Elevated urinary epithelial membrane antigen: novel evidence of the association between H. pylori and bladder cancer progression

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Abstract

More investigations are required to evaluate the association between H. pylori infection and bladder cancer (BC) aggressiveness. This study aimed to evaluate this association using a comparison of urinary epithelial membrane antigen (EMA) levels between H. pylori infected and non-infected BC patients. Using enzyme linked immunosorbent assay (ELISA), urinary EMA was detected in H. pylori-infected and non-infected BC patients with different tumor outcomes. H. pylori infection was more prevalent in BC (65.3%) patients compared to patients with benign bladder cystitis (33.3%). Elevated urinary EMA levels were associated with BC (3.8±0.3 vs. 1.7±0.1 µg/mL). Among H. pylori infected patients, high concentrations of urinary EMA were also related to BC aggressiveness including MIBC (4.2±0.2 vs. 2.7±0.2 µg/mL; OR=11.5) and high histological grades (4.5±0.3 vs. 2.9±0.2 µg/mL; OR=12.3). In conclusion, elevated urinary EMA in patients infected with H. pylori compared to non-infected BC patients,

this study may confirm the significant association between H. pylori and BC aggressiveness.

***Keywords*:** Urinary epithelial membrane antigen; worse outcomes; Bladder cancer; Infection; *H. pylori*.

1. Introduction

Helicobacter pylori (*H. pylori*) is a common spiral-shaped gram-negative bacteria (Sabbagh et al., 2019). In more than half of the global population, *H. pylori* is present and it can cause varied diseases ranged from peptic ulcer to gastric cancer (GC) (Crowe, 2019; Cho and Jin, 2022). While *H. pylori* is confirmed to be causative factor of some gastric disorders such as GC, gastritis and mucosa-associated lymphoid tissue (MALT)-lymphoma, its involvement in gastrointestinal and other systems is being thoroughly reported (Gravina et al., 2020).

Worldwide, bladder cancer (BC) is one of the most frequent tumors (Zhang et al., 2023). Although patients with nonmuscle-invasive BC (NMIBC) display favorable survival rates, about 50% of them may relapse during follow-up, while up to 20% progress to muscle-invasive BC (MIBC) (Kim et al., 2023). As invasion existent give significant therapeutic and prognostic implications for BC patients (Ripoll et al., 2021), accurate understanding of factors associated with disease progression and worse outcomes is imperative (Kim et al., 2023). Although MALT lymphoma has been found to be arising in the bladder (Bates et al., 2000), additional investigations are needed to evaluate the association between *H. pylori* and BC aggressiveness.

This study aimed to evaluate urinary epithelial membrane antigen (EMA), protein that found on the body exterior cell surface and is used as established BC biomarker (Attallah et al., 2015), levels in BC patients and to assess their relation with the disease progression and tissues invasion as a new evidence that prove the association between *H. pylori* and BC aggressiveness.

2. Materials and Methods

2.1. Patients

A total of 230 (95 BC, 75 benign bladder cystitis, 60 healthy) subjects were prospectively collected from Urology and Nephrology Center, Mansoura University. BC diagnosis was mainly according to urine cytology and, in few cases of suspicious results, bladder biopsy was taken for histopathological screening. Patients with benign bladder cystitis were having hematuria or inflammatory smears. BC patients were classified based on tumor stage and differentiating grade. Patients with pelvic tumors, another urological tumor history or recent urological surgery were excluded. Study protocol was in consistent with 1975 Helsinki ethical guidelines and approved by Mansoura University Hospitals Ethical committee.

2.2. Diagnosis of *H. pylori*

Despite antibodies detection in *H. pylori* diagnosis, we used here a very sensitive and specific immunoassay for H. pylori antigen (58 KDa) detection as described by Attallah et al. (Attallah et al., 2004; Attallah et al., 2022), considering active infection.

2.3. Detection of Urinary EMA

Urine levels of EMA were measured using enzyme-linked immunosorbent assay (ELISA) according to previously described protocol of Attallah et al. (Attallah et al., 2015; Attallah et al., 2014). Briefly, urine, after dilution in 50mM carbonate/bicarbonate buffer (pH 9.6), were added to microtiter plates at 4°C overnight. Non-binding sites were blocked with 0.1% bovine serum albumin. Mouse monoclonal specific antibodies against EMA diluted in phosphate buffer saline (PBS; pH=7.4) was added and incubated at 37°C for two hours. Then after washing, alkaline phosphatase labelled goat anti-mouse antibody (Sigma, USA) diluted in PBS-T20 was incubated at 37°C for one hour. After second washing, p-nitrophenyl phosphate substrate (1 mg/mL) was added and incubated for ten minutes at 37°C. The developed colour intensity was obtained by measuring the absorbance at 450 nm (Plate reader, Σ960, Metretech Inc, Germany). For EMA concentration measurement, a standard curve (0.6-37.5 µg/mL) was produced from dilution series of previously purified EMA.

2.4. Statistical analysis

All statistics were done by SPSS Inc., Chicago v.20. Quantitative and qualitative parameters were described as mean±SD and absolute numbers (percentages), respectively. Appropriately, variables comparisons between groups were assessed by Student's t-test or chi-squared test. Odds ratios (OR) of elevated EMA in *H. pylori* infection for BC severity were measured. $P<0.05$ was statistically significant.

3. Results

3.1. Patients' characteristics

Mean age of BC patients was 61.7±11.2 years and there was a predominance of male gender (72/95). The benign (54/75 were males; 58.8±11.4 years) and healthy (51/60

were males; 55.5±8.2 years) controls were age- and sex- matched (P>0.05) with BC patients. BC patients were classified based on histological grade (22 with Grade I and 73 with Grades II-III) and tumor invasion (32 with NMIBC and 63 with MIBC).

3.2. Elevated EMA levels revealed that infection was associated with BC progression

Despite healthy individuals, *H. pylori* infection was more prevalent in BC (65.3%) patients compared to patients with benign bladder cystitis (33.3%) (Figure 1). Moreover, elevated urinary EMA levels were associated with BC (3.8±0.3 µg/mL) patients compared to benign controls (1.7±0.1 µg/mL) (Table 1). In BC patients, great concentrations of urinary EMA were also related to the disease aggressiveness including MIBC (4.2±0.2 *vs.* 2.7±0.2 µg/mL) and high tumor histological grades (4.5±0.3 *vs.* 2.9±0.2 µg/mL) among *H. pylori* infected patients. These elevated EMA (> 2 µg/mL) levels were associated with higher risk to develop advanced tumor stages (OR=11.5) and high grades (OR=12.3) (Figure 2).

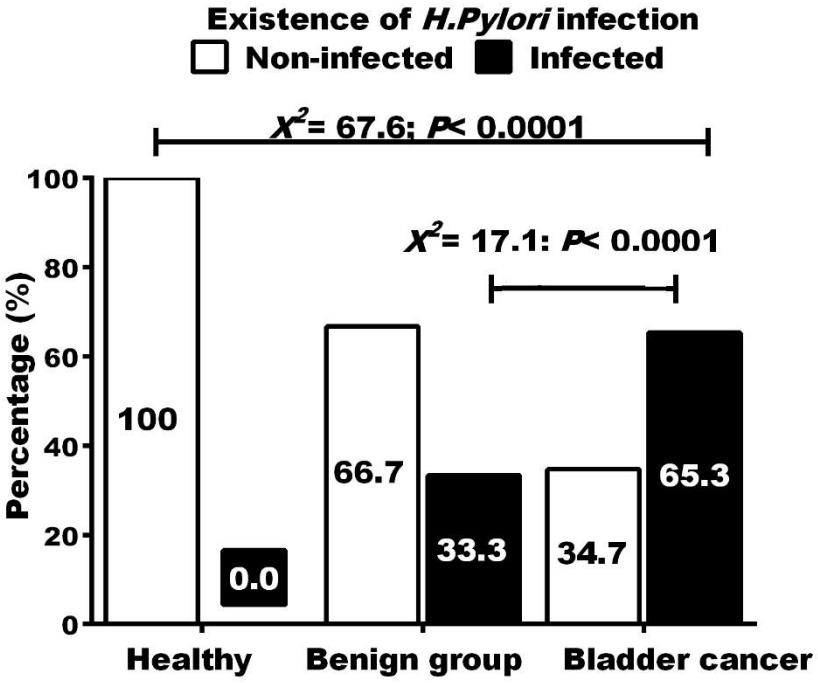

Figure 1. *H. pylori* antigen detection rates among BC patients compared to patients with benign bladder diseases and healthy controls.

Table 1: Urinary EMA among H. pylori infected BC patients

Categories	**Level of EMA (µg/mL)**	***P value***
Benign group	1.7±0.1	0.0001
Bladder cancer	3.8±0.3	
Tumor stage		
NMIBC, stages < T2	2.7±0.2	0.0001
MIBC, stages ≥T2	4.2±0.2	
Tumor histological grade		
Low-grade (GI)	2.9±0.2	0.0001
High-grade (GII-III)	4.5±0.3	

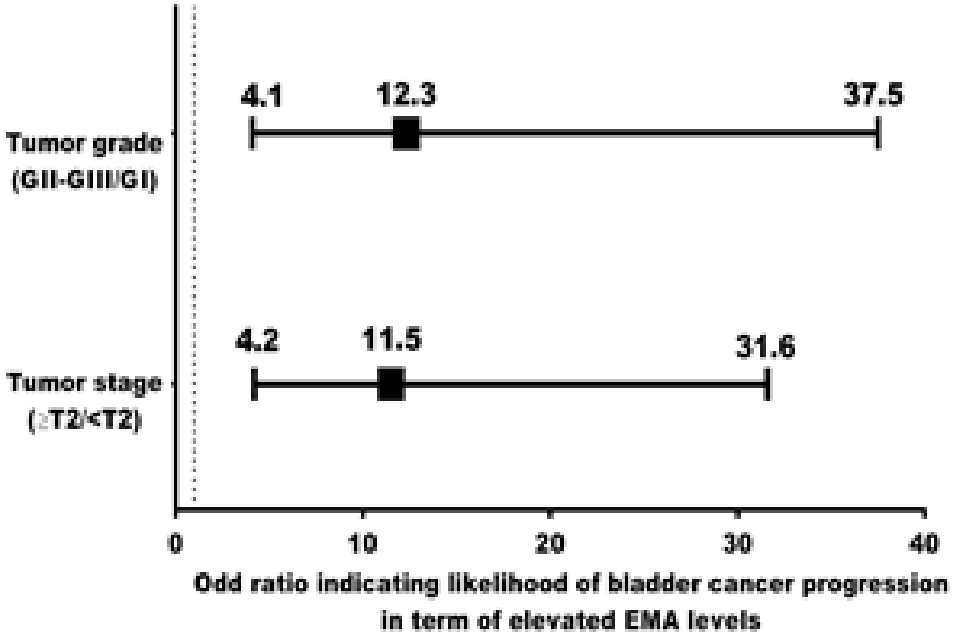

Figure 2. Adjusted risks to develop advanced tumor stage and grade in case of elevated urinary EMA and *H. pylori* infection. This is represented bydds ratio (with 95% confidence intervals (CI)). Elevated EMA was >2.0µg/mL according to receiver-operating characteristic curve.

4. Discussion

As it has been found to cause different gastrointestinal disorders including chronic atrophic gastritis, duodenal ulcer and gastric lymphoma and adenocarcinoma, *H. pylori* infection is found also to be related to other extra-intestinal and -gastric diseases (Hassan et al., 2015). Thus, it is a focus of attention nowadays (Al-Marhoon, 2008). According to the World Health Organization, *H. pylori* has been designated as a definitive carcinogen (Al-Marhoon, 2008). Beside the exact mechanism related to tumor development through bacterial infection is not well understood, the role of *H. pylori* infection in BC development is still a matter of debate (Al-Marhoon, 2008). In this study, we aimed to evaluate this association in terms of the relation between the bacterial infection and elevated urinary EMA, as an established tumor marker, in BC progression.

In this study, *H. pylori* infection was found to be more prevalent in BC (65.3%) patients compared to patients with benign bladder cystitis (33.3%). Among *H. pylori* infected patients, elevated EMA urinary levels were associated with BC (3.8±0.3 µg/mL) development (levels of benign controls were 1.7±0.1 µg/mL) and worse outcomes including MIBC (4.2±0.2 vs. 2.7±0.2 µg/mL; OR=11.5) and high tumor grades (4.5±0.3 vs. 2.9±0.2 µg/mL; OR=12.3).

EMA expression was reported immunohistochemically in BC to be marker for disease development and progression (Asamoto et al., 1989; Takashi et al., 1987).

Despite normal urothelial epithelium and using monoclonal antibody, positive EMA staining in transitional cell carcinomas could be divided into highly stained cells, slightly stained tumor and strongly EMA positive cells. Also, urinary EMA was reported to be a promising non-invasive BC marker that is associated with worse out comes of the disease (Attallah et al., 2015). Thus, in this study the elevated EMA in BC patients who infected with *H. pylori* infection may reveal that the bacterial infection is associated with tumor aggressiveness.

H. pylori-infected patients are closely related to chronic inflammations that consequently connected to cancer development (Hassan et al., 2015). Schematically, there are 2 pathways have been suggested to elucidate the relation between cancer and inflammation. In the intrinsic pathway, hereditary adjustments that progress to neoplastic transformation tiger inflammatory mediators' production; in the extrinsic pathway, the main push that purposes the growing in cancer hazard is chronic inflammation (Hassan et al., 2015, Segura-López et al., 2015).

Some studies reported that MALT lymphoma arising in the bladder (Kempton et al., 1997). Among patients with bladder MALT lymphoma, a history of chronic cystitis is common (Al-Maghrabi et al., 2001). Thus, an association of infectious agents with chronic antigenic stimulation and consequently tumor occurrence has been postulated (Al-Marhoon, 2008). In the same line, by eradication of *H. pylori* infection, MALT lymphoma regression has been found (van den Bosch et al., 2002). Also in patients with chronic bladder cystitis, *H. pylori* vaccines (intravesical vacA-based) has been hypothesized to protect against bladder MALT lymphoma (Pastuszka et al., 2007).

5. Conclusions

In the light of elevated urinary EMA in bladder cancer patients infected with *H. pylori* compared to non-infected BC patients, this study may confirm the significant association between H. pylori and BC development. It is also used the urinary EMA to evaluate the association between the bacterial infection and the disease aggressiveness. More studies are needed to investigate the exact biological pathways of this relation.

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Conflicts of Interest

None

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