Kirkuk University Journal /Scientific Studies (KUJSS)
Volume 10, Issue 3, September 2015 , p.p(14-27)
ISSN 1992 - 0849

# Prevalence of Entameba histolytica and Entameba dispar among the immune suppressed patients (cancer patients ) after receiving chemotherapy treatment in Azadi Teaching Hospital

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**Received date: 22 / 6 / 2014** **Accepted date: 8 / 3 / 2015**

## ABSTRACT

***Amebiasis caused by Entamoeba histolytica is a third leading causes of death in worldwide. Little is known about its occurrence of parasite in subjects with cancer patients after receiving chemotherapy. This study aimed to determine, the frequency of E. histolytica in cancer patient admitted to oncology department in Kirkuk Teaching Hospital . Fresh stool specimens collected from 93 patients, their age group ranged from 1-90 years during Febrauary -2013to Feb 2014. E. histolytica /E.dispar were determined in 10/93 (10.7%) by microscopic examination while 33/ 93 (35.5%) detected by the E. histolytica / E. dispar olyclonal ElLISA tests. E. histolytica/ E. dispar infection were higher in patients with hematogenous cancer (64.4%) as compare with solid tumor (30.8%) .***

***Keywords:*** *E. histolytica , E. dispar , Cancer, diarrhea and abdominal disorder.*

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Volume 10, Issue 3, September 2015 , p.p(14-27)
ISSN 1992 - 0849

# الاصابات اميبا الحالة للنسيج واميبا المتحولة في مرضى مثبطين مناعيا (مرضى السرطان) بعد أخذهم علاج الكيمياوي في مستشفى ازادي التعليمي

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تاريخ استلام البحث: 22 / 6 / 2014 تاريخ قبول البحث: 8 /3/ 2015

## الملخص

**داء الأميبات الناجمة عن المتحولة الحالة للنسج هو مسبب الثالث للوفيات في العالم. لا يعرف الكثير عن وقوعه في مرضى السرطان بعد بروتوكول دورة العلاج الكيميائي للمرضى السرطان . تهدف هذه الدراسة إلى تحديد نسبة الإصابة اميبا الحالة للنسيج في مرضى السرطان الذين أدخلوا قسم الأورام لمستشفى كركوك التعليمي. تم أخذ 93عينة براز من مرضى السرطان، تراوحت أعمارهم بين 1–10 إلى 81–90 من الفترة شباط –2013لغاية شباط 2014. تم تحديد أميبا حال النسيج في 10 (10.7٪) من خلال الفحص ألمجهري في حين أن 33 (35.5٪) شخصت ا اميبا حالة للنسيج /واميبا المتحولة بالكت اليزا. أظهرت الدراسة انتشار أعلى 64.4%في السرطانات الدموية مقارنة بالسرطانات ت الصلبة.**

**الكلمات الدالة :** اميبا الحالة للنسيج، اميبا المتغيرة، السرطان، الإسهال و الاضطرابات المعوية .

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## 1.INTRODUCTION

The fecal-oral spread protozoan parasite *E. histolytica* is an important human pathogen. Normally, this parasite resides and multiplies in the large bowel and can persist there for months and years causing only an asymptomatic luminal gut infection .However, occasionally *E. histolytica* penetrates the intestinal mucosa, which leads to ulcerative colitis or it disseminates to other organs, most commonly to the liver, where It induces abscess formation. Cysteine peptidases are considered to play a major role for the pathogenicity of *E. histolytica* as suggested by Maccarlane & Singh 2006 [1]. The genus Entamoeba contain the many species including *E. histolytica, E. dispar, Entamoeba moshkovski Entamoeba polecki, E. coli and E. hartmanni* reside in the human intestinal lumen. *E. histolytica* is the only species definitely associated with pathological sequelae in humans; the others are considered nonpathogenic [2,3]. Although recent studies highlight the recovery of *E. dispar and E. moshkovskii* from patients with gastrointestinal symptom [4].

There is still no definitive evidence of a causal link between the presence of these two species and the symptoms of the host [5]. Clinical features of amebiasis range from asymptomatic colonization to amebic dysentery and invasive extra intestinal amebiasis, which is manifested most commonly in the form of liver abscess . Approximately 50 million peoples have invasive Amoeba , resulting in 100,000 deaths per year Although the parasite has a worldwide distribution, recorded high prevalence rates of more than 10% of the population have been reported from various developing countries [6]. *E. histolytica*- related diarrheal illnesses have recently been reported to have a negative impact on the growth of children Despite the availability of effective therapy, morbidity and mortality associated with amebic infection have persisted, suggesting that interventions designed to limit or to eliminate disease are ineffective[7]..

As humans appear to be the only host, an appropriate control program could potentially eradicate amebiasis. New approaches to the identification of *E. histolytica* are based on detection *of E. histolytica* specific antigen and DNA in stool and other clinical samples. Several molecular diagnostic tests, including conventional and real-time PCR, have been developed for the detection and differentiation of *E. histolytica, E. dispar*, and *E. moshkovskii* in clinical samples.

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These molecular methods have led to a reevaluation of the epidemiology of amebiasis in terms of prevalence and morbidity, particularly in those geographical areas with high endemic rates [8].

## 2.MATERIAL AND METHODS

A total of 93 stool samples were collected from patients complaining from malignant cancer with abdominal disorder (abdominal pain and diarrhea ) were used in the present study. The samples were collected from patients with cancer during their attendance to Oncology Department at AL-Azadi Teaching Hospital from period February 2013 to February 2014. Patients age were ranged from 1-10 to 81-90 years .

### 2.1 Sample collection:-

#### 2.1.1.Stool collection:-

A fresh stool sample was collected from each patient using disposable plastic container .Then examined using double wet preparation as see with light microscopy using. Small amount (0.5 ml - 3 ml) of stool specimens were stored in sterile screw cap containers and kept at -20°C using deep freez until being examined by ELISA.

### Examination of stool specimens

### Direct (Wet Mount) Examination

Stool samples were examined by wet mount preparation to detect the trophozoites and/or cysts of *E. histolytica / E. dispar*. Two slides were prepared for each sample, using a clean grease free slides, a small drop of normal saline was placed on the slide and mixed with a small pea size after well mixing of the sample using a wooden stick, a clean cover slid then placed and the specimen examined using light microscopy under low and high power magnification. The identification of the parasite was done by direct wet mount using normal saline 0.9 %, buffered methylene blue, and lugol's iodine 1 % (WHO, 1991)( ).

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**ELISA FOR E. HISTOLYTICA / E. DISPAR STOOL ANTIGEN[9].**

**(Diagnostic Automation Co. Germany)**

The Diagnostic Automation Inc. ELISA stool antigen assay was performed on 93 of stool samples from patients with cancer and 10 control stool specimens (microscopy negative for *E. histolytica / E. dispar* trophozoite and/or cyst) and the test performed according to manufacturing company.

## 3.RESULTS

The result presented in this study were based on the analysis of 93 patients with malignant carcinoma , receiving course of chemotherapy and complaining from abdominal disorder compared with 10 apparently healthy individual considered as a control, Table (1). The rate of *E. histolytica* infection in cancer patients was 35.5%(33/93 patients). Thus more than 1/3 of patients with cancer are prone to develop infection with *E. histolytica / E. dispar*.

Solid cancer form 21.5%(20/33patients) of the infected patients with *E .histolytica / E. dispar* while hematogenous cancer form 13.9%(13/33 patients. The frequency of infection was with not significant (X2=0.01, P≥0.05) difference between solid cancer and hematogenous *cancer*, Table (2).

Microscopical wet mount show positivity rate of 10.75% (10/93) for *E. histolytica / E. dispar* . The trophozoite stage was only found in one patient 1(10%) with positive Table (3)

Table (4) showing frequency of Amoebiasis among patients with cancer and high rate 20.4% was recorded among patients aged from 41 to 50 years followed by 17.2% among those aged from 51-60 years .The male / female ratio was 1.1:1; ( 49/44). The high frequency rate patients was in male 52.6% (49/ 93 cases). The age group frequency distribution in regard to gender was with high significant differences for both gender (X2= 14.814, P= 0.004). In addition, Cancer patients were predominant in age group of (51-60) year (14/44; 31.8) of male, while in female patients the predominant cases were in the age group of (41-50) and (61-70)year, Table (5).

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**Table (1):** Number and percentages of cancer patients (infected and non-infected) with *E. histolytica* patients and control groups

| Study groups | Number | Percentage |
|---|---|---|
| Cancer patients infected with *E. histolytica /E. dispar* | 33 | 35.5% |
| Cancer patients not infected with *E. histolytica/E. dispar* | 60 | 64.5% |
| Total | 93 | 100% |
| Control group | 10 | |

**Table (2):** patients distribution according to type of cancer

| Patient group | Type of cancer | |
|---|---|---|
| | Solid cancer No(%) | Hematogenus cancer No(%) |
| Cancer patients infected with *E. histolytica/E. dispar* | 20(30.8%) | 13(46.4%) |
| Cancer patients non infected with *E .histolytica/E. dispar* | 45(69.2% | 15(53.6%) |
| **Total** | 65(69.9%) | 28(30.1%) |

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**Table (3):** Frequency of *E. histolytica / E. dispar* stages in positive cases using Microscopy.

| Stages | No. positive<br>Total | Positive % |
|---|---|---|
| **Trophozoite** | 1/10 | 10 % |
| **Cyst** | 9/10 | 90 % |
| **Total** | 10/10 | 100 % |

**Table (4):** Age Distribution among patients group.

| Age groups | Female (%) | Male (%) | Total% |
|---|---|---|---|
| **(1-10)year** | 2(4.5%) | 4(8.16%) | 6(6.45%) |
| **( 11-20 )year** | 3(6.8%) | 6(12.24%) | 9(9.67%) |
| **(21-30 )year** | 4(9%) | 6(12.24%) | 10(10.75%) |
| **(31-40 )year** | 4(9%) | 5(10.20%) | 9(9.6%) |
| **( 41-50 )year** | 9(20.45% | 10(20.4%) | 19(20.43%) |
| **( 51-60 )year** | 14(31.8%) | 2(4.08%) | 16(17.20%) |
| **( 61-70)year** | 5(11.3% | 10(20.4%) | 15(16.12%) |
| **( 71-80 )year** | 3(6.8%) | 4(8.16%) | 7(7.5%) |
| **( 81-90 )year** | 0 | 2(4.3%) | 2(2.15%) |
| **Total** | 44(47.31%) | 49(52.68%) | 93(100%) |

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**Table (5):** Distribution of *E. histolytica / E. dispar* among cancer patients to the age groups in relation to age and gender.

| Age Groups | Female(%) | Male(%) | Total |
|---|---|---|---|
| **(1-10)year** | 1(50%) | 1(50%) | 2 |
| **( 11-20 )year** | 0(0% | 2(100%) | 2 |
| **(21-30 )year** | 3(50%) | 3(50%) | 6 |
| **(31-40 )year** | 4(66.66%) | 2(33.33%) | 6 |
| **( 41-50 )year** | 2(40%) | 3(60%) | 5 |
| **( 51-60 )year** | 2(40%) | 3(60%) | 5 |
| **( 61-70)year** | 2(50%) | 2(50%) | 4 |
| **( 71-80 )year** | 1(33.33%) | 2(66.66%) | 3 |
| **( 81-90 )year** | 0 | 0 | |
| **Total** | 15(45.45%) | 18(54.54%) | 33(100%) |

## 4.DISCUSSION

Immunosuppression is an important clinical entity in studying of infection and epidemiology of vgrious types of infection in developing countries. The epidemiological characteristics of infections were well studied in immnocompetent individuals, however, still there is a gap in information regarding the epidemiological characteristic of infections in immunocomromized individual.

Microscopical wet mount show positivity rate of 10.7% for *E. histolytica / E. dispa*r the most predominant E. *histolytica /E. dispar* stage found in positive cases was the cyst stage (90%). This finding agreed to that reported by Redondo etal[10]. in Mexico who found 95% of cystic

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stage for *E. histolytica/E. dispar*. In addition, the present study finding was about similar to that reported in Ethiopia (Desta Haftu etal) [11], who reported a rate of 10.5% for cyst and 2.6% for trophozoite. However, the present study finding not agreed with finding (Ibraheem) who found that trophozoite was the common parasite stages[12].

*E. histolytica* infection rate was significantly different between age groups ($X^2$=14.314, P= 0.004) and predominant infection rate was in the age groups of 21-30year and 31-40 years. This pattern of *E. histolytica* infection in cancer patients not goes with that reported in immunocompetent individuals as the infection rate with *E. histolytica* shifted to children age groups [10,13,14]. The present study show that frequency distribution of *E. histolytica* positive case not show significant difference between male (54.5%) and female (45.5%). This study shows that higher incidence of cancer cases was in the age of 41-60 years (37.6%) . In addition, the difference in cancer incidence between age groups in our study population was significant. This finding suggest the influence of age on cancer incidence in the studied population. This study indicated that *E. histolytica /E. dispar* infection was detected in 35.5% of patients with cancer.

In literature, the prevalence of *E. histolytica* infection varies globally and with a range of 1% in developed countries 1992)[15]. In Iraq, the reported studies suggest a prevalence rate of 20.7% to 29.5%. But a study performed in Basrah suggest a prevalence of 27.7%in adult (Al-shaheen etal 2007)[16].

Subjects with some type of immuncompromised status and those receiving immunosuppressive chemotherapy have an increased incidence of parasitic infection including *E. histolytica* (Botero 2003) [17]. The present study *E. histolytica* / *E. dispar* incidence rate was higher to that reported by Botero etal (2003)[17], as they found incidence rate of 9.91% in immunocompromised patients. However, the above incidence rates were including *E. histolytica/E. dispar*, but in this study *E. histolytica* incidence rate was 10.7% and then *E. dispar* forms 24.8% which was nonpathogenic.

A study performed in Saudia Arabia (AL-Megrin 2010) [18] reported incidence rate of 5.2% for E. histolytica in immunocompromised patients. In addition, Mohanad etal (2002) [19] reported a rate of 1.7% for *E. histolytica* in Northen in India. However, the present study *E.*

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*histolytica* infection rate was relatively higher than that previously reported for western Nepal (27.7%)[20],. Bogota, Colombia (25.2%)(21) (Florez etal 2007), Ethiopia (24.8%) (Assefa etal 2009) [22]. In recently reported study (Jegeda etal 2014)[23] *E. histolytica* infection was reported in 5.7% among HIV/AIDS. Patients in Nigria . However *E. histolytica /E. dispar* infection rate in HIV/AIDS patients was influenced by sexual behavior (Lowther etal 2000)(24). Furthermore, in a pediatric immunocompromised population, the infection rate was 4.7% (Idris 2010)[25].. Although infected host with *E.histolytica* deploys a robust immune 1response, the parasite has developed a remarkable number of mechanisms to evade these attacks [26]. The diversity in *E. histolytica* infection rate in immunocompromised individual as this study indicated and as reported in literature are due to different factors. The factors that influence variation in infection rate with *E. histolytica* in immunocompromised subjects include personal difference in innate immunity, ability of parasite strains to evade host immune response, variability of host inflammatory response that contribute to tissue damage, and host genetics[27,28]. Parasitic infection severity, natural course and manifestation was modified by the compromise in host immune response. Cancer was associated with immune deficiency and this subsequently enhance the emergence of infection. Since the suppression is not presented as entity, thus there is a differences in infection rates with *E. histolytica* in different reported studies settings. In previous reported study [29], suggest that in animal model, the presence of commensal *Clostiridia.* Related bacteria in gut is protective during *E. histolytica* infection. This finding may add explanation for host response differences to *E. histolytica* infection and geographical variation in infection rate in immunocomptent and immunocompromised subjects. Amoebiasis or unknown factors related to infection with *E. histolytica* may stimulate the proliferation of Lymphoma cells [30]. Although the present study finding indicated that tumor type may influence *E. histolytica* infection rate, other study not reported a statistical significant differences between the different type of immunocompromised condition.

From the results of the present study., it concluded that *E. histolytica /E. disare* predisposing factor of cancer patients.

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