Ministry of Higher Education and Scientific Research Middle Technical University Technical Institute/Kut



Epidemiology and Clinical Features of

Entamoeba histolytica

A Search

Submitted to the Board of the Department of Medical Laboratories Part of the Requirements for obtaining a Technical Diploma Certificate

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1445 A.H

2024 A.C



بِسْم الله الرّحْمنِ الرَّحِيمِ

يرفع الله الذين امنوا منكم والذين اوتوا العلم درجات والله بما تعملون خبير

صدَقَ الله العَلي العَظِيم سورة المجادلة الآية الحادية عشر



اقرار المشرف

اشهد ان اعداد هذا البحث الموسوم:

Epidemiology and Clinical Features of Entamoeba histolytica

الذي تقدم به طلبة الدبلوم التقنى كل من:

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قد جرى تحت اشرافي في قسم تقنيات المختبرات الطبية / المعهد التقني كوت كجزء من متطلبات نيل درجة الدبلوم التقني في تقنيات المختبرات الطبية وعليه ارشحه للمناقشة

المشرف م.م حيدر عبد الله كاظم 2024/4/28

اقرار لجنة المناقشة

نحن اعضاء لجنة المناقشة نشهد باننا اطلعنا على البحث الموسوم

Epidemiology and Clinical Features of Entamoeba histolytica

المقدم من الطلبة:

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والمقدم الى مجلس قسم تقنيات المختبرات الطبية / المعهد التقني كوت.

وقد ناقشنا الطلبة في محتوياته وفي ما له علاقة به. ونقر بانه جدير بالقبول كجزء من متطلبات نيل درجة الدبلوم التقني في تقنيات المختبرات الطبية.

اعضاء اللجنة العلمية

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رنيس القسم

عضوا نام ر عبر اللم لعقد حالى عضوا

رليس اللجنة

Dedication

To my parents more than anyone else Who are responsible for what I have become Dearest brothers and sisters

with loyalty....

Abstract

Entamoeba histolytica (E. histolytica) is a unicellular, protozoon parasite of humans. Infection with *E. histolytica* may be the cause of a variety of symptoms, beginning from no symptoms to severe intestinal and extraintestinal diseases. Amoebiasis is thought to be one of the most commonly known parasitic diseases affecting millions of people worldwide. Transmission is often associated with contaminated food and water. Laboratory diagnosis of the parasites is usually based on microscopy and serological methods.

This study performed on (381) patients from different age groups and from both genders who attended to parasitology lab of Al karama teaching hospital in wasit province where only (15 %) of this patients were diagnosis as *E. histolytica* positive infection. The descriptive data showed that (58 %) of *E. histolytica* infection in males and (42 %) in females. Moreover, the results demonstrated that the incidence of infection increased in age group (50 \leq) years (27%) and decreased in age group (40-49) with percentage of (9 %).

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Chapter One Introduction

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1.1. Introduction

Entamoeba histolytica (*E. histolytica*) is a unicellular, protozoon parasite of humans. It moves by a jelly-like tongue-like protrusion of the cytoplasm "pseudopodium." Infection with *E. histolytica* may be the cause of a variety of symptoms, beginning from no symptoms to severe fulminating intestinal and/or life threatening extraintestinal disease ⁽¹⁾.

There are at least six species of the genus Entamoeba that can be identified in human stools. These are *E. histolytica*, *E. dispar*, *E. mshkoveskii*, *E. hartmanii*, *E. polecki, and E. coli. Entamoeba histolytica* is the only species associated with disease⁽²⁾.

The rate of infection by this parasite differs among countries based on socioeconomic status, sanitary conditions and population. Its endemicity is high in poor and socio-economically deprived communities in the tropics and subtropics. The transmission and distribution of the infection is enhanced by environmental, socioeconomic, demographic, and hygiene-related behaviour among individuals. Transmission is often associated with contaminated food and water ⁽³⁾.

Clinical features of amoebiasis ranges from asymptomatic colonization to amoebic dysentery and invasive extraintestinal amoebiasis which is manifested most commonly in the form of liver abscess. The intestinal symptoms can range from mild diarrhea to dysentery with blood and mucus in stool. Laboratory diagnosis of the parasites is usually based on microscopy and serological methods including enzyme linked immunosorbent assay (ELISA)⁽⁴⁾.

1.2 Aims of the study:

To study the epidemiology, diagnosis and treatment of *Entamoeba histolytica* and some specific aspects of its pathogenesis.

Chapter Two

Literature Review

2.1 Epidemiology

Amebiasis is responsible for around 100,000 deaths/year, mainly in Central and South America, Africa, and India, as well as for a significant rate of morbidity manifested as invasive intestinal or extraintestinal disease. Infection with *E. histolytica* has been estimated to be as high as 50% in some developing countries as South and Central America, Africa and Asia ⁽⁵⁾.

Factors as poverty, low socio-economic standards including bad sanitation, improper water supply, and overcrowding contribute positively to the increased rates of transmission of the parasite and disease ⁽⁴⁾.

2.2 Morphology and Biology

Entamoeba histolytica occurs in the following forms, the trophozoites, precyst, cyst, metacyst, and metacystic trophozoite. The trophozoite is about 10–60 μ m in size with a clear finely granular cytoplasm and a spherical nucleus that shows aggregation of chromatin beads at the nuclear membrane and a centrally located karyosome. The trophozoite is the active stage that moves, feeds, and divides ⁽⁶⁾.

Amoebae are anaerobic and have no mitochondria. The pathogenic amoeba may be haematophagous, with ingested RBCs in its cytoplasm. The nonpathogenic ameba feeds on bacterial microbiota and intestinal contents ⁽⁶⁾.

In fresh-stool examined under the microscope, the trophozoite moves actively by a fingerlike protrusion of the ectoplasm "pseudopodium," into which the cytoplasm is pulled moving the whole body of the organism in its direction. In order to encyst, the trophozoites rounds up, discharges the undigested food, and becomes a precyst. The precyst contains collections of cigarshaped ribosomes, called chromatoid bodies, as well as a glycogen food vacuole. All are extruded as the cell shrinks to become a mature cyst. In the process of becoming tetranucleated, the nucleus of the cyst undergoes two mitotic divisions. Chromatoid bodies and glycogen vacuoles cannot be seen at this stage and the mature cyst has an average size of around 12-13 µm. Encystation never occurs outside the body or in the tissue ⁽⁷⁾.

Cysts pass in human feces and can remain viable in damp soil for up to 8 days and in water for 9 to 90 days according to temperature. Mature cyst is the infective stage and infection occurs by ingestion of these cysts in contaminated water or food ⁽⁸⁾.



Figure 1: Life cycle of *E. histolytica* ⁽⁷⁾.



Figure 2: Mature *Entamoeba histolytica* Cyst (Mature Cysts have 4 Nuclei) ⁽⁵⁾.



Figure 3: Trophozoites of *Entamoeba histolytica* with Ingested Erythrocytes ⁽⁸⁾.

2.3 Pathophysiology

Amoebiasis is thought to be one of the most commonly known parasitic diseases affecting millions of people worldwide. The combination of intestinal symptoms with the microscopic finding of haematophagous forms of amebae in feces is an indication of an attack of the intestinal epithelium by pathogenic amebae. The amebae use their lectins to adhere to the cell surface sugars "galactose and N-acetylgalactosamine" and a 260-kd surface protein that contains 170 and 35 kD subunits. Immunoglobulin A (IgA) against amebic lectin may result in recurrence of attacks. Amebae begin to secrete their lytic enzymes including pore-forming proteins, lipases and the cysteine proteases, which initiate a process of cell necrosis and apoptosis in the affected colonic epithelial cells. Immune cells become attracted to the damaged colonic cells and become destroyed by the lytic enzymes into the surrounding tissue, initiating an ulcer in the affected area. Amebic ulcer in the colonic epithelium is typically flask shaped ⁽⁹⁾.

In case trophozoites reach a blood vessel during tissue destruction, blood will appear in stool causing the typical amebic dysentery. Some trophozoites can pass into the portal vessels to reach the liver, evading the complement-mediated lysis in blood, where they can produce similar pathological effect in the liver cells ending in a typical abscess ⁽¹⁰⁾.

Blood route or direct spread by rupture may carry *E. histolytica* trophozoites to other organs in the body other than the liver as the lungs causing pleuropulmonary disease, peritoneum causing peritonitis, pericardium leading to pericarditis, to the brain causing a brain abscess and/or may spread to the genital and urinary system causing genitourinary infection $^{(10)}$.

2.4 Clinical Picture

Infection may be asymptomatic in most of the cases; however, severe fulminating disease may occur after an incubation period of 7–28 days from exposure to infection. Mild symptoms may be in the form of abdominal cramps, diarrhea with passage of 3–8 soft stools/day, or passage of stool with mucus and occasional blood. There may be fatigue, excessive gasses, rectal pain "tenesmus," and weight loss ⁽¹¹⁾.

In heavy infection, the patient may show, fever, abdominal tenderness, vomiting and bloody diarrhea of about 10–20 motions/day. Cases with amebic liver abscess usually show sub-acute onset with fever and pain in the right upper quadrant of the abdomen. Diarrhea may be an associated symptom in 30% and Jaundice in about 10% of cases. There may be previous history of dysentery few months before ⁽¹²⁾.

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Diagnosis needs the combination of clinical, radiological and immunological methods. The liver abscess is usually solitary and mostly in the right lobe of the liver with high titer of antibodies against *E. histolytica*, detected in more than 99% of cases. The aspirated pus is thick, brownish, and typically called "anchovy sauce" ⁽¹¹⁾.

Other site for extraintestinal spread of *E. histolytica* includes the pulmonary amoebiasis. Infection may reach the pleural cavity and lungs by the haematogenous route or after perforation of a hepatic abscess through the diaphragm. Brain abscess may also develop via the heamatogenous route, while the cutaneous infection can occur at site of aspiration of a liver abscess ⁽¹³⁾.

Ameboma It is one of the rare complications of colonic infection with pathogenic *E*. *histolytica*. It occurs as a result of deep invasion of the wall of the colon by invasive *E*. *histolytica* trophozoites with extensive formation of granulation tissue and the development of tumor-like mass. The most common sites of ameboma are the cecum and ascending colon. It is manifested by intestinal obstruction and bleeding $^{(13)}$.

2.5 Laboratory Diagnosis

2.5.1 Microscopy Method

Identification of haematophagous trophozoites in fresh stool smears is the primary step for the identification of intestinal amebiasis and is an indication of infection. Because of irregular output of the parasite stages in stool, a single stool analysis is not usually positive. To further improve the sensitivity of the microscopic examination, it is indicated to examine two or three stool specimens on different days, or to do microscopic examination of the second motion after a saline purge. Microscopy is also used to examine aspirates from liver or lung abscess and the parasite can only be visualized in about 20% of cases ⁽¹⁴⁾.

2.5.2 Culture Method

Culture is a method of diagnosis, however it is not easy to perform and less sensitive than microscopic examination with a success rate of 50–70%. The technique is considered very sophisticated and is used mainly for research studies ⁽¹⁵⁾.

2.5.3 Antigen and Antibody Detection

Detection of coproantigen of *E. histolytica* by ELISA is a sensitive and specific test that depends on the use of monoclonal antibodies kits. It is considered more sensitive than light microscopy for detection of infection with *E. histolytica* ⁽¹⁶⁾.

Antibodies are detected in 99% of cases with amoebic liver abscess. ELISA, the most commonly used worldwide, is a sensitive and specific test that can differentiate *E. histolytica* from *E. dispar*. False negative results can occur within the first 7–10 days after infection ⁽¹⁶⁾.

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2.5.4 Molecular Techniques

The identification of *E. histolytica* is now using a wide variety of PCR-based assays. Some PCR techniques can successfully and simultaneously differentiate all the Entamoeba spp. from fecal samples in one test ⁽¹⁵⁾.

2.6 Treatment

Metronidazole is recommended by the WHO as the drug of choice for treatment of cases with amebiasis. Metronidazole has an antimicrobial effect against the anaerobic bacteria and protozoa as *E. histolytica* ⁽¹⁷⁾.</sup>

Tinidazole has been approved by the US Food and Drug Administration (FDA), for treatment of both intestinal and extraintestinal amebiasis. It is more effective in comparison with metronidazole with less dosage, less duration of intake and less adverse effects ⁽¹⁷⁾.

2.7 Control

Avoid the poor personal hygienic practices, such as neglecting washing hands before eating, especially after working with the soil. It is also of great importance to wash raw vegetables before using in salad. Food handlers should be inspected regularly and those infected should be prevented from food handling until proved non-infective. Treatment of the infected family members and health education for proper sanitary conditions are of prompt importance ⁽¹⁸⁾.

Chapter Three

Patients and Methods

Patients and Methods

3.1. Patients

This study performed on (381) patients from different age groups and from both male and female who attended to the parasitology lab of Al karama teaching hospital in wasit province for examination and diagnosis of *E. histolytica* infection where only about (15 %) of this patients were diagnosis as *E. histolytica* positive infection.

3.2. Study Groups

The study groups were divided as the following:

3.2.1. Age groups:

The age distribution divided into six groups as the following:

- 1. **First group:** patients age were (10 <) years.
- 2. Second group: patient age range between (10-19) years.
- 3. Third group: patient age range between (20-29) years.
- 4. Fourth group: patient age range between (30-39) years.
- 5. **Fifth group:** patient age range between (40-49) years.
- 6. **Fifth group:** patient age range were $(50 \ge)$ years.

3.2.2. Gender Group:

The gender group divided into two groups as the following:

- 1. Male group: include all males from different age distribution.
- 2. **Female group:** include all females from different age distribution.

3.2. Methods:

3.2.1. Stool Examination:

All cases in this study were examined and diagnosis by using stool examination method for *E. histolytica* infection.

3.2.2. Data Collection Form:

العمر	الجنس	Male	عدد	العدد الكلي	عندان وتأريخ الصفحة	رقم
		Female	المصابين	للحالات	طوران وتاريخ الصلك	الصفحة
						1
						•
						2
						3
						1
						-+
						F
						Э



Results



The results of the present study showed that only (15 %) of patients that precipitate in this study were infected by *E.histolytica* as shown in figure (1).

Figure (1): Percentage of infected patients in this study.

4.1. Gender distribution of *E.histolytica* infection

By comparing the infection by *E.histolytica* between male and female groups as in figure (2) the results shown that the percentage of infected males was (58%), while the percentage of female group was (42 %).



Figure (2): Gender distribution of *E.histolytica* infection.

4.2. Age distribution of *E.histolytica* infection

The result of age distribution of *E.histolytica* infection shown that the highest percentage of infection was in age group of $(50 \le)$ years with (27%) and the percentage of infection in age group of (29-29) years was (20%) and the percentage in age group (10 >) was (17%), while the lower percentage of infection was in groups of (40-49) years with percentage of (9%) as shown in figure (3).



Figure (3): Age distribution of *E.histolytica* infection.

The results in figure (4) shown that the highest percentage of *E.histolytica* infection in males were in age group of $(50 \le)$ years with percentage (26 %), however, the lower percentage was in age group (40-49) and (10-19) years with (9 %).



Figure (4): Male age distribution of *E.histolytica* infection.

According to the results in figure (5) which shown the female age distribution, the highest percentage of infection in females in age groups of (50 \leq) with percentage of (28 %), while the lower percentage of infection in females were in groups of (10 >) and (40-49) with percentage of (8 %).



Figure (5): Female age distribution of *E.histolytica* infection.

Chapter Five DISSCUSION

Discussion

Regarding gender distribution, the data of this study revealed that male patients infected with the parasite were higher than females with 58% and 42% respectively. These data are in consistent with Hamza, *et.al* who recorded infection of *E. histolytica* was higher in males (55.2%) than in females (44.8%), and there was no correlation between genders (P> 0.05) (19). The results of the present study are contrast to Al-Damerchi *et al.*, who reported elevated infection rate of amoebiasis in females rather than males (19,16).

In terms of age distribution, the result generated in this research found that the highest infection rate was in age group of $(50 \le)$ years while in children was in children below 10 years, these data is in agreement with Hamza, *et.al* data that recorded similar infection rate in patients below 5 years (20).

Added to this, Ngui *e al.*, study illustrated that infection rates by age categories, it was found that adults (23.9%) had higher rates than children (15.3%) (20,18). The data confliction indicated the effect imposed by this parasite on children below five as they are more vulnerable to infections due to the fact that their habits of manipulating things around them may increase the risk of acquiring the infective form more readily.



Conclusions

Amebiasis continues to be one of the most important health problems in tropical and subtropical countries. It occurs as a result of infection with the pathogenic strain of *E. histolytica*. Infection may be asymptomatic or may be fulminating with intestinal and extraintestinal tissue invasion. The prevalence of amebiasis all over the world has dropped significantly after improvement of the diagnostic methods that could differentiate *E. histolytica* from the other nonpathogenic strains. Cases could be treated successfully with metronidazole or tinidazole. Travellers are at risk of catching infection in exotic countries due to lack of proper health facilities.

Recommendations

The following suggestions are recommended:

- 1. Travelers to endemic areas should take precautions regarding food and water, such as drinking only bottled or boiled water and avoiding fresh products that has already been peeled or washed with local water.
- 2. Contacts of infected individuals should practice good hand hygiene.
- 3. There is no available vaccine at this time to protect against *E. histolytica* infection. Development of this vaccine is still a work in progress.



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الخلاصة

الاميبيا الحالة للنسج هو طفيلي اولي وحيدة الخلية. قد تكون الإصابة بالاميبيا الحالة للنسج سببًا لمجموعة متنوعة من الأعراض ، بدءًا من عدم وجود أعراض إلى الأمراض المعوية الشديدة والامراض غير المعوية. يُعتقد أن داء الأميبات هو واحد من أكثر الأمراض الطفيلية المعروفة التي تؤثر على ملايين الأشخاص في جميع أنحاء العالم. غالبًا ما يرتبط الانتقال بالأغذية والمياه الملوثة. عادة ما يعتمد التشخيص المختبري للطفيليات على الفحص المجهري والطرق المصلية.

اجريت هذه الدراسة على (381) مريضا من مختلف الفئات العمرية ومن كلا الجنسين الذين حضروا الى مختبر الطفيليات في مستشفى الكرامة التعليمي في محافظة واسط حيث تم تشخيص (15%) فقط من هؤلاء المرضى على انهم مصابون بعدوى الاميبيا الحالة للنسج. اظهرت البيانات الوصفية ان (58%) من حالات الاصابة بالاميبيا الحالة للنسج في الذكور و (26%) في الاناث. كما اظهرت النتائج ان نسبة حدوث الاصابة ارتفعت في الفئة العمرية (20%).



وزارة التعليم العالي والبحث العلمي الجامعة التقنية الوسطى المعهد التقني في الكوت

الوبائية والصفات السريرية للأميبيا الحالة للنسج

بحث مقدم الى قسم تقنيات المختبرات الطبية- المعهد التقني/ كوت وهي جزء من متطلبات نيل شهادة الدبلوم في التقنيات المختبرية الطبية

من قبل الطلبة

فاطمة حيدر فاضل

فاطمة عدنان هادي

فاطمة على منعم

فاطمة رشيد عزيز

فاطمة الزهراء ادريس

فاطمة نعيم كاظم

كاظم حامد كاظم

بإشراف الاستاذ

م.م حيدر عبد الله كاظم

2024م

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