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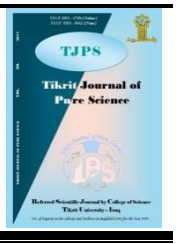

Detection of *Entamoeba histolytica* and *Giardia lamblia* in children with diarrhea in Tikrit city

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Abstract

Background: *Entamoeba histolytica* are very common protozoan parasites in Iraq and are causative agents of gastrointestinal diseases especially among children.

Objective: To show the distribution of *Entamoeba histolytica* and *Giardia lamblia* among children aging 6-12 years old in Tikrit City.

Materials and Methods: A cross sectional study was carried on (1100) school children attended Tikrit Primary Heath Care Centers for period from February 2010 to June 2012. For each child the clinical examination was carried on by physicians in addition to anthropometric measurements, laboratory investigations including haematological, biochemical and general stool examination.

Results: Stool samples were collected from 1100 children from both sexes, the rate of infections were: *E. histolytica* 9.3%, *G. lamblia* 8.9%.The overall rate of *E. histolytica* and *G. lamblia* infections was highest among 6-7 years (20.7%) followed by >10-12 (18.7%) and >7-10 years (17.2%) years. The distribution of both infections did not vary significantly between sexes. The anthropometric measurements in infected children were lower than non-infected controls, although haemoglobin concentration in both infections did not vary from non-infected children, while the white blood cells were higher among infected children in both infections than non-infected ones. In all infected groups, the neutrophils were lower while monocytes were higher than non infected controls. The eosinophils and lymphocytes were lower in *E. histolytica infected children*.

The mean values of alkaline phosphatase, total protein and albumin did not differ between infected and non-infected ones. The globulin value in *Giardia lamblia* was lower than non infected ones. The concentration of zinc in serum of all infected children was lower than non-infected ones. In both infections, T-rosette and B lymphocyte were lower while the Null cells were higher in infected children than non-infected ones.

Conclusions: The rate of both infections in Tikrit city was high. The highest rate of infections was among 6-7 years. The infections did not lead to anemia, but increased total leucocytes count and Null cells but decreased anthropometric measurements, T-rosette cells, B lymphocytes and zinc concentration

Introduction

Amoebiasis is a common problem of human civilization, it is caused by parasitic protozoan infection, most common in developing countries especially among immunocompromised individual and also distributed among monosexual. It is acquired by ingestion of food or water containing the cyst form of the parasite *Entamoeba histolytica* [1].

Entamoeba histolytica is known as pathogenic intestinal ameba, its range of symptoms varies and depends on location of parasite in the host and the extent of tissue infection. Among symptomatic

intestinal amoebiasis, patients exhibit nondescript abdominal symptoms or may complain of more specific symptoms including diarrhea, abdominal pain and cramping, chronic weight loss, anorexia, chronic fatigue and flatulence. Secondary bacterial infection may develop after the formation of flask-shaped amebic ulcers. Some cases develop into amebic dysentery [2].Extra-intestinal amebiasis may take place in the liver, lung, pericardium, spleen, skin and brain. Venereal amebiasis may occur [3&4]. In Iraq, the distribution of amoebiasis were fluctuated depending on sample size, seasonal assessments, and laboratory techniques and other factors (5, 6, 7, 8, 9 & 10).

Giardiasis is a disease caused by *Giardia lamblia* occur worldwide and may infect up a third of the population in developing countries. Approximately about 200 million of people in the world have clinically manifested giardiasis, with 500.000 New cases per year [11]. It is transmitted by swallowing cysts in contaminated food and water or fecal-oral contact. While the parasite can be spread in different ways, water (drinking water and recreational water) is the most common mode of transmission [12]. Giardiasis varies in severity, the disease can be acute or chronic and asymptomatic carriers are common. Children are more frequently affected than adults, although all ages may acquire the infection [13].

Following incubation period of 2 to 3 weeks, water and smelly diarrhea, abdominal cramps, flatulence and anorexia, sometimes accompanied by nausea may occur. Fat soluble vitamin and folic acid deficiencies , weight loss, malabsorption syndrome as well as steatorrhea may occur [14]. In Iraq, there are different reports about distribution of giardiasis in various parts of country with different rate of infection (15, 16, 17, 18, 19, &20).

The present study was carried on to show the distribution of *E. histolytica* and *G. lamblia*

Materials and Methods:

Study population: The present study was done in Tikrit city on 1100 children aged from 6-12 years old (445 males and 655 females). Those were children attended to the clinical centers during the time of the study suffering from diarrhea. All the children were exposed to complete clinical examination in the three Medical Centers and private medical laboratories, including laboratory investigations. The study started from beginning of February 2010 until end of June 2012. A special questionnaire form was arranged to collect information, using direct interviewing to the eligible adults for the individuals, and with children parents.

Anthropometric measurements: The body weight of children were measured by electronic balance distributed by (WHO/UNICEF to primary health care's) with minimal clothing. The height of the children measured to the nearest 0.5cm with a vertical measuring scale fixed to the metal bar connected to the beam balance. The skin fold thickness (SFT) was measured using Harpenden skin fold caliper of a constant pressure of 10gm/sq.mm was used to serve as an index of subcutaneous fat. The mid upper arm circumference (MUAC) was measured to the nearest 0.1 centimeter with a non-elastic fiber glass measuring tape, which was placed gently but firmly round the middle of the left upper arm to avoid compression of the soft tissue [21].

Collection of the specimen: One-three stool samples were collected from each patient in a tightly covered wide mouth plastic disposable container. These were labeled with sample numbers, date and name of the patient. The sample was examined within 1 hour from collection to detect the parasitic infection. The samples were examined by direct wet mount method and formal-ether concentration techniques [13].

Blood samples were obtained, about 10 ml from each patient, 9 ml of blood without EDTA and 1 ml of blood added with EDTA-was drawn intravenously from each subject. The former was used for T-and B-lymphocyte counts, biochemical tests, and zinc concentration. The blood with EDTA used for hemoglobin estimation, total and differential WBC count.

Hematological parameters: The hemoglobin estimation was done by Sahli method, the test is done by dilution of 0.02 ml. of blood with 2 ml of 0.1 mol/hydrochloric acid converting the hemoglobin to acid hematin.

The white blood cell numbers were counted under light microscope (10X and 40X) using Neubauer counting chamber. It was estimated by diluting of whole blood in Turk's solution (glacial acetic acid, 1% with 1-2 drop methyl blue to give purple colour). The differential leucocytes count was made by counting 200 leukocytes in blood smears, stained with Leishman' s stain. These were differentiated into neutrophils, eosinophils, basophils, lymphocytes and monocytes and the percentage of each was calculated using oil lens microscope (100X).

Biochemical tests: The total serum protein and albumin were measured by using kits from Randox Laboratories Ltd., U.K. The value of serum globulin was estimated by subtracting the value of serum albumin from the total protein. The alkaline phosphatase (ALP) was estimated by colorimetric method using kit of bioMerieux sa Marcyl Etoile-France.

The serum zinc level was estimated using atomic absorption spectrophotometer (Varian, Australia) after dilution of serum samples with deionized distilled water 1:4 to bring the metal concentration within the working range of atomic absorption spectrophotometer by direct aspiration of the samples.

Statistical analysis: Both Chi-square and student's t-test were used according to the requirement of the test [22].

Results

The overall rate of *E. histolytica* and *G. lamblia* infections among school children was 18.2 %. The frequency of *E. histolytica* 9.3%, the highest rate was among 6-7 years (11.3%) followed by >10-12 (10.9%) and >7-10 (7.8%). In *G. lamblia* was 8.9 %, the highest rate was among 6-7 (9.4%), and >7-10 years (9.3%), the lowest was among >10-12 years (7.8%). The overall rate of both infections, was (20.7%) among 6-7 year , followed by >10-12 years (18.7%) and >7-10 years (17.2%).

Table (1) Distribution of *E. histolytica* and *G. lamblia* infections according to age of children

Age Year	No. examined	*E. histolytica*		*G. lamblia*		Total	
		No. +ve	%	No. +ve	%	No.	%
6-7	159	18	11.3	15	9.4	33	20.7
>7-10	621	49	7.8	58	9.3	107	17.2
>10-12	320	35	10.9	25	7.8	60	18.7
Total	1100	102	9.3	98	8.9	200	18.2

Table 2 shows distribution of *E. histolytica* and *G. lamblia* infections according to gender. In *E. histolytica* infection, the rate of male vs female was 13.0/ 6.7; in *G. lamblia* 10.1/8.1. Statistically there was no significant difference between gender in both infections.

Table (2) Distribution of *E. histolytica* and *G. lamblia* infections according to gender of children

Age Year	No. examined	*E. histolytica*		*G. lamblia*	
		Male	Female	Male	Female
6-7	159	10	8	7	8
>7-10	621	25	24	28	30
>10-12	320	23	12	10	15
Total examined	1100	58	44	45	53
		5.27%	4%	4.1%	4.8%
Total boy 445		13.0		10.1	
Total girl 655			6.7		8.1

Table 3, shows the anthropometric measurements values (mean weight, height, skin fold thickness and mid upper arm circumference) among children infected with *E. histolytica* and *G. lamblia* infections were lower than non-infected controls of different age groups. The difference between infected and non infected groups in all parameters in both infections was statistically significance (P<0.05).

Table (3) The anthropometric measurements values among *E. histolytica* and *G. lamblia* infected children Mean ±S.D.

Parasites	Age	Infection	Mean Weight kg	Mean height cm	Mean SFT mm	Mean MUAC mm
E. histolytica						
	6-7	+ve	18.8±0.4	110.3±1.2	8.0±0.7	16.6±2.6
		-Ve	19.0±0.4	111.6±1.5	8.9±1.3	17.3±0.8
	>7-10	+ve	25.5±0.8	129.4±1.2	9.3±1.8	19.4±0.7
		-ve	26.6±1.7	130.4±0.8	10.3±1.4	20.5±1.2
	>10-12	+ve	43.5±0	142.7±2.2	11.5±1.1	19.5±2.5
		-ve	44.0±1.6	144.0±1.5	12.7±1.3	20.6±0.9
G. lamblia						
	6-7	+ve	19.8±1.6	110.4±1.2	8.5±1.3	17.5±0.6
		-ve	20.5±1.3	111.0±0.6	9.5±0.8	17.8±0.9
	>7-10	+ve	23.2±0.8	128..4±1.5	9.7±0.3	18.5±1.0
		-ve	23.5±0.4	129.5±1.3	10.1±0.7	18.8±0.4
	>10-12	+ve	42.5±0.7	140.0±1.4	11.4±0.8	21.3±2.1
		-ve	43.5±1.5	142.0±1.7	12.3±1.4	22.8±1.8

+ve=Infected -ve=Not infected

Table 4, shows the haemoglobin concentration (g/dl)and total white blood cells count among children infected with *E. histolytica* and *G. lamblia* and non-infected children . Although the haemoglobin concentration in infected children was lower than non infected ones in both infection, but statistically there was no significant difference between infected and non-infected children; the haemoglobin value was within normal range. The total white blood cell counts in infected groups was significantly higher than non-infected ones (<0.05) and also its value was within normal range.

Table (4) The hemoglobin concentration and white blood cells count among *E. histolytica* and *G. lamblia* infected children.

Infection	Infected	Hemoglobin g/dl	Sig.	WBC count Cell/10^3/Mcl	Sig
E. histolytica	Yes	11.6±1.50	NS	9.44±1.67	<0.05
	No	12.97±2.87		9.04±2.7	
G. lamblia	Yes	11.37±1.24	NS	9.24±1.67	
	No	12.94±2.17		8.30±1.77	<0.05

Table 5, shows the differential white cell counts among children infected with *E. histolytica and G. lamblia* and non-infected children. In *E. histolytica* infected children, the neutrophil, eosinophil and lymphoocyte counts were lower while the basophil and monocyte counts were higher than non infected ones. In *G. lamblia* infected group the neutrophil count were lower while the lymphocyte and monocyte counts were higher than non infected ones, The basophil and eosinophil count did not vary significantly between infected and non infected ones. Statistical analysis using student t-test was carried on to show significant difference between any two groups (P<0.05).

Table (5) Differential leucocytes count among *E. histolytica* and *G. lamblia* infected children

Group	Infect-Ion	Neutrophil %	Eosinophil %	Basophil %	Lymphocyte %	Monocyte %
E. histol-ytica	Yes	50±4.04**	1.8±0.7*	0.6±0.4**	34±1.6**	8.0±3.7**
	No	52±4.7	2.0±0.9	0.5±0.3	37±2.1	6.0±0.5
G. lamblia	Yes	51±3.6**	2.1±2.4 NS	0.6±0.9NS	40±1.5**	4.0±1.3**
	No	53±4.0	2.0±1.9	0.5±1.2	39±1.4	3.0±1.2

* P<0.05 **P<0.01

Table 6, shows some biochemical parameters among infected and non-infected children. The alkaline phosphatase, total protein and albumin values did not differ significantly between infected and non-infected children. Although the globulin value did not vary significantly between children infected with *Entamoeba histolytica,* and non-infected children but in *G. lamblia* infected children was lower than non-infected children (P<0.01).

Table (6) Biochemical parameters among *E.histolytica* and *G. lamblia* infected children
Mean± S.D.

Parasites	Infected	ALP	Total protein	Albumin	Globulin
E. histolytica	Yes	93.67±5.64	5.37±1.47	3.60±0.80	1.77±1.30
	No	95.67±6.94	5.30±0.74	3.64±1.24	1.67±1.10
G. lamblia	Yes	96.34±4.74	5.20±0.97	3.67±1.57	1.54±1.40<0.5
	No	97.67±4.34	5.60±1.40	3.87±1.54	1.74±1.77

Table 7, shows significant difference of zinc concentration (µmol/L) in serum of children with *E. histolytica* and *G. lamblia* infected and non-infected children. Serum zinc concentration in all infected children was significantly lower than non-infected ones (P<0.05).

Table (7) Serum zinc concentration among children infected with *E. histolytica* and *G. lamblia*
Mean±S.D.

Infection	Infected	Non-infected	T-test
E. histolytica	5800±0.75	62.00±0.95	P<0.05
G. lamblia	57.50±1.30	63.5±1.00	P<0.05

Table 8. shows the T rosette forming cells, B lymphocyte and Null cells count among *E. histolytica* and *G. lamblia* infected children and non-infected ones. In infected children, the T-rosette and B lymphocyte were lower while the Null cells was higher than non-infected children.

Table (8). Values of T rosettes forming cells, B-lymphocyte and Null cells (%) among *E. histolytica* and *G. lamblia* infected children.
Mean±S.D.

Parasites	Infection	T rosette cells	B lymphocyte	Null cells county
Entamoeba histolytica	Yes	51±1.7	19±5.5	30.0±3.6
	No	54±3.4	20±6.1	26.6±4.2
Giardia Lamblia	Yes	51±3.0	19±5.4	30.0±4.8
	No	55±3.7	20±5.6	25±5.5

t-test=0.01

Discussion

The overall rate of *E. histolytica* and *G. lamblia* among 1100 children aged from 6-12 years old were 18.2%. This reflects that protozoan infections are common in Tikrit. Salman et al. [20] examined 266 found the overall rate of parasitic was 64.28% in 171 stool samples, this rate was contributed five protozoan parasites: *E. histolytica* 94(34.21%), *G. lamblia* 47(17.66%). These infections are endemic worldwide and are a major public health problem specially in tropical and subtropical countries [23]. Comparing the rate of infections with those from different parts of the country and neighboring countries, showed a considerable difference could be found in prevalence of infections. The differences can be explained by the influence of level of sanitation, environmental conditions, human behavior, laboratory diagnosis used for detection of infections and health education among school children [24]. Transmission of these protozoan occur via fecal-oral route either directly or indirectly via eating or drinking focally transmitted food and water, in addition, asymptomatic carriers of these infections are a constant risk for transmission in a community [18].

High infection rates were found in children aged from 6-7 and >7-10 years old and low infection rates were found among children aged from >10-12 years. There were no significant difference between infected and non-infected ones in relation to age group. This finding is almost identical to that reported by [18] in Baghdad City, who reported that the rate of intestinal parasites among children was 57.9%., the highest prevalence (71.4%) was recorded among 6-11 years age group followed by age group 12-18 years (55.1%), while the group below than 2 were the less affected group (30.6%). In Dohuk [15] examined 1261 stool specimens of children, found the age group 10-12 years had the highest rate (81.2%) and 7-9 years the lowest (22.9%). In Kadhmiyah Hospital in Baghdad [8] the rate of *E. histolytica* was 9.80% and *G. lamblia* 1.51%. In Kirkuk, [19] examined 417 stool samples from internal displaced persons living in schools, houses under construction and rented accommodation and houses in Kirkuk; recorded the rate of *G. lamblia* was 10.31%, *E. histolytica* 1.67%. They showed high rate among those aging from 1-10 years and no significant difference between males and females.

Regarding the distribution of infections according to gender, although the rate of both infection in males were greater than females, but statistically there was no significant difference between sexes. In Baghdad City, it has been reported that there was no significant difference between gender and infectivity rate of *E. histolytica* and *G. lamblia* in children aging from 1 month to 2 years (8).

In Kirkuk [9] examined 943 stool samples of children attended Kirkuk Pediatric hospital, she found the rate of *E. histolytica/E.diapar* was 30.22%, the highest rate was among 5-6 years (43.96%). Infection rate among illiterate children was higher than educated ones. *G. lamblia is recognized as the most common intestinal protozoan parasite of human beings in Iraq [25] especially in Kirkuk* [26].

In Duhok city- Iraq, [15], examined 451 stool samples from primary school children, they found the rate of *Giardia lamblia* infection was 41.2%, which was higher among boys (41.6%) than girls (35.6%). In Baghdad, [18], found the prevalence of *G. lamblia* was (31.)%), the rate of intestinal parasites was slightly higher in males (59.9%) than females (56.2%), but statistically there was no significant difference between sexes. The higher rate of infection in males is due to males tend to spend their time in street, and playing in sand but females tend to spend most of time in homes. This finding is contrast to [27] who found the rate of intestinal parasites was higher in females than in males.

The lower anthropometric measurements in infected children indicate that protozoan infections lead to growth retardation, this finding is in agreement with that reported by [28] in Hawija district, Kirkuk governorate and also with [29] who studied the prevalence of *B. hominis* in Turkey and in Tikrit City [10].

In the present study, although the hemoglobin concentration was lower in all infected children groups than non-infected ones, but statistically there was no significant difference between infected and non-infected groups.

The lower hemoglobin concentration among infected children reflect that protozoal infections affect the general health of those children.

The increase in white blood cells during protozoal infections is also reported in *B. hominis* infection [10].

The white blood cells main function is to fight infection defend the body by phagocytosis against invasion by foreign organisms, and to produce or at least transport and distribute antibodies in the immune response. In this study the white blood cells count was higher among infected children than non-infected ones. This finding is also reported in other study in Baghdad [8] in *G. lamblia* and *E. histolytica* [30].

Blood serum contains large amounts of protein. Two major groups of proteins in blood serum are albumin and globulins. A total serum protein test measures the total amount of protein in blood serum as well as the amount of albumin and globulin. Because the total protein represents the sum of albumin and globulin. It is important to know which protein fraction is high or low than the total protein. In this study the values of serum total protein and immunoglobulin did not differ between infected and non-infected children. Similar finding is reported by [10] in *B. hominis* infection. Decrease albumin may also be explained by malnutrition or a low protein diet.

Giardia lamblia and *Entamoeba histolytica* were related to delirious consequence may result from protein energy malnutrition. In the present study the total protein and albumin were significantly lower in both infections [31]. In Tikrit [32] recorded significantly lower level of serum total protein and albumin in *G. lamblia* infected patients comparison to control. In Erbil, [5] recorded no significant difference in levels of total serum protein and albumin in infected and non-infected groups (6.82+0.59, 3.8+o.3) (7.23+0.4, 3.82+0.27 gm/dl) respectively. In Kalar town [31], showed that the concentration of serum globulin did not vary significantly between *E. histolytica* infected and non-infected children.

The value of alkaline phosphatase, total protein, albumin did not vary significantly between infected and non-infected children. Although the the globulin value did not vary significantly between children infected with *E. histolytica* but in *G. lamblia* infected children was lower than non infected ones. It has been reported that alkaline phosphatase and leucocyte counts elevated in amoebic liver abscess [33]

Higher concentration of serum globulin among *G. lamblia* infected patients than non-infected ones is identical to study reported by [34] who recorded higher concentration of total globulin in serum in *G. lamblia* infected 2.57gm/dl compared to control 1.83gm/dl. They found that the concentration of serum globulin did not vary significantly between children infected with *E. histolytica* and non-infected ones.

Zinc is essential trace element for a wide range of biochemical, immunological and clinical infections. Zinc deficiency is due to lack of intake of animal protein, high dietary phytate that limit zinc absorption and intake, in addition to increased fecal losses during diarrhea [10]. The lower zinc concentration in infected children than non infected ones is in agreement with [35] who reported decrease in zinc level in serum of children aged 6 years infected with diarrhea. This is also identical to that reported in *G. lamblia* infection in Turkey [36] *and in B. hominis* infection among primary school children in Baghdad [37].

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إيجاد طفيلي الزحار الاميبي وجيارديا لامبيليا في الأطفال المصابين بالاسهال في مدينة تكريت

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الملخص

طفيلي الزحار الاميبي من الطفيليات الشائعة في العراق وواحد من العوامل المسببة للامراض المعوية وخاصة بين الأطفال.

الهدف : استبيان توزيع طفيلي الزحار الاميبي *Entamoeba histolytica* و جيارديا لامبليا *Giardia lamblia* بين الأطفال في عمر 6-12سنة في مدينة تكريت.

المواد وطرائق العمل: أجريت الدراسة على 1100 طالب من المراجعين للمراكز الصحية في مدينة تكريت للفترة من شباط 2010 لغاية حزيران 2012 مع الفحوصات السريرية والتشخيص المختبري (الفحوصات الدموية والكيموحيوية وفحص البراز العام).

النتائج: من مجموع 1100 طفل من كلا الجنسين كانت نسبة الإصابة ب امبيا الزحار 9.3% وجيارديا لامبيليا 8.9% وكان نسبة الإصابة الكلية للطفيليين كان اعلى لدى الاعمار من 6-7 سنة (20.7%) تلتها 10-12 سنة (18.7%) و 7-19 سنة (17.2%). وان انتشار الإصابتين لم تختلف معنويا بين الجنسين.

بالرغم من ان تركيز الهيموكلوبين لم يختلف معنويا عن غير المصابين ولكن كريات الدم ابيضاء كانت اعلى لدى الأطفال المصابين. في جميع المجاميع المصابة كان الخلايا البيضاءالعدلةNeutrophils اوطأ نسبة بينما الخلايا الأحادية النواة Monocytes كان اعلى من مجموعة السيطرة. في كلتا الإصابتين ان خلايا T-rosette و B-Lymphocytesكان اوطأ بينما Null cells كان اعلى في الأطفال المصابين عن غير المصابين.

الاستنتاجات: ان نسبة الإصابة لكلا الطفيليين في مدينة تكريت كانت عالية وان اعلى نسبة إصابة كان لدى الاعمال من 6-7 سنوات وان الإصابتين لم يؤدي الى فقر الدم ولكن أدى الى زيادة المجموع الكلي لعدد كريات الدم البيضاء وخلايا Null cells ونقصان عدد خلايا T-rosette cellsوتركيز الخارصين.