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ORIGINAL PAPER

Fatih Atuğ M.D. · Levent Turkeri · Ozlen Atuğ
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Detection of *Helicobacter pylori* in bladder biopsy specimens of patients with interstitial cystitis by polymerase chain reaction

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Abstract The cause of interstitial cystitis (IC) is still unknown. Several features suggest that it may be an infectious disease and it has compelling similarities to chronic gastritis. The identification of *Helicobacter pylori* as the cause of chronic gastritis focused attention on this organism. Many studies have been done investigating the role of *H. pylori* in the etiology of IC. Previous studies mostly determined the presence of *H.pylori* with antibodies in the serum samples of IC patients, but these methods may lead to false positive or negative results. We therefore investigated the presence of *H. pylori* in bladder biopsy specimens by using polymerase chain reaction (PCR), which is accepted as the most sensitive and specific test for detecting this organism. A total of 32 patients with IC were enrolled into the study. The PCR assay was performed on cold cup bladder biopsies of IC patients. Both positive and negative controls were included in each set of PCR reactions. Gastric biopsy specimens of peptic ulcer patients with proven *H. pylori* infection were used as positive controls. Bladder biopsies of all IC patients were negative for *H. pylori* DNA. PCR showed the presence of *H. pylori* in the positive controls in each cycle demonstrating that the PCR assay was working properly. Thus, there is no evidence that IC is the result of *H. pylori* infection. This study does not negate the possibility that other infectious agents may play a role in the etiology of IC.

Keywords Polymerase chain reaction · Interstitial cystitis · *Helicobacter pylori*

F. Atuğ (✉) · L. Turkeri
Marmara University School of Medicine,
Urology Department, Istanbul, Turkey

O. Atuğ
Marmara University School of Medicine,
Gastroenterology Department, Istanbul, Turkey

C. Cal
Ege University, School of Medicine, Urology Department,
Izmir, Turkey

F. Atuğ
Department of Urology, Dicle University School of Medicine,
Diyalbakir, Turkey
E-mail: Fatihatug@hotmail.com
Tel.: +90-932-3135593
Fax: +90-412-2488440

Introduction

Interstitial cystitis (IC) is a chronic bladder disease of unknown etiology. The disease presents itself predominantly in women and is characterized by urinary urgency, frequency, nocturia and suprapubic pain. IC was described more than 100 years ago, but in spite of significant research efforts the statement made by Hanash and Pool from 1969 that "the cause is unknown, the diagnosis is difficult and the treatment is temporary and palliative " is still very much valid [1].

Although there are many theories, the etiology of IC remains obscure. Acute onset of symptoms, predominance in women, inflammatory changes seen on histopathological examination and the presence of immunoglobulin A deposits in the bladder epithelium suggest that IC may be caused by an infectious organism. The possibility of infectious agents playing a role in the etiology of IC has been investigated for a number of years. Early studies were directed towards attempts to cultivate the infectious agents on routine culture media and microscopic examinations of urine or bladder tissue specimens for the presence of microorganisms. So far, however, no microorganism has ever been identified as the etiologic agent of IC [2]. Inability to culture microorganisms routinely from IC patient's urine does not negate the role of difficult or impossible to culture microorganisms in this disease.

During the last decade, with the use of polymerase chain reaction (PCR), bacterial DNA sequences have been detected in the bladder biopsies and urine specimens of patients with IC. However, cultures from biopsies and urine samples of IC patients by routine techniques were unable to identify any microorganisms. It is hypothesized that this is due to the adherence or invasion of bacteria into the bladder wall [3]. Recent research has focused on utilizing molecular techniques to search for the presence of microorganisms in urinary bladder tissue, particularly intracellular or cell associated organisms.

In the last 15 years, *Helicobacter pylori* has been identified as the cause of chronic gastritis. This was not previously associated with any infectious etiology. The discovery of *H. pylori* as the cause of gastric and duodenal ulcers has provided strong support for the concept that some inflammatory diseases of unknown origin may have a microbial etiology. Bladder biopsies of IC patients frequently show an inflammatory type of reaction analogous to chronic gastritis [4]. Moreover, Isogai et al. succeeded in inoculating *H. pylori* into the bladders of mice, which caused an intense inflammatory reaction [5].

The aim of this study is to determine the role of *H. pylori* in the etiology of IC using PCR to detect the presence of this organism in bladder biopsies of patients. This is the first study in which PCR technology is used to search the evidence of *H. pylori* in IC patients.

Materials and methods

A total of 32 women with an average age of 51.4 years having a 4–25 year (mean 8 years) history of symptoms and fulfilling the diagnostic criteria established by the United States National Institutes of Health, were enrolled in the study [6]. A PCR assay was performed for the detection of *H. pylori* on cold cup biopsy specimens from the patients.

All patients underwent cystoscopy under general anesthesia. Two separate cold cup biopsies were obtained from the bladder wall of IC patients using sterile techniques. The biopsy specimens were stored at −70°C until total DNA was extracted. Positive and negative controls were included in each set of PCR reactions in order to ensure that the PCR system was working correctly and to avoid false negative reactions.

Biopsy specimens of peptic ulcer patients with a proven *H. pylori* infection were used as positive controls. After DNA isolation, PCR was performed on Clo test positive biopsies obtained from proven duodenal ulcer patients. Clo test (Delta-West, Perth, Australia) is a diagnostic test which relies on urease activity in gastric biopsy specimens and hence indirectly determines the presence of *H. pylori*. DNAse-RNAse free water lacking target DNA, was used as a negative control to demonstrate the purity of the system and eliminate the possibility of contamination.

DNA extraction

DNA was extracted from gastric and bladder biopsies. The samples were incubated in lysis buffer for 6 h at 37°C. Genomic DNAs were then extracted from lysed bladder tissues. The DNA isolation method from the bladder tissues of IC patients was a modification of the technique described by Mégraud et al. [7].

PCR protocol

We used a modification of the PCR protocol described by Monteiro et al. [8]. This protocol has been used on more than 2,000 gastric biopsies over the last few years. PCR was carried out in the following manner. Amplification was performed in a reaction volume of 50 µl consisting of 2.5 µl of sample DNA, 2.5 µl of each primer and 45 µl of reaction mixture. The primers used were proposed for the urease C gene of *H. pylori* as recommended by Labigne et al. [9]. The oligonucleotide sequence of this primer pair was: A = 5′AAGCTTT TAGGGGTGTTAGGGGTTT3′, B = 5′AAGCTTACT TTCTAACACTAAACGC3′.

The negative control, containing no DNA, and the positive control containing DNA derived from *H. pylori* positive gastric biopsies, were prepared for each set of experiments. Initial denaturation was carried out for 5 min at 94°C. Next, 41 cycles of amplification were performed in an automatic thermal cycler. Each cycle consisted of three steps of 1 min each: a denaturation step at 94°C, an annealing step at 50°C and an extension step at 72°C. After amplification, the tubes were maintained at 4°C until the samples were analyzed.

Analysis of the PCR products

The PCR amplified products were analyzed by agarose gel electrophoresis. Samples were subjected to electrophoresis on a 1% agarose gel. The gel, which was stained with ethidium bromide (1 µg/ml), was examined under UV fluorescence for the presence of the amplified DNA. A sample was considered positive when a band of 294 bp was detected on the gel.

Results

The bladder biopsies of all IC patients were negative for *H. pylori* by PCR. Positive controls in each cycle demonstrated that the PCR was working properly, and negative controls ruled out the probability of false positives.

The results of a PCR amplification are shown in Fig. 1. Lanes 1–6 show the results for IC patients. Lanes 7–8 show positive controls (gastric biopsies) and lane 9 shows a negative control. The two gastric biopsies had

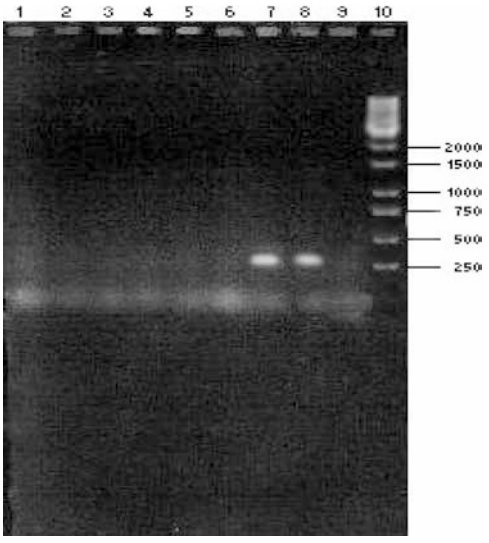

Fig. 1 PCR amplification of DNA products from one group of interstitial cystitis patient's bladder biopsies. *Lanes 1–6*: *Helicobacter pylori* negative interstitial cystitis patients. *Lanes 7* and *8*: *H. pylori* positive controls show the amplification of the 294 bp fragment. *Lane 9*: negative control. *Lane 10*: molecular weight marker (1 kb DNA ladder)

an amplified fragment of 294 bp which was scored as positive for the presence of *H. pylori*.

PCR did not detect any *H. pylori* DNA in any of IC patients.

Discussion

Although the etiology of IC is unknown, several features suggest that it may be an infectious disease. Patients with IC are twice as likely to have a history of urinary tract infection as normal individuals, and are 10–12 times as likely to have a history of childhood urinary tract disorders [5]. In addition, the predominance in women with the syndrome, the usually acute onset of symptoms, the inflammatory changes seen on histopathological examination, and the presence of immunoglobulin A deposits in bladder epithelium suggest that IC may be caused by an infectious organism [5].

The first molecular evidence demonstrating the presence of bacterial DNA in the bladder tissue of IC patients was reported in 1993. Domingue and colleagues used general 16S rRNA bacterial primers with a PCR approach to search for the presence of bacteria in bladder biopsies from patients with IC [10]. They demonstrated gram negative bacterial DNA in bladder biopsies from 29% of patients with IC. Molecular evidence of bacterial DNA in these patients indicates that bacteria present in the bladder are not culturable on ordinary media, even with meticulous methods. The authors suggested that if the microorganisms are intracellular, cell-associated or adhere rigidly to the bladder mucosa, urinary cultures may not adequately reflect the microbial content of the tissue [10].

A few years later, Heritz and colleagues identified bacterial genes with the PCR method in bladder biopsies and urine specimens. However, the biopsies and urine cultures in both IC and controls were negative in the study. They hypothesized that bacteria can not be cultured by conventional methods in IC due to the adherence or invasion of bacteria into the bladder wall [3]. In another study, Domingue and colleagues treated a group of IC patients with rifampicin and ciprofloxacin [11]. All patients showed a gradual improvement in their symptomsfrom the second to the fourth week. One group of patients demonstrated long-term cure and required no further treatment of their IC symptoms. The authors implied that such a prolonged response strongly suggests that a secondary or placebo effect had not occurred [11]. Furthermore, it is interesting that these antibiotics are very effective drugs for the eradication of *H. pylori*.

Although microorganisms may not be causative agents, their presence may lead to immune and host cell responses that can exacerbate the inflammatory response. Bladder biopsies from patients with IC frequently show an inflammatory type of reaction. Typically, there are inflammatory and ulcerative changes in bladder biopsies, despite an inability to recover any infectious agent from the urine.

H. pylori is the most common infection worldwide. In developed countries, infections occurs in more than 50% of adults, while developing countries have infection rates reaching 90%. The highest incidence is seen in communities with poor sanitation. Most people infected with *H. pylori* are asymptomatic, suggesting that other factors are also necessary for the development of *H. pylori* associated gastrointestinal disease [12].

During the last 10 years it has been demonstrated that *H. pylori* may exist outside of the gastric mucosa. It has been detected by PCR in the feces and saliva of patients with gastritis [13, 14]. Fecal-oral and waterborne modes of transmission may lead to perianal and vaginal colonization in women. This anatomical localization may allow bacteria to enter the bladder [13, 14].

In 1994, Isogai and colleagues inoculated *H. pylori* into the bladders of mice and caused an intense inflammatory reaction. They demonstrated that *H. pylori* may invade the bladder but they did not evaluate the long-term effect on the bladder [5].

Recently, English and colleagues observed a patient with IC whose symptoms improved dramatically after treatment for *H. pylori* infection. They decided to perform a study to determine the incidence of *H. pylori* antibodies in women with IC [15]. They found *Helicobacter* seropositivity in 22% of patients with IC and 35% in their control group. No statistical difference was detected between the two groups. They suggested that the incidence of infection with *H. pylori* is not increased in IC, and it is unlikely to be a causative factor. However, clinical studies investigating the role of *H. pylori* in IC patients using antibodies may suffer from major drawbacks.

Utilizing PCR technology, investigators found that people with *H. pylori* negative gastric samples have *H. pylori* DNA in their saliva, and all had a marked systematic antibody response [12]. In addition, it was observed that *H. pylori* antibody titers decrease after the eradication of the bacteria with antibiotics. Cullen and colleagues demonstrated that patients who were treated with antibiotics had at least a 50% fall in antibody titer in 3–4 months. Also, after a 12 month follow-up, 85% of patients became serologically negative [16]. Therefore, studies investigating the incidence of *H. pylori* antibodies may not accurately reflect reality for IC patients.

PCR is the most sensitive and objective method for the detection of *H. pylori*, and is accepted as the gold standard for detection in biopsy specimens by numerous investigators [17, 18]. Lage and colleagues compared PCR and other diagnostic methods for the detection of *H. pylori* and found that PCR is at least as sensitive as culture for the detection of infection in patients [18]. According to the literature, PCR was found to be highly specific and sensitive for *H. pylori* identification [19, 20]. In another study, Fabre and colleagues compared PCR with culture, rapid urease test (clo test) and histopathological tests for the detection of *H. pylori* in gastric biopsy specimens. They found PCR to be the most sensitive and specific test in their study [20].

This study uses PCR for the detection of *H. pylori* in tissue samples from 32 patients. PCR amplification results from all patients with IC were negative. Positive controls were detected in each set of PCR reactions, indicating that the negative result is a true result.

Recently, Agarwall and Dixon investigated the presence of *H. pylori* nucleic acid in fresh and paraffin-embedded biopsy specimens but they did not find any evidence of *H. pylori* DNA [21].

All previous studies have evaluated the incidence of *H. pylori* serologically. To the best of our knowledge, this is the first study using PCR technology on IC patients, and we found that bladder tissue specimens such patients were consistently negative for *H. pylori* DNA.

Conclusion

The role of an infectious agent in the etiology of IC is still undetermined. Today, the general consensus is that the etiology is multifactorial. Because of the presence of chronic inflammation in patients with IC, an infectious etiology has frequently been considered. Moreover, infection may lead to immune and host cell responses that could initiate or exacerbate an inflammatory state. This study does not negate the possibility that infectious agents may play a role in the etiology of IC, but a role for *H. pylori* appears to be negated.

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