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Research Article

Prevalence of Intestinal Infections with *Entamoeba histolytica* Among Patients of Makkah Hospitals, Saudi Arabia: A 5-Year Retrospective Study

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Abstract

Background: Amoebiasis, a disease caused by the enteric protozoan parasite *Entamoeba histolytica* (*E. histolytica*), the most important parasitic etiology of acute diarrhea. The present study was carried out to determines the prevalence of *E. histolytica* among patients of Makkah hospitals, Ministry of health, Saudi Arabia for five years (2014-2018).

Materials and Methods: A retrospective analysis of the recorded intestinal amoebiasis infections among patients visiting Al-Noor Specialist Hospital, Heraa General Hospital, Maternity and Children Hospital and King Abdul-Aziz Hospital, Makkah, Saudi Arabia, from 2014 to 2018. A total of 55,108 in- and outpatient were examined for intestinal amoebiasis infection.

Results: 1289 patients out of 55,108 were infected with *E. histolytica* with prevalence rate 2.3%. The highest percentage of positive cases were found in Al-Noor Specialist Hospital (5.7%) followed by Heraa General Hospital (3.99%), King Abdul-Aziz Hospital (Al Zahir) (0.7%) whereas only (0.4%) of examined samples were positive for *E. histolytica* in Maternity and Children Hospital. In addition, the occurrence of intestinal amoebiasis is higher among adults than children. Furthermore, the number of positive *E. histolytica* samples was decreased from 2014 to 2018.

Conclusions: The present study revealed that intestinal amoebiasis are still detected in the patients of Makkah hospitals and still a public health problem in Makkah, Saudi Arabia. Improving the diagnostic procedures to detect *E. histolytica*, as well as performing regular epidemiological surveys about intestinal parasitic infections especially *E. histolytica* are required to develop effective prevention and control strategies.

Keywords: Microscopic Examination; *Entamoeba histolytica*; Makkah; Saudi Arabia

Abbreviations

KSA: Kingdom of Saudi Arabia; WHO: World Health Organization; *E*, *Entamoeba*; ALA: Amoebic Liver Abscess; ELISA: Enzyme Linked Immunosorbent Assay; GE, Gastroenteritis; IHA: Indirect Hemagglutination; ICT: Immunochromatographic; CDC: Center of Disease Control; CT: Computed Tomography; IFA: Indirect Immunofluorescent; RBCs: Red Blood Cells

Introduction

Entamoeba histolytica is the causative agent of amebiasis and is globally considered a leading parasitic cause of human mortality. Approximately 50 million people have invasive disease, resulting in 110,000 deaths annually especially in tropical and sub-tropical countries including Saudi Arabia [1].

Although the parasite has a worldwide distribution, high prevalence rates of more than 10% of the population have been reported from various developing countries such as Central and South America, Africa, and the Indian subcontinent due to inaccessibility of safe water, inadequate nutrition, eating unwashed fruits or vegetables and unhygienic food handling practices of food handlers [2].

The genus *Entamoeba* contains many species, six of which (*E. histolytica, E. dispar, E. moshkovskii, E. 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 non-pathogenic [1].

In organisms isolated from a patient with amoebic dysentery, Charcot-Leyden crystals and blood is the most common finding in the acute stage and this feature is diagnostic for *E. histolytica* [3].

Transmission of amoebiasis occurs mainly via the fecal-oral route or drinking contaminated water containing *Entamoeba* cyst [4]. In the external environment, cysts are relatively resistant to disinfection by chlorination. Inside the body, gastric acidity is lethal to trophozoites but has no effect on *E. histolytica* cysts [5]. Cyst formation occurs only within the intestinal tract. The cysts are infective and represent the mode of transmission from one host to another [3]. After cyst ingestion, no changes occur in an acidic environment; however, once the pH becomes neutral or slightly alkaline, the encysted organism becomes active, with the outcome being four separate trophozoites in the large intestine. Clinical features of amebiasis due to *E. histolytica* range from asymptomatic colonization to amoebic dysentery and invasive extraintestinal amebiasis, which is manifested most commonly in the form of liver abscesses [5]. The emerging trophozoite invades the colonic mucosa causing clinical symptoms, including amebic colitis, watery, bloody, or mucous diarrhea and dysentery, abdominal cramps and tenderness, weight loss and rarely the formation of a tumor like granulation mass (ameboma) [6]. Trophozoites migrate through the bloodstream affecting the liver causing amoebic liver abscess (ALA) as well as the lung causing pulmonary amoebiasis and other organs [7]. The majority of ALA manifestations present with fever, right upper quadrant pain, respiratory disease with a cough [5,8]. However, about 90% of amebic infections are asymptomatic and self-limiting [9].

The gold standard method for diagnosing intestinal amebiasis is microscopic examination of stool samples for detecting *E. histolytica* cysts and trophozoites using normal saline and iodine. The presence of Charcot-Leyden crystals and blood is the most common finding in the acute stage. In addition to the RBCs, macrophages and polymorphonuclear cells (PMNs) can also be seen on microscopy in cases of amoebic dysentery [3,8]. During microscopic examination of intestinal tissues, biopsies may reveal broad based flask shaped ulcer [5,6]. Additional diagnostic methods include isoenzyme analysis, detection of *E. histolytica*-specific antibodies and specific antigen in stool and other clinical samples as well as several molecular-based tests have been developed for diagnosis of *E. histolytica* by clinical laboratories [3,8,10].

Extraintestinal amoebiasis is confirmed by positive serological tests such as indirect hemagglutination assay (IHA), indirect immunofluorescent assay (IFA) and enzyme linked immunosorbent assay (ELISA) [3,8]. However, lower percentage of ALA patients may demonstrate *E. histolytica* parasites in hepatic fluid or in the necrotic material at the margin of the abscess. Diagnosis can be achieved by abdominal ultrasound or computed tomography (CT) scan [3,8,11].

Saudi Arabia is considered as one of the largest countries that receive many expatriate workers from different regions in the world known to be endemic for numerous diseases including intestinal parasitic infections [12]. *E. histolytica* among the common identified intestinal protozoan parasites in the examined patients from different regions of Saudi Arabia such as Hail [12], Riyadh [4,13], Abha (Asir) [14], Al-Medinah [15] and Taif [16] but there is a scarcity of information about the frequency and

distribution of intestinal amoebiasis in Makkah region. Therefore, this retrospective study aims to fill this gap in knowledge and to elucidate the situation regarding the prevalence of intestinal amebiasis among in- and outpatients of Makkah Hospitals during the period from 2014 to 2018 using the data records of Makkah hospitals as well as to assist in intestinal amoebiasis prevention efforts.

Materials and Methods

Study region and population

The present hospital-based retrospective study was carried out by analyzing medical records of 55,108 patient samples (inpatient and outpatient departments) were collected from the Health Affairs Department, Makkah region and included in this study between 2014 and 2018. These samples represent data collected from four general governmental hospitals in Makkah (Al-Noor Specialist Hospital, Heraa General Hospital, Maternity and Children Hospital and King Abdul-Aziz Hospital (Al-Zahir). Information regarding age groups of patients (adults <12 and children ≤12), year of analysis and result of stool analysis (positive or negative for *E. histolytica*) were included in this research.

The Makkah Province is one of the 13 provinces of Saudi Arabia. It is the third-largest province by area at 153,128 km^2 (59,123 sq mi) and the most populous with a population of 7,715,338 of 2018, of which 4,516,577 were Saudis and 3,198,761 were foreign nationals. Makkah features a hot desert climate. Most of the central and eastern portions of the province are desert, with the Hejaz mountains [17] (Figure 1).

Figure 1: Map of Saudi Arabia: Available online: https://www.worldometers.info/maps/saudi-arabia-map (accessed on 15 September 2023).

Samples collection and examination

Samples were collected in sterile plastic containers, carefully labelled and transported to the Microbiology lab. The stool specimens were examined macroscopically for detecting the colour, consistency, the presence of mucous and blood. In addition, the direct microscopic examination using normal saline and iodine was used to detect the protozoan parasites. In organisms isolated from a patient with dysentery, Charcot-Leyden crystals and red blood cells (RBCs) is the most common finding in the acute stage and this feature is diagnostic for *E. histolytica* [3,8].

Data collection

The data sets of stool samples examination for a total 55,108 in- and outpatients of Makkah Hospitals during the period from 2014 to 2018 were collected from the hospital information system database department based on prior permission from the administration officials of the hospital.

Ethical approval

Research Ethics Training Curriculum Certificate was acquired for all candidates included in this study. Patients' data were primarily collected from the department of health affairs, Makkah region. All required eligibility forms including search approval form, data share agreement form, non-disclosure agreement form and minimal risk informed consent form were filled and signed by all candidates.

Data analysis

The collected data were yearly classified to determine the prevalence of *E. histolytica* infection per year. The frequency distributions of collected data were calculated and presented in tables and graphs according to hospitals, years, age group (adults and children), positive and negative cases. All statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) program version number 26. Two tests were used: student t-test is used to compare two means to assess whether they are from the same population, presuming that both groups are normally distributed and have relatively equal variances. Student t-test was applied to assess the differences between hospital and year were included in this study. Correlation test is statistical technique that can show whether and how strongly pairs of variables are related. The correlation test was carried out to assess the relationship between adult age group and positive cases. A P-value of <0.05 was expressed statistically significant.

Results and Discussion

Results

Prevalence of *E. histolytica* in different hospitals

A total 1289 (2.3%) samples were confirmed positive for *E. histolytica* infection of 55,108 patient samples using microscopic stool analysis (Table 1). The highest percentage of positive cases were found in Al-Noor Specialist Hospital (5.7%), followed by Heraa General Hospital (3.99%), King Abdul-Aziz Hospital (Al Zahir) (0.7%) whereas only 0.4% of examined samples were positive for *E. histolytica* in Maternity and Children Hospital. There is a statistically significant difference in terms of number of positive samples among Makkah hospitals (P < 0:05).

Annual occurrence of E. histolytica positive cases

The prevalence of *E. histolytica* during the period of study (2014 – 2018 G) is shown in Table 2. The number of positive samples was decreased from 2014 to 2018 G. However, the decrease was not statistically significant.

Prevalence of E. histolytica in adults and children

The percentage of positive *E. histolytica* cases was higher among adults (66-84%) than children (16-34%). There is a statistically significance relationship between adult age group and confirmed positive samples (P <0:05), indicating that the occurrence of disease is higher among adults than children (Figure 2).

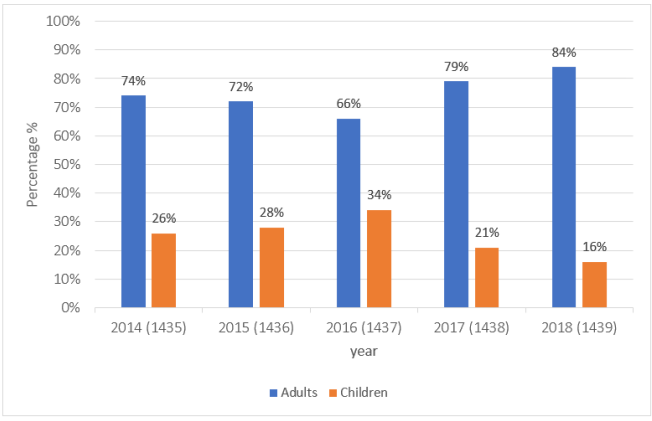

Figure 2: Prevalence of *E. histolytica* infection among age groups.

Hospitals	No. of samples	No. of positive (%)
Al-Noor Specialist Hospital	15,189	869 (5.7%) *
Heraa General Hospital	4,359	174 (3.99%)
King Abdul-Aziz Hospital (Al Zahir)	32,629	234 (0.7%)
Maternity and Children Hospital	2,931	12 (0.4%)
Total	55,108	1289 (2.3%)

Table 1: Prevalence of *E. histolytica* among patients in Makkah hospitals.

* P < 0.05 vs other Hospitals.

Year	Total samples	Total positive (%)
2014	11,316	293 (2.6)
2015	11,277	273 (2.4)
2016	10,304	250 (2.4)
2017	11,241	254 (2.3)
2018	10,970	219 (2)

Table 2: Annual occurrence of *E. histolytica* positive cases from 2014-2018 G.

Discussion

Entamoeba histolytica is often the most prevalent pathogenic protozoan parasites in the intestine and still a major community health concern in the Middle East, particularly in the Arab countries such as Qatar [18], United Arab Emirates [19], Iraq [20], Yemen [21], Sudan [22], Palestine [23] and Jordan [24], High level of *E. histolytica* motility and phagocytosis is responsible for the parasite invading different tissues of the host [7,9,25]. The findings of the present study revealed that out of 55,108 samples examined for *E. histolytica,* 1289 (2.3%) were positive. Our findings showed an agreement with the results studies in Saudi Arabia such as a study by Alqarni., *et al*. [26] where *E. histolytic* accounted for 2.7% among food handlers in Belgarn (2.97%) among food handlers in Jeddah [27], 4.1% of the schoolchildren in Abha (Asir) [14], 4.7% of the patients in Al-Noor Specialist Hospital, Makkah [28], 6% of the symptomatic children in Jeddah [10], 8.8% in Riyadh [29]. Furthermore, a relatively high frequency of *E. histolytica* infection,

(23%) among food handlers in Al-Medinah [15] as well as (83%) among adult diarrheic patients visiting King Fahd hospital in Jeddah [30].

Compared to other countries, the prevalence of intestinal infections with *E. histolytica* in Mozambique (2.0%) [31], Iran (3.7%) (Yesigat., *et al*. 2020) [32], Turkey (69.9%) [33] and Ethiopia (12.8%-70.8%) [2,34-36].

Differences in the prevalence of intestinal amoebiasis in the current studies and other studies in Saudi Arabia as well as other countries due to sociodemographic differences as well as the diagnostic techniques used.

In the present study, the highest positive *E. histolytica* infection cases were found in Al-Noor Specialist Hospital, (5.7%). This rate is extremely related to that published in a previous study conducted in Al-Noor Specialist Hospital in Makkah (4.7%) [28]. Furthermore, a recent report revealed that the amoebiasis prevalence among suspected patients in Security Forces Hospital, Makkah city (2.3%) [37]. Whereas the lowest number of positive samples for *E. histolytica* infection in the current study were found in Maternity and Children Hospital with total number of (12/2,931, 0.4%). Our findings on the prevalence of *E. histolytica* showed an agreement with the results of Amer., *et al*. [13] where *E. histolytic* accounted for 0.27% among in-and outpatients visiting King Fahd Medical City, Riyadh, KSA. This might be due to the fact that Maternity and Children Hospital only receive samples from children and pregnant women. On the other hand, in a previous study of infants and children among Ibn Sina College Hospital and Al-Jedaani Hospital in Jeddah, *E. histolytica* parasite was the most predominant infection associated with gastroenteritis (GE) (20%) [38].

The occurrence of intestinal amoebiasis is higher among adults than children in the current study. Our findings showed an agreement with the results of a study in Jeddah [30], Riyadh [13] and Makkah [38]. In addition, Omar., *et al*. [14] stated that the prevalence of *E. histolytica* was found to increase with age. Furthermore, this observation is similar to other community-based studies such as Addis Ababa, Ethiopia, whereas the dominant parasite from food handlers were *E. histolytica* (70.8%) [36]. The high prevalence among adults could be attributed to the close contact with the contaminated environment through their tendency to spend most of their times outside their houses and to eat the fast foods from the restaurants. On the other hand, in Bangladesh, where diarrheal diseases are the leading cause of childhood death, approximately 50% of children have serological evidence of exposure to *E. histolytica* by 5 years of age [39].

Therefore, measures including regular medical check-up especially for food handlers, improved safe food handling practice, improved personal hygiene and environmental sanitation as well as improved facility utensils sanitation to develop effective prevention and control strategies.

The present study included a limited number of patient variables; it would have been preferable if more variables such as sociodemographic information includes gender, seasons, nationality, residence location, educational level, environmental factors such as water supply and sewage disposal need to be included in the future survey which may have produced a very precise description of prevalence of intestinal parasites in the community.

Conclusions

Intestinal amoebiasis are still a public health problem in Makkah, Saudi Arabia. Improving the diagnostic procedures to detect pathogenic amoebae, conducting concentration methods, and introducing other techniques such as antigen and antibody detection as well as molecular assays to improve work performance and parasite identification sensitivity. Furthermore, performing regular epidemiological surveys about intestinal amoebiasis to develop effective prevention and control strategies. Educational campaigns about the pathogenesis and transmission of amoebiasis are required to educate the population about the disease and control its transmission. Food of unknown origin as well as unhealthy water must be avoided. Food handlers must have valid heath certificates enabling them to handle food and carry out periodic health examination. Individual sanitary practices such as hand washing, and personal hygiene should be improved.

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Conflict of Interest

The authors declare that there is no conflict of interests.

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