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Potential association between *Helicobacter Pylori* infection and bladder cancer progression

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Investigating the relation between *Helicobacter Pylori* (*H. Pylori*) and bladder cancer (BC) can potentially prevent future BC progression. *H. Pylori* is considered as a class I carcinogen, which is responsible for many disorders as gastritis, duodenal ulcers, gastric adenocarcinoma, also some extra gastrointestinal tract diseases. We aimed to evaluate the relation between *H. Pylori* infection and BC progression by evaluating *H. Pylori* antigen in BC patients in comparison with patients with benign bladder diseases and healthy controls. Moreover, we studied the relation between elevated *H. Pylori* antigen levels and BC patient in terms of ages, sex, BC types and BC stages and grades. A total of 57 BC patients, 54 patients with benign bladder diseases and 75 healthy individuals were included. *H. Pylori* antigen was detected using western blot and ELISA. In contrast to healthy individuals, sharp band corresponding to *H. Pylori* antigen (58-kDa) was obtained in sera of patients with bladder diseases who infected with *H. Pylori*. *H. Pylori* antigen detection rates increased in BC patients (56.1%) than patients with benign bladder conditions (31.5%). Among BC patients, *H. Pylori* antigen detection rate was significantly increase (P= 0.023) with the patients ages. According to tumor aggressiveness features, *H. Pylori* detection rates was significantly higher in late stage than early stage (P =0.007) and higher in high grade compared to those with low grade (P = 0.01). Furthermore, infected people with *H. Pylori* were more likely to have sever tumor features including late stages and high grades than those without *H. Pylori*-infection with odd ratio (OR=7.6 and 5.4 respectively) which may indicate that *H. Pylori* infection increases the risk of disease progression. In conclusion, patients who under BC treatment should consider *H. Pylori* diagnosis to prevent future disease progression as *H. Pylori* infection were shown to be associated with tumor progression.

Keywords: *Helicobacter Pylori* antigen, *H. Pylori* infection, Bladder cancer, Progression

INTRODUCTION

Bladder cancer (BC) is the world's 9th most commonly diagnosed malignant tumor and the 13th most common cause of death related to cancer with about annually 429000 new cases and 165000 deaths reported in worldwide (Jacyna et al. 2019). In Egypt, it is the 3rd common tumor after liver and breast cancers (Khateeb et al. 2017). According to the demographic data and continuous increase in BC incidence in previous years, it reports that the number of BC cases in 2035 might double compared to 2012 (Jacyna et al.2019). That is also because of its association with many risk factors such as smoking, schistosomiasis, exposure to environmental cancerogenic, genetic mutations and some

medical conditions (Cumberbatch et al. 2018).

Infection and chronic inflammation have been established as risk factors for cancer and malignancy. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are produced under inflammatory conditions from inflammatory cells and epithelial cells, resulting oxidative stress which causes DNA damage and DNA mutation (Kawanishi et al. 2017; Murata, 2018). There are several infectious agents are classified as class I carcinogen such as *Helicobacter pylori (H. Pylori)* (Butcher et al. 2017). *H. Pylori* infection is highly common in human, infecting nearly half population in the world. *H. Pylori* is a gram negative bacterium, spiral shaped, flagellated and belongs to *Helicobacteraceae* family (Frost et al. 2019; Kira and Isobe, 2019). *H. Pylori* may cause gastrointestinal tract disorders such as chronic gastritis, duodenal ulcer, gastric ulcer, gastric adenocarcinoma or gastric mucosa-associated lymphoid tissue (MALT lymphoma) and also some extra gastrointestinal disorders as iron deficiency anemia, pernicious anemia, neurological diseases and metabolic disorders (Toyoshima et al. 2017; Tsay and Hsu, 2018) . Colonization of *H. Pylori* with human stomach is an important gastric adenocarcinomas risk factor (Knekt et al. 2006). Combined analysis of some prospective studies reported positive strong association between non-cardia gastric adenocarcinoma and *H. Pylori* antigens seropositivity (Kamangar et al. 2006). Moreover, several studies have reported that *H. Pylori* may have a role in progression of bladder cancer (Al-Marhoon, 2008).

In this study, we, therefore, aimed to identify *H. Pylori* antigen in bladder cancer patients in comparison with patients with benign bladder inflammation and healthy controls. Also, to evaluate association between *H. Pylori* infection and bladder cancer by comparing antigen detection rates between bladder cancer patients and all non-cancer individuals using ELISA. Also, to evaluate the relation between *H. Pylori* infection and progression of the disease by evaluating antigen serum levels in patients with different stages and grades.

MATERIALS AND METHODS

Subjects

This study included 111 Egyptian patients who were diagnosed with bladder diseases from Mansoura University, (Urology and Nephrology Center) and (Clinical Oncology and Nuclear Medicine department), Mansoura, Egypt. There were categorized into two groups, group 1 consisted of 57 patients (45 male and 12 females) with bladder cancer aged between 20-86 years with mean age ± SD of 55.2±11.4 years. Of BC patients, 48 were diagnosed by histopathology with transitional cell carcinoma (TCC) and 9 with squamous cell carcinoma (SCC). Group 2 included 54 patients (39 male and 15 females) with non-cancerous bladder cystitis (benign growth) aged between 36-75 years with mean age ± SD of 52.6±11.0 years. Moreover, a group of 75 healthy individuals (68 male and 7 females) aged between 21-50 years with mean age ± SD of 48.3±5.5 years were included as negative controls group. We have got an informed consent from each individual according to the guidelines of Mansoura University Hospitals and Helsinki declaration Ethics and Scientific Committees.

Identification of *H. Pylori* antigen in serum samples

By sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), proteins of the serum samples were separated according to the method of Laemmli (1970). Following electrophoretic separation, protein bands were transferred by Western blotting onto nitrocellulose membrane (0.45 mm pore size, Sigma, USA) according to Towbin et al. (1979). Then, they were immunostained using monospecific antirabbit for *H. Pylori* antigen (ABC Diagnostics, New Damietta, Egypt) and anti-rabbit IgG alkaline phosphatase conjugate (Whole molecule, Sigma) according to (Attallah et al. 2004).

Quantitation of *H. Pylori* antigen serum levels

H. Pylori antigen levels were quantified using ELISA. Serum samples is diluted (1:400) in coating buffer (pH 9.6, 0.1 M carbonate/bicarbonate buffer), then 50 µl per well of diluted serum coated in a microtiter plate overnight at 4°C. After blocking with BSA (0.7% in coating buffer), 50 µl /well of *H. Pylori* monospecific antibody (1:100 in PBS) was added and incubated at 37 °C for two hours. Then, 50 µl /well of anti-rabbit IgG alkaline phosphatase conjugated (1:700 in PBS) was added. An enzyme detection system composed of nitrophenyl phosphate substrate (50 µl /well) was added. Color intensity at 405 nm was a function of *H. Pylori* antigen concentration.

Statistical analysis

H. Pylori antigen serum levels were expressed as mean ± standard deviation (SD),

whereas categorical variables were expressed as percentages or numbers. To compare *H. Pylori* infection proportions, Pearson's chi-squared (X^2) test was used. Differences in *H. Pylori* antigen serum levels among the included subjects' groups and between different tumor features were assessed by *ANOVA* or *t*-test as appropriate. *P* values <0.05 were considered statistically significant. SPSS (SPSS Inc., Chicago, IL) and GraphPad Prism (GraphPad, San Diego, CA) programs were used for all statistical analyses.

RESULTS

Bladder cancer was associated with *H. Pylori* infection

Sharp band was observed at 58-kDa in serum samples of patients with bladder diseases who infected with *H. Pylori* but no reaction with serum samples of healthy individuals was observed (Figure 1A). *H. Pylori* antigen was detected in 56.1% (32/57) of BC patients and 31.5% (17/54) of patients with non-cancerous bladder cystitis (benign) conditions. No case of 75 healthy controls was positive for *H. Pylori* antigen (X^2 =53.6, *P*<0.0001) (Figure 1B).

H. Pylori antigen levels were associated with BC patient age, gender and cancer type.

As shown in (Figure 2A), among bladder cancer patients, *H. Pylori* antigen detection rate was significantly increase (*P*=0.023) with the patients ages (30.8%, 52.0% and 78.9% for age of 20-39 years, 40-59 years and ≥60 years; respectively). *H. Pylori* antigen was detected in males (60.0%) more than females (41.7%) but the difference was not significant (*P* =0.255) (Figure 2B). In terms of BC type, *H. Pylori* antigen was detected in TCC patients more than SCC (58.3% and 44.4%, respectively) without significant differences (*P* = 0.441) (Figure 2C).

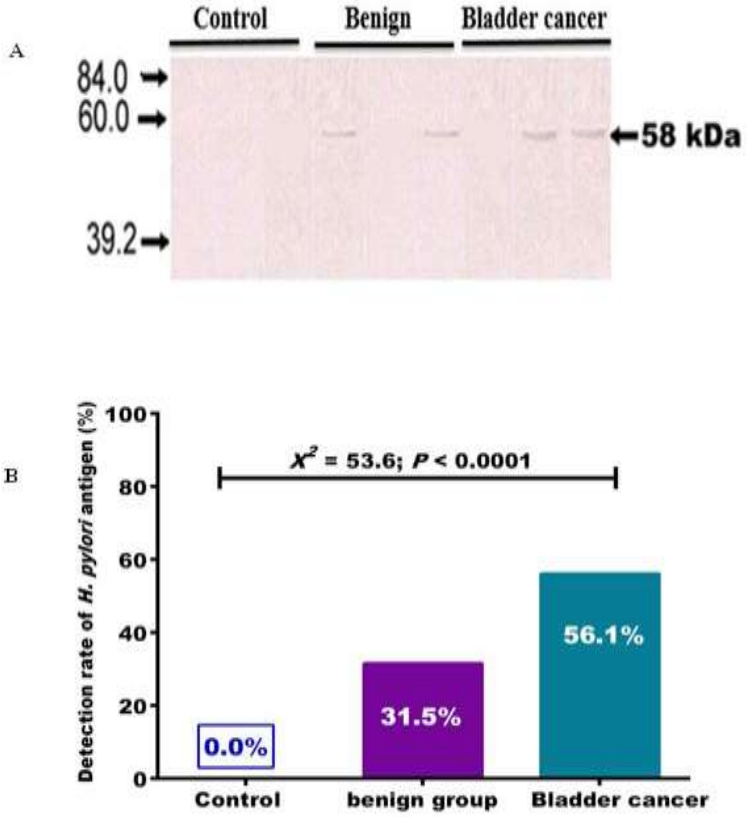

Figure 1: *H. Pylori* infection and bladder cancer. (A) Western immunoblot analysis for *H. Pylori* antigen. Molecular weight marker (sigma) included Bovine serum albumin (84.0kDa), Ovalbumin (60.0kDa) and carbonic anhydrase (39.2kDa). (B) detection rates of *H. Pylori* antigen among three groups.

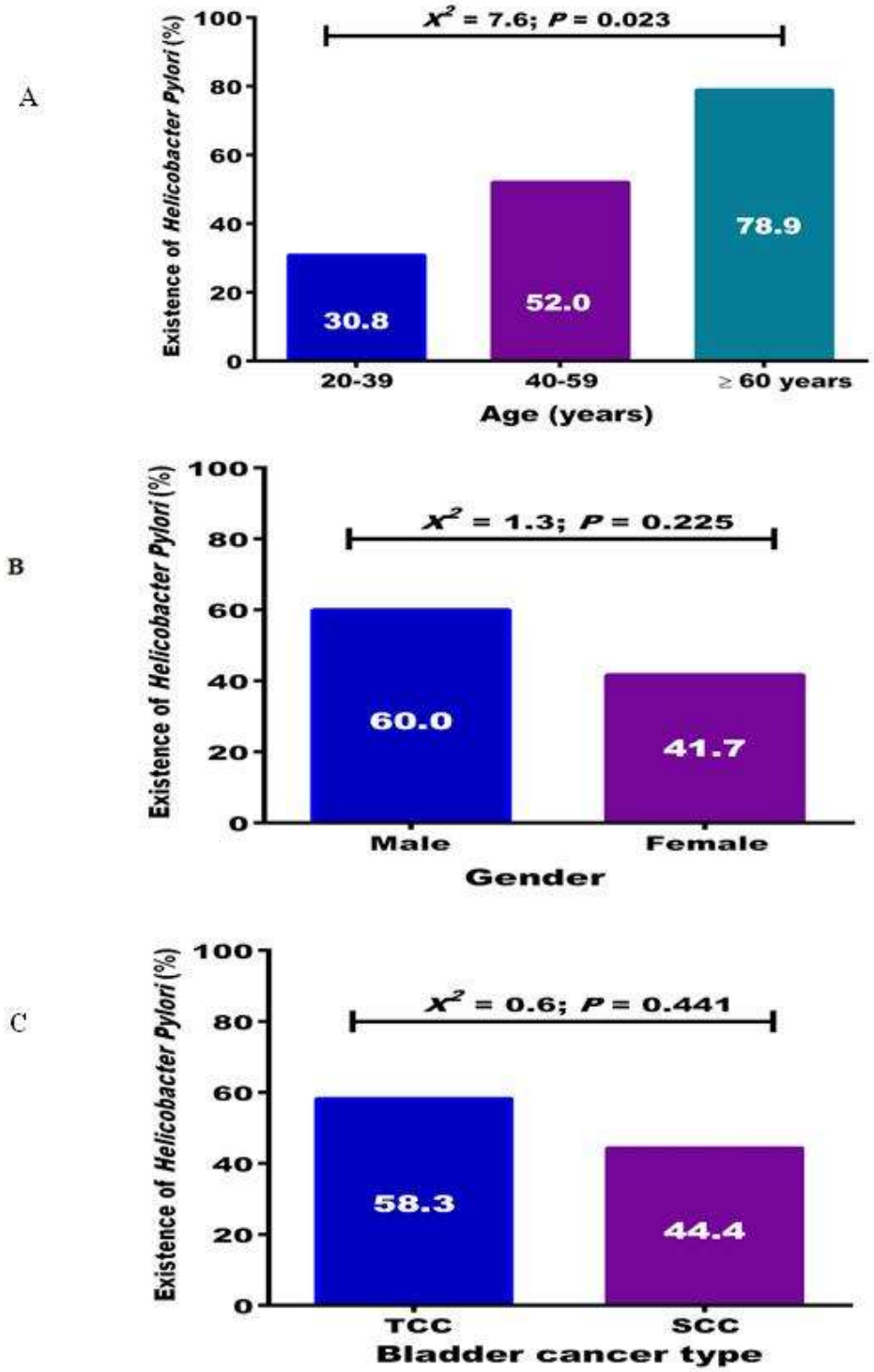

Figure 2: Co-existence of *H. Pylori* infection and bladder cancer in terms of age (A), gender (B) and (C) bladder cancer type: TCC and SCC.

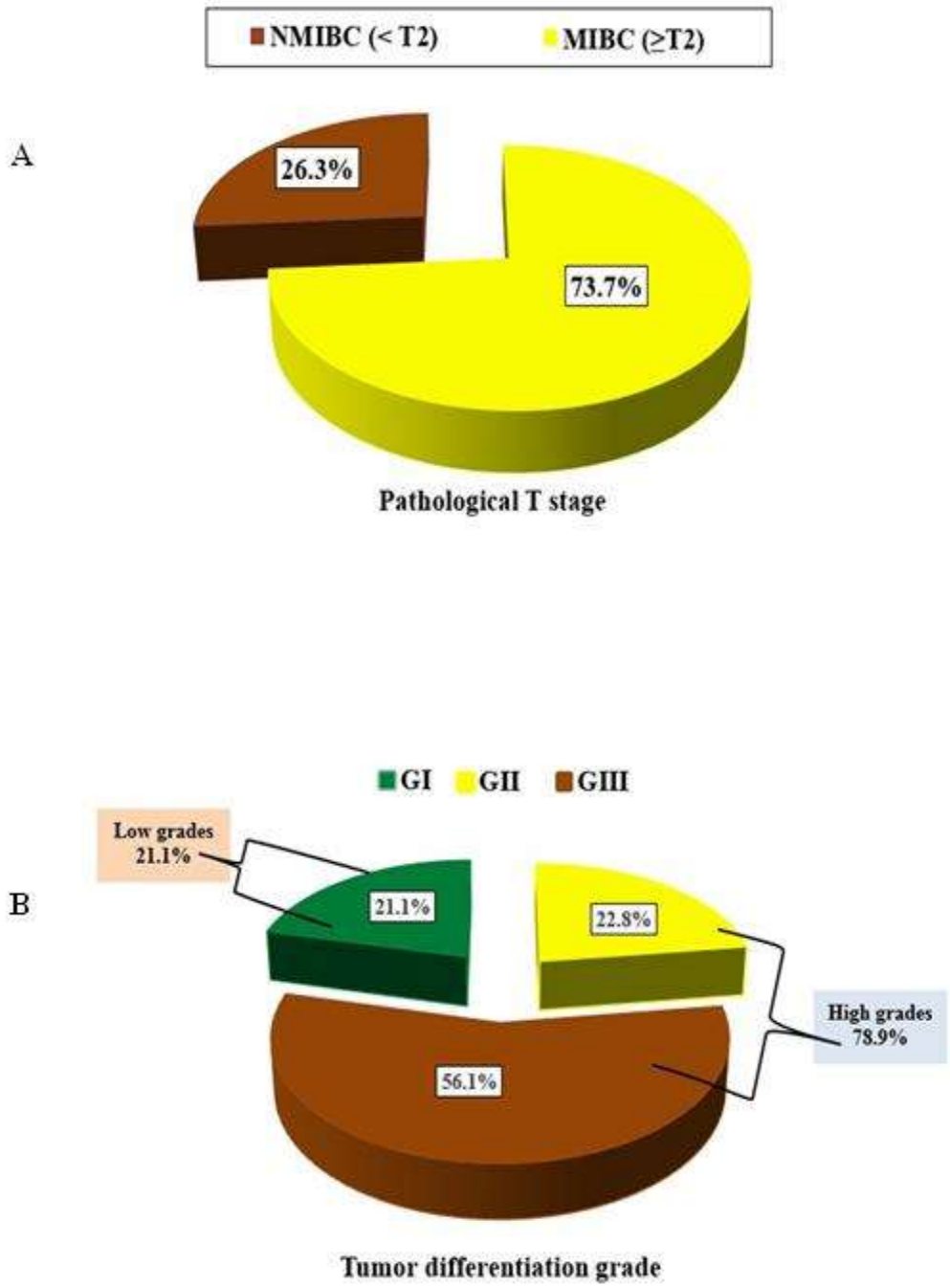

Figure 3: Classification BC patients according to (A) tumor stages, (B) tumor grades.

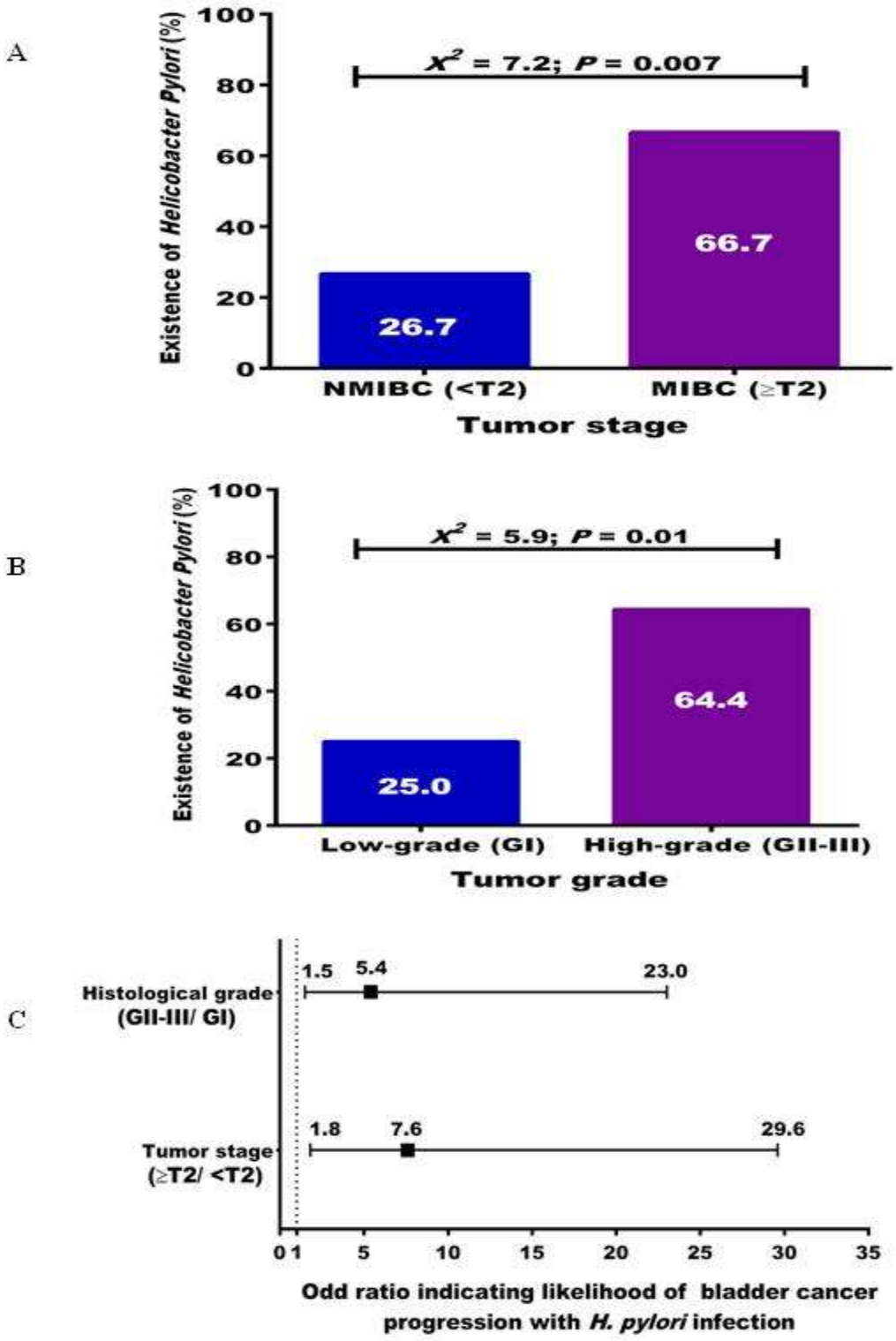

Figure 4: Detection rate of *H. Pylori* antigen among bladder cancer patients according to (A) tumor stage: NMIBC and MIBC, (B) histological grade, (C) the likelihood of bladder cancer progression with *H. Pylori* infection.

Elevated *H. Pylori* antigen were associated with BC progression

In (Figure 3), BC patients were classified according to tumor stages and grades. The detection rate of *H. Pylori* antigen in patients with late tumor stages [≥T2 muscle invasive bladder cancer (MIBC)] were higher (*P* =0.007) than early stages BC patients [<T2 non-muscle invasive bladder cancer (NMIBC)] (Figure 4A). Also, *H. Pylori* antigen detection rate was significantly high (*P*=0.01) in BC patients with high grade GII-III compared to patients with low grade GI tumors (Figure 4B). Patients with *H. Pylori* infection were more likely to have sever tumor features including late stages and high grades than those without *H. Pylori* infection (OR=7.6 and 5.4 respectively) (Figure 4C).

DISCUSSION

H. Pylori was identified in the past ten years as one of the main causes of gastric and duodenal ulcers. Beside gastrointestinal disorders, *H. pylori* is considered to have a role in cardiovascular disorders, neurological diseases, metabolic disorders, kidney diseases and pancreatic cancer (Atuğ et al. 2004; Lu et al. 2018). Several molecular studies have demonstrated the role of infectious microorganisms in chronic prostatitis and bladder cancer, also have revealed the existence of *H. Pylori* genetic DNA in prostatic tissues (Al-Marhoon et al. 2015). However, the association between *H. Pylori* infection and bladder cancer remains unclear.

In this study for the first time, we identified *H. Pylori* antigen using western blotting in bladder diseases at 58- KDa. similar *H. Pylori* antigen molecular weights were previously reported (Attallah et al. 2004). Here, BC patients also tended to have significantly (*P*< 0.0001) higher *H. Pylori* infection rates compared to patients with benign bladder disorders. The detection rate of *H. Pylori* antigen among bladder cancer patients was significantly increase (*P*=0.023) with the patients ages. Although the detection rate was found to be higher in terms of sex and BC type, the difference was statistically nonsignificant (P =0.255, P = 0.441; respectively)

For the first time in this study, *H. Pylori* antigen detection rate was significantly high (*P* =0.007) in patients with late tumor stages [≥T2 (MIBC)] compared to early stages BC patients and the detection rate of *H. Pylori* antigen was significantly higher in high grade GII-III BC patients than with low grade GI tumors (*P*=0.01).

In 1993, bacterial DNA was firstly found in tissue of interstitial cystitis patients. *H. Pylori* was inoculated into mice bladders in 1994 and caused an intense inflammatory reaction .Increasing evidences revealed that *H. Pylori* has been found to induce cystitis, which may further lead to bladder cancer, these growing evidences are linking *H. Pylori* infection with many urological diseases (Atuğ et al. 2004; Abdollahi et al. 2016).

A study reported that there is an association between bladder cancer and peptic ulcer (Michaud et al. 2004). Moreover, the major risk factor of peptic ulcer is *H. Pylori* (Sverdén et al. 2019). None of the previous studies regards the association between bladder cancer progression and *H. Pylori* infection. Here for the first time, we evaluate the effect of *H. Pylori* infection on the BC progression in terms of staging, grading and also patient ages. In this study elevated *H. Pylori* antigen detection rates were related with tumor aggressiveness features as late tumor stages and high grades.

P53 (tumor suppressor p53) is one of the most important biomarkers for diagnosis gastric cancer which codes for a protein and regulates the cell cycle (Ghatak et al.2018). *H. Pylori* has an ability to suppress P53 by activation AKT1 that resulting in phosphorylation and activation of HDM2, then p53 degradation in epithelial cell of gastric, increasing the risk of gastric cancer. Also, P53 mutation is responsible for 50% of BC cases (Wei et al.2010; Wu et al. 2019).

CONCLUSION

The results of this study urge oncology physicians, treating BC patients, to consider early detection of *H. Pylori* among those patients to prevent future tumor progression. Further studies are needed to identify carcinogenesis mechanisms induced by *H. Pylori*, Specifically bladder carcinoma.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

ACKNOWLEGEMENT

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AUTHOR CONTRIBUTIONS

Attallah AM, El-Far M and Omran MM were chief investigators who conceptualized and designed the study. They equally participated in

all parts of this final manuscript. Ashraf M was investigator who collected data from the literature, collected samples and carried out the work. All authors read, reviewed and approved the final manuscript.

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