ARTICLE / INVESTIGACIÓN

Study the relationship between *Helicobacter pylori* and bladder cancer

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Abstract: Given that bladder cancer is one of the most common cancers, and *Helicobacter pylori* infection also has 30-80% prevalence in different communities, this study investigates the role of *H. pylori* in developing bladder cancer; From December 2013 to February 2020, 200 patients with bladder tumors who underwent bladder tumor resection through the urethra in Kamkar-Arabnia Hospital were included in this study. *H. pylori* Ab, IgA, and IgG tests were first requested from all patients. If their antibodies were positive, other periodic tests including creatinine-sodium-potassium, Prothrombin Time (PT), Prothrombin Time Test (PTT), and International Normalized Ratio (INR), urinalysis, and culture were taken. The obtained results were analyzed using SPSS software version 25, and in the chi-square test, P <0.05 was considered a significant level; (3) Results: Based on laboratory findings, 66.5% of patients were *H. pylori* + (p <0.05). The result of the PCR test was positive in 4% of all patients. Besides, 6% of patients who tested positive for *H. pylori* Ab also showed positive PCR tests. Further studies are needed to investigate the association between *H. pylori* infection and bladder tumors to evaluate the proper role of *H. pylori* in tumors of the urinary system, especially the bladder and prostate, which have not been treated or reduced by treating *H. pylori*.

Key words: *Helicobacter pylori*, Bladder cancer, Urinary system, Polymerase Chain Reaction.

Introduction

Most published research points to several factors that cause cancer, such as toxins, drugs, smoking, and obesity. However, there are few studies on cancer development through bacterial infections. Besides, the mechanisms of cancer through bacterial infections are not well understood. *H. pylori* are the first known bacterium to cause stomach cancer and may also be associated with cancer out of the human stomach[1]. Therefore, there is a lot of focus and attention on *H. pylori* infection nowadays. This bacterium, which lives in the upper gastrointestinal tract, is found in half of the world's population. Its prevalence in geography, ethnicity, age, and socio-economic factors is very high in developing countries and less in developed countries[2,3]. *H. pylori* is a gram-negative flagellate, microaerophilic, and helical bacterium that causes gastritis and can eventually cause stomach cancer[4-7]. Studies have recently shown that this bacterium also causes organs outside the digestive system[6,8,9]. Other studies suggest that *H. pylori* may cause bladder and prostate inflammation or involve other organs[10]. On the other hand, vitamin D3 deficiency may also cause prostate cell proliferation and cancer[11]. *H. pylori* infection causes chronic inflammation that can lead to metaplasia, dysplasia, and cancer[6,10,12]. *H. pylori* infection is one of the risk factors in cancer development. However, its presence does not mean the definitive development of cancer[13]. The World Health Organization identifies it as a class 1 carcinogen because *H. pylori* in the stomach increase cancer risk by six times[5,14]. Given that bladder cancer is one of the most common cancers and *H. pylori* infection also has 30-80% prevalence in different communities[5], this study investigates the role of *H. pylori* in developing bladder cancer.

Materials and methods

Ethical consideration

The ethical committee approved this research's Qom University of Medical Sciences principles, Qom, Iran (Ethical number: IR.MUG.REC.1395.69). Additionally, Written consent was obtained from all patients included in the study.

Study design and patients

In a dissertation study from December 2013 to February 2020, 200 patients with bladder tumors referred to Kamkar-Arabnia Hospital, Qom, Iran, were assessed. After confirming the bladder tumor by cystoscopy[15], patients underwent resection of the bladder tumor through the duct[16].

Antibody detection

First, all patients underwent *H. pylori* antibody (Ab), Immunoglobulin A (IgA), and IgG tests[17]. For this purpose, peripheral blood was collected to determine anti-H pylori IgG and IgA serum levels. ELISA method (Accubind®, USA) was used to determine serum anti-H pylori IgG and IgA levels. The serum samples were diluted to 1/100. Other steps were performed according to the instructions of the manufacturer.

Complementary tests

After antibody detection, other periodic tests, including creatinine-sodium-potassium PT, A partial thromboplastin time (PTT), Prothrombin Time Test and INR (PT/INR), urinalysis, and culture were performed on patients with positive antibodies. Furthermore, Polymerase Chain Reaction (PCR) test was performed after the surgery[18].

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DNA extraction

The removed bladder tissue was immediately frozen in liquid nitrogen and stored at -80 °C until the experiment. A maximum of 25 mg of tissue was divided into small pieces and placed in a 1.5 mL microcentrifuge tube. Genomic DNA was extracted from the bladder tissues using the DNA extraction and purification kit (Qiagen, Valencia, CA). DNA was extracted directly from each tissue sample and used as a template to identify the specific *H. pylori* 16S rRNA gene. The quality and purity of extracted DNA samples were checked by gel electrophoresis and NanoDrop device (MA, USA), respectively[19-21].

PCR procedure

H. pylori was identified using the 16S rRNA-based PCR (primers: HP-F: 5'-CTGGAGAGACTAAGCCCTCC-3' and HP-R: 5'-ATTACTGACGCTGATTGTGC-3')[22]. PCR circumstances and volumes were done according to described method[22]. Briefly, PCR was performed in of 50 µL volume. Ingredients were amplified in a device (Eppendorf Co., Germany) at several temperatures, including 1 cycle at 94 °C for 2 min, 30 cycles of 30 s at 95 °C, 30 s at 60 °C, and 30 s at 72 °C and another one cycle of 8 min at 72 °C. Positive control was H. pylori 26695. The negative control was PCR-grade water (Thermo Fisher Scientific). Electrophoresis was performed by agarose gel (2.5%) at 120 V for 30 min. UVI doc system was applied for gel visualization[23,24].

Data analysis

Obtained data were analyzed by SPPS.V.25 according to the Chi-square test. P <0.05 was level of significancy[25,26].

Results

Table 1 shows the demographic characters of the studied patients and the distribution of *H. pylori*. The distribution of male and female patients amongst the examined population was 65% and 35%. One-hundred and thirty-three out of 200 (66.5%) examined samples were positive for *H. pylori*. Findings revealed that 85% of samples had positive homogeneity. Additionally, 87.5% of patients were cytologically positive. Besides, 82.5% of patients were diagnosed using ultrasonic technology. The majority of examined patients were educators (29%), followed by the farmer (28%) and labor (18.5%). Of the 200 patients in the study, 89 patients had high blood pressure (p <0.05) and were taking aspirin (ASA). There were 120 smokers (P <0.05). However, 25% of patients had no risk factor.

Figure 1 shows the age distribution of patients examined in the present study. The mean age was 67 years, and the ratio of men to women was 1.9. The prevalence of bladder cancer was significantly higher in men than in women (P <0.05). 61-80 years old men have the highest cancer incidence (P <0.05).

Table 2 shows the histopathological features of the bladder cancer examined in the present research. Transitional cell cancer (TCC)was the most commonly identified cancer type (87.5%), while adenocarcinoma (2.5%) was the less commonly identified.

Table 3 shows the German immune lab test findings for the detection of *H. pylori* amongst examined samples. A total of 133 out of 200 (66.5%) cases were recognized as *H. pylori*-positive using the German immune lab test (P <0.05).

Table 4 shows the PCR results for the detection of *H. pylori* in diverse kinds of bladder cancers. The result of the PCR test was positive in 4% of all patients. Besides, 6% of patients who tested positive for *H. pylori* Ab also showed positive PCR results.

Discussion

Infectious diseases have been considered health-threatening issues in the last centuries[27-40]. Studies have shown that common organisms in saliva include three specimens of *H. pylori*, Campylobacter, and Neisseria cinerea, which are also present in the gastrointestinal tract. In vitro, these samples can catalyze many drugs and cause long-term gastrointestinal infections by causing nitrosamine compounds and gastric cancer[41]. However, in a study conducted by Heidari in Qom (2020), it was found that vitamin D3 deficiency plays a role in causing Benign prostatic hyperplasia (BPH) and possibly cancer[11]. Also, in the study of Alireza Abdollahi *et al.* on 126 patients, 33.3% had prostatitis with pelvic pain, and 84 patients in the control group had no symptoms. All were positive for *H. pylori* and antibodies, although they had no prostatitis symptoms, detrimental to *H. pylori* in inflammatory prostate disease[13]. In this study conducted in Iran, many of these bacteria were identified in the prostate, BPH, and prostate cancer. *H. pylori* were examined by immunohistochemistry (IHC) and PCR, and the results were determined by DNA sequencing. However, *H. pylori* have been reported positively in one case immunohistochemistry[13]. In the study by Michaud *et al.* (2004), men with gastric tumors had a higher prevalence of bladder cancer, but cancer risk was not higher in patients with a duodenal ulcer[42]. Gastric ulcers were significantly more common among patients with gastric cancer than renal cancer. The gastric/duodenal ulcers proportion in the gastric group was 6.5, and the renal cancer group was 0.33[42]. In this study, *H. pylori* were identified as a risk factor for gastric and duodenal cancer. This bacterium causes stomach ulcers in the duodenum due to high acidity, but in the stomach, low acid production, gastritis, and ulcer disorders cause poor absorption of antioxidants, oxidative stress, and high levels of nitrates. Nitrates and nitrosamine compounds are also known as bladder carcinogens[42]. Due to this condition, it also occurs in the bladder as it does in the stomach. In this study, the condition closest to *H. pylori* was not correlated with gastric cancer, which seems to include a direct carcinogenic effect of the bacterium in the other studies was associated with *H. pylori* in 70-80% cases was positively correlated to gastric cancer[42]. Oral sex is one of the most common sex practices in the world. *H. pylori* transmitted via the act of sex through the urethra may lead to infection[42]. Several studies have shown that the transfer of bacterial metabolites of *H. pylori* can play a role in developing urinary tract cancers[43]. Matsumoto's study showed that *H. pylori* infection caused Hodgkin's lymphoma, regressed by *H. pylori* treatment. Finally, further studies are needed to prove the role of *H. pylori* infection directly or indirectly in various tumors, including the urinary syste[44].

Infection and chronic inflammation have been recognized as essential predisposing factors for carcinogenesis and tumors. International agency for cancer (IARC) research has estimated that approximately 11% of cancers are related to infectious diseases like bacteria, viruses, and parasites. Human cancer is caused by infectious agents such as *H. pylori*, Human papillomavirus (HPV), Epstein-Barr virus (EBV), Sickle hemoglobin (HbS), and Hepatitis C virus (HCV), and human immunodeficiency viruses (HIV)[14]. Chronic inflammations are accountable for about 25% of cancer cases. Environmental issues, including HCV, HPV, HBV, and *H. pylori* infections, may be accountable for around 65-80% of gastric cancers, 80% of hepatocellular cancers, and 90% of cervical cancers[14]. Under inflammation conditions, reactive oxygen species (ROS) and reactive nitrogen species (RNS) are made from inflammatory

Demographic characters	Frequency (%)
Total population	200
Male	130 (65%)
Female	70 (35%)
***H. pylori*-positive**	133 (66.5%)
***H. pylori*-negative**	67 (33.5%)
Homogeneity-positive	170 (85%)
Non-homogeneity	30 (15%)
Cytology-positive	175 (87.5%)
Cytology-negative	25 (12.5%)
Ultrasonic-positive	165 (82.5%)
Ultrasonic-negative	35 (17.5%)
Job title	
Educator	58 (29%)
Farmer	56 (28%)
Labor	37 (18.5%)
Hygienics	4 (2%)
Mechanic	15 (7.5%)
Driver	11 (5.5%)
Housewife	19 (9.5%)
High blood pressure with aspirin taking	89 (44.5%)
Smoking	120 (60%)
Without risk factor	50 (25%)

Table 1. Demographic characters of the studied patients and distribution of H. pylori.

and epithelial cells. These agents are capable of causing damage to various cellulars such as nucleic acids, proteins, and lipids. In this regard, *H. pylori* infection could affect the chronic bladder inflammation related to releasing large amounts of pro-inflammatory and vasoactive substances such as IL-1, IL-7, IL-1, and TNF-a or eicosanoids (leukotrienes, prostaglandins) and acute-phase proteins involved in the number of inflammatory diseases[10]. Besides, *H. pylori*-induced cytotoxin promotes intracellular survival of bacterium, modulates host immune responses, and induces autophagy because *H. pylori* as an intracellular microorganism invade and replicate in the cells. Compared to the translocation of *H. pylori* from the oral cavity to the stomach, *H. pylori* may reach the bladder through to the urethra, contaminated by saliva, and so on[45].

In the study by Heidari *et al.*[11], *H. pylori* infection was present in the urinary secretions of 8-hydroxy-2-deoxyguanosine, which causes DNA damage. In the 24-hour urine study of the subjects, 8ohdG was significantly higher in *H. pylori*-infected individuals than in the control group. This study found that 8ohdG is one of the most abundant lesions in DNA. The 8ohdG generated by ROS is caused by a radical oxygen attack on DNA and interferes with DNA repair[43]. This bacterium is significantly involved in developing bladder cancer and causes inflammation of the stomach, duodenum, and gall bladder[4]. *H. pylori's* role in developing lower gastric lymphoma has also been identified[44]. Recently, the association of this bacterium with urinary tract infection has been confirmed[13]. In a pilot study in patients with chronic prostatitis and pelvic pain, more people had a positive *H. pylori* antibody than in the control group[45, 46]. However, it is not clear exactly how *H. pylori* are transmitted and why some individuals become symptomatic, and some do not[4]. Bacteria are likely to be transmitted through feces, mouth, saliva, and contaminated water and food[47]. *H. pylori* is present in several locations, including adhesions to epithelial cells or inside vacuoles in epithelial cells. This bacterium stays in the lipid tissue and carbohydrates around the membrane

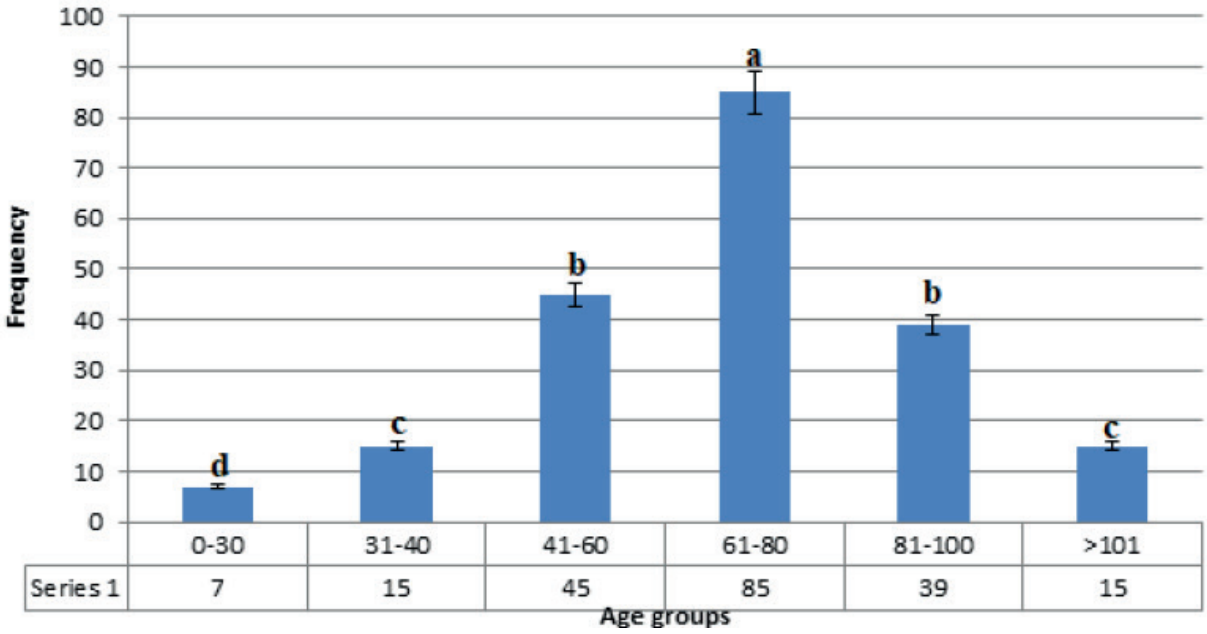

Figure 1. Age distribution of patients with bladder carcinoma. Dissimilar letters in each column show statistically significant differences (P <0.05).

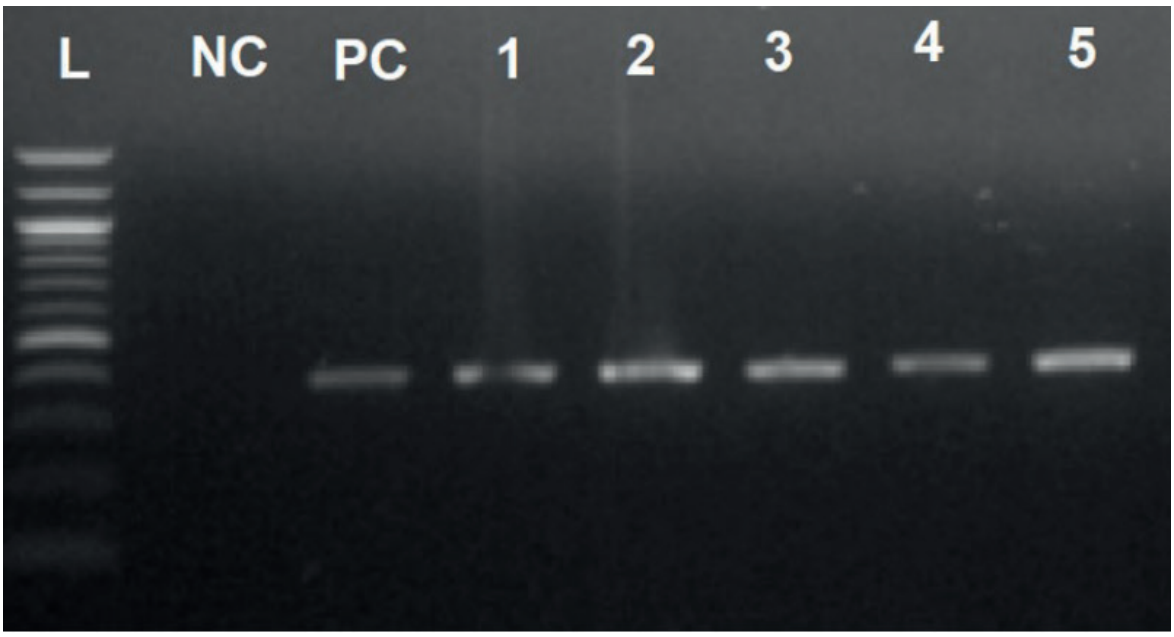

Figure 2. PCR electrophoresis. L: Ladder (100 bp), NC: Negative control, PC: Positive control, 1-5: Positive samples for H. pylori (446 bp).

by creating adhesio[45]. The reason that suggests the role of *H. pylori* in the development of bladder disease is the observation of *H. pylori* in other organs associated with other cancers, and this case has been identified for a long time[48].

Bladder malt lymphoma also resolves after *H. pylori* treatment[45,49]. Because *H. pylori* can cause infection into the bladder and prostate mucosa through the urethra (oral sex, anal sex, etc.) it causes chronic inflammation and the process of prostate and bladder cancer[45]. Al-Marhoon, in various studies that have examined the association between *H. pylori* and urological diseases, has stated that the most crucial reason for the role of *H. pylori* in causing chronic inflammation is that it leads to lymphoma. In a study by Shria Kumar *et al.* on 371,813 people diagnosed with Helicobacter pylori infection, treatment of *H. pylori* infection reduced gastric cancer risk only if eradication was successful[50]. As shown in the study of Heidari *et al.*, BCG injection effectively treated interstitial cystitis[51]. Bacillus Calmette-Guerin (BCG) injection can also be used to treat high-grade bladder cancers[52]. In this study, we had eight positive PCR cases of bladder tumors. Considering that there were 133 positive antibody tests in the study, the small number of patients due to infection is not conclusive. Of course, laboratory and individual disorders may play a role. Although, laboratory and individual disorders may play a role in this regard.

Type of cancer	No (%) patient
Transitional cell cancer (TCC)	175 (87.5)
Squamous cell carcinoma (SCC)	15 (7.5)
Adenocarcinoma	5 (2.5)
Mix	5 (2.5)

Table 2. Histological features of bladder cancer.

Table 3. Findings of the German immune lab test.

Total cases	*H. pylori* +	*H. pylori* -
200	133 (66.5%)	67 (33.5%)

	TCC	SCC	Adenocarcinoma	Mix
PCR +1	3	5	-	-

Table 4. PCR results for the detection of H. pylori in diverse bladder cancers.

Conclusions

The current research showed that *H. pylori* infections might predispose factors for bladder cancer. In this regard, a total of 133 out of 200 (66.5%) cases were recognized as *H. pylori*-positive using the German immune lab test (P <0.05). Additionally, 6% of patients who tested positive for *H. pylori* Ab showed positive PCR results. Further studies are needed to investigate the association between *H. pylori* infection and bladder tumors. These studies should investigate the proper role of *H. pylori* in tumors of the urinary system, especially the bladder and prostate, which have not been treated or reduced by treating *H. pylori*.

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