Republic of Iraq

Ministry of Planning

Central Organization for Standardization and Quality Control

Quality Control Department

Food Industries

The prevalence of anemia among children infected with *Entamoeba histolytica* in Baghdad

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This study was conducted from October 2018 until April 2019 in different hospitals and primary health care center. Eighty one children (n=81) were participated in this study, their ages were between (1-12) years old.

The participants were divided into two groups based on their microscopic general stool examination. The first group were diagnosed as *E.histolytica* infected children (n=47).While no pathogens were detected in the stool samples of the second group, they considered as healthy control children (n=34). Blood samples were taken from all subjects and were tested for haemoglobin, ferritin, iron, total binding iron capacity (TBIC) and mean corpuscular volume (MCV). Nutritional status was evaluated for all children aged ≥ 5 years old.

Results showed that the prevalence of anemia was higher in children infected with *E.histolytica* compared with the control. Both of haemoglobin and iron levels were significantly lower in infected children comparing with control. Although ferritin level was lower in infected group non- significantly. Both gender and age are significantly related to low serum iron levels. Significant relation were noticed between each of type of anemia and nutritional status with the *E. histolytca* infection. Microcytic anemia was seen in the majority of *E.histolytica* infected children as well as severe malnutrition were highly reported.

These findings indicate that anemia is more expected to be present in children infected. Therefore, efforts should be focused to health education of populations at high risk of both anemia and *E.histolytica*.

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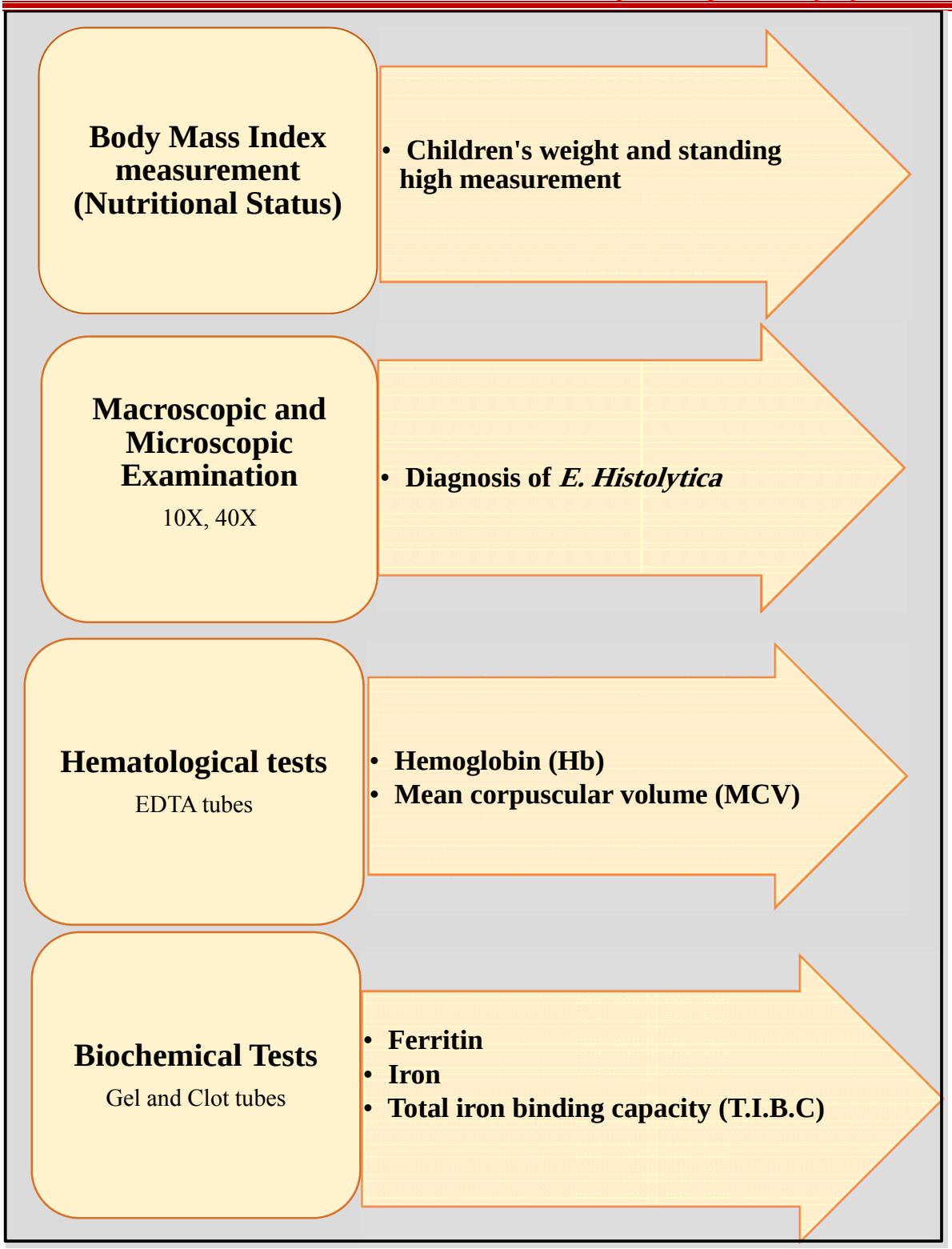
This study was an analytical case control study conducted from October 2018 until April 2019. The population in this study was children from both genders in some hospitals, primary health care centers and public primary schools in Baghdad, Iraq.

Sampling included 81 participants who matched inclusion criteria and had no exclusion criteria. The inclusion criteria were children aged ≤12 years, who were willing and had their parents' agreement to participate in this study. They also must not have been taking anti- helminth or anti-protozoa medication in the previous 24 weeks prior to the study and had no history of asthma, atopic dermatitis, immunodeficiency, malignancies, rheumatic disease, and other infections.

Exclusion criteria were obesity and stool and blood samples that were impaired or missing. The study protocol was approved by the Ministry of Health and Environment, Baghdad, Iraq. A permission from primary school authority were also approved.

The 81 participants were divided into two groups based on their microscopic general stool examination. Stool samples were preserved in 10% formalin before transporting to the laboratory, where they were examined under the light compound microscope. Diagnosis was confirmed by finding trophozoites or cysts of *E.histolytica*. The first group was diagnosed as *E.histolytica* infected children (n=47), while no pathogens were detected in the stool samples of the other group which was considered as the healthy control group (n=34).


Body Mass Index measurement (Nutritional Status)
• Children's weight and standing high measurement
Macroscopic and Microscopic Examination
10X, 40X
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EDTA tubes
• Hemoglobin (Hb)
• Mean corpuscular volume (MCV)
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1. Determination the possible correlation between anemia and intestinal parasitic infection with *E. histolytica* in Iraqi children.

2. Estimation of some blood parameters (hemoglobin concentration, mean corpuscular volume, ferritin, iron, total iron binding capacity.

3. Nutritional status evaluation by measurement of body mass index (BMI) in children infected with (*E. histolytica*) parasite compared with control healthy group.

Anemia is abnormal haemoglobin level in blood resulted from various influences, which is the most common cause is iron deficiency (IDA)[1]. Childhood anemia is considered as global public health challenge which is often numerous potential etiologies [2]. IDA anemia is the most public micronutrient deficiency that is affected nearly 35% of the world's population and 1.2 billion individuals worldwide [3, 4].

Many influences contributing to anemia, which are irregularly eating habit, poor maternal attention and acquired infection mainly (intestinal parasitic infections) that showed to be very common among school children [5].

Parasitic infection included protozoa and helminthes with different strategies of infection. The protozoan parasites is resulting in symptomatic diseases in (50 million) person and death rate in 100,000 people [6].

Helminthic infections cause anemia by dropping iron uptake from the intestine [7], while protozoan parasites impact anemia by destructing the intestinal mucosa which affects the absorption of micronutrients, such as iron [8]. Such mechanisms correspondingly affect hosts' nutritional grade and then change their immune system [9]. *E. histolytica* is intestinal protozoan parasites, widely prevalent, causing serious public health issues especially in developing countries [10].

This pathogen is causes amoebic colitis, amoebic dysentery, and amoebic liver abscess, resulting in 100,000 deaths annually [11]. The differences in prevalence of *E. histolytica* parasite may due to several factors, including: nutritional, environmental, geographical, socio-economic conditions, as well as demographic and health related behaviour [10].

In Iraq, many papers presented different prevalence rates of *E. histolytica* and its association with age and gender of patients [10, 12]. But little is known about its relation with anemia especially in children.

1. Literature Review

1.1 *E. histolytica* Taxonomy

According to [13] *E. histolytica* is given taxonomy rank as following:

- **Kingdom**: Protozoa
- **Subkingdom**: Neozoa
- **Phylum**: Amoebozoa
- **Class**: Entamoebidea
- **Order**: Euamoebida as *Entamoeba histolytica, E. coli, E. dispar, E. hartmanni, E. gingivalis, E. moshkovskii, E. chattoni (=E. polecki), Endolimax nana, Iodamoeba buetschlii*.

1.2 *E. histolytica* morphology and Life Cycle

The life cycle of *E. histolytica* is very simple and consists of two stages:

❖ **Trophozoite**: The trophozoite is an active, invasive, feeding, and proliferating stage, its diameter ranged between (10 to 50 μm), it is containing a single nucleus with a central karyosome and thin peripheral chromatin

❖ **Cyst:** It is infective, resist stage, its diameter ranged between (10 to 15 μm). The quadrinucleated cyst contains four or fewer nuclei and it can resist environment for weeks, acidity and chlorination and desiccation [14].

E. histolytica is monoxenous that it does not need an intermediate host, and its entire life cycle is completed only in one host, that is commonly humans, However, *E. histolytica* may be infect monkeys, some other animals like primates have been infected [15].

Transmission of infection occur after intake of the infectious cyst. This most generally arises from fecal contaminated hands, water, food, fly, and newly by sexual contact [16].

After ingestion, the cysts pass through the stomach and small intestine where excystcysts occur and the send out trophozoites are reached to the large intestine and undergo binary fission to multiplying and making more trophozoites. Trophozoites can penetrate the mucus layer of the large intestine and may invade the intestine and pass to extra- intestinal areas as the liver via hepatic portal circulation or spread to sites such as the lung and brain through blood streams. Encystation takes place in the colon, Cysts and trophozoites are shed off in stools to external environment [14].

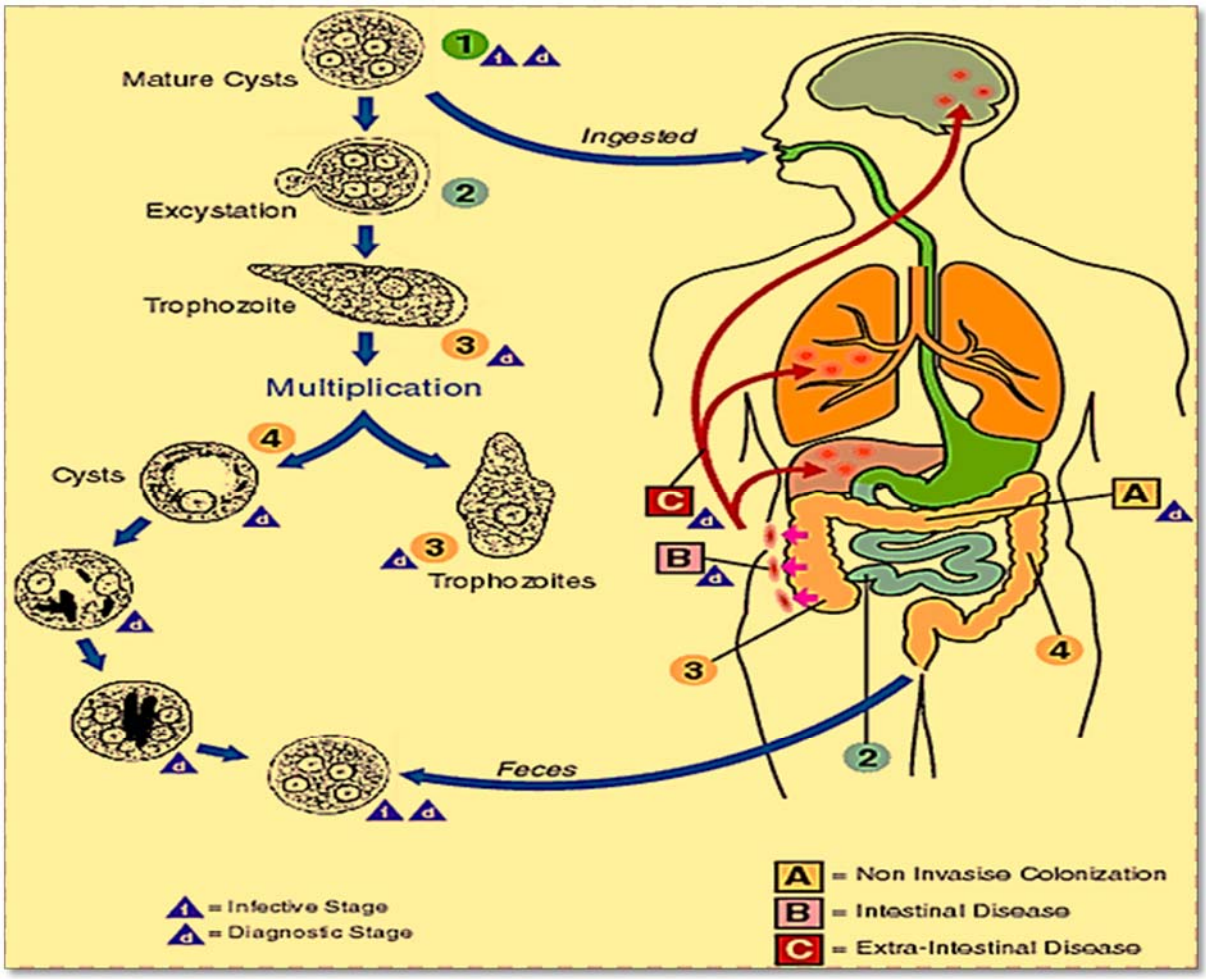

Figure (1-1): Life cycle *E. histolyica* [17]

1.3 Species and host rang

Entamoeba species are divided according to characters of intestinal parasite such as the trophozoites and cyst size, nuclei numbers in mature cysts, the component and location of the nucleus [18].

There are six various species belonging to Genus *Entamoeba* which have been detected in lumen of human intestine (*E. moshkovskii, E. dispar, E. coli, E. histolytica, E. polecki and , E. hartmanni*) [19]. These species are considered as commensal, with the exception of species of *E. histolytica* which is absolute pathogenic [20].

1.4 Amoebiasis

Amebiasis is considered endemic disease developing parts of Central and South America, Asia and Aria. This disease caused by infection with the intestinal protozoan parasite *E. histolytica* [6]. Most of Amoebifria. moebiasis intestinal protozoan infections occur without symptoms, in some cases the trophozoites invasive the intestinal mucosa and causes dysentery, ulcers and liver abscess. The complication of the disease may be threat life of the infected people [21].

A- Intestinal Amoebiasis

Intestinal *Entamoeba* infections have a wide range of symptoms which usually occur during weeks after cyst ingestion. Manifestation of can range for moderate diarrhea to dysentery with mucus and blood [18]. Amoebic Colitis occur in the ascending colon or cecum, one of its indications is watery or bloody diarrhea with presence of abdominal cramps, weight loss and pain/tenderness, and amebic colitis may also presented similar to inflammatory bowel disease (IBD) [22]. Commonly, ameboma formation because of partial or not treated amoebic colitis and it occurs in patients with persistent chronic infection [23]. Manifestation that life's patient threat of amebic colitis including fulminant infection, that result in large areas of colonic involvement with puncture and peritonitis, toxic megacolon, or bowel necrosis [22].

B- Extra- Intestinal Amoebiasis

Generally, invasion of *Entamoeba* leads to amoebic liver abscess but in some complicated cases, it may extend to other body organs such as pleuropulmonary, genitourinary, cerebral, renal, and cutaneous sites [24]. Pulmonary amoebiasis illness happens as complication of infection or by ruptured amebic liver abscess and presenting with lung abscess or pneumonitis. Intraperitoneal rupture is rarely occur but can threat patient life if the pericardial involvement. Occasionally, trophozoites may spread hematogenously to extra- intestinal sites and the central nervous system [25].

1.5 Anemia

A- Definition

Anemia is defined as un typical hemoglobin (Hb) concentration in blood that impacts about 40% school children in developing countries [26]. The most usual cells in body are red blood cells which involved hemoglobin that have essential role in transporting of oxygen molecules to brain and body [27].

Economic and social conditions like poverty, improper sanitation, and reduced education level for parents, unsuitable dietary system, low intake and prevalence of anemia has sometimes associated with community [10].

In infants and children, Iron deficiency anemia is serious health challenge for many considerable reasons; first: nutrition in infants is usually iron insufficient; second: Iron is wanted at highest level during childhood; third : Iron deficiency anemia at this young age may lead to complication such as neurodevelopmental and cognitive deficits, which may be irreversible [28].

B- Diagnosis and Treatment

Anemia concerning disease is produce by abnormality in red blood cells count such as (pernicious anemia and erythrocytosis); or it induced by abnormality in red blood cells size such as (microcytic anemia and megaloblastic anemia) [29]. The most commonly type on anemia in children is microcytic anemia by cause of iron deficiency [1].

During childhood, Iron status estimated by special iron biomarkers such as serum iron, total iron binding capacity, transferrin saturation and erythrocyte protoporphyrin, serum ferritin concentrations [30].

Serum ferritin value symbolized total iron store in the body and it concentration is influenced by gender and age. It's present in low concentrations is considered as previous and highly specific indicator of iron deficiency [31].

Iron is necessary micronutrient has effective presentation in all human body cell [32]. It has different crucial biological process and metabolic function involving in oxygen transporting (as heme in hemoglobin molecule) energy generation, and DNA (Deoxyribonucleic acid) biosynthesis [33].

Treatment used for iron deficiency is oral ferrous sulphate which considered as the cheapest description and the most successes therapy [28].

2. Materials and Methods

2.1 Materials

2.1.1 Laboratory equipment: The laboratory equipment used in the present study were listed in table (2-1).

Equipment	Company	Origin
Celltac Es Mek-7300K	Nihon Kohden	Germany
Centrifuge	Hettich	Germany
Dimension X pand plus	Siemens	Germany
Electronic scale	India Mart	India
Horizontal microplate shaker	Green Bio research	USA
Immulite 2000 Xpi Immunoassay System	Siemens	Germany
Light microscope	Novex	Netherland
Mixers- rotators tubes	Shandong Bio &Media	China
Refrigerator	Desmon	India
Timer	Volac	England
Wooden Stadiometer	CMS equipment Ltd.	UK

Table (2-1): Equipment

2.1.2 Laboratory plastic wares.

The plastic wares used in the present study are shown in table (2-2).

plastic wares	Company	Origin
Beaker 500 Ml	Simax	USA
Centrifuge tube (15 ml)	Abdos	India
Disposable Pasteur pipette 3Ml	Abdos	India
Disposable syringes 5 Ml	Abu Dhabi Medical Devices Co.	UAE
Gel & clot activator Tube 6.0 Ml	Sanli	China
Glass Funnel	Simax	USA
Glass rod	Simax	USA
K_2EDTA tube 0.5 ml (pediatric tube)	Sanli	China
Micropipette 100- 1000	Human	Germany
Non- sterile Eppendorf tube 1.5 Ml	Bioneer	Korea
Pipette tips 1 ml	AFCO	China
Plane tube 10 ml	Biozek medical	Netherlands
Screw tube	Biozek medical	Netherlands
Tourniquet	Medeco	UAE

Table (2-2): laboratory plastic wares

2.2 Methods

2.2.1 Data Collection, Anthropometry and Nutritional status

Interviews with parents were performed based on a prepared questionnaire to obtain information about background characteristics of children as well as the children's past and present illnesses. Body weight and height were measured using standardized procedures [34] and recorded as the midpoint of duplicate measurements. Children's weight was measured by an electronic scale (IndiaMart, India) and their standing height was measured with a wooden stadiometer (CMS equipment Ltd, UK).

The children had minimum clothing and no shoes. Body Mass Index (BMI), which is the weight in kilograms of a person divided by the square of the height in meters, was used to evaluate the nutritional status of the children, which was classified into severe malnutrition (BMI < 15.9 kg/m^2), moderate and mild malnutrition (BMI = 16-18.4 kg/m^2) and normal (BMI = 18.5- 25 kg/m^2) [34]. The nutritional status was evaluated only for children aged ≥5 years old.

2.2.2 Sample collection

- **Stool collection**

After adequate instructions were given to the children parents, they were provided with clean and dry screw capped plastic tubes to collect stool samples and they were asked to bring it in the next morning with guidance to bring samples as soon as possible. All stool specimen were received, labeled and divided into two groups depend on their microscopic general stool examination.

- **Blood collection**

About (4-5 ml) of blood samples were taken using a venipuncture technique, about (0.5 ml) of blood samples were added immediately into labelled tube containing (EDTA) for anticoagulant and kept in cold container. The rest of blood samples were added into labelled, vacuumed, gel& clot activator tube and left for 30 minutes at room temperature to clot before all samples were put in centrifuge set

at 3000 rounds per minute (rpm) for 10 minutes, each serum sample was transferred by sterile micropipette into 3 sterile Eppendrof tubes for following different tests to avoid freezing and thawing that may influence the accurate of result. All blood samples analyzed less than 12 hours after blood collection.

2.2.3 General stool examination

All samples were transferred to the laboratory in screw cap plastic container. Samples were marked with patient identification (ID) number, part of feces was separated for direct general stool examination.

- **Macroscopic examination**

The feces were observed for color, consistency, and presence of blood, mucus in cases of intestinal parasites.

- **Microscopic examination**

A- Direct normal saline (0.9% NaCl) wet smear preparation

The feces was prepared to be examined under light microscope by adding one drop of normal saline (0.9% solutions NaCl) on a clean glass slide and mixed with small amount of feces by wooden stick, then it was covered by using glass cover slide and examined for parasites presence.

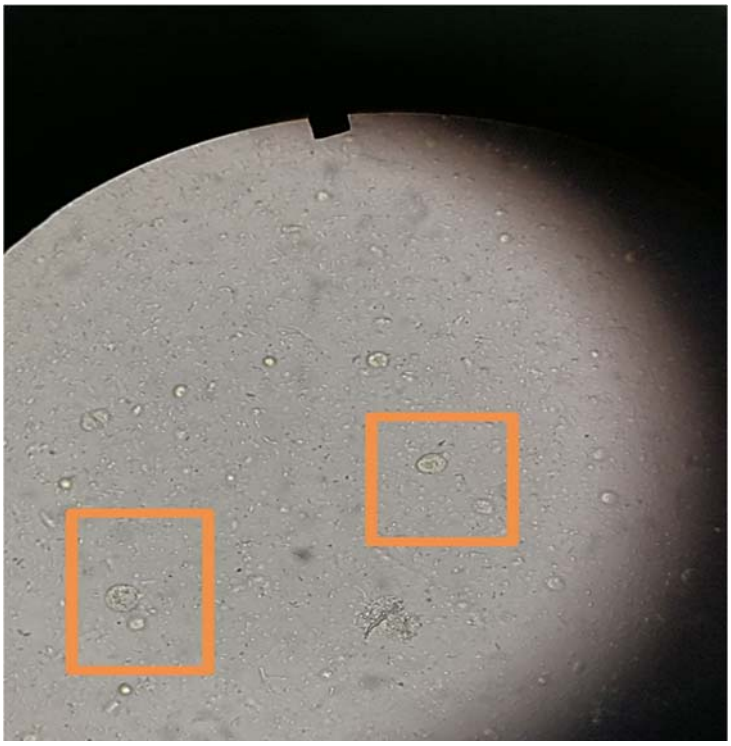

Figure (2-1): A- *E. histolytica* microscopic diagnosis

B - Lugols- Iodine wet smear preparation

The same previous procedure was repeated with mixing one drop of Lugols iodine (iodine stain) and examined for parasites presence.

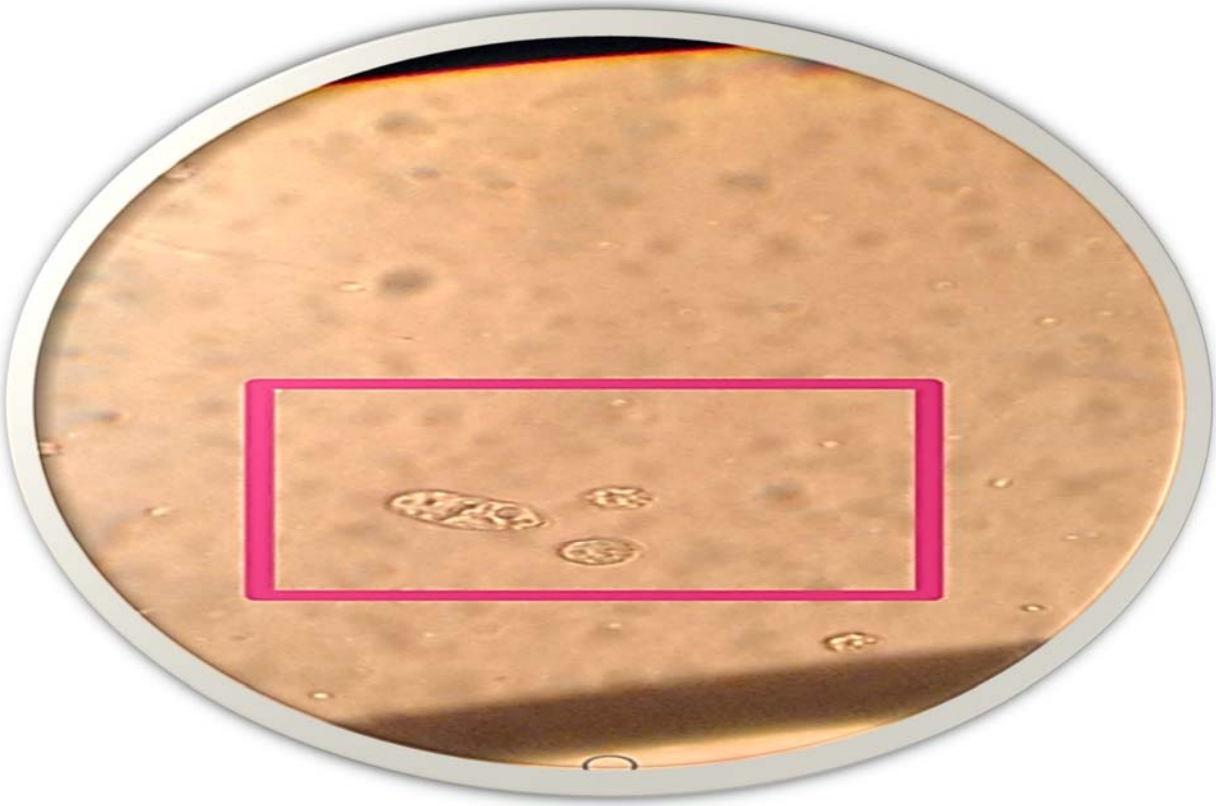

Figure (2-1): B- *E. histolytica* microscopic diagnosis

2.2.4 Hematological tests

- **Complete blood count**

For aneima diagnosis in human blood, hemoglobin level (Hb) and mean corpuscular volume (MCV) were measured by Celltac Es MEK-7300K (NIHON KOHDEN/ Germany) automatic hematology analyzer [35].Only (30 µl) of complete blood preserved in EDTA tube was pipetted into cuvette and applied to the system.

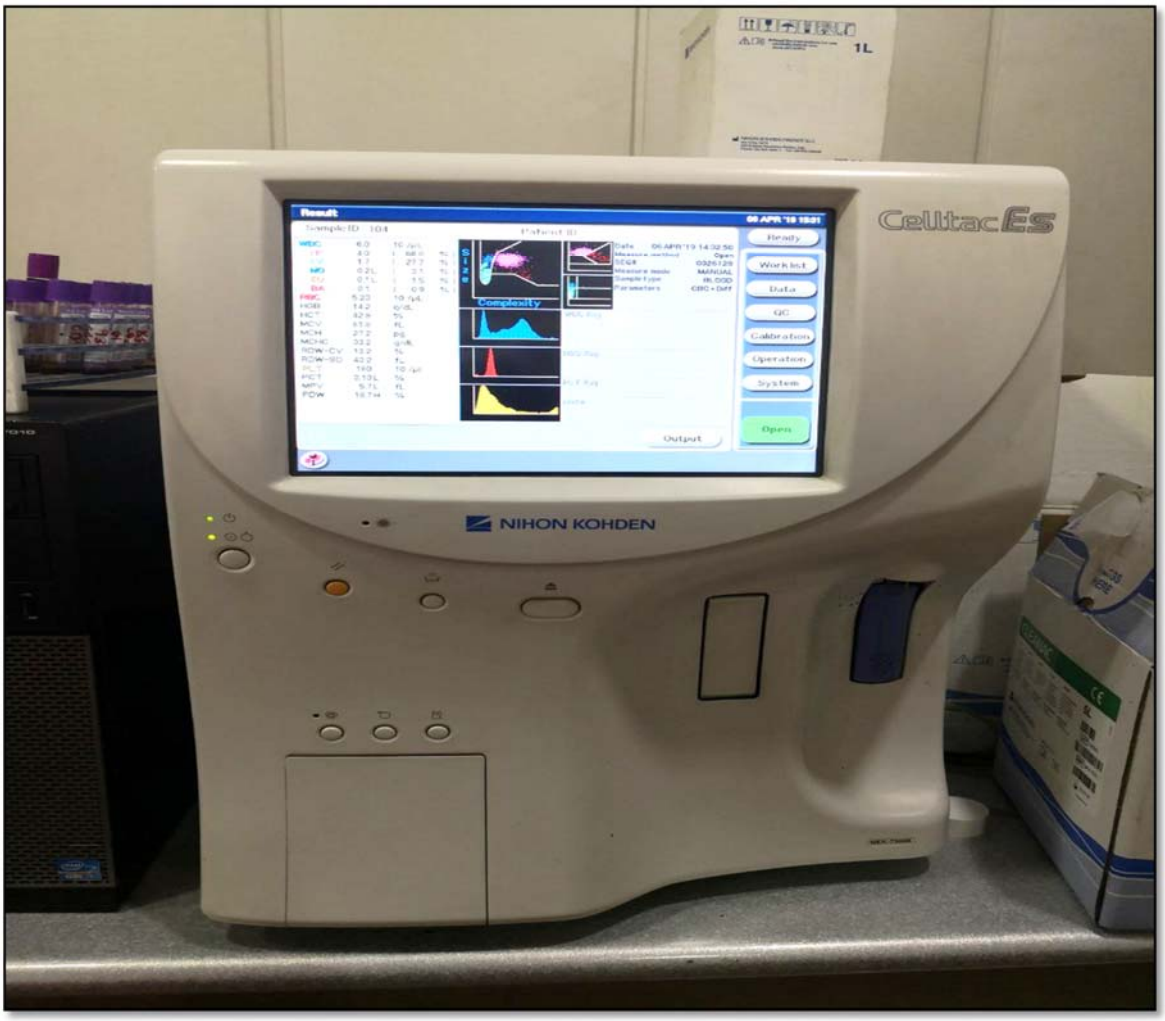

Figure (2-2): Complete blood count by (Celltac Es MEK-7300K)

2.2.5 Biochemical tests

- **Iron and total iron binding capacity (TIBC)**

In this study biochemical test quantitative measurement for iron and total iron binding capacity (TIBC) in the serum were measured by Dimension X pand plus (Siemens/ Germany) which is automated chemistry analyzer system. Sampling, reagents delivery, mixing, processing and printing of results are automatically performed by Dimension® system, sample serum size was (25µl) transferred into cuvette and provided into the system.

Figure (2-3): T.I.B.C measurement by (Dimension X pand plus)

- **Ferritin**

In this study biochemical test Quantitative measurement for ferritin was measured by IMMULITE 2000XPi Immunoassay System. (Siemens/ Germany) which is automated immunoassay analyzer system. Sampling, reagents delivery, mixing, processing and printing of results are automatically performed, only (10 µl) of serum sample was added into cuvette and applied in to the system.

Figure (2-4): Ferritin measurement by (IMMULITE 2000XPi Immunoassay System)

2.2.6 Statistical Analysis

All the collected data were analyzed using SPSS 16.0 (SPSS Inc., Chicago, IL, USA). Data is defined in the distribution of frequencies then analyzed using Chi-Square (χ^2) to determine whether there is an association between each of anemia, type of anemia, and nutritional status with *E.histolytica* infection. Then, all data variables, including haemoglobine, ferritin and serum iron were analyzed using student t-test to evaluate whether there is any statistically significant difference between *E.histolytica* infected children and the control group. The differences were considered significant with p-value< 0.05 (CI 95%)[36].

Results:

Eighty one child were enrolled in this study. The age distribution was 1 to 12 years. Twenty six child were between 1 to 3 years old, 29 were between 4 to 6 years old, 14 were between 7 to 9 years old, and 12 were between 10 to 12 years old. Forty nine were male and 32 female. Results showed that the mean (SD) hemoglobin was 121.79 g/L (1.36) with a range between 82 g/l and 145 g/l. The mean (SD) of hemoglobin 118 g/L (12.8) was significantly lower (p=0.014) in *E.histolytica* infected children compared with the mean of hemoglobin 125 g/L (13.7) in control group. Figure (3-1).

The prevalence of anemia was higher in patients infected with *E. histolytica* (29.7%) comparing with the control subjects who showed less prevalence rate of anemia (23.52%). Statistical analysis indicated that there was no significant difference in the prevalence of anemia between *E. histolytica* infected children and control group (p>0.05). Anemia was largely reported among those aged (1-3) years old for both *E. histolytica* infected children and control subject. The statistical analysis showed no significant relation between age and prevalence of Anemia in both *E. histolytica* infected children and control subjects among all age groups (p>0.05) Table (3-1). Moreover no significant relation was noticed between gender and prevalence of anemia in both *E. histolytica* infected children and control subject (p>0.05) Table (3-2).

Results showed that the mean (SD) of ferritin level was lower 16.4 µg/mL (24.7) in *E. histolytica* infected children compared with the mean of ferritin 23.6 µg/mL (17.9) in control group. Figure (3-2). Although non-significant differences were noticed in ferritin level between *E. histolytica* infected children and control group. While low ferritin level was significantly (p=0.002) high distributed in *E. histolytica* infected children. Results showed that 30 out of 47 (63.82%) *E. histolytica* infected children had low ferritin levels while the low ferritin levels were noticed only in (29.4%) of the control group. No significant differences were noticed regarding ferritin level between *E. histolytica* infected children and control group for all age groups except in age group (4-6) years old. Table (3-3). In this age group low ferritin level was significantly

(p=0.02) high distributed in *E.histolytica* infected children. While non-significant relation was noticed between gender amd low ferritin levels. Table (3-4)

The mean (SD) of serum iron was significantly lower (p=0.0001) in *E. histolytica* infected children 23.5 µg/mL (16.7) compared with the mean of serum iron 65.5 µg/mL (26.7) in control group .Result showed that (93.6%) *E. histolytica* infected children had low iron level while the low iron level were noticed only in (29.4%) of the control group. Low serum iron level was largely reported among those aged (7-9) and (10-12) years old for both *E. histolytica* infected children and control subject (Table 3-5). The statistical analysis showed significant relation between age and low serum iron level in both *E. histolytica* infected children and control subjects (p>0.05). As well as significant relation was noticed between gender and low serum iron level in both *E. histolytica* infected children and control subject (p>0.05). (Table 3-6).

Results also showed that the mean (SD) of TIBC was lower in *E. histolytica* infected children 349 µg/dL (92) compared with high TIBC level 364 µg/dL (78.4) in control group. No significant differences (p>0.05) were noticed in TICB between *E. histolytica* infected children and control subjects.

Table (3-7) illustrates the distribution of anemia according to MCV values among cases of *E.histolytica* and control group. Microcytic anemia (MCV<82 fL) was seen in the majority of *E. histolytica* infected children compared with less cases in control group. On the other hand Normocytic anemia (MCV =82-98 fL) was noticed in the majority of control group compared with less cases in *E.histolytica* infected children. No cases of Macrocytic anemia (MCV>98fL) were seen among both infected and control groups. Statistical analysis showed that there was significant relation (p<0.05) between type of anemia and the infection with *E. histolytica*.

Nutritional status was evaluated in this study only for children aged more than 5 years old. Results showed that 23 out of 24 (95.9%) *E.histolytica* infected children had severe malnutrition (BMI < 15.9 kg/m^2) compared with 62.5% of control group who showed severe malnutrition. No normal nutritional status (BMI = 18.5-25 kg/m^2) was noticed in *E. histolytica* infected children while 1 case with normal nutritional status

was reported in control group Table (3-8). Statistical analysis showed that there was significant relation ($p<0.05$) between the nutritional status and the infection with *E. histolytica*.

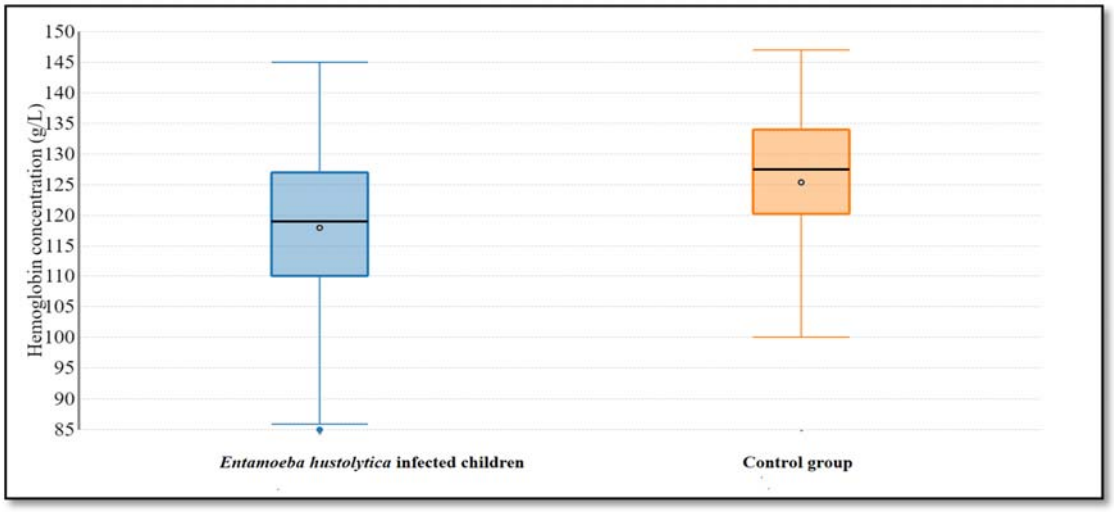

Figure (3-1): Haemoglobin concentration (g/L) in *E.histolytica* infected children and control group

Age group/years	Cases with *E. histolytica*		Control group		
	No. of tests	Prevalence of anemia (Hemoglobin concentration	No. of tests	Prevalence of anemia (Hemoglobin concentration	P-value
1-3	20	8 (40%)	6	2 (33%)	0.25
4-6	18	4 (22.2%)	11	3(27%)	0.75
7-9	4	1 (16%)	10	2 (20%)	0.82
10-12	5	1 (20%)	7	1 (14.2)	0.79
Total	47	14 (29.78%)	34	8 (23.52%)	0.24

Table (3-1): Prevalence of anemia, based on haemoglobin concentration, related to age for patients infected with *E.histolytica* and control group

Gender	Cases with *E. histolytica*		Control group		
	No. of test	Prevalence of anemia (Hb concentration)	No. of test	Prevalence of anemia (Hb concentration)	P-value
Male	26	10 (38.46%)	23	4 (17.93%)	0.1
Female	21	4 (19.4%)	11	4 (36.36%)	0.2
Total	47	14 (29.78%)	34	8 (23.52%)	0.24

Table (3-2): Prevalence of anemia, based on haemoglobin concentration, related to gender for patients infected with *E.histolytica* and control group

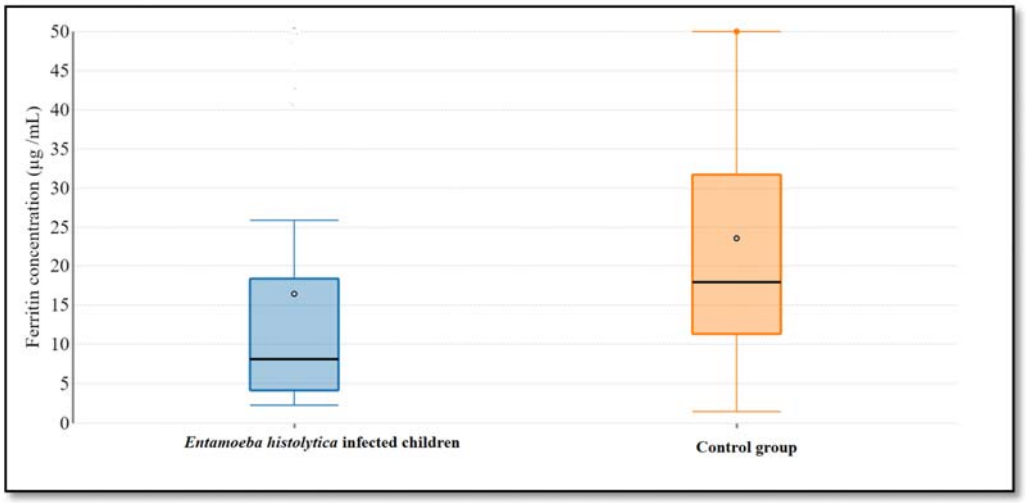

Figure (3-2): Ferritin concentration (µg /mL) in *E.histolytica* infected children and control group

Age group/years	Cases with *E. histolytica*		Control group		
	No. of tests	Low ferritin level (<12µg/mL), %	No. of tests	Low ferritin level (<12µg/mL), %	P-value
1-3	20	14 (70%)	6	1 (16.6%)	0.4
4-6	18	11(61.1%)	11	2 (18.1%)	0.02
7-9	4	2 (50%)	10	4 (40%)	0.7
10-12	5	3 (60%)	7	3 (42.8%)	0.8
Total	47	30 (63.82%)	34	10 (29.4%)	0.002

Table (3-3): Low ferritin level related to age for patients infected with *E.histolytica* and control group

Gender	Cases with *E. histolytica*		Control group		
	No. of test	Low ferritin level (<12µg/mL), %	No. of test	Low ferritin level(<12µg/mL), %	P-value
Male	26	16 (61.5%)	23	8 (34%)	0.6
Female	21	14 (66.6%)	11	2 (18.18%)	0.2
Total	47	30 (63.82%)	34	10 (29.4%)	0.002

Table (3-4):- Low ferritin level related to gender for patients infected with *E.histolytica* and control group

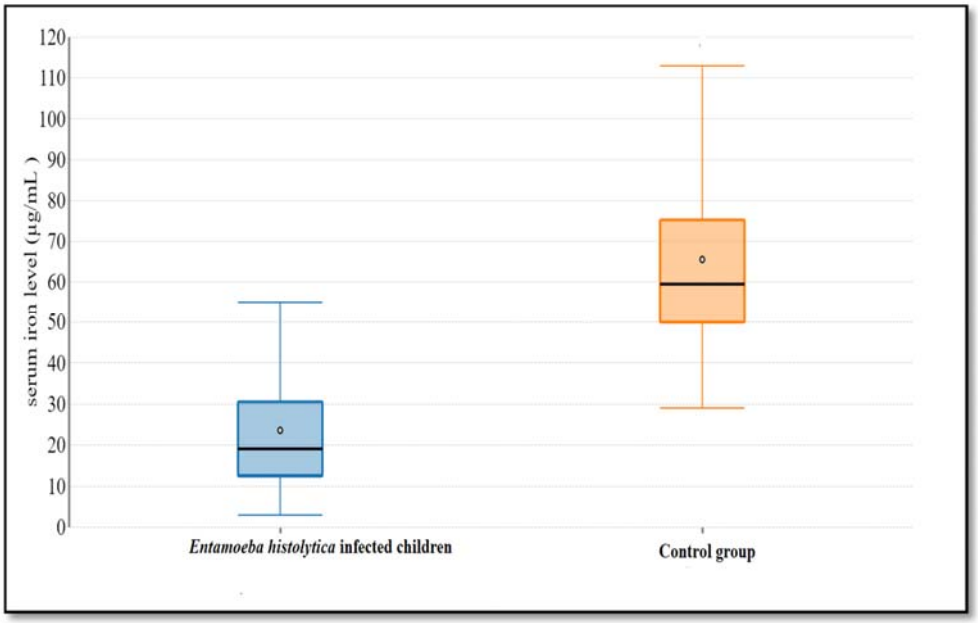

Figure (3-3): Serum iron concentration (µg /mL) in *E.histolytica* infected children and control group

Age group/years	Cases with *E. histolytica*		Control group		
	No. of tests	Low serum iron level (<50µg/mL), %	No. of tests	Low serum iron level (<50µg/mL), %	P-value
1-3	20	19 (95%)	6	1 (16.6%)	0.00006
4-6	18	16 (88.8%)	11	3 (27.27%)	0.0007
7-9	4	4 (100%)	10	3 (30%)	0.017
10-12	5	5 (100%)	7	3 (42.85%)	0.03
Total	47	44 (93.6%)	34	10 (29.4%)	0.00

Table (3- 5): Low serum iron level related to age for patients infected with *E.histolytica* and control group

Gender	Cases with *E. histolytica*		Control group		
	No. of test	Low serum iron level (<50µg/mL), %	No. of test	Low serum iron level (<50µg/mL), %	P-value
Male	26	25 (96.1%)	23	8 (34.7%)	0.000004
Female	21	19 (90.4%)	11	2 (18.18%)	0.0004
Total	47	44 (93.6%)	34	10 (29.4%)	0.00

Table (3-6): Low serum iron level related to gender for patients infected with *E.histolytica* and control group

Type of anemia based on MCV	Cases with *E. histolytica*	Control group
Microcytic anemia MCV<82 fL	39 (82.9%)	3(8.82%)
Normocytic anemia MCV =82-98 fL	8 (17.1%)	31(91.17%)
Macrocytic anemia MCV>98fL	0	0
Total	47	34
X^2= 43.45; P-value=0.00		

Table (3-7): The types of anemia based on MCV for patients infected with *E.histolytica* and control group

Nutritional status based on Body mass index	Cases with *E. histolytica*	Control group
Severe malnutrition (BMI < 15.9 kg/m^2)	23 (95.9%)	15 (62.5%)
Moderate, Mild malnutrition (BMI = 16–18.4 kg/m^2)	1 (4.1%)	8 (33.3%)
Normal (BMI = 18.5-25 kg/m^2)	0	1 (4.1%)
Total	24	24
X^2= 8.129; P-value=0.017		

Table (3-8): Nutritional status for patients infected with *E.histolytica* and control group. Only the children more than 5 years are included.

❖ Discussion

Anemia is the global health problem that affects many people in each socioeconomic status, all over the world. In Iraq, anemia is an important public health issue mainly in children and adolescents [37]. Nonetheless, there was no national data on the prevalence of anemia among children. This prevalence rate among children could be associated with poor nutrition and some other factors [38].

Results showed that the hemoglobin level was significantly lower in *E.histolytica* infected children compared with the hemoglobin level in control group. As well as the prevalence of anemia, depending on hemoglobin level, was higher 29.78% in *E.histolytica* infected children compared with control group. The parasite interacts to the mucous of the small intestine that make villous atrophy in different level, besides triggering inflammatory infiltrate and crypt hypertrophy. These processes disrupt the enterocytes and change bile acid metabolism that affects the absorption of most nutrients which are essential for body function, such as vitamin, iron, zinc, and folic acid [39].

Such results are similar to previous Iraqi study for children in Baghdad showed that hemoglobin concentration was significantly lower in *E. histolytica* infected patients (10.36±1.58) g/dl versus control group who showed high concentration of hemoglobin (12.87±0.95) g/dl [40]. Other study found a relationship between anemia and parasitic infections in Mosul province, north of Iraq [41].

The results of the current study showed that anemia was largely reported in children aged (1-3) years old compared to school children. However, this finding might be attributed to the benefit of school children having a good education regarding taking good food supplemented with iron and other minerals [42]. Therefore, the hemoglobin levels in school children are likely to be with in normal values. The increased risk of anemia in children below the age 3 years is consistent with the results from other countries [43, 44].

Serum ferritin reflects total body iron stores, thus ferritin consider an early and highly specific parameter that used in diagnosis of iron deficiency, in the absence of an associated disease [31]. In this study, it has been found that ferritin level was lower in *E. histolytica* infected children compared with the ferritin level in control group. Low ferritin levels were significantly high prevalent in *E. histolytica* infected children (63.82%) compared with less prevalence rate (29.4%) of low ferritin level in control group. The low ferritin level in children infected with *E. histolytica* is likely due to the ability of the trophozoite of this parasite to utilize ferritin as an iron source [45].

These results were in agreement with the results of other Iraqi study illustrated that ferritin was higher in non- infected children with *E. histolytica* while the infected children showed low levels of ferritin [46].

On the other hand our results were disagreed with the results of [26] who showed that no correlation were noticed between ferritin and protozoa parasitic infection ($p > 0.05$). The differences between the findings in the present study and other are likely due to age, the species of parasite and the sample size.

Serum iron was significantly lower in *E. histolytica* infected children compared with the serum iron in control group. Result showed that (93.6%) *E. histolytica* infected children had low iron level while the low iron level were noticed only in (29.4%) of the control group. One causes of anemia is gastroenterological disorders led to malabsorption and inflammation [47]. *E. histolytica* pathogenesis including inflammation, as well as it causes amebic colitis manifestations like diarrhea, which may be watery or bloody which is likely cause weight loss [22]. That may be explain the low serum iron levels in patients as host defense manner due to its pathogenesis.

This result was consistent with other study which has been conducted that the serum iron level decreased significantly ($p<0.01$) in children with giardiasis and amoebiasis compared to the uninfected group [48]. Furthermore, other study [49] showed significant differences for serum iron between children with and without some Intestinal parasites. In contrast, Indonesian study was conducted among school children showed no significant relation between gastrointestinal parasitic infection and low

level of serum iron [26]. These differences between studies are likely due to the differences in age of children included in each study and the type of nutrition.

These results were confirmed by TIBC test which showed that the infected group had less TIBC level than normal, which is an indicator of too little iron in the blood of infected children with *E. histolytica.*

Results showed that microcytic anemia was seen in the majority of *E. histolytica* infected children compared with less cases in control group. While Normocytic anemia was noticed in the majority of control group compared with high percentage in *E. histolytica* infected children. No cases of Macrocytic anemia were seen among both infected and control groups. Microcytic anemia caused by iron deficiency, is the most common type of anemia during childhood, whereas macrocytic anemia is rare in children [50].

Similar results study included school- age children showed patients with protozoan parasite had microcytic anemia (32%) although there were no significant correlation between protozoan infection and the mean corpuscular volume ($P>0.05$) [26].This results are likely be a good indicator of the relation between anemia and infection with gastro intestinal parasite.

Results showed that (95.9%) of *E. histolytica* infected children had severe malnutrition (BMI < 15.9 kg/m^2) compared with control group who showed less percentage of malnutrition. The higher rate in severe malnutrition in children infected with *E. histolytica* is likely be associated with the parasite pathogenicity and the manifestation of Amoebic colitis that characterized by diarrhea, which may be bloody and watery present with abdominal cramps, and weight loss [22].

This result is in complete agreement with other previous study which showed that malnutrition underweight in *E. histolytica* infection according to WFH (Weight For High) [48]. Additionally, agreed with [34] results who showed that children with lower body weight and lower height were more infected with intestinal parasites than children with higher anthropometric parameters.

Conclusion:

1. Anemia is more expected to be present in children with Amoebiasis.
2. *E.histolytica* parasite have ability to to alter some blood parameters such as Haemoglobin, Iron, Ferritin and Mean corpuscular volume (MCV).
3. Possible correlation between severe malnutrition and *E.histolytica* infection in children.

Recommendations:-

1. Our findings may provide some guidelines for investigators assessing management approaches for the control both anemia and infection with *E. histolytica* in Iraq.
2. More studies are needed to investigate the correlation between other gastro intestinal parasites and anemia.

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الخلاصة

اجريت الدراسة في الفترة مابين تشرين الاول من العام 2018 الى نيسان من العام 2019 في عدد من المستشفيات و مراكز الرعاية الصحية الاولية، حيث تم شمول (81) طفل تراوحت اعمارهم بين (1-12) سنه.

تم تقسيم الاطفال ال مشمولين بالدراسة الى مجموعتين اعتمادا على نتائج فحص عينات البراز مجهرياً. ضمت المجموعة الاولى 47 طفلا شخصوا باصابتهم بالاميبا الحالة للنسيج في حين ضمت المجموعه الثانية 34 طفلا لم تشخص لديهم اي اصابة معوية وتم اعتبارهم مجموعة السيطرة. جمعت عينات الدم من جميع الاطفال المشمولين بالدراسة للتحري عن كل من الهيموكلوبين، الفيرتين، الحديد، سعة الارتباط بالحديد و حجم الكرية الوسطي كما تم تقييم الحالة الغذائية للاطفال المشمولين بالدراسة من عمر خمسة سنوات فاكثر.

أظهرت النتائج ان انتشار فقر الدم كان عاليا لدى الاطفال المصابين بالاميبا الحالة للنسيج مقارنة بمجموعة السيطرة. كما ان مستويات كل من الهيموكلوبين و الحديد قد انخفضت بشكل معنوي، بينما لم يكن الانخفاض معنويا" في مستوى الفيرتين لدى الاطفال المصابين بالطفيلي.

كما ربطت النتائج بين كل من العمر والجنس مع مستويات الحديد المنخفضة. اضافة الى وجود علاقة معنويه بين كل من نوع فقر الدم و الحالة الغذائية مع الاصابة بالطفيلي حيث وجد ان فقر الدم صغير الكريات هو النوع الاعلى نسبة"، كما ان حالة سوء التغذية الحاد كانت واسعة الانتشار لدى الاطفال المصابين.

نتائج هذه البحث تشير الى ان فقر الدم يمكن ان يكون متوقعا بصورة كبيره لدى الاطفال المصابين. وعليه فان الجهود يجب ان تتركز على التعليم و الثقيف الصحي لدى الاشخاص الاكثر خطرا للتعرض الى فقر الدم او الخمج بالاميبا الحالة للنسيج.

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انتشار فقر الدم بين الاطفال المصابين بطفيلي الاميبا الحالة للنسج *Entamoeba histolytica* في بغداد

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