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Human amoebiasis in Multan, Punjab, Pakistan

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To determine the overall prevalence of human amoebiasis at Nishtar Hospital Multan, Punjab, stool samples (n = 766) were collected from patients visiting Nishtar Hospital Multan. Temporary mounts were prepared for determine amoebiasis. Out of 766 patients, 66 were infected with *Entamoeba histolyticalEntamoeba disparlEntamoeba moshkovskii.* The overall prevalence of parasites was 8.61%. It was more prevalent in females (9.46%) as compared to males (8.15%). The relationship between sex and human amoebiasis was non-significant (P>0.05). The prevalence of amoebiasis was highest in age group of 33 to 48 years (16.67%) and lowest in 49 to 63 years (3.45%). The difference was statistically significant (P<0.05). The high prevalence may be attributed to low sanitary conditions in the region. The protozoan parasites did not show gender specific differences.

Key words: Prevalence, age, sex, amoebiasis, humans.

INTRODUCTION

Amoebiasis is still one of major health problem and predominantly affects individuals of lower socioeconomic status who live in developing countries (Simonetta et al., 2002). It is estimated that *Entamoeba histolytica* may infect half a billion people annually, with 100,000 deaths worldwide (Villalba-Magdaleno et al., 2007).

However, the true distribution of amoebiasis is not clear in most countries. This has been particularly complicated by the existence of different species morphologically identical but genetically different; namely *E. histolytica*, which is pathogenic, *Entamoeba moshkowskii* and *Entamoeba dispar*, which are non-pathogenic species (Diamond and Clark, 1993). The differentiation of *E. histolytica* and *E. dispar* is necessary to avoid unnecessary treatment of patients infected with the non-pathogenic *E. dispar* and to estimate the real prevalence of *E. histolytica* (Tanyuksel and Petri, 2003). Currently, microscopy, immunoflorescence (IFA), polymerase chain reaction (PCR) and serological methods including enzyme-linked immunosorbent assay (ELISA), indirect hemagglutination assay (IHA) and latex agglutination are used for the laboratory diagnosis of amebiasis (Petri et al., 2000). The diagnosis of intestinal amoebiasis is still mostly based on the microscopical detection of organisms in stool samples (Schunk et al., 2001).

Many people infected with *E. histolytica* are asymptomatic and never develop clinical symptoms (Clark, 1998). *E. histolytica* trophozoites can both lyse host cells and induce their suicide through programmed cell death (Knoll et al., 2011). The bacterial flora of the intestine also plays an important role in its virulence and is major cause of amebic colitis, liver abscess and significant morbidity and mortality worldwide. Humans are the natural reservoir of *E. histolytica* and infection occurs via faecal-oral transmission (Singh et al., 2004). The prevalence of amoebiasis varies with the population of individuals affected, differing between countries and between areas with different socioeconomic conditions. Some up to 50% of the population is affected in regions with poor sanitary conditions (Al-Harthi and Jamjoom, 2007).

It has been reported that 10% of the world's population are infected by *Entamoeba* species, in which pathogenic *E. histolytica* constitute 10% of these infections and the remaining 90% are infected by non-pathogenic *E. dispar*

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Table 1. The relationship between sex and *E. histolytica/E.dispar/E.moshkovskii* in humans.

Gender	Number of hosts examined	Number of hosts infected	Prevalence (%)
Male hosts	491	40	8.15
Female hosts	275	26	9.46

The difference is statistically non-significant (P>0.05).

(Braga et al., 2001). However, a recent study highlighted the existence of another species of *Entamoeba* known as *E. moshkovskii* which can also cause infection among humans (Hamzah et al., 2006).

Several microscopy-based diagnoses on the prevalence of the *E. histolytica/E. dispar*/*E. moshkovskii* complex are performed worldwide (Al-Harthi and Jamjoom, 2007). Considerable work has been done in various parts of world (Braga et al., 2001) and in Pakistan (Siddiqui et al., 2002). So keeping in view the importance of amoebiasis, the project was designed to examine the overall prevalence, relationship between sex, age and amoebiasis in humans.

MATERIALS AND METHODS

The present study was carried out at Nishtar Medical College and Hospital, Multan, Punjab province of Pakistan from July 2009 to July 2010. Multan is the oldest city in Asian subcontinent. It lies between latitude 30.2 N, 71.45 E, and lies at an altitude of 710 ft. It has dry climate with very hot summers and mild winters. Its average rainfall is 127 mm with highest temperature of 54°C and the lowest recorded temperature is -1°C. The number of inhabitants is about 5 million.

A total of 766 stool samples were collected from patients visiting Nishtar Hospital, Multan. The collected materials were transferred to bottles containing 5% formalin. The specimen bottles were labeled with host name, sex and age.

The formalin-ether concentration technique (FECT) was used for detecting *E. histolytica* (Cable, 1985) in brief; the preparation process was as follows: a stool sample of approximately 1 g was places into a tube containing 10 ml of formalin. The sample was mixed thoroughly, and then the stool solution was filtered using a funnel with gauze and centrifuged for 1 min at 500 g. Supernatants were removed with a pipette, and 7 ml saline solution were added and mixed with a wooden stick. Three milliliters ether were then added and the tubes closed with rubber stoppers and shaken well. The rubber stoppers were then carefully removed and the tubes were centrifuged for five minutes at 500 g. The supernatant was discarded and the entire sediment was examined for the presence of protozoa using a microscope. The results are expressed in percentages and the values between different groups were compared by using Chi Square test.

RESULTS AND DISCUSSION

During the present study, the overall prevalence of *E. histolytica* was 8.62%. The prevalence of amoebic disease has been reported in various parts of the world. Lower prevalence rate was recorded as 2.2% from infants and young children, aged less than five years in Jeddah, Saudi Arabia by using the commercially available ELISA kit (El-Sheikh and El-Assouli, 2001), 0.78% in Iran by using direct and formalin-ether concentration methods (Hooshyar et al., 2004), 5.5% in agricultural areas in Beit Lahia, Gaza strip (Braga et al., 2001) and 3.95% in Bangladesh (Alzain, 2006). High prevalence recorded was 48.86% in rural area of Karachi, Pakistan by using microscopic study (Khanum et al., 2010), 27% in Northern Pakistan by examination of fecal samples within 4 h by temporary mounts in order to diagnose the parasites (Siddiqui et al., 2002), 13.8% in a community of 246 inhabitants located at the South-eastern border of the state of Morelos, Mexico in the state of Morelow, Mexico (Waqar et al., 2003), 72% in Abeokuta, Nigeria and 30% prevalence was recorded in patients of 0 to 76 years old visiting National Reference General Hospital N'Djamena, Chad Republic by direct microscopic examination (Ramos et al., 2005).

The above comparison shows that prevalence of *E. histolytica* is variable in different studies. This may be explained on the basis that the prevalence of amoebiasis depends on many ecological, physiological, behavioral and nutritional factors, for example, hazardous health situation facilities, low income status, contaminated food and water, lack of health education and hygienic domestic condition (Hamit et al., 2008).

Relationship between sex and *E. histolytica/E.dispar/E.moshkovskii* in humans

The prevalence of *E. histolytica/E.dispar/E.moshkovskii* in male was 8.15% and in females was 9.46% (Table 1). The difference was statistically non-significant (P>0.05), but the female showed higher prevalence tendency than males. Work has been done on the prevalence of *E. histolytica* with respect to gender. Similar findings of more prevalence was recorded in females (1.25%) than males (1%) in Mexico (Das et al., 2006) and (29.4%) in females (19.4%) in males in Brazil (Gonzalez et al., 1995). Similar findings were also reported in Iran, female hosts showed more infection of 1.6% as compared to males with 1.16% in Degema, Nigeria (Braga et al., 1996) and in Tanzania (Nyenke et al., 2008).

Usually, females are more immune to parasitic infections but the immunity of female could be broken down under certain circumstances. During the present study, females showed higher prevalence of *E. histolytica/E. dispar/E. moshkovskii.* This could be due to hormonal fluctuation in females during various stages of reproductive cycle that may affect their immunity and help

Table 2. The relationship between age and *E. histolytica/E.dispar/E.moshkovskii* in humans.

Age groups (years)	1-16	17-32	33-48	49-63
Number of hosts examined	437	198	102	29
Number of hosts infected	31	17	17	1
Prevalence (%)	7.09	8.59	16.67	3.45

The statistically difference was significant ($p<0.05$).

opportunistic parasites to establish (Mazigo et al., 2010). Moreover, pregnancy also decreases immunity in the expectant mother and makes them more susceptible to various parasitic diseases (Roberts et al., 2001). Women do washing of clothes and cooking the more. This may increase exposure to waterborne diseases and may explain the increased prevalence of disease in women (Jamieson et al., 2006). Feeding habits like eating sweet things and patronizing food vendors are observed as risk factors contributing to the intestinal parasitic infections among the women (Meinhardt, 2006). Female's personal hygiene like not washing hands before eating and after defecation, use of ordinary papers for cleaning after defecation and house hold practices are additional risk factors for the higher prevalence in female care takers (Amuta et al., 2010).

Relationship between age and *E. histolytica/E.dispar/E.moshkovskii* in humans

The prevalence was maximum (16.67%) in age group of 33 to 48 years and minimum (3.45%) in age group of 49 to 63 years as shown in Table 2. The age of the hosts play an important role in the prevalence rate. Similar findings was reported in Brazil (Azar et al., 2009) and highest prevalence (15.4%) in age group of >45 years and minimum (7.5%) in age group of 0 to 4 years in Tanzania (Braga et al., 1996).

In the present study, the middle age group showing higher prevalence than other groups may explain why the lower immunity in this age group makes it more susceptible to parasitic infections or could be behavioural/occupational. The immune system decreases with normal aging and with stress. Stress in older adults provides evidence that these processes contribute to effects that mimic, exacerbate and possibly accelerate the effects of aging on immunity (Hawkley and Cacioppo, 2004). Moreover, under-nutrition fundamentally affects the cell mediated immune response. The lack of calcium, magnesium, iron, zinc, copper, iodine and selenium has been associated with signs of immune deficiency (Fekete and Kellems, 2007). This age group was probably exposed to the causative organism during homosexual activity (Ohnishi et al., 2004). The high rates of oral-anal and oral-genital sexual practices by this group could also be the major factor for high prevalence (Stark et al., 2008).

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