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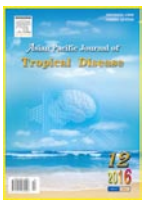

Epidemiological investigation doi: 10.1016/S2222-1808(16)61164-7

Studying the prevalence of *Campylobacter jejuni* in adults with gastroenteritis from northwest of Iran

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ARTICLE INFO

Article history:
Received 15 Jun 2016
Received in revised form 18 Jul, 2nd revised form 20 Sep 2016
Accepted 15 Oct 2016
Available online 26 Oct 2016

Keywords:
Campylobacter jejuni
RT-PCR
Gastroenteritis
Campylobacteriosis

ABSTRACT

Objective: To investigate the prevalence of *Campylobacter jejuni* (*C. jejuni*) in the patients with gastroenteritis.

Methods: This descriptive and analytical study included all adult patients with acute diarrhea admitted to the University Hospital of Zanjan Province who were enrolled in a one-year period from 2013 to 2014. Stool samples were checked for white blood cells (WBC) and lactoferrin, then samples with WBC $\leqslant$ 5 positive for lactoferrin were selected for amplification of *mapA* gene of *C. jejuni* by RT-PCR assay.

Results: In this study, 864 patients (410 men and 454 women) with acute diarrhea were enrolled, of which about 718 patients had WBC less than 5 and 146 patients had WBC more than 5 in the stool exam. All inflammatory diarrhea samples were tested for lactoferrin and 111 cases of the samples tested were positive for lactferrin. A total of 40 samples out of 111 were positive for *C. jejuni* by RT.

Conclusions: The finding of this study showed that the prevalence of inflammatory diarrhea and diarrhea caused by *Campylobacter* in this study was high. This need for education and awareness in this area, as well as appropriate treatment is too important.

1. Introduction

Infection with *Campylobacter jejuni* (*C. jejuni*) is one of the most common causes of bacterial acute gastroenteritis worldwide[1]. *Campylobacter* species are important cause of morbidity caused by diarrheal illness especially in childhood in developing countries. *Campylobacter* enteritis due to *C. jejuni* and *Campylobacter coli* is the only form of campylobacteriosis of major public health importance. Campylobacteriosis is endemic in developing countries and the major sources of human infections are foods and environmental contamination[2].

In other words, the occurrence of gastroenteritis caused by human *Campylobacter* has been mainly resulted from the consumption of contaminated food and animal products, especially poultry. Due to the high prevalence of *Campylobacter* in these animals, person-to-person transmission is very rare[3,4]. Domestic and companion animals as well as wild birds are known as reservoirs for *Campylobacter* species, and shedding of the bacteria from them leadding to contamination of the environment. Risk factors for getting *Campylobacter* in developing countries comprise the presence of an animal in the cooking area, lack of piped water and exposed garbage in cooking areas[4].

Campylobacter species are Gram-negative bacteria that have a spiral or curved shape. The bacteria grow quite slowly. About 72–96 h is required for primary isolation from clinical samples and isolation from blood can take even longer[5]. The rate of *Campylobacter* infections worldwide has been grown, with the number of cases often above those of shigellosis and salmonellosis[6]. This increase demands a clearer understanding of the epidemiology of *Campylobacter* infection.

Usually, developing countries do not have national surveillance programs for campylobacteriosis. Therefore, the values of the number of cases for a population do not exist[7]. Most estimations of incidence in these countries are from laboratory-based surveillance of pathogens responsible for diarrhea. The isolation rates of *Campylobacter* in developing countries range from 5% to 20%[8].

The clinical spectrum of *Campylobacter* enteritis ranges from a watery, non-bloody and non-inflammatory diarrhea to a severe inflammatory diarrhea with fever and abdominal pain[8,9]. But,

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Foundation Project: Supported by the research deputy of Zanjan University of Medical Sciences (Grant No. A-12-342-12).
The journal implements double-blind peer review practiced by specially invited international editorial board members.

most typically infection with *C. jejuni* lead to an acute, self-limited clinical spectrum. Clinically, campylobacteriosis is indistinguishable from acute gastrointestinal infections created by other bacterial pathogens, such as *Shigella*, *Salmonella* and *Yersinia* species[10].

The features stated of gastrointestinal sickness in developing countries are fever, watery stool, vomiting, abdominal pain, dehydration and the presence of fecal leukocytes. Patients are also often underweight and malnourished[11-14].

In laboratory studies, red blood cells and fecal leukocytes are found in the stools of 75% of infected persons[15,16]. The peripheral white blood cell (WBC) count may be slightly elevated. Diagnosis of *Campylobacter* enteritis is confirmed by obtaining cultures of the organism from stool samples.

PCR and ELISA for detecting *Campylobacter* DNA and antigens in stool samples have been developed and may become useful in the diagnosis of *Campylobacter* and other infectious diseases[17-22].

Genotyping methods such as ribotyping and pulsed-field gel electrophoresis have high ability to recognize *Campylobacter* DNA in stool samples very well[23]. Other methods of direct detection of *Campylobacter* in the clinical samples such as screening using DNA or amplification by PCR have been successfully used in the research studies[24]. In 2012, El-Adawy *et al.* reported that multiplex PCR diagnostic tools are fast, inexpensive and sensitive for *Campylobacter*[25].

Considering the increased rate of global incidence of campylobacteriosis and since there is no sufficient data in this field in the developing countries such as Zanjan Province in the northwest of Iran, this study was designed to investigate the prevalence of these organisms in the patients with gastroenteritis.

2. Materials and methods

2.1. Subjects

This descriptive and analytical study included all adult patients with acute diarrhea admitted to the University Hospital of Zanjan Province who were enrolled in a one-year period from 2013 to 2014. The research project has been approved by the Research Ethics Committee of Zanjan University of Medical Sciences, according to the Declaration of Helsinki (http://www.ufrgs.br/HCPA/gppg/helsin5.htm). Acute diarrhea means increased water content in the stools or faulty absorption of water or active secretion of water by the intestine in less than 14 days. Some cases such as taking antibiotics before sampling, having the history of the diarrhea diseases (such as cancer, irritable bowel, colitis, infection, *etc*.), lack of satisfaction in the study and with the age less than 12 years were excluded.

Among those who participated in the study, stool samples were collected. All samples were frozen at the central laboratory at –80 ℃ until for RT-PCR use. Samples obtained from subjects with inflammatory criteria (WBC $\geqslant$ 5) had been selected and isolated through fecal lactoferrin detection. Lactoferrin detection in stool had been confirmed for diagnosis of inflammatory bowel disease (IBD)[26]. Intestine inflammation could be caused by bacterial infection or IBD which was directly related to the activity and severity of disease. The aim of this study was to rule out IBD, and the rest of the patients whose WBC count was $\geqslant$ 5 in stool exam had been tested for lactoferrin. The positive samples for lactoferrin were selected for bacterial infection. Although this test in infants fed with breast milk caused false positive results, cross-reaction with lactoferrin in cow's and goat's milk did not occur, and therefore according to the survey of adults, no problem had been found in this study[26]. The positive samples for lactoferrin were selected for *C. jejuni* diagnosis by RT-PCR.

2.2. DNA extraction and RT-PCR assay

Genomic DNA from stool samples was extracted by AccuPrep Stool DNA Extraction Kit (Bioneer, South Korea) according to kit manual. PCR reactions were performed in 20 μL total volume by CJF (5′-CTGGTGGTTTTGAAGCAAAGATT-3′) and CJR (5′-CAATACCAGTGTCTAAAGTGCGTTTAT-3′) primers[27]. The primers amplified the *mapA* gene (X80135) of *C. jejuni* with amplicon size of 95 bp. The reaction mixture contained 10 pmol/L of each primer and EvaGreen qPCR Master Mix contained 1 unit of Taq DNA polymerase, 0.2 mmol/L of each of deoxynucleotide triphosphates, 1.5 mmol/L of $MgCl_2$ and 100 ng of DNA as template. The amplification program was as follows: initial denaturation at 95 ℃ for 10 min, followed by 50 cycles of denaturation at 94 ℃ for 10 s, annealing at 55 ℃ for 30 s and extension at 72 ℃ for 30 s. RT-PCR curve acquisition and analysis were performed on Rotor-Gene 6000 (Corbett, Australia).

3. Results

In this study, 864 patients (410 men and 454 women) with acute diarrhea were enrolled, of which about 718 patients had WBC less than 5 and 146 patients had the WBC more than 5 in the stool test. All inflammatory diarrhea samples were tested for lactoferrin and 111 cases of the samples tested were positive for lactferrin. A total of 40 samples out of 111 were positive for *C. jejuni* by RT.

Demographic data including age, gender, location and season with the PCR-positive infections in each group was indicated in Table 1.

Table 1
Demographic data of patients and frequency of *C. jejuni* confirmed by RT-PCR.

Variable		Numbers (%)	Number of PCR positive (%)
Age	12–20	73 (8.4)	0 (0.0)
	21–30	226 (26.2)	13 (31.7)
	31–40	207 (24.0)	11 (26.8)
	41–50	183 (21.2)	9 (22.0)
	51–60	121 (14.0)	7 (19.5)
	> 60	54 (6.2)	0 (0.0)
Gender	Man	410 (47.5)	18 (46.3)
	Female	454 (52.5)	22 (53.7)
Address	City	678 (78.5)	32 (80.5)
	Village	186 (21.5)	8 (19.5)
Getting season	Spring	308 (35.6)	16 (39.0)
	Summer	408 (47.2)	19 (48.8)
	Autumn	118 (13.7)	5 (12.2)
	Winter	30 (3.5)	0 (0.0)

Among the total samples, 618 cases were (71.6%) non-inflammatory diarrheal cases (WBC < 5) and 246 (28.4%) were inflammatory cases (WBC > 5), respectively. All 246 cases (28.5%) with inflammatory view were investigated for lactoferrin, of which 111 samples (45.1%) were positive for lactoferrin. All the 111 samples were analyzed by PCR for *C. jejuni* of which 40 cases (6.36% in total 7.4%) were positive for *C. jejuni* target gene.

Among patients who were positive for lactoferrin, 42 subjects (37.8%) were men and 69 (62.2%) were female. Among the patients positive for *C. jejuni* by RT-PCR, 18 patients (46.3%) were male and 22 patients (53.7%) were female.

4. Discussion

In the present research, the 864 patients with acute diarrhea during the study were enrolled, of which 618 were non-inflammatory diarrhea (WBC < 5) and 246 cases were inflammatory diarrhea (WBC > 5). Inflammatory diarrhea samples were tested for bacterial diarrhea by lactoferrin examination and 111 cases showed lactoferrin then these samples were tested by RT-PCR for *C. jejuni* target gene amplification, of which 40 samples were positive for *C. jejuni*.

From 864 patients, 410 cases (47.5%) were male and 454 patients (52.5%) were female. Since there was little difference between men and women in terms of acute diarrhea, when lactoferrin was tested, most people who were positive for lactoferrin were women (62.2%). However, among patients with positive for *C. jejuni* by RT-PCR, there was no significant difference between men and women ($P > 0.05$).

In one study conducted in Semnan Province of Iran by Jazayeri *et al*., the prevalence of *C. jejuni* was 12.4% and most affected patients were in the age group less than 10 years of age (45.1%)[28].

Norouzi *et al.* in 1999 in Babol district of Iran studied prevalence of *Campylobacter* species in children less than 7 years old with acute diarrhea[29]. From 260 samples, 20 cases were positive for *campylobacter* spp. and 12 cases (4.6%) were positive for *C. jejuni*. In our study, those people who were positive for lactoferrin had ages of 12 to 30 years old (32.4%). Among patients that were positive for *C. jejuni* by PCR, patients also had ages of 12 to 30 years old. These findings indicate that diarrhea due to *C. jejuni* in comparison with other causes of diarrhea, is more common in early age group and the problem could be related to their living style. It is suggested that further studies are needed to investigate the prevalence of *Campylobacter* diarrhea at early age. Among patients with diarrhea, the most were from urban areas (78.5%) and this pattern was repeated in cases positive by PCR and lactoferrin (respectively 78.4% and 80.5%). We expected high cases with diarrhea from rural area because of higher hygiene levels in urban areas. However, this observation can be attributed to the less referrals from rural area to the urban centers. In other words, rural patients with acute diarrhea do not go to urban centers and are obtaining services in rural health centers.

In terms of the seasonal significance, the most predominant peak was observed in the summer (47.2%), spring (35.6%), autumn (13.7%) and winter (3.5%), respectively. Given the prevalence of diarrheal diseases in summer and spring, in our study this pattern was followed in the cases positive for *C. jejuni* which indicates that the incidence of acute diarrhea due to *Campylobacter* also follows the seasonal pattern of other types of diarrhea.

As expected, a large percentage of cases of acute diarrhea were non-inflammatory and probably due to viral infection (71.6%). However, in this study, the cut off for the WBC count for bacterial diarrhea was considered $\geqslant 5$ if this level was considered $\geqslant 10$, and the cases with bacterial diarrhea could be increased.

In a study conducted by Riaz *et al.* in one year (2005–2006), 454 patients with diarrhea were investigated, as the prevalence of *Campylobacter* was reported 5.2%[30].

In another study conducted in 2006 by Irajian *et al.* in Semnan, Iran, the prevalence of *C. jejuni* was 12.4%[28]. Most cases were in the age group under 10 years old.

Totally, 111 out of 246 inflammatory samples (45.1%) were positive for lactoferrin and this marker is indicating the inflammatory nature of these. Of the 111 cases, 40 patients (36.6%) were positive for *C. jejuni* (4.7% of total samples). Other infectious agents or other acute inflammatory conditions such as IBD can cause the rest of inflammatory diarrheas.

In one study that investigated over 30 000 stool cultures, the frequency of the most common bacterial pathogens causing diarrhea was as follow: *Campylobacter* was 2.3%, Salmonella was 1.8% and Shigella was 1.1%[31].

In the study of Norouzi *et al.*, the prevalence of *Campylobacter* in children less than 7 years old with acute diarrhea was investigated[29]. From 260 samples, 20 patients had *Campylobacter* and 12 (4.6%) cases have been affected by *C. jejuni*.

Differences between prevalence of *C. jejuni* in this study with other studies may be related to seasonal differences and acute diarrhea causing agents in different regions.

The finding of this study showed that the prevalence of inflammatory diarrhea and diarrhea caused by *Campylobacter* in this study is high. This needs for education and awareness in this area, as well as appropriate treatment is too important.

It is suggested that more studies that are comprehensive are needed to investigate the causes of other types of acute diarrhea via proprietary methods. These finding can be helpful in the regional management of diseases.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

We thank the research deputy of Zanjan University of Medical

Sciences for financial support of this project (Grant No. A-12-342-12).

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