Study of the relationship between *Entamoeba histolytica* and *candida* species infection among cancer patients in Kirkuk city /Iraq

Ali Ibrahim Mohammed salih 1, Sadia Shahab Hamad2, Bari lateef Mohammed3

1,2,3 College of science/university of Kirkuk/Iraq

Ibrahimali1994385@gmail.com

Abstract

The current study was conducted among immunocomromised cancer patients who attended the Oncology and Hematology Hospital in Kirkuk/Iraq and some private laboratories since November of 2019 until November of 2020 to detect the prevalence of *E. histolytica* and *candida* spp, a total of 142 afresh stool samples were collected to determine the incidence of *E. histolytica* parasite and *candida* spp. The overall infection rate of *E.histolytica* was 33.8%, and for the *candida* spp was 72.5%. The results showed that the parasite infection rate in males was 35% and it was higher than females which amounted 33.3%. without significant difference between them. While the infection rate with *Candida* spp was 74.4% in females which was higher than the males that amounted 60% without significant difference between them.

Introduction

Cancer is defined as a mass that grows and enlarges in size. The tumor may be a locally benign tumor that does not cause damage or a malignant tumor that locally increases in size and can spread to the body (Croce, 2008). Cancer patients undergo intensive chemotherapy with cytostatic and immunosuppressive drugs, in addition to radiotherapy in an attempt to destroy the tumor. And its attacks the immune system, that decrease the patient's immunity, which expose the patient to opportunistic diseases (Hierhozler, 1992).

*Entamoeba histolytica* is an intestinal parasitic protozoan. It is an anaerobic intestinal parasite that causes about 50 million infections and a mortality rate more than 100,000 worldwide annually (Zlobl, 2001; Fotedar *et al*., 2007). It
colonizes and invades the intestines, causing amoebiasis that lead to colitis and is the third most common cause of death among parasitic diseases, after malaria and schistosoma (Fotedar & Chadee, 2010; Ali, 2015; Ghasemi et al. 2015). The parasite *E. histolytica* spreads worldwide especially in tropical and subtropical regions (Al-Areeqi et al., 2017). *Candida* species are opportunistic fungal pathogens that cause severe infections in immunocompromised patients. After increasing in the number of immunodeficiency patients, the number of life-threatening Candidiasis has increased (Borst, 2002). More than 200 *Candida* species has been classified, but only a few number causes diseases in humans (Warnock, 2007). *Candida* species constitute one of the main groups of fungi causing dermatological and systemic diseases (Bramono et al., 2006).

**Materials and methods**

This study concerned cancer patients performed in Kirkuk city/ Iraq, A total of 142 stool samples were collected from patients with cancer of both gender and ages were ranged from less than 10 years old to more than 70 years old during their attendance to laboratories of the Oncology and Hematology Hospital in Kirkuk and some private laboratories since November of 2019 until November of 2020, to determine the prevalence and epidemiology of amoebiasis and candidiasis.

A fresh stool sample were collected from patients using disposable plastic container. the samples were divided into three portion the first portion were examined by the direct wet mount and floating methods to detect the trophozoites and/or cysts of *E. histolytica*. Two slides were prepared for each sample, using a clean slides, a small drop of normal saline was placed on the slide and mixed with a small peace size after well mixing of the sample using a wooden stick, a clean cover slide then placed and the specimen examined using light microscopy under x40 and x100 power magnification (WHO, 1991), the second portion of stool specimens (0.5-3 mg) was preserved in sterile screw cap
containers that contain potassium dichromate (K₂Cr₂O₇) at -20°C using deep freez until being examined by ELISA for E. histolytica / E. dispar stool antigen (Diagnostic Automation, INC. Suit, Netherlands)

While the third portion of the sample it was cultivated on culture media for detecting the Candida spp such as saproide dextrose agar, HiChrome Candida Differential then using serum culture for germ tube formation test that’s specific to diagnose Candida albicans species.

Results and Discussion

Immunosuppression is an important clinical condition in studying of various types of infection and epidemiology in developing countries, those who receiving immunosuppressive chemotherapy have an increased incidence of parasitic and fungal infection including E. histolytica and Candida spp. The result of present study that based on cancer patients showed 46 positive samples for Microscopical wet mount method and the rate was 32.4% and 48 samples were positive for ELISA test with an infection rate amounted 33.8%. While 103 samples were positive for Candida spp with a rate reached 72.5% as shown in Table (1).

<table>
<thead>
<tr>
<th>The total number of examined samples</th>
<th>E.histolytica</th>
<th>Candida spp</th>
</tr>
</thead>
<tbody>
<tr>
<td>positive samples</td>
<td>negative samples</td>
<td></td>
</tr>
<tr>
<td>E.histolytica</td>
<td>E.histolytica</td>
<td></td>
</tr>
<tr>
<td>DIRECT</td>
<td>ELISA</td>
<td>DIRECT</td>
</tr>
<tr>
<td>No.</td>
<td>Rate%</td>
<td>No.</td>
</tr>
<tr>
<td>46</td>
<td>32.4</td>
<td>48</td>
</tr>
</tbody>
</table>

Table (1): The percentage of amoebiasis and candidiasis in cancer patients

The present study finding was close to that reported by (Mustafa et al., 2015) in Kirkuk, that recorded an infection rate reached 35.5% for the Entamoeba
*Histolytica* in immunosuppressed patients. This finding agreed to a study in Basrah city by (Khaleel et al., 2020) who reported a rate of 20.2% in cancer patients. In addition, the present study finding was agreed with a study reported by (Asefa et al., 2009) in Ethiopia. Who reported a rate of 24.8% in immunosuppressed patients. Wowever the present study was relatively higher than that previously submitted in Nigeria by (Jegede et al., 2014), reported incidence rate of 5.7% in immunosuppressed patients and also was close to the study done by (Florez et al., 2008) which the infection rate was 13% in immunosuppressed patients in Colombia, and in Riyadh, Saudi Arabia the study done by (Al-Megrin et al., 2014) which indicate the infection rate 5.2% in the immunosuppressed disease.

The difference in the infection rates of *E. histolytica* in immunosuppressed persons is back to various factors, including the innate immunity that differs from one person to another and the ability of the parasite to evade the immune response of the host, which contributes in tissue damage. The rates of infection are different in the published research, because cancer is so related to immunodeficiency, and this promotes the parasitic infection, as the innate immunity is unable to repel the parasite.

While the results of the current study for *Candida spp* was close to that conducted by (Salah et al., 2020) in the city of Baghdad on cancer patients which recorded a rate reached 87.6%. These results was higher than the study reported by (Pappas et al., 2010) in America where the rate of infection was 53%, and to a study conducted by (Simadibrata et al., 2004). The reason of increasing in infection rates is that the samples were collected from cancer patients while undergoing chemotherapy that suppresses immunity and leads to a decrease in the body’s defenses against candidiasis, as well as causing an imbalance in the attribution of commensal bacteria in the digestive system.
which allows the yeast to grown and invasive the gastrointestinal tract without any competitor.

The amount of infection among males recorded 14 cases infected with the parasite and the rate reached 35%, while the infected females was 34 cases with a rate reached 33.3%, and the results showed no significant difference. As for the *Candida spp* infection among males was 24 cases with a rate amounted 60%, and a rate of 77.4% for 79 females. And the results showed no significant difference. As shown in the table(2).

**Table (2): Rate of amoebiasis and candidiasis in cancer patients according to gender**

<table>
<thead>
<tr>
<th>Gender</th>
<th>The total number of examined samples</th>
<th>positive samples <em>E.histolytica</em></th>
<th>positive samples <em>Candida spp</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No.</td>
<td>Rate%</td>
</tr>
<tr>
<td>Males</td>
<td>40</td>
<td>14</td>
<td>35</td>
</tr>
<tr>
<td>Females</td>
<td>102</td>
<td>34</td>
<td>33.3</td>
</tr>
<tr>
<td>Total</td>
<td>142</td>
<td>48</td>
<td>33.8</td>
</tr>
<tr>
<td>Statistical Analysis</td>
<td>T-test</td>
<td>T=2.24, P-value=0.26 NS</td>
<td>T= 5.57 , P-value=0.113 NS</td>
</tr>
</tbody>
</table>

The results of current study was close to a study reported in Kirkuk by (Mustafa *et al.*, 2015) that recoded a rate of 52.86% for *E.histolytica* among males while recorded a rate of 47.31%. Also the study agreed with a study reported by (Khaleel *et al.*, 2020) in Basrah among cancer patients that recorded a rate of 54.4% compared with females rate that amounted 45.6%. As well as the present study agreed with a study done by (Lehman LG *et al.*, 2013) that reported that the infection with *Candida spp* was higher among the females than the males, the study also agreed with a study reported by (chung *et
that recorded a high percentage of *Candida spp* in females compared to males among cancer patients.

This difference in infection rates according the gender is due to the variance in their immunity as the females have an immunity more deficient than the males because of recurrent pregnancies and abortion also taking antibiotic for frequent inflammations such as urinary tract infection these drugs influence the bacterial balance in the digestive system which leads to several problems such as parasitic and fungal infections.

As for the distribution of parasitic infections according to the types of cancer the study showed that the rate of parasitic infection in patients with hematogenous cancer was 44.1% and it was higher than patients with solid cancers that amounted 29.2%, and the results showed no significant difference. This study agreed with a study reported by (Mustafa *et al.*, 2015), where the rate of infection in hematogenous cancer was 46.4%, and in solid cancers was 30.8% as shown in Table (3). As for the *Candida spp* infection the rate of infection was higher in patients with solid cancers that amounted 75.7%, and less in patients with hematogenous cancer, which amounted 65.1% as shown in Table (3), and the results showed no significant difference, the study agreed with a study submitted by (Chung *et al.*, 2017) that shown the incidence of *Candida spp* in solid cancers was higher than the hematogenous cancer.
Table (3): Percentage of amoebiasis and candidiasis in cancer patients, according to type of cancer

<table>
<thead>
<tr>
<th>Cancer Type</th>
<th>The total number of examined samples</th>
<th>Positive samples E.histolytica</th>
<th>positive samples Candida spp</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>Rate%</td>
<td>No.</td>
</tr>
<tr>
<td>hematogenous cancer</td>
<td>43</td>
<td>19</td>
<td>44.1</td>
</tr>
<tr>
<td>Solid cancers</td>
<td>99</td>
<td>29</td>
<td>29.2</td>
</tr>
<tr>
<td>Total</td>
<td>142</td>
<td>48</td>
<td>33.8</td>
</tr>
<tr>
<td>Statistical Analysis</td>
<td>T- test</td>
<td>T=-2.04</td>
<td>p-value=0.29</td>
</tr>
</tbody>
</table>

The reason behind the high percentage of *candida spp* in solid cancer is that this type usually required a surgical intervention that needs to stay in hospital for several days and this a main reason to made the patients more susceptible to infections because of polluted air and food an using polluted surgical instrument during the surgery.

The study indicated that *C. albicans* was the more dominant species with a rate amounted 70% compared to other species, as shown in Figure (1), followed with a rate 6.8% for *C. krusi* and *C. Parapsilosis*, and 2.9% for *C. glabrata*.

This study agreed with (Gammelsrud et al., 2011), where the highest rate of infection is recorded with *C. albicans* and the rate reached 63% in children with cancer. It is also agreed with a study reported by (Vijayalakshmi et al., 2016), where the highest percentage of *C. albicans* was recorded, and also is similar to (Otašević et al., 2018), where the highest percentage of *C. albicans* is
recorded. The *Candida albicans* prevalence is due to its ability to produce hemolysin and obtain iron that is the most important factors for its survival and causing infection in the host tissues as with absence of free iron it goes to acquired it from iron-containing compounds such as hemoglobin. Also, it has the ability to form biofilms which gives it the ability to resist anti-fungals and resist the process of phagocytosis by the phagocytic cells of the host. Its spread is also attributed to its ability to form the germ tube through its ability to penetrate the layer of epithelial cells lining the body and tissues and reach the bloodstream as well as its importance in feeding, and also to its ability to form chlamydial spores as a kind of protection when nutrients decrease from the media. The all above are the important virulence factors that gives this species the capability to dominate

![Figure (1): The percentage of Candida spp](image)

The following curve shows that the relationship between the two infections is a strong inverse relationship. It shows that the high infection of the parasite reduces the infection of the *Candida spp*. This is perhaps due to the high ability of the cyst to resist the unsuitable conditions by surrounding with a smooth chitin wall. This gives it the ability to withstand until the relevance conditions

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are provided, to get out of the cyst and settle in the intestines and compete the yeast and other micro-organisms for nutrients. The trophozoite phase of the parasite has the ability to secrete enzymes and decompose the mucous membrane of the large intestine and goes deep inside the intestinal wall, making it protected and able to multiply and continue.

**Figure (2): The relationship between *Entamoeba histolytica* and *Candida* spp infections**
References


• **Hierhozler, J. C. (1992)**. Adenoviruses in the immunocompromised host, clinical Microbiology Reviews. 5: 262-274.


