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The Effect of Infection on Some Intestinal Parasite on Some Hematological Parameters for Diarrhea Patients in Zakho City, Kurdistan Region, Iraq

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ABSTRACT

This study was carried out in Emergency hospital of Zakho / Duhok, Iraq from january2019 to december2019. A total of 515 Patients of both sexes from all ages were eligible for this study. The chosen patients had single infection either with *Entamoeba histolytica* or *Giardia lamblia. Entamoeba histolytica* was diagnosed in 122(23.6%) stool samples from the total number examined No significant differences were appeared between males (23.8%) and females (23.5%) infected with *E. histolytica.* Age group 16-25 years had significantly higher rate of infection (28.8%) followed by 1-15 years with rate of (24.6%). The lower rate was for 36-45(14.2%). High rates of the *E. histolytica* positive samples contained trophozoite and cyst (50%), followed by trophozoite stage (26.3%), while cyst stage was found in (23.7%). *G. lamblia* prevalence was significantly lower compared with *E. histolytica*. It was diagnosed in 96 (18.6%) stool samples of 515 samples examined. significant differences were recorded between the females (16.9%) and males (20.1%). Age group 1-15 years had significantly higher rate of infection (21.5%). The lower rate was for 36-45(12.3%). *G. lamblia* was more prevalent as trophozoite (47.9%), trophozoite and cystic (35.5%) than cystic stage alone (16.6%). The samples containing trophozoite and cystic stages of *E. histolytica* had the most apparent significant effect on all hematological parameters, and the highest rate of abnormality was seen in HB levels and WBC levels, as well as for *G. lamblia* was seen the highest rate of abnormality in HB levels and WBC levels of The samples containing trophozoite and cystic stages.

Keywords: Entamoeba histolytica, Giardia lamblia, Hematological parameters, Iraq.

1. INTRODUCTION

Diarrhea is a major public health problem worldwide, especially in children. One in ten child deaths result globally from diarrheal disease before their fifth birthday, resulting in about 800 000 fatalities worldwide annually, most occurring in sub-Saharan Africa and south Asia (Liu et al. 2010)1. In South Asia, Diarrhea accounts for 26.1% of childhood deaths with a peak incidence in their early years of Life (Walker et al. 2012)2. The incidence of diarrheal diseases varies greatly with the seasons and a child's age. The rate is highest in the first two years of life and declines as a child grows older. In Bangladesh, one third of the total child death burden is due to diarrhea (Victora et al. 1993).

Every year, a rural child suffers on average from 4.6 episodes of diarrhea, from which about 230,000 children die (Piechulek et al. 2003. Intestinal amebiasis caused by the protozoan *Entamoeba histolytica* is the third-greatest parasitic disease responsible for death in the world after malaria and schistosomiasis (Stanley,2003). Giardiasis, caused by *Giardia lamblia*, is a frequent cause of diarrhea that can have a negative impact on growth and development of children (Simsek, et. al, 2004). Amebiasis can result in dysentery, malabsorption and extra intestinal complications; giardiasis is associated with acute diarrhea, steatorrhea and lactose intolerance (Stanley, 2003, Simsek, et. al, 2004). The relationship between chronic parasitic intestinal infection and anemia has been thoroughly investigated and confirmed in several studies (Brooker, et. a,1999, Stephenson, et. al,2000). In particular, infection with hookworm species can cause severe anemia due to continuous blood loss from blood-sucking adults and bleeding mucosal ulcer (Stephenson, et.al,2000)

The prevalence of intestinal parasites differs from one region to another and its prevalence related to several factors such as geographical factors, climate, poverty, malnutrition, high population density, and personal and community hygiene. Also, optimal conditions for the growth and spread of intestinal parasites, the absence of potable water and the poor state of health play a major role in the spread of these parasites. Research has indicated that school-age children are most vulnerable to intestinal parasitic diseases, and their effect on children is different and dangerous (3).

Most of the parasitic infections transmitted through food and contaminated water. These two sources are one of most important factors leading to increased intestinal parasites. More than 72 species of parasites have been isolated from water and food, most of which are humanly infected (4, 5, 6& 7). Intestinal parasites are an important cause of many pathological effects such as Diarrhea, Abdominal pain, Anorexia Weight loss, Flatulence, Vomiting, Nausea, Fever, and Bowel blockage (8). Some intestinal parasites can also impede the absorption of digested nutrients such as carbohydrates, proteins, vitamins, and minerals important for human health (9) .

Anemia occurs in children due to iron deficiency, but the parasitic infection is another because of it. Hemoglobin is the red dye that found within the red cell. Losses occur in certain pathological conditions including parasitic infection, malnutrition, blood loss, and chronic infection, which expressed as anemia. White blood cells are one of the essential components of blood in peripheral blood, protecting against germs, parasites, tumors, and other diseases. Five types of white blood cells differ in size, proportions and functions that are as follows: neutrophil eosinophils, basophils cells, lymphocytes and monocytes (10).

The *E. histolytica* and *G. lamblia* infection lead to iron deficiency (65). The presence of *E. histolytica* in the intestines causes gastrointestinal disturbances, through the trophozoite, which attaches to the intestinal villi and absorbs nutrients (66). It also secretes the proteins that analyze the host's tissues and cells and feed on red blood cells (67). In addition, parasite infection may lead to necrosis of the intestinal mucosa, leading to the deterioration of absorption of essential substances and tissue damage; Intestinal parasites are strongly associated with the development of anemia because they cause malabsorption and undernutrition (68). The Giardia parasite causes damage to the gastrointestinal mucosa, causing malabsorption syndrome, especially vitamins (Vitamin B12) and iron (69, 70 & 71). Besides, other essential nutrients because it makes a barrier to the passage of these substances from the intestinal cavity to the bloodstream. The effect of the dwarf worms on the amount of hemoglobin, through the competition of the host on food, depending on the number and density of this parasite. The pinworm has little effect compared to other parasites because they are found in the small intestine and do not feed on blood but feed on secretions or host products and do not cause any damage to the intestinal wall (72).

The prevalence of E. *histolytica* is widespread worldwide, especially in the tropical and subtropical regions such as Iraq (48). The wet climate provides the conditions for the maturity of the cysts of this parasite (49) and then their transmission to humans (50). It also transmitted from person to person directly and indirectly (fecal-oral) through food and water contaminated with mature cysts (10 & 51). As well as the lack of potable water that may be contaminated with cysts of this parasite, that not affected by the chlorination process for sterilizing drinking water Some insects such as domestic flies play a role in transporting this parasite. The increase in infection in children (7). is due to the slow development of their immune system and their lack of knowledge of good health habits (36). The cause of *G. lamblia* parasites infection is due to the direct transmission of it by contaminated water and with mature cysts. Animal waste and farming practices are important sources of water and food pollution (52), as food well as a lack of interest in personal hygiene (53)

The aim of the present study was the detection of intestinal parasitic infection (*Entamoeba histolytica* and *Giardia lamblia*) in human population, and find out the effect of the two parasitic infections on some hematological parameters.

2. MATERIALS AND METHOD

2.1 Microscopic Examination:

From January2019 to December2019. this study was carried out in Emergency hospital of Zakho a total of 515 Patients of both sexes from all ages whom suffering from diarrhea were chosen for this study. Stool samples from each patient was collected in a clean, dry, tight fit cover and examined within half an hour. in parasitology lab. In the hospital. The samples were examined for the presence of the *Entamoeba histolytica* and *Giardia lamblia* parasites. Microscopic examination: All stool samples were examined microscopically by direct wet mount method with normal saline and iodine with high power (40x) for detection of the trophozoite and cyst stage of *Entamoeba histolytica* and *Giardia lamblia*.

2.2 Hemoglobin Estimation:

Five ml of venous blood sample was collected from each infected patient. 1.5 ml of the blood was collected to EDTA tube for hemoglobin estimations. The graduated tube was filled to the 20 mark (on % scale) with N/10 HCL. The blood was drawn by using hemoglobin pipette to the 0.02 ml (20µl). The tip of the pipette was wiped with cotton so that no blood may left that stick on its outside. The blood expelled into the tube containing HCl solution, a small amount of an acid was sucked into the pipette and expelled again into the tube. The content mixed quickly but gently with a glass-rod stirrer and leaved for 10 minutes. Distilled water (or HCl solution) was added drop by drop, mixed between each addition until the color matches with the standard. The amount of the solution in the graduated tube was read. The calibration gives the Hb concentration as percentage.

2.3 White Blood Cell Count:

For calculation of the total white blood cells count, the blood was diluted with a fluid which lyses red blood cells, but spares white blood cells and nucleated red cells. The pulp of the finger was tabbed with a sterile lancet and the first drop of blood wiped away. When a big enough drop of blood formed, the tip of the pipette was applied to the drop and blood sucked up to the 0.5ml mark. The diluting fluid draw up till the 11 mark - till glass bubble was full. Dilution would be 1: 20. The pipette was holed horizontally and rotate vigorously between finger and thumb for mixing. The fluid contained within the stem of the pipette

was let out. The pipette was touched to the side of the Neuberger counting chamber. The chamber was filled with the diluted blood. wait two minutes to allow the cells to settle. The WBCs were counted. As WBCs are larger cells and are found in less amounts than RBCs, the larger squares of the counting chamber are used. No. of WBC in 1mm3 of undiluted blood=10Z x 20 / 4.

2.4 Statistical Analysis

For finding the differences according to different parameter analyzed by SPSS for windows, was used for statistical analysis of these samples by identified to percentages.

3. RESULTS

The results of the study in table1, Shows some significant differences of *E. histolytica* and *Giardia lamblia* distribution between the males and females. The incidence of *E. histolytic* (23.6%) in this study was significantly higher than *G. lamblia* (18. 6%).The results of this study had showed no significant differences between the rate of *E. histolytica* in males (23.8%) and females (23.5%), also shows the frequency of *G. lamblia* infection in relation to sex, no significant differences were noted between the distribution of the parasite in females (16.9%) and males (20.1%).

Table 1. Distribution of *E. histolytica* and *Giardia lamblia* according to sex

Parasite	*E. histolytica*			*Giardia lamblia*		
sex	Male	Female	Total	Male	Female	Total
Examined No.	243	272	515	243	272	515
ve No.+	58	64	122	49	46	96
%	23.8	23.5	23.6	20.1	16.9	18.6

Regarding age group in table2. the rate of *E. histolytica* was significantly highest in 16-25 years (28.8%), followed by 1-15 years (24.6%) and 26-35 year (23.4%) while the 36-45 age group showed lower infection rate (14.2%)Regarding the results of *G. lamblia* incidence according to age groups, also showed significant differences were appeared in the parasite distribution in relation to age groups., The rate of *G. lamblia* was significantly highest in 1-15 years (21.5%), followed by 26-35 years (19.5%) and 16-25 year (17.6%) while the 36-45 age group showed lower infection rate (12.3%).

Table 2 Distribution of *E. histolytica* and *Giardia lamblia* according to age groups

Parasite	*E. histolytica*					*Giardia lamblia*				
Ages	1-15	16-25	26-35	36-45	Total	1-15	16-25	26-35	36-45	Total
Examined No	215	118	98	84	515	204	125	97	89	515
ve No.+	53	34	23	12	122	44	22	19	11	96
%	24.6	28.8	23.4	14.2	23.6	21.5	17.6	19.5	12.3	18.6

Table 3 showed that the mixed infection with trophozoite and cystic stages were most apparent stage in *E.*

histolytica infection. These stages were found in 61 positive stool samples (50%) followed by trophozoite stage in 32 samples (26.3%), while the cystic stage was found in 29 positive samples (23.7%). Also shows differences between the samples containing trophozoite and Cyst+trophozoite stages of *G. lamblia* comparing with those containing cystic stages alone. The most apparent stage was the trophozoite (47.9%), followed by cyst+ trophozoite (35.5%), while the cystic stage was found in only 16.6% of the positive samples.

Table 3. Distribution of *E. histolytica* and *Giardia lamblia* stages

Parasite	*E. histolytica*				*Giardia lamblia*			
Stages	Trophozoie	Cyst+trophozoite	Cyst	Total	Trophozoite	Cyst+trophozoite	Cyst	Total
ve No.+	32	61	29	122	46	34	16	96
%	26.3	50	23.7	100	47.9	35.5	16.6	100

Table 4 shows the effect of *E. histolytica* and *G. lamblia* infections on hematological parameters. It indicates that samples containing trophozoite and cystic stages of *E. histolytica* had the most significant effect on all hematological parameters, and the highest rate of abnormality was for HB levels, followed by those samples having *E. histolytica* trophozoite and *E. histolytica* cyst, while shows the highest rate of abnormality was for WBC levels. In *G. lamblia* the samples containing trophozoite stage had the most significant effect on all hematological parameters, and the highest rate of abnormality was for HB levels, followed by those samples having *G. lamblia* trophozoit +cyst and *G. lamblia* cyst, also shows the highest rate of abnormality was for WBC levels.

Table 4. Normal and abnormal hematological parameters regarding to *E. histolytica* and *G. lamblia* infections N'=normal, AB=abnormal, HB= Hemoglobin, WBC= White blood cell count.

Parasite stages	+ve No.	N.WBC	AB.WBC	N.HB	AB. HB
E. histolytica trophozoit	32	13	19	14	18
E. histolytica cyst	29	12	17	13	16
E. histolytica trophozoit +cyst	61	25	36	27	34
G. lamblia trophozoit	46	21	25	19	27
G. lamblia cyst	16	9	7	8	8
G. lamblia trophozoit +cyst	34	18	16	19	15

4. DISCUSSION

This study had concluded evaluation the effect of *Entamoeba histolytica* and *Giardia lamblia* parasites on some hematological parameters for diarrhea patients, in Emergency hospital of Zakho, the incidence of *E. histolytic* (23.6%) in this study was significantly higher than *G. lamblia* (18. 6%). This may due to the greater longevity of cysts in environment conditions. Identical results were found by other authors (Al-Kaycee, & Sultan 2008, Shaheen, et. al, 2007), frequency rate of 29.2%for *E. histolytica* comparing with those of *G. lamblia* (15,0%) was found by (Shaheen, et. al,2007), Prevalence of *E. histolytica/dispar* and *G. lamblia* were, respectively, 18.8% and 13.9%(Ouattara, et. al,2010). But Matthys, et. al, had reported

equal rates (26.2, 25.9 %) for the two parasite (Matthys, et. al, 2011). Geographical variation of different countries and the endemicity of the parasites in these different areas may led to these variations. The results of this study had showed no significant differences between the rate of *E. histolytica* in males (23.8%) and females (23.5%), this result may be because these groups equally involved in out and indoor activities which might lead to the parasite transmission in both groups. This This was agreed with that reported by Raza and Sami how record rate of 16, 19 % for each of males and females respectively (Raza, & Sami 2009). the frequency of *G. lamblia* infection in relation to sex, no significant differences were noted between the distribution of the parasite in females (16.9%) and males (20.1%). This result is in disagreement with (Al-Saeed & Issa, 2006) who indicate 14.6 % in males and 35.6% in females. The rate of *E. histolytica* was significantly highest in 16-25 years (28.8%), followed by 1-15 years (24.6%) and 26-35 year (23.4%) while the 36-45 age group showed lower infection rate (14.2%)This result is in agreement with Hamad and Ramzy whom reported rate 45- 52.38 % in 1-12 years old, which may be attributed to defecation practices because these groups of children are fully independent in toilet use and are more involved in both outdoor activities and feeding (Hamad&Ramzy, 2011) the results of *G. lamblia* incidence according to age groups, also showed significant differences were appeared in the parasite distribution in relation to age groups., The rate of *G. lamblia* was significantly highest in 1-15 years (21.5%), followed by 26-35 years (19.5%) and 16-25 year (17.6%) while the 36-45 age group showed lower infection rate (12.3%). InAl-Saeed1and Issa study the infection rate was highest in the age group 1–15 years, they attribute that perhaps because parents are responsible for their hygiene (Al-Saeed& Issa, 2006). The present results are similar to studies of intestinal parasitoids in Dyala and Basrha (Al-Khaysee, & Sultan 2008, Shaheen, Z..et, al 2007) the mixed infection with trophozoite and cystic stages were most apparent stage in *E. histolytica* infection. These stages were found in 61 positive stool samples (50%) followed by trophozoite stage in 32 samples (26.3%), while the cystic stage was found in 29 positive samples (23.7%). This may because the trophzoite is responsible for acute cases which require emergent treatment, while Cyst+trophozoite found in chronic cases which is responsible for recurrent diarrhea. Similar result recorded by Al-Khaysee and Sultan, they found the most *E. histolytica* positive samples were for those containing trophozoite and cyst stage (Al-Khaysee& Sultan, 2008). Also shows differences between the samples containing trophozoite and Cyst+trophozoite stages of *G. lamblia* comparing with those containing cystic stages alone. The most apparent stage was the trophozoite (47.9%), followed by cyst+ trophozoite (35.5%), while the cystic stage was found in only 16.6% of the positive samples (Al-Khaysee & Sultan, 2008) found no significant differences between the positive samples containing *G. lamblia* stages.

The results of the current study showed significant changes in hematological parameter of patients with intestinal protozoan infection (*E. histolytica* and *G. lamblia*). it was observed increase in the Hb level and an increase in theWBC level, this result was similar to those of (16), how studied the effect of intestinal parasites on the blood picture as our study agrees with (17). A study in Sudan conducted to find out the effect of *E. histolytica* on some hematological parameters, showed a decrease in the rate of Hb and an increase in WBC in people with infect by E. *histolytica* parasite. A study of (2) in India was conducted to find out impact of giardiasis on hematological profile of infected children. The results of his study showed a significant decrease in mean values of Hb from (11.15) to (10.05) in males and (11.36) to (10.08) in females. In case of WBC count, a significant increase in the total number of WBC was observed in a case of infected children from (7.96) to (9.38), with respect to differential leukocyte count, Eosinophils

showed a marked increase in their number in infected individuals (5.0), in comparison to uninfected ones (2).

The effect of *E. histolytica* and *G. lamblia* infections on hematological parameters. It indicates that samples containing trophozoite and cystic stages of *E. histolytica* had the most significant effect on all hematological parameters, and the highest rate of abnormality was for HB levels, followed by those samples having *E. histolytica* trophozoite and *E. histolytica* cyst, while shows the highest rate of abnormality was for WBC levels In *G. lamblia* the samples containing trophozoite stage had the most significant effect on all hematological parameters, and the highest rate of abnormality was for HB levels, followed by those samples having *G. lamblia t*rophozoit +cyst and *G. lamblia* cyst, also shows the highest rate of abnormality was for WBC levels. Evidence from community studies indicate the role of *E. histolytica* and *G. lamblia* infection in causing iron deficiency anemia has been confirmed by other studies (Al-Naemi, et. al, 2011, Juma'a, 2006). Chronic giardiasis can interfere with the growth of children by impaired nutrient digestion (fat and vitamin), anemia and lactose intolerance associated with growth impairment [Stanley, 2003, Simsek, et. al, 2004, Juma'a, 2006).

5 CONCLUSION

The current study showed the prevalence of intestinal protozoa infection in Emergency hospital of Zakho / Duhok, Iraq, and the prevalence rates of intestinal protozoa infection were (23.6%) more common than *Entamoeba histolytica*, followed by *Giardia lamblia* (18.6). The results of the current study showed significant changes in the results of hematological criteria with people intestinal protozoan infection (*E. histolytica* and *G. lamblia*). where it is observed increase in the Hb level and an increase in the level of (WBC),

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