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# The Effect of *Entamoeba Histolytica* and *Giardia Lamblia* Infection on Some Human Hematological Parameters

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**Abstract:**
This study was carried out in Al Haweeja General Hospital/Kirkuk, Iraq from June2013to February2014. A total of 207 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 diagnosedin 84 (40.5%) stool samples from the total number examined. No significant differences was appeared between males (43.8%) and females (37%) infected with *E. histolytica*. Age group 1-10 years had significantly higher rate of infection (60%) followed by 11-20 years with rate of 34%. The lower rate was for 41-50 (16%). High rates of the *E. histolytica* positive samples contained trophozoite and cyst(47.6%), followed by cystic stage (27%), while trophozoite stage was found in 25% of samples.*G. lamblia* prevalence was significantly lower compared with *E. histolytica*. It was diagnosed in 15 (7.2%) stool samples of 207 samples examined. No significant differences were recorded between the females (8%) and the males (6%). Also no significant differences appeared between the different age groups. *G. lamblia* was more prevalent as trophozoite (46.6%), trophozoite and cystic (40%) than cystic stage alone (13.3%). 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, followed by these samples having *E. histolytica* cyst alone , also significantly high rate of abnormality was for PCV levels. The lowest rate was for samples containing *G. lamblia* stages. Eosinophil showed significantly high rate with *G. lamblia* trophozoite compared with the control. Lymphocyte showed significantly high rate with *E.histolytica* (trophozoite+cyst) compared with the control. No significant differences were noted with the other types of WBCs (neutrophil, monocyte, basophil) inall cases of infections compared with the control.
**Keywords:** *Entamoeba histolytica*, *Giardia lamblia*, Hematological parameters, Iraq.

## Introduction :

Parasitic infections are a major public health problem worldwide, particularly in developing countries (http:esa.un.org). Intestinal parasitic infections are most common and with high prevalence in Iraq (Hamad & Ramzy( 2011**,** Al-Saeed1& Issa 2006, Al-Khaysee, & Sultan 2008**,** Raza, & Sami 2009, AL-Shaheen,et.al, 2007**)**.Numerous protozoon species parasitize the intestine of humans, and some of them are *Giardia lamblia* and *Entamoeba histolytica*, cause a remarkable quantified disease burden, particularly in the humid tropics (AL-Shaheen,et.al, 2007, Ouattara., et. al,2010). Epidemiologic studies and public health interventions, however, more often focus on helminths (e.g., *Schistosoma mansoni*, *Ascaris lumbricoides*, *Trichuris trichiura*, and hookworm) than intestinal protozoon infections (Okhuysen, 20018). This may be partially explained by the fact that the microscopic diagnosis of intestinal protozoa is more difficult, hence requiring experienced, highly skilled technicians for correct identification and differentiation of intestinal protozoon cysts, and is more time consuming than the microscopic detection of helminth eggs in human stool samples. Amoebiasis due to infections with the intestinal protozoon *Entamoeba histolytica* results in 40,000–100,000 deaths each year (Stanley, 2003), and giardiasis due to Giardia intestinalis might affect 200 million people per annum (Minenoa, & Avery, 2003). However, the burden of pathogenic intestinal protozoan infections remains to be determined, which is a challenge due to the paucity of up-to-date epidemiological data (Hotez, et. al, 2009).

Parasitic infections are governed by behavioral, biological, environmental, socioeconomic and health systems factors. Local conditions, influence the risk of infection, disease transmission and associated morbidity and mortality (Al-Khaysee, & Sultan 2008,Raza, & Sami 2009). In 2002, WHO estimated the number of people infected by digestive tract parasites at 3.5 billion (WHO (2001) . 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, malabsorbtion and extraintestinal 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 andbleeding mucosal ulcer (Stephenson, et. al,

2000). In a study from Brazil, eosinophil counts were higher in individuals co-infected with intestinal helminths and ectoparasites than in individuals with only intestinal helminths (Heukelbach,et. al,2006). In Iraq especially Kirkuk, relativelyfew studies have been done on the hematological altration of intestinal parasites, therefore 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.

**Materials and methods**:

**Patient selection:**

From June to October 2012 this study was carried out in Al Haweeja General Hospital. A total of 207 Patients of both sexes from all ages whom suffering from diarrhea and abdominal pain were chosen for this study.

**Stool specimen collection:**

Stool samples from each patients 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.

**Stool examination:**

Macroscopic examination: The stool samples were examined with naked eyes before microscopically examination for color, consistency, blood mucous.

Microscopic examination: All stool samples were examined microscopically by direct wet mount method with normal saline and lugols iodine with high power (40x) for detection of the trophozoite and cyst stage of *Entamoeba histolytica* and *Giardia lamblia* (AL-Shaheen,et.al, 2007).

**Blood collection:**

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 and PCV estimations.

**Packed Cell Volume (PCV) estimation:**

**Is** the volume of erythrocytes expressed as fraction of the volume of whole blood in a sample. This was done by allowing the blood to enter a capillary tube leaving at least 15 mm unfilled. the capillary tube then sealed, centrifuged in micro centrifuge for 5 minutes at 2700 rpm. Height of the erythrocyte using haematocrit reader was read and express result as a percentage or liter/liter (Heukelbach,et. al,2006).

**Hemoglobin estimation:**

The graduated (Sahli's Adams) 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 sahli 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 match's with the standard. The amount of the solution in the graduated tube was read. The calibration gives the Hb concentration as percentage (Daniel, 2011).

**White blood cell count and differential count :**

**Calculation of the total white blood cells count:** For this, 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 Neubauer 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 (Heukelbach,et. al,2006). No. of WBC in $1mm^3$ of undiluted blood=10Z x 20 / 4.

**Differential leukocytes count:** The differential count is obtained by counting the number of white cells on the thin leishman stained fixed peripheral blood films. The was done using a chart as follows, this chart has rows. A field is chosen under the microscope, in the 'body' area of the blood film, white cells are counted in the field eg. 6 neutrophils, 3 lymphocytes, 1 monocytes A similar count is done in 9 more fields while filling the above chart according to the different numbers of white cells contained, bringing the total number of cells counted, to 100. The total number of each type of white cell was then taken as the percentage. To obtain the absolute count, these percentages should multiplied by the total white cell count. The same procedures was repeated for five healthy person whom used as control (Daniel, 2011).

**Statistical analysis**: For finding the differences according to different parameter chi-square ($X^2$) test was used for statistical analysis of these samples by statigraphic program.

**Results anddiscussion:**

The results of the study intable1, showed no significant differences of *E. histolytica* and *Giardia lamblia* distribution between the males and females .

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

| **Parasite** | *E. histolytica* | | | *Giardia lamblia* | | |
|---|---|---|---|---|---|---|
| **sex** | **Male** | **Female** | **Total** | **Male** | **Female** | **Total** |
| Examined No. | **105** | **102** | **207** | **105** | **102** | **207** |
| +ve No. | **46** | **38** | **84** | **7** | **8** | **15** |
| % | **43.8** | **37.2** | **40.5** | **6** | **8** | **7.2** |

This study had concluded evaluation the effect of *Entamoeba histolytica* and *Giardia lamblia* parasites on some human hematological parameters,in Al haweeja General Hospital. The incidence of *E. histolytica*(40.5%) in this studywas significantly higher than *G. lamblia*(7.2%). This may due to the greater longevity of cysts in environment conditions.Identical results were found by other authors (Al-Khaysee, & 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 showedno significant differences between the rate of *E. histolytica* in males (43.8%) and females(37.2%), 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 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). Table1also shows the frequency of *G. lamblia* infection in relation to sex, no significant differences was noted between the distribution of the parasite in females (8%) and males (6%).This may be due to same reasons mentioned above.This result is in disagreement with ( Al-Saeed & Issa, 2006) who indicate 14.6 % in males and 35.6% in females.

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

| Parasite | *E. histolytica* | | | | | | | *Giardia lamblia* | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Ages in years | < 1 | 1-10 | 11-20 | 21-30 | 31-40 | 41-50 | total | ≤1-15 | 16-30 | 31-45 | 46-50 | total |
| Examined No. | 22 | 90 | 23 | 36 | 24 | 12 | 207 | 119 | 52 | 26 | 10 | 207 |
| +ve No. | 6 | 54 | 8 | 8 | 6 | 2 | 84 | 9 | 2 | 2 | 1 | 15 |
| % | 27 | 60 | 34 | 22 | 25 | 16 | 40.5 | 8 | 3.8 | 7.6 | 10 | 7.2 |

Regarding age group , the rate of *E. histolytica* was significantly ($p<0.05$) highest in 1-10 years (60%), followed by 11-20 years (34%) and <1 year (27%), while the 41-50 age group showed lower infection rate (16%) table 2. This result is in agreement withHamad 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, 2012).Regardingthe resultsof *G. lamblia* incidence according to age groups, no significant differences were appeared in the parasite distribution in relation to age groups table 2.The ≤ 1 year old group had a low rate (8%) of infection comparing with those of*E. histolytica*. 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 parasitosis in Dyala and Basrha (Al-Khaysee, & Sultan 2008, Shaheen, et. al, 2007).

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

| Parasite | *E. histolytica* | | | | *Giardia lamblia* | | | |
|---|---|---|---|---|---|---|---|---|
| Stages | Trophozoite | Cyst+trophozoite | Cyst | total | Trophozoite | Cyst+trophozoite | Cyst | total |
| +ve No. | 21 | 40 | 23 | 84 | 7 | 6 | 2 | 15 |
| % | 25 | 47.3 | 27.3 | 100 | 46.6 | 40 | 13.3 | 100 |

Table 3 showed that the mixed infection with trophozoite and cystic stages weremost apparent stage in *E. histolytica* infection. These stages were found in 40 positive stool samples (47.6%) followed by cystic stage in 23 samples (27.3%), while the trophozoite stage was found in 21 positive samples (25%). This may because thetrophzoite is responsible for acutecases 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).table 3alsoshows differences between the samples containing trophozoite and Cyst+trophozoitestages of *G. lamblia* comparing with those containing cystic stages alone. The most apparent

stage was the trophozoite (46.6%),followed by cyst+ trophozoite (40%) , while the cystic stage was found in only 13.3% of the positive samples.(Al-Khaysee & Sultan, 2008) found no significant differences between the positive samples containing *G. lamblia* stages.

Table 4 Microscopic finding in relation to parasitic infections

| Parasite | Pus+ | Pus ++ | RBC+ | RBC ++ | Bacteria+ | Bacteria ++ | Bacteria +++ |
|---|---|---|---|---|---|---|---|
| *E. histolytica* t. | 15 | 5 | 10 | 11 | 2 | 1 | 1 |
| *E. histolytica* c. | 4 | 3 | 6 | 5 | 9 | 2 | 1 |
| *E.histolytica* t+c | 17 | 20 | 2 | 36 | 6 | 4 | 2 |
| *G. lamblia* t. | 2 | 3 | 1 | 4 | 1 | 1 | 3 |
| *G. lamblia* c. | 1 | 1 | 0 | 0 | 1 | 1 | 0 |
| *G. lamblia* t+c | 4 | 2 | 2 | 4 | 0 | 2 | 4 |

T =trophozoite, C= cyst

The results in table 4 indicated significant differences between the microscopic finding with the parasites. The most significant effect was for samples containing trophozoite and cystic stages of *E. histolytica*. The highest rate of was for those samples containing pus and RBC cells , followed by those samples having *E. histolytica* trophozoite alone. The lowest was for samples containing *G. lamblia* stages.This is somehow agree with AL-faydawi & Daher,Hegazi whom found the positive samples with *Entamoeba histolytica* allwere diarrheic with blood (AL-faydawi & Daher, 2001,Hegazi, et.al,2013).

Table 5 Normal and abnormal hematological parameters regarding to *E. histolytica* and *G. lamblia* infections.

| Parasite stages | +ve No. | N.WBC | AB.WBC | N.HB | AB.HB | N.PCV | AB.PCV |
|---|---|---|---|---|---|---|---|
| *E .histolytica* t. | 21 | 10 | 11 | 9 | 12 | 8 | 13 |
| *E. histolytica* c. | 23 | 12 | 11 | 9 | 14 | 8 | 15 |
| *E. histolytica* t+c | 40 | 16 | 24 | 12 | 28 | 10 | 30 |
| *G. lamblia* t | 7 | 4 | 3 | 5 | 2 | 5 | 2 |
| *G. lamblia* c | 2 | 1 | 1 | 2 | 0 | 2 | 0 |
| *G. lamblia* t+c | 6 | 2 | 4 | 2 | 4 | 1 | 5 |

T =trophozoite, C= cyst, N=normal, AB=abnormal, HB= Hemoglobin, PCV= Packed Cell Volume.

Table 8 shows the effect of *E. histolytica* and *G. lamblia* infections onhematological parameters. It indicate 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* cyst alone, and the highest rate of abnormality was for PCV levels. The lowest was for samples containing *G. lamblia* stages. 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).

Table 6 Differential WBC count in relation to parasitic infection

| WBC type / Parasite | L% M±S.D | N% M±S.D | M% M±S.D | B% M±S.D | E% M±S.D |
|---|---|---|---|---|---|
| Control | 35±3.8 | 57±7.2 | 3±3.7 | 1±0.6 | 4±0.6 |
| *E. histolytica* c. | 35±0.45 | 56±2.6 | 2±3.31 | 1±0.5 | 6±1.1 |
| *E.histolytica* t. | 35±2.5 | 55±3.3 | 1±3.11 | 2±0.9 | 7±0.98 |
| *E. histolytica* t+c | 38±4.7 | 52±3.4 | 2±3.8 | 1±0.9 | 7±1.0 |
| *G. lamblia* t. | 36±2.3 | 57±2.0 | 1±3.1 | 1±0.5 | 5±1.9 |
| *G. lamblia* c. | 34±1.0 | 57±1.5 | 2±2.1 | 1±0.2 | 6±1.2 |
| *G. lamblia* t+c | 34±3.2 | 56±2.6 | 1±3.9 | 1±0.5 | 8±1.08 |

T =trophozoite, C= cyst, *L=Lymphocyte, N=neutrophil, B=basophil ,M=Monocyte, E=Eosinophil,*

The differential WBC count in relation to parasitic infection (table 6) had significant effect, eosinophil showed high rate with all stages of the two parasitic infections compared with the control. Lymphocyte showed significantly high rate with *E. histolytica* (trophozoite+cyst) compared with the control. Neutrophil, monocyte and basophil showed no significant variations in all cases of infection compared with the control.Eosinophil was elevated in all types of infection comparing with the control, but the highest rate was for those samples infected with the trophozoite+cyst stages for the two parasites. This due to that thelymphocyte andeosinophil are elevated during parasitic infection as immune response against the parasites(Heukelbach, et. al,2006).The role of leucocytosis and eosinophil in parasitic infection have been improved previously (Heukelbach, et.

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