

**The Relationship Between The Infection With *E. histolytica* and
Some Blood Parameters**

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Abstract

Background: Infection with *Entamoeba histolytica* is a major health problem in many tropical and subtropical areas of the world, especially in developing countries. The factors that control the pathogenesis of *E. histolytica* are not completely understood. However, key features are the ability of the organism to lyse host cells and cause tissue destruction, with induced immune responses occurring in invasive disease. Up to now, few reports about blood alterations induced by *E. histolytica* infection have been described.

Objective: The aim of the present study was to evaluate the relationship between the infection with *E. histolytica* and some blood parameters

Patients and methods: A total of 320 male and female patients presented with infected diarrhea to the Central Teaching Hospital for Pediatric and some Medical Centers in Baghdad city – Iraq were examined, during the period from April to October, 2014. The age of patients ranged from 1-50 years. Forty five healthy parasite-free individuals used as a control group. Stool samples were collected from patients for direct microscopic examination, then the blood parameters evaluated were (RBCs, HB, PCV, MCV, MCH, MCHC, PLT, TLC, Neutrophile, Lymphocyte and mixed cells) in the patient's blood, using Sysmex instrument.

Results: In this study 60 patients out of 320 patients confirmed to be positive for *E. histolytica*. The results showed a significant decrease ($P < 0.05$) in RBCs count, level of Hb, PCV, MCV and MCHC in *E. histolytica* infected patients in comparison to healthy control group. In addition, the total leucocyte count and differential type of neutrophil, lymphocyte and mixed cells (monocyte, eosinophile, basiphile) increased significantly ($P < 0.05$) in patients infected with *E. histolytica* in compared to healthy control.

Conclusion: The present study confirmed the existence of a relationship between the infection with *E. histolytica* and changes in blood parameters indices involved in anaemia.

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This may be an evident proof that parasitic infections have an adverse effect on haemoglobin, packed cell volume, and mean cell haemoglobin concentration, and immune response.

Keyword: blood parameters, *Entamoeba histolytica*, parasitic infection

العلاقة بين الخمج بطفيلي اميبا الزحار وبعض المعايير الدموية

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الخلاصة

تمهيد: الإصابة بأميبا الزحار مشكلة صحية كبيرة في العديد من المناطق الاستوائية وشبه الاستوائية من العالم، خصوصاً في الدول النامية، العوامل التي تُسيطر على نشوء المرض لم تُفهم بالكامل. على أية حال، ان قدرة الطفيلي على تحليل خلايا المضيف والتسبب بتدمير الانسجة المصابة وبالتالي حث الاستجابة المناعية تعد الميزات الرئيسية للأمراضية، و لحد الآن هناك القليل من الدراسات حول التغيرات الدموية المستحثة من قبل اميبا الزحار.

الهدف: هدف الدراسة الحالية هو تقييم العلاقة بين الإصابة (الخمج) بطفيلي اميبا الحالة للنسيج وبعض المعايير الدموية

المرضى والطرق: ما مجموعه 320 مريض يُقدّمون إلى المستشفى التعليمي المركزي للأطفال وبعض المراكز طبية في مدينة بغداد – العراق تم فحصهم، أثناء الفترة من نيسان إلى تشرين الأول، 2014. وقد كانوا بعمر من 1 إلى 50 سنة (ذكور وإناث) و 45 حالة صحية خالية من الطفيلي اعتبرت مجموعة سيطرة. تم جمع عينات الخروج من المرضى للقيام بالفحص المجهرى المباشر، كما درست معايير الدم (RBCs, HB, PCV, MCV, MCHC, mixed cells) في دم المرضى باستخدام جهاز Sysmex.

النتائج: في هذه الدراسة وجد 60 مريض من مجموع 320 مصابين بأميبا الزحار و 45 حالة صحية خالية من الطفيلي اعتبرت مجموعة سيطرة اكتشفت بالفحص المجهرى، اظهرت النتائج انخفاض معنوي ($P < 0.05$) في كريات الدم الحمر ومستوى وحجم الكريات المرصوصة ودلائل كريات الدم الحمر المتمثلة بمعدل حجم الكرية MCV وتركيز خضاب الدم داخل الكرية

MCHC. في المرضى المصابين بأميبا الزحار مقارنة بمجموعة السيطرة بينما نتائج عدد كريات الدم البيض

وانواعها (lymphocyte, neutrophil)

(basophile, monocyte eosinophil) اظهرت زيادة معنوية ($P < 0.05$) في المرضى المصابين بأميبا

الزحار مقارنة بمجموعة السيطرة.

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الاستنتاج: أكدت الدراسة الحالية وجود علاقة بين الخمج الطفيلي اميبيا الزحار والتغيرات في معايير الدم مما يؤدي إلى فقر الدم. هذا ربما دليل واضح على ان الاصابة بالطفيلي تؤدي الى تأثير مضاؤ على مستوى وحجم الكريات المرصوصة ودلائل كريات الدم الحمر المتمثلة بمعدل حجم الكرية وحث الاستجابة المناعية.
الكلمات المفتاحية: معايير الدم، اميبيا الزحار، الخمج الطفيلي

Introduction

Intestinal parasitic infections represent a large and serious medical and public health problem in developing countries (1). The infection varied from one area to another depending on the degree of personal and community hygiene, sanitation and climatic factors (2).

In particular, *Entamoeba histolytica* is an intestinal protozoan parasite; it is the third leading cause of death in humans after malaria and schistosomiasis (3).

The World Health Organization (WHO) estimates that the *E. histolytica* is a major cause of morbidity worldwide, causing approximately 50 million cases of dysentery and 100,000 deaths annually (4). It is distributed worldwide and represents a serious health threat in tropical and subtropical developing regions, as well as in the developed country (3). The high prevalence of infection is due to fecal contamination of food and water supply (5).

In Iraq, reports showed a different incidence levels of *E. histolytica* infection ranges from (2.92%) to (41.25%) (6, 7).

Entamoeba histolytica, the etiologic agent of amoebic colitis and liver abscess, causes human infections on a global scale, resulting in significant human suffering (5). This corresponds to invasion of the intestine when active trophozoites invade and penetrate the intestinal muscular wall and simultaneously feed on red blood cells. Amoebic dysentery ensues when trophozoites continue to erode the intestinal epithelium, leading to the formation of flask-shaped ulcers (8). Thus, amoebae with ingested erythrocytes, although seen rarely are a key diagnostic feature of *E. histolytica* when found in the stool of a person experiencing amoebic dysentery. The onset of these symptoms occurs over a period of several weeks (8). Low birth-weight, low productivity in adulthood, stunted growth, low hemoglobin concentration, chronic loss of blood and iron are related to parasite infection (9). Besides disrupting growth, iron deficiency increases susceptibility to infections, interferes with mental

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activity leading to apathy, irritability and lowers powers of concentration as well as a reduced learning capacity (10).

The relationship between parasitic infection and anemia is a pathogeno-physiologic type. It is recognized that certain factors play important roles, and include: the strain and number of the parasite, the size and site, metabolic processes of the parasite, age and level of immunity at the time of infection, immune responses to the infection, presence of co-existing diseases or conditions which reduce immune responses, malnutrition, and the life style of the infected person (11).

Relatively, few studies have been done on the hematological changes in patients with intestinal protozoa, therefore the aim of the present study was to evaluate some hematological parameters, including blood parameters as well as the WBCs count and differential types changes in patients with *E. histolytica* and compared it with data obtained from a sex and age-matched parasite-free control group.

Materials and Methods

Patient selection:

A total of 320 male and female patients with diarrhea visited the Central Teaching Hospital for Pediatric and some Medical Centers in Baghdad city – Iraq were selected, during the period from April to October, 2014. The age of patients ranged from 1 - 50 years and 45 healthy, sex and age- matched parasite-free consider to be a control group

1-Stool Examinations

Stool samples collection:

Stool samples from each patient were collected in a clean, dry, tight fit cover containers and examined within half an hour in parasitology lab. The samples were examined for the presence of *E. histolytica* .

Stool samples Examinations

1-Macroscopical Examination

It was performed by observing grossly the consistency of stool samples, presence of blood, mucus and other substances.

2- Microscopical Examination

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Direct Method

From each stool samples, smears with normal saline and lugols iodine were examined. Two direct smears were examined from each fecal sample, by preparing two clean dry microscope slides, one with normal saline and the other with lugols iodine solutions. By using clean wood stick, the stool specimen was touched in different sites, especially where streaks of blood or pus were noticed, then mixed thoroughly with each drop of normal saline and lugols iodine solutions on the prepared slides, then each slide was covered with a cover slip. The smear was examined thoroughly under the low (x10) and high (x40) powers of the microscope .

Blood Examinations

Blood Samples Collection:

Blood samples were drawn from the 60 patients that infected with *E. histolytica* out of the 320 patients and 45 healthy parasite free as control, by using disposable syringes 2ml. one ml of the collected blood was put in EDTA tube for blood parameters and WBCs count and differential by using Sysmex instrument, America.

Procedure

Ten μ l of blood sample was placed in the aspirator of the instrument and the blood sample was aspirated.

Statistical analysis: Statistical Analysis System- (SAS, 2010) was used to evaluate the effect of different parameters in this study. Chi-Square test was used to significant compare between different groups (patient and healthy control) (12).

Results

In this study, 60 out of 320 patients (18,75%) were confirmed to be positive for *E. histolytica*. Forty five healthy sex- and age- matched parasite-free persons were used as a control group.

Blood parameters

The statistical analysis of the results has shown a significant decrease ($P < 0.05$) in a count of red blood corpuscular, hemoglobin concentration, packed cell volume, mean corpuscular volume and mean corpuscular hemoglobin concentration and a significant increase ($P < 0.05$) in mean corpuscular hemoglobin .While the platelets count was non- significant ($P > 0.05$) in patients with *E. histolytica* infection compared to the healthy control group, (Table 1).

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Table (1): Comparison between patient infected with *Entamoeba histolytica* and healthy control regarding blood parameters

Parameter	Patients N=60	Healthy Control N=45
RBCs X10 ⁶ /mm ³	*3.423 ± 0.041	4.352± 0.099
Hb g/dL of blood	*8.124 ± 0.124	12.543±0.772
PCV (%)	*31.942 ± 0.798	36.223±0.358
MCV (mm ³)	*72.054 ± 0.689	80.332±0.789
MCH(pg) /cell	*24.548 ± 0.301	22.389±0.235
MCHC (g/dLof RBCs)	*23.082 ± 0.345	27.356±5.254
PLT X10 ³ /mm ³	324.520±7.123	324.482±7.145

* Significant difference (P<0.05) between patients and control group .

Differential Leukocyte count

The result of total leucocyte count and differential type of neutrophil, lymphocyte and mixed cells (monocyte, eosinophile, basiphile) showed a significant increase (P < 0.05) in patients infected with *E. histolytica* in comparison to healthy control group (Table 2).

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**Table (2): Compare between patient infected with *E.histolytica* and healthy control in
WBCs count and differential**

Parameter	Patients N=60	Healthy Control N=45
TLC (X103/ mm ³):	*9.178 ± 0.213	6.321±0.568
Neutrophile	*55.589 ± 0.189	50.748±0.423
Lymphocyte	*25.638 ± 0.154	20.587±0.048
Mixed cells(Monocyte, Eosinophile, Basiophile)	*9.852 ± 0.085	6.555±0.052

* Significant difference (P<0.05) between patients and control group .

Discussions

Infection with *E. histolytica* is a major health problem in many tropical and subtropical areas of the world, especially in developing countries (13).The factors that control the pathogenesis of *E. histolytica* are not completely understood. However, key features are the ability of the organism to lyse host cells and cause tissue destruction, with induced immune responses occurring in invasive disease (14).

The present results have revealed a significant decrease in RBCs count, concentration of Hb and PCV in patients with *E. histolytica* infection compared to healthy control group. This result may be due to the fact that this parasite causes digestive disturbance, also release the trophozoite motile feeding stage which adheres to villi of intestine and suck the chime from villi (15) and secretes proteolytic enzymes that dissolve host tissues and host cells and engulfs RBCs (10

In addition, the infection with *E. histolytica* leads to necrosis of intestinal mucosa causing damage and degeneration of absorption sites of necessary substances, beside the bleeding associated with this process. The infection with intestinal parasites has an important effect on values of blood (16). For these, the intestinal parasites are strongly associated with

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the development of anemia as they cause malabsorption, nutritional deficiencies and gastrointestinal blood loss (17).

The results have shown a decrease in MCV and MCHC in patients infected with *E. histolytica* when compared to healthy control group. A decrease in MCV may be due to a decrease in Hb inside RBCs caused by *E. histolytica* infection; decrease in MCHC is caused by iron deficiency anemia that lead to decrease in formation of Hb in RBCs . In contrast, the platelets count did not show a significant increase in the individuals infected with *E. histolytica* when compared with the healthy individuals in the control group. This may suggest that *E. histolytica* activates platelets, and the degree of their activation determines their morphologic parameters (18).

The data of this study showed a significant increase in WBCs and this may be explained by the increase in the number of neutrophil, lymphocyte and mixed cells (monocyte, eosinophil, basophile). Because the infection with the pathogenic *E. histolytica* produces a marked immune response which results in the development of protective immunity (19). The role of leukocytosis and eosinophil in parasitic infection has been proved (20).

The decreasing level of Hb which is associated with increasing the number of eosinophils and total W.B.Cs numbers related to the ability of *E. histolytica* to alter blood constituents. This result of high density of this protozoan that leads to mal- absorption of necessary nutrients for the formation of blood components (16). In human intestinal epithelial cell inflammatory response, the literatures reflect the role of eosinophil in the pathogenesis and protective immunity against protozoan parasites.

Conclusion

The present study confirmed the existence of a relationship between the infection with *E. histolytica* and changes in haematological parameters indices that lead to anemia. This is perhaps an evident proof that the parasitic infection have an adverse effect on hemoglobin, packed cell volume, mean cell hemoglobin concentration and immune response.

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