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Prevalence of parasites in patients with gastroenteritis at East of Mazandaran Province, Northern Iran

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Abstract. Parasitic gastrointestinal infections are one of the most important health problems in the developing countries, which lead to the onset of intestinal disease particularly diarrhoea. Due to the particular geographic situation in the Mazandaran province, individuals are infected with various intestinal parasites. The aim of this study was to determine the prevalence rate of enteropathogenic parasites in the patients with gastroenteritis living at the east of Mazandaran province (Sari, Nekah and Joybar cities), northern Iran. This descriptive study was carried out from September 2009 to March 2010. Faecal samples were collected by randomized cluster method from 962 patients with gastroenteritis who were refered to the Health Service Centers of Sari, Neka and Joybar cities. All data about the patients were recorded in questionnaire. Stool specimens were examined by direct wet mounting, formol-ether concentration, and Ziehl-Neelsen acid fast stain and Auramin Phenol fluorescence (APF) method for the investigation of *Cryptosporidium* and *Isospora*. Prevalence of intestinal parasites and their relationship with gender, age, and season were investigated, and the obtained data were analyzed with χ^2 test using the SPSS software (16.0). Out of 962 patients with gastroenteritis, overall infection was 9.1%; *Giardia lamblia* (4.1%) with the highest and *Enterobius vermicularis* (0.2%) with the lowest prevalence rate. Prevalence rate of other parasites were as follow: *Cryptosporidium*, 0.1%; *Entamoeba histolytica*, 0.1%; *Chilomastix mesnili*, 0.1%; *Entamoeba coli*, 1.2%; *Blastocytis hominis*, 1.8%; *Trichostrongylus* spp., 0.4% and *Hymenolepis nana*, 0.9%. Findings showed that *Giardia* is the most common cause of intestinal infection at the east of Mazandaran province, and could be defined as the most important parasitic agent of gastroenteritis. On the other hand, infection with enteropathogenic parasites as compared with the previous reports showed significant decline, which reveals the coverage of health education, increase of public knowledge on the parasitic diseases and sanitation of living environment.

INTRODUCTION

Gastroenteritis is inflammation of the stomach, small and large intestines (Jones *et al.*, 2007), and is one of the most common diseases in man (Okitsu-Negishis *et al.*, 2004). It can affect individuals of any age and is a significant health risk (Jones, 2003). It is the second most common cause of death in adults, and the cause of childhood death (Zamir *et al.*, 2006). Among children lower than 5 years of age there are more than 700 million cases of gastroenteritis per year. Mortality rates of 3.5 to 5 million have been estimated to be associated with gastroenteritis annually, mostly observed in developing countries (Wilhelmi *et al.*, 2003).

Infectious agents of pediatric gastroenteritis can be classified into three broad classes: bacterial, viral and parasitic. Many protozoan parasites live in the gastrointestinal tract, infecting some 3.5 billion individuals worldwide (Johary *et al.*, 2010). Parasitic intestinal infections are major public health problem throughout the world. They are important causes of mortality and morbidity in developing countries and infection occur through direct ingestion of infective eggs or

cysts, contaminated unwashed hands, mechanical vectors and contaminated water. Infection is closely related to low socio-economic status, poor sanitation, inadequate medical care and absence of safe drinking water (Feenstra *et al.*, 2000).

Four species of intestinal parasitic infections with particular importance are *Giardia lamblia*, *Cryptosporidum parvum*, *Entamoeba histolytica* and *Blastocystis hominis* (Al-Bwardy *et al.*, 1988). *Giardia lamblia* is a parasite with worldwide distribution and common in moist and warm climates. Giardiasis is an important unresolved health problem in the developing countries which is related to improper sanitation and management of supplied water (Addiss & Mathews, 1991). In a study in Brazil, the prevalence rate of *G. lamblia* among diarrhoeic children ten years of age or younger was found to be 9.9% (Mariadas, 2007). In another study on the diarrhoeal patients, the most predominant parasite species was *G. lamblia*, 2.5%; *E. histolytica*, 0.4%; *C. parvum*, 0.4%; and mix infection, 0.1% (Jeong-Weon, 2009). Most infections with giardiasis are asymptomatic, but some with subacute or chronic diarrhoea and intestinal irritation, a condition which leads to malabsorption and nutritional deficiency, particularly in children (Brown & Neva, 1983; Dubey *et al.*, 2000). *Cryptosporidium parvum* is one of the most commonly enteric parasites in the immuno-compromised and immuno-competent individuals worldwide, it occurs in up to 7% of the children with diarrhoea in the industrialized countries and up to 12% of the children with diarrhoea in the developing countries (Leav *et al.*, 2003). In Mazandaran, northern Iran, its prevalence in HIV/AIDS patients was 10% (Daryani, 2009). Amebiasis affects about 500 million people worldwide, and its prevalence rate is more in the developing countries (Anderson, 2000). In Turkey the prevalence of parasites causing this disease was high among people living in unhygienic condition (Aksoy *et al.*, 2005). In Pakdasht, Tehran Province, the highest infection rate (41.5%) was related to protozoan parasites (Shahbazi *et al.*, 2003). A study in Ardabil, Iran, showed the infection rates of *G. lamblia*, *B. hominis* and *Entamoeba coli* to be 14%, 10% and 4.1%, respectively (Daryani *et al.*, 2003). *Blastocystis hominis* is the most common human intestinal protozoa throughout the world. Reports suggested a pathogenic role of *B. hominis* in causing intestinal inflammation.

Most studies on gastroenteritis have focused on viral and bacterial infections, while gastroenteritis where intestinal protozoan parasites may have played a role has not been well studied. The main objective of this study was to determine the prevalence of intestinal parasites in the faeces of gastroenteritis patients.

MATERIALS AND METHODS

In this study, faecal samples were collected from 962 gastroentritis patients referring to 6 health service centers of eastern Mazandaran (Sari, Nekah and Joybar cities) by randomized cluster method from September 2009 to March 2010 and transferred to the laboratory of parasitology at the Sari Medical College or the Iran Pasteur Institute, Amol Branch. After observing the consistency and recording the features of faeces, each specimen was examined by direct wet mounting, formol-ether concentration, and Ziehl-Neelsen acid fast stain and Auramin Phenol fluorescence (APF) methods for investigating *Cryptosporidium* and *Isospora*.

Ziehl Neelson's acid fast staining

The faeces smears fixed with 96% ethanol, were stained with carbol fuschin, then washed in tap water, rinsed with 3% acid alcohol, later stained with 0.5% malachite green (5 min), and observed under light microscope (at 1000 × magnification).

Auramin Phenol fluorescence method (APF)

The smear was fixed with 96% ethanol, then stained with auramin (15 min), washed with water and rinsed with 3% acid alcohol till the auramin color disappeared. The smear was then washed with water, and stained with 0.5% potassium permangnate (1-3 min). Finally the

smear was washed with water, air dried and mounted with glycerin buffer and observed under fluorescence microscope (Perch *et al.*, 2001; Nahrevanian & Asmar, 2006, 2008).

For analysis purposes patients were allocated in three age groups as follow: 0-10 yr, 11-20 yr and above 21 yr. In this survey, viral or bacterial causes were not evaluated.

RESULTS

A total of 962 stool specimens were collected from gastroenteritis patients. Of these specimens, 58.7% were collected from males, and 41.3% from females. The overall parasitic infection rate in the gastroenteritis patients was 9.1% (5.2% male, 3.9% female). Presence of helminthiasis was observed in 15 (1.5%) patients, protozoa in 73 (7.6%) persons. *Giardia lamblia* with 4.1% in protozoa and *H. nana* with 0.9% in helminithes had the highest infection rates. Prevalence of other parasites is shown in Table 1.

As depicted in Table 1, there was not a sex related significant difference in the prevalence between various parasites ($P=0.28$, $\chi^2=9.75$). Though the highest infection rate was observed in the age group above 21 years (10.9%), chi square test did not show any significant difference in relation to parasitic infection in different age groups ($P=0.2$, $\chi^2=20.39$) (Table 2).

DISCUSSION

Despite of development in health cares, parasitic infections remained as one of the most common cause of diseases in the developing countries. *Cryptosporidium* and *Isospora*, though considered a health problem, but generally are not investigated in the routine laboratory examinations. Due to the above mentioned conditions, we studied the parasitic infections among patients with gastroenteritis at the east of Mazandaran province and determined the probable rate of parasitic infections in the patients with gastroenteritis. In this regard, the overall prevalence rate of all parasitic infections was found to be 9.1%. Infection in males (5.2%) was higher than females (3.9%). In another study on 802 gastroenteritis patients in Babol and Babolsar cities, northern Iran, the prevalence rate of entropathogenic parasites was 3.4% (Ghorbannya, 2007). In a study on 6252 elementary school students at Mazandaran province, northern Iran, prevalence rate indicated 57.1% (Asmar, 2000). The different finding could be

Table 1. Prevalence of various parasitic infections in the gastroenteritis patients by sex

Gender / Parasites	Male		Female		Total	
	Number	%	Number	%	Number	%
Cryptosporidium	1	0.1	0	0	1	0.1
Giardia lamblia	21	2.2	19	1.9	40	4.1
Entamoeba histolytica	1	0.1	0	0	1	0.1
Entamoeba coli	6	0.6	6	0.6	12	1.2
Hymenolepis nana	5	0.5	4	0.4	9	0.9
Trichostrongylus spp.	2	0.2	2	0.2	4	0.4
Blastocystis hominis	14	1.4	4	0.4	18	1.8
Enterobius vermicularis[1]	0	0	2	0.2	2	0.2
Chilomastix mesnili	0	0	1	0.1	1	0.1
Total	50	5.2	38	3.9	88	9.1

($\chi^2=9.75$, $df=8$, $P=0.28$)

[1]This parasite was determined using direct examination (no scotch tape method)

Table 2. Prevalence of parasitic infections in the patients referring with gastroenteritis by age

Age group (year)	Number of study cases	The subjects infected with different parasites	
		Number	%
0–10	268	14	5.2
11–20	207	21	10.1
Above 21	487	53	10.9
Total	962	88	9.1

(χ^2=20.39, *df*=16, P=0.203)

explained by the fact that older individuals practice better hygiene and avoid using contaminated water/food which are the main control measures of preventing common intestinal parasite infections.

A study in Iran, showed a prevalence rate of intestinal parasites between 8.4% and 29.7% in general population; the reason for such high rate of infection was due to drinking of contaminated water, and improper knowledge of hygiene (Hamedi *et al.*, 2005). In the Republic of Korea, the prevalence of *Cryptosporidium* varied according to different localities, for example a prevalence of 1% (among non HIV patients), (Lee *et al.*, 2005), and 3.3% among villagers in several rural areas (Yu *et al.*, 2004) were reported. Also Park *et al.* (2006) and Seo *et al.* (2001) reported the prevalence of 1.5% and 1.9%, respectively. The data of the studies indicate different prevalence rates of *Cryptosporidium* infection which are attributed to the living standards, hygienic condition, low socio-economic status and using of unsafe water (Iqbal *et al.*, 1999). Seasonal variations of human cryptosporidiosis also have been reported and epidemiological studies have demonstrated that acute infection with *Cryptosporidium* sp. among children and infants is more prevalent in the developing countries (> 5%) than the developed countries (<1% to 3%), (Iqbal *et al.*, 1999).

The prevalence of *G. lamblia* in subjects aged 16-20 years old was recorded in 20.3% (Taherkhani *et al.*, 2009). The prevalence rate of infection in children was reported as follow: *E. vermicularis* (33.8%), *G. lamblia* (26.2%), *Hymenolepis nana* (4.8%), *Ascaris lumboricoides* (3%) and *E. histolytica* (2.3%) in Damghan, Iran (Heidari & Rokni, 2003). The observed multiple infection could be explained by the facts that many species of protozoa have the same mode of transmission. *Giardia lamblia* as most prevalent parasite can spread in water, and possibly food and person to person.

Study on the children with diarrhoea in Egypt, revealed the prevalence of parasitic infections rate was 46% (EL-Mohammady *et al.*, 2006). Research on 127 diarrhoeic children in New Delhi (India) indicated a parasitic infection rate of 46.5% (Kaur *et al.*, 2002). A study on 217 students in Vietnam showed that the highest rate of helminth infection (67%) belonged to *Trichuris trichiura* and the most common protozoan, was *E. coli* with prevalence rate of 8% (Uga *et al.*, 2005). Another study on prevalence of protozoan infections in ulcerative colitis patients in Mexico City reported the rate of 24% (Jesus *et al.*, 2010).

A study showed that 38.5% of school children in northern Iraq were infected with *G. lamblia* (Al-Saeed & Issa, 2006). A research in Addis Ababa (Ethiopia) on diarrhoeal patients detected *G. lamblia* in 6.3% and *H. nana* in 0.5% of individuals under study (Adamu *et al.*, 2005). In a research in Turkey on the 196 patients with gastrointestinal symptoms, pathogenic parasites were detected in 11 (5.6%) samples whereby *G. lamblia* was found in 7 (3.57%) and *H. nana* in 1 (0.51%), *B. hominis* in 4 (2.04%) (Tuncay *et al.*, 2008).

Data on the frequency of parasitic enteropathogenes among young children with acute diarrhoea in Saudi Arabia revealed *G. lamblia* infection in 1% of the patients under study (Johary *et al.*, 2010). Epidemiological data in different parts of Iran indicated 0-3.6% prevalence rate of intestinal helminth parasitic infection (Asghari *et al.*, 2002). In the present survey we could identify the prevalence rate of *E. vermicularis* was only 0.2%, which is related to the improper application of the recommended procedure. The differences in the prevalence rate of

parasites reported in different studies may be attributed to the different groups of studied populations and the years the surveys were performed. Comparison of our data with the above mentioned studies revealed low prevalence rate of intestinal parasitic infection in the eastern townships of Mazandaran province.

As the methodology of the identification is similar in all studies, the probable low rate of infection in the region in only gastroenteritis patients of all age groups may be attributed to education, public hygiene and drinking of public water supply.

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