lakes or the area of drinking water intake. Also, factors such as no running water and not following the hygienic rules of the water, improperly chlorination of water lead to *E. histolytica* infection.[46]

The distribution of *E. histolytica* according to the months of the year shown in Table 5 proved that the highest prevalence rate of *E. histolytica* infection was observed in September (19.5%), then followed by October (15.9%) but the lowest infection rate of samples was in November (11.1%) of the same year 2021, with no significant difference. A study performed by (Al-Hifi et al.,) also supported our result.[47] Results from the availability of the proper environmental factors for parasites growth as intestinal parasites are dominant at a tropical area like the need for fast consumption of drinking water during September increase the properly of drinking contaminated water, which contains the infective stage of these parasites,[45] moreover, some behavioural factors could be involved, for example, the consumption of drinks with ice, ice cream and raw fruits in ice is associated with *E. histolytica* infection.[48] Our results were also clarified by researchers who emphasized the importance of environmental factors (temperature, wind, humidity) in the spread of intestinal parasites and they also reported the lowest percentage of infection during November mentioning that cold weather decreases parasite infection throw killing the infection stages (cyst).[47,49]

Table 6 explains the prevalence of *E. histolytica* infection according to the maternal educational status of the children and the higher infection rates were among illiterate mothers (19.8%) and who had school education (13.3%) compared to (9%) with college education, statistically significant difference ($P = 0.037$).[23] in their study also reported the prevalence of *E. histolytica* to decrease with rising maternal educational level. This finding is in agreement with the findings of studies in other developing nations, for example researchers found that the knowledge, perception, and behaviour of mothers helped design and implement an effective community based intestinal parasites control program.[50] Morever other study documented that the education of mothers was the best predictor of health and nutrition inequalities among children in rural Uganda.[51] Furthermore a study in Iran showed that the better the educational level of the mothers lead to the lower parasitic infection rate in children.[52,53]

Cytokine results of the present study as shown in Table 7 demonstrated elevated serum levels of the three cytokines (IL-17, IFN -γ and TNF-α) in symptomatically infected children with *E. histolytica* in comparison to the control negative group.

Regarding IL-17, elevated serum levels in infected children were recorded in comparison to the control group. This is because *E. histolytica* disrupts the mucosal barrier in a sequential process of adherence to intestinal epithelial cells by a parasite Gal/GalNAc lectin, followed by the killing of the epithelial cells in a nibbling process termed amoebic trogocytosis, leading to penetration of the epithelium and destruction of submucosal tissue.[54] *E. histolytica* induces the production of pro-inflammatory cytokines, including interleukin-17.[55,56] One of the mechanisms that lead to cytokine production is the activation of the inflammasome.[57,58]

Regarding IFN-γ, significant differences were observed (P-value = 0.04) which is in agreement with the study of which was performed on 250 amoebic patients in the Thi-Qar government.[44] Also, in a cohort study performed on 138 children similar results were documented.[59] Lastly, TNF-α has a highly significant difference statistically ($P < 0.001$) in the current study which is in agreement with.[60]

The first line of immune defence against *E. histolytica* is stomach acid which can kill the trophozoites while amoebic cysts are very resistant.[61] Cysts exocyst in the lumen of the intestine then, Trophozoites attach to intestine tissues leading to disruption of the muscle layer which facilitate the invasion of tissues.[62] This stage leads to the discharge of powerful cytokines to employee immune cells to the location of invasion.[63] Both cytokines TNF-α and INF-γ inducted by IL-12 from natural killer cells activate macrophages to discharge ROS and NO that destroy the parasite.[44]

Advanced TNF-α creation was lately shown to associate with *E. histolytica* diarrhea.[54] TNF-α triggers cytotoxic activity by stimulating neutrophils and macrophages to release ROS and NO to fight the parasite and stimulates phagocytosis. TNF-α is also, one of the pro-inflammatory cytokines which mediate inflammation and stimulate acute phase proteins.[8]

This study agrees with Peterson et al., (2011) study which establish an association between higher TNF-α creation and amoebic dysentery and an over-aggressive immune response from TNF-α cause improved inflammation and therefore diseases.[54] As well as in agreement with the study which performed on 31 patients suffering from amoebic colitis and 31 patients as healthy control, serum level IFN-γ was higher in patients with amoebic colitis compared with control.[64] Also, a cohort study from Bangladeshi children, reported that an elevated level of TNF-α can increase the risk of first and recurrent *E. histolytica*-related diarrheal infections, proposing that the production of TNF-α play a role in future susceptibility to *E. histolytica* diarrhea and pathogenesis of amoebiasis. High levels of TNF-α protein expression was observed in children who had *E. histolytica* diarrhea compared with those who had no infection.[54]

Table 8 and Figure 4 demonstrated that the peak of serum concentration of the three cytokines (IL-17, IFN-γ and TNF-α) were in those children who were infected with the parasite 1 to 3 days (24–72 hr.) suffered from diarrhea before their serum was taken for our investigations. The peak were 20, 24 and 12 (pg/ml) respectively. Several studies were in agreement with this result as Kwant et al., (2004),[65] documented the peak serum level of IFN-γ gamma which were after 24 hr. of infection. Also, another study reported the peak of TNF-α were during 72 hr. after infection.[66]

Conclusion

We can conclude the following points from the current study:

1. The prevalence of amoebic infection was (16.1%) in Sulaymaniyah province among symptomatic children based on the microscopic diagnosis.
2. Higher rates of infections were seen in males than in females without significant difference.
3. Higher significant difference recorded in rural children than urban as well as higher in those who use general water than well water and other water sources for drinking.
4. Based on this study maternal educational status has reversed relationship with amoebic infection, as increasing educational level will decrease amoebic infection.
5. Significant difference observed between the age and prevalence rate of infection.
6. Prevalence rate of amoebic infection is affected by the months of the year without a statistically significant difference.
7. The immunological assessment of IL-17 showed that there was no significant difference between infected and control individuals, while the rest of IFN- γ and TNF-α were documented significant and highly significant differences respectively.

Recommendations

1. More research into the epidemiology and prevalence of amoebiasis is needed to aid future studies and the development of effective diagnosis, prevention, and treatment methods.
2. To avoid amoebiasis infection, good hand-washing, avoiding faecal-oral contact, good food preparation, and drinking clean water are all critical.
3. To prevent the spread of the invasive virulent strain of *E. histolytica*, patients with acute amoebiasis should have their feces properly disposed of, especially in rural areas.
4. Advising parents to take measures to control and reserve in providing water and food necessary for children.
5. Focusing on public health education and preventive health system.

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Conflict of Interest

The authors declare no conflict of interest to this current study.

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