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Original Article

Study of Immuno-physiological Alterations Induced by *Helicobacter Pylori* Infection Among Population in El-Baida City, Libya

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ABSTRACT

Background. This work was carried out to investigate the changes in hematological and some physiological parameters among patients with H. pylori Admitted in El-Beida City, Libya. **Methods.** This study was carried out in 11 consecutive months (Oct 2018 to Aug 2019). A total of 181 adult patients were divided into two groups upon presence and absent of antibodies against to H. pylori. The positive group had 34 (60.7%) males, 102 (81.6%) females and the negative group had 22 (39.3%) males and 23 (18.4%) females. **Results**. Highest prevalence with positive H. pylori infection was noted in age group 20-50 years. Overall, 181 patients, 70.166 % cases were found positive with H. pylori in serum and 32.6% of cases were positive with H. pylori in stool. Most red blood parameters were significantly decreased in patients compared to control subjects. The numbers of white blood cells and percentage number of neutrophils were increased significantly in positive cases of *H. pylori* infection. A percentage value of lymphocytes was decreased significantly in positive cases. Results of platelets parameters were decreased in all subjects. In term of type of blood groups, the highest prevalence among individuals with blood group A, B and O. Mean levels of liver and kidney function parameters in the sera were in normal range all patients. In the current study, association of *H. pylori* infection with smoking, marital status and allergic disease were studied. Over positive cases 70.58 % of these cases were smoking. Upon marital status, *H. pylori* prevalence was higher in married subjects (65%) as compared to single subjects. And prevalence of non-allergic was higher in negative subjects with *H. pylori* as compared to positive subjects. **Conclusion.** H. pylori infection is usually lifelong therefore a positive test, physiological alteration usually denotes active infection unless the patient has received eradication therapy.

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INTRODUCTION

Helicobacter pylori (*H. pylori*), a gram-negative, helical bacilli that live in the gastric epithelium was first isolated in 1983 [1]. It is transmitted via the fecal-oral, gastro-oral, or oral-oral routes [2, 3]. It is able to thrive in the gastric environment due to urease [4], motility [5], and adherence to gastric epithelium [6, 7], which allow it to neutralize gastric acid, penetrate through the mucus layer to the gastric epithelium, and colonize. Although infection with *H. pylori* persists without treatment, the majority of infections do not lead to symptoms or gastrointestinal disease (8, 9). In the 30 years that have elapsed since its discovery, more than 50 extragastric manifestations of *H. pylori* infection have been reported, involving a range of medical specializations including cardiology,

dermatology, endocrinology, gynecology and obstetrics, pneumology, neurology, odontology, ophthalmology, otorhinolaryngology, pediatrics, and hematology [10]. This human pathogen is known to induce several gastric disorders, but may also be associated with extragastric diseases like anaemia, dyspepsia, and some immunological disorders [11, 12]. It is important to note that of the extragastric diseases described in the medical literature that are possibly associated with *H. pylori*, only three such conditions have been incorporated into the guidelines: iron deficiency, vitamin B12 deficiency, and primary immune thrombocytopenic purpura. A negative association between *H. pylori* infection and asthma development has been observed. Studies with both children and adult subjects [13, 14] have reported a lower prevalence of *H. pylori* infection in asthmatic patients.

Earlier studies reported association between *H. pylori* presence in the liver and disease progression in those with viral chronic hepatitis and cirrhosis [15, 16]. In developing countries its prevalence reaches 75% in populations <20 years of age, and 90-100% in populations >50 years of age, without differences in gender distribution. It has been directly associated with gastroduodenal peptic ulcer, cancer, gastric lymphoma and with other entities such as nonulcer dyspepsia, lymphocytic gastritis, Menetrier's disease and protein-losing enteropathy [7].

Among Libyan population, the prevalence of *H. pylori* infection is not well known [17, 18]. The rate of infection in a group of dyspeptic patients reported to be around 82% [19]. Infection acquired early in childhood and reached up to 94% in older age [20]. Prevalence of *H. pylori* was significantly increased in association with marital status, education and low socioeconomic status [20, 21]. There is a lack recorder about this infection. In addition, no detailed study on such infection is being recorded so far in El-Baida City in Northeast of Libya. Therefore, the aim of this study was designed to study immuno-physiological alterations induced by *H. pylori* infection among population in El-Baida City, Libya.

METHODS

The study protocol was reviewed and approved by Bioethics Committee at Biotechnology Research Center (BEC-BTRC) with Ref No: BEC-BTRC 08-2018. Inclusion criteria involve: patients agreement participation in the study.

The study was performed between Oct 2018 to Aug 2019 at different private labs (Al-Raze Lab, Al-Mejher Lab and Al-Azher Lab) with Zoology department at Omar Al-Mukhtar University. One hundred and eighty one patients were enrolled in this study. All subjects (who included in this study) were diagnosed with gastrointestinal symptoms related to presence of *H. pylori*. Blood and stool samples were collected from each patient. At same time: questionnaire was answered from each patient including: age, sex, smoking habit, marital status and presence/absence of allergic.

Two blood samples were taken from each patient. One sample (2 ml) for all CBC tests was performed by automatic blood cell analyzer (XP-300 Automated Hematology Analyzer, Sysmex American, Inc. CBCs were performed on EDTA as anti-coagulated samples. The other sample (2 ml) was centrifuged at 3,000 rpm for 10 minutes to obtain serum. These serum samples were stored at −20°C until studied for other parameters. Host ABO and Lewis antigen phenotypes were determined with a macroscopic tube agglutination technique using commercially available murine anti-A, anti-B, anti-AB, anti-Lea and anti-Leb blood grouping reagents (Bioscot, Edinburgh, UK).

Level of IgG/IgM antibodies against *H. pylori* were investigated by enzyme-linked immunosorbent assay (ELISA) kit (Euroimmun Lübeck, Germany). Serum specimens were analyzed as described by the kit procedure. Level of IgG/IgM antibodies against *H. pylori* in stool were investigated by ELISA (Connex GmbH, Martinsried, Germany) method in stool specimens that were stored at −20°C. The results were analyzed spectrophotometrically. Absorbance was read at 450/650 nm.

Liver function parameters were estimated in all subjects by a commercially available test kits method on automatic analyzer from Biotechnologies, Germany with the manufacturer's instructions strictly adhered to using spectrophotometers (Humalyzer Junior). Determination of creatinine was based upon the Jaffe reaction with de-proteinization creatinine, in alkaline picrate solution forms a color complex), and serum urea which was determinate enzymatically by kits from Biomarhreb, Germany using spectrophotometer (Humalyzer Junior) for both tests [22] .

Statistical analysis was carried out in Minitab software 17. Statistical significance was assessed using two samples T- test analysis after detection normal distribution to the data and appropriate $P < 0.05$ consider significant. The numerical data were shown as number and percentage. To find the significant difference between the observed variable studied, Pearson Chi-Square Test for Association was used, P value was taken as level of significance at <0.05.

RESULTS

A total number of 181 patients (56 males (30.9%), 125 females (69.1%) were enrolled in this study. Upon this study 181 patients who positive with *H. pylori* infection was found 136 (75.12 %) and negative with *H. pylori* infection was 45 (24.86 %). Over all 181 patients who positive with *H. pylori* infection was found 34 (60.7%) and 102 (81.6%) for male and female respectively, as compared to negative subjects with *H. pylori* that found 22 (39.3%) and 23 (18.4%) for male and female respectively with significant deference between gender as shown in Table 1.

Results from distribution of cases upon smoking behavior in each gender separately were shown in same Table. In male subjects who were positive with *H. pylori* infection, 70.58 % of these cases were smokers and 28. 42% of them were non- smokers. Significant deference was found between percentage number smoking/non-smoking and positive cases for male subjects (P=0.000). Prevalence of non-allergic was higher in negative subjects with *H. pylori* as compared to positive subjects. At same time, *H. pylori* prevalence was higher in female subjects 11.76% as compared to male subjects (5.88%). Non-significant deference was found between percentage number of allergic/non-allergic and positive cases for male/female subjects (P=0.02). *H. pylori* prevalence was higher in married subjects 60.78% and 64.7 % for female and male respectively as compared to single subjects. Non-significant deference was found between percentage number single/married and positive cases for male/female subjects (P=0.06) (Table 1).

Results from distribution of cases upon age groups in each gender separately, (as each group consist of 10 years intervals) were shown in Table 2. Highest prevalence with positive *H. pylori* infection was noted in age group 20-30 years followed by age groups 31-40 and 41-50 years in both gender. Lowest prevalence was observed in the age group of >60 years for both genders.

Table 1. Number and percentage of (sex, smoking habits, allergic condition, and marital status) among positive/negative H. pylori infection subjects

Items	Female No of cases (%)		Male No of cases (%)		P Value
	Negative with *H .Pylori*	Positive with *H. pylori*	Negative with *H .Pylori*	Positive with *H. pylori*	
Sex	23 (18.4)	102 (81.6)	22 (39.3)	34 (60.7)	0.002
Smoking	0	0	10 (45.5)	23 (70.6)	0.000
Non-Smoking	23 (100)	102 (100)	12 (54.5)	11 (29.4)	
Allergic	20 (87)	12 (11.8)	19 (87)	2 (5.9)	0.02
Non-Allergic	3 (13)	90 (88.2)	3 (13)	32 (94.1)	
Single	6 (26.1)	40 (39.2)	6 (27.3)	12 (35.1)	0.06
Married	17 (73.9)	62 (60.1)	16 (72.8)	22 (64.4)	

Table 2. Age distribution of male and female subjects

Age (Years)	Female No of cases (%)		Male No of cases (%)	
	Negative with *H. Pylori*	Positive with *H. pylori*	Negative with *H. Pylori*	Positive with *H. pylori*
<20	3 (13.04)	9(8.82)	3(13.63)	0
20-30	6(26.08)	43(42.15)	4(18.18)	13(38.23)
31-40	4(17.39)	27(26.47)	5(22.72)	12(35.29)
41-50	2(8.69)	16(15.68)	6(27.27)	5(14.70)
51-60	6(26.08)	7(6.86)	4(18.18)	3(8.82)
61-70	1(4.34)	0	0	0
>71	1(4.34)	0	0	1(2.94)

Total cases were tested for comparison between level of IgG and IgM antibodies against *H. pylori*

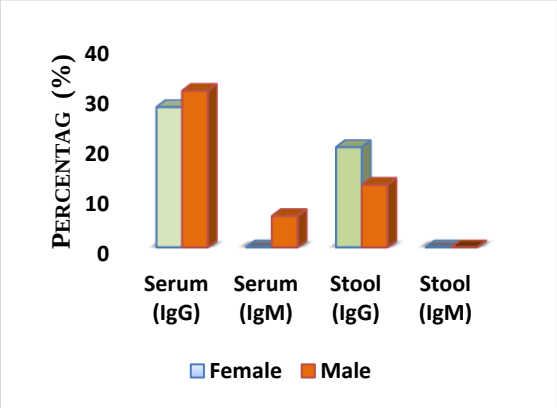

Figure 1. Percentage number of positive cases for levels of IgG and IgM against H. pylori from stool and serum

From stool and serum samples: The highest percentage of level of IgG antibody was found with serum followed by stool (Figure 1). While level of IgM antibody was found with serum followed by stool for female and male respectively. These levels were not significantly deference between level of two antibodies in each gender (P =0.306).

Comparison between percentage numbers of cases upon level of anti- *H. pylori* (IgG) antibodies in stool and serum samples among 181 subjects was illustrated in Table 3. Data from samples of stool/serum was divided to three categories upon level of IgG antibody against *H. pylori* infection: negative, equivocal and positive. Overall 181 patients, 32.596 % cases were found positive with *H. pylori* in stool and 3.9% of cases were equivocal. Overall 181 patients, 70.166 % cases were found positive with *H. pylori* in serum and 4.97 % of cases were equivocal with *H. pylori* infection. Percentage numbers of cases upon level of IgG anti-*H. pylori* was significantly deference in positive cases (serum or stool) comparison to negative cases.

Table 3. Levels of anti-H. pylori (IgG) antibodies of positive/negative cases in stool and serum samples

Three categories upon level of Antibody against *H. pylori*		Level of antibody (U/ml) Mean ±SEM	Number of case (%)
Negative < 15 U/ml	Stool	5.47± 0.38*	115 (63.5)*
Equivocal 15-20 U/ml		16.1±0.68*	7 (3.9)*
Positive >20 U/ml		63.9±3.90	59(32.596)
Negative <0.75 U/ml	Serum	0.49±0.019*	45(24.862)*
Equivocal 0.75-1 U/ml		0.79±0.017*	9(4.972)*
Positive >1 U/ml		3.06±0.12	127(70.166)

Data are expressed as mean ± SEM. Within each level of antibody in serum/stool separately, means with different superscript (*) were significantly different at p<0.05. Where means without superscripts mean that there is no significant difference (p>0.05).

Values derived from CBC, including differential cell counts were recorded for each patient who positive cases of *H. pylori* infection compared to the values of patient who negative cases of *H. pylori* infection. Generally, the results were shown a lot of similarity between both genders in terms of effects. RBC counts were studied, and the results are shown in Table 4. The levels of HGB concentrations in the female and male who positive with *H. pylori* infection were significantly decreased compared to negative subjects with *H. pylori* infection. The presence of *H. pylori* had significant effect on the percent HCT. Values of MCH, MCHC and RDW (%) were also significantly lower in patients when compared with negative *H. pylori* subjects.

The WBC and Granulocytes were increased with significantly difference in female and male positive subjects with *H. pylori* (Table 5). However, percentage a level of Lymphocytes (%) was significantly

decreased both gender with positive *H. pylori* infection when compared with negative *H. pylori* infection subjects. Results of platelets are shown in Table 5. The mean PLT (× 103/µl) number and the mean platelets volume MPV (%) in patients with *H. pylori* were decreased in both female and male subjects. Meanwhile PDW (%), PCT (%) and P-LCR (%) were also decreased without significant deference in both female and male subjects with *H. pylori* infection compared to negative subjects respectively.

Results of different blood groups were shown in Table 6. Overall subjects, the results were shown similarity with positive cases with *H. pylori* infection in both genders in term of type of blood groups. The lowest prevalence of was shown among subjects with blood group AB, and the highest prevalence among individuals with blood group A, B and O. Rh-positive subjects with *H. pylori* infection was higher than Rh-negative subjects at same blood group in both genders.

Table 4. Values of RBC, HGB, HCT, MCV. MCH, MCHC and RDW in positive/negative H. pylori infection subjects

Parameters	Female		Male	
	Negative with *H .Pylori*	Positive with *H. pylori*	Negative with *H .Pylori*	Positive with *H. pylori*
RBC (×10⁶/µl)	4.595±0.10	4.389±0.045	5.065±0.076	5.010±0.15
HGB (g/dl)	13.11±0.34	11.88±0.19*	15.44±0.32	13.94±0.45*
HCT %	38.28±0.74	35.78±0.38*	43.38±0.70	39.77±1.1*
MCV (fL)	83.72±1.4	81.94±0.83	85.73±0.99	80.8±2.2**
MCH (pg)	28.63±0.61	27.16±0.34**	30.50±0.44	28.12±0.76*
MCHC (g/dL)	34.16±0.32	32.84±0.39*	35.56±0.31	34.81±0.34
RDW (%)	19.6±5.4	15.81±0.32	13.13±0.22	13.83±0.70

Data are expressed as mean ± SEM. Within each parameter in each gender separately, means with different superscript (*) were significantly different at p<0.05. Where means without superscripts mean that there is no significant difference (p>0.05).

Table 5. Values of (WBC, Lymphocytes and neutrophil) and (PLT, MPV, PDW and PCT) in positive/negative H. pylori infection subjects.

Parameters	Female		Male	
	Negative with *H .Pylori*	Positive with *H. pylori*	Negative with *H .Pylori*	Positive with *H. pylori*
WBC (×10³/µl)	6.71±0.42	8.23±0.32*	7.64±0.41	9.64±0.69*
Lymphocytes (%)	31.8±2.9	22.4±1.6*	31.44±2.0	24.4±2.3*
Neutrophil (%)	45.2±2.8	48.8±4.5	55.2±3.3	56.2±3.9
PLT (10³/µl)	277.8±9.8	256.1±13	264.2±16	249.9±14
MPV(fL)	15.6±2.0	11.93±1.6	16.4±3.8	12.4±2.6
PDW (%)	12.11±0.81	11.46±0.38	11.43±0.54	9.97±1.3
PCT (%)	0.27±0.029	0.293±0.041	0.22±0.073	0.2235±0.017

Data are expressed as mean ± SEM. Within each parameter in each gender separately, means with different superscript (*) were significantly different at p<0.05. Where means without superscripts mean that there is no significant difference (p>0.05).

Table 6. Blood group distribution among positive/negative H. pylori infection subjects

Blood groups	Female No of cases (%)		Male No of cases (%)	
	Negative with *H .Pylori*	Positive with *H. pylori*	Negative with *H .Pylori*	Positive with *H. pylori*
A-	0	7(6.86)	1(4.54)	2(5.88)
A+	10(43.47)	39(38.23)	3(13.63)	17(50)
B-	1(4.34)	4(3.92)	0	2(5.88)
B+	3(13.04)	18(17.64)	1(4.54)	3(8.82)
AB-	1(4.34)	1(0.98)	0	0
AB+	1(4.34)	4(3.92)	1(4.54)	2(5.88)
O-	2(8.69)	5(4.90)	3(13.63)	2(5.88)
O+	5(21.73)	24(23.52)	9(40.90)	6(17.64)

The effect of the presence of *H. pylori* infection on serum (AST, ALT and ALP) and (creatinine and urea) were shown in Table 7. All mean level values were in normal range in both gender. The results of the effect of symptoms related to *H. pylori* infection on kidney function parameters (creatinine and urea) were in normal range in female and male patients.

Table 7. Levels of (AST, ALT, and ALP) and (creatinine and urea) among positive/negative H. pylori infection subjects

Parameters	Female		Male	
	Negative with *H .Pylori*	Positive with *H. pylori*	Negative with *H .Pylori*	Positive with *H. pylori*
AST (U/L)	37.2± 2.6	37.95± 1.4	38± 2.7	42.3± 2.2
ALT (U/L)	36.6± 3.1	33.75± 2	37.1± 3.9	41.5± 3.2
ALP (U/L)	166.1±9.8	155± 9.1	168± 9.9	185.4±10
Creatinine (µg/dl)	0.78± 0.027	0.76± 0.026	0.80 ± 0.28	0.84± 0.026
Urea (mg/dl)	29.27± 1.6	28.05± 1.7	29.4± 1.7	29.2± 1.8

Data are expressed as mean ± SEM. Within each parameter in each gender separately, means with different superscript (*) were significantly different at p<0.05. Where means without superscripts mean that there is no significant difference (p>0.05).

DISSCUSION AND CONCLUSION

H. pylori represent one of the most common and medically important infections worldwide [23]. However, the prevalence varies greatly among countries and among population groups within the same country [24). It occurs in at least half the world's population [25]. 181 was the total number of patients who were diagnosed with symptoms related to presence of *H. pylori* daring months of study at El-Baida City.

In this study, both of genders appear to be equally exposed to *H. pylori* infection, since quite increase infection in female than males. This result agrees with other studies in Libya [17, 20, 26, 27] and in Egypt [28]. Disagree also with a meta-analysis showed that male gender is an issue related to increased prevalence for the infection of *H. pylori* [29]. The relationship between gender and *H. pylori* infection has been controversial in other studies [30, 31]. A systematic review with meta-analysis carried out in 2018 reported that no significant difference was observed between the two genders in worldwide *H. pylori* prevalence [32]. The role of sex to put males at significantly higher risk of *H. pylori* infection compared to females was observed in many previous studies at different countries [33-35]. Higher prevalence rates among females were similarly previously reported in many studies [25, 26, 34, 35].

The influence of lifestyle on prevalence of *H. pylori* infection remains controversial. Among the lifestyle habits common among male subjects; smoking showed a high association with *H. pylori* infection. Other authors also reported that smokers were at higher risk of acquiring *H. pylori* infection [26, 28, 36-38]. Smoking was implicated to promote virulent infection in individuals by inducing the expression of virulent genes, including cag A [39]. On the other hand, in most studies, there was no significant association between smoking and *H. pylori* infection [40, 41].

Upon marital status, *H. pylori* prevalence was higher in married subjects as compared to single subjects. In contrast of other studies from different society which reported that marital status, positively correlated with positive H.pylori seroprevalence, in Germany [42], in Turkey [38] and in Libya [20, 25-27]. This observation may refer to the modes of transmission of *H. pylori* or to stress in lifestyles.

In the current study, prevalence of non-allergic was higher in negative subjects with *H. pylori* as compared to positive subjects and this in line with previous study [43]. An issue to be considered in studies showing negative associations between *H. pylori* and various atopic and allergic diseases is that *H. pylori* positivity is linked with more crowded living conditions and poor hygiene in infancy [44].

Within the age range of 20 to 70 years, highest prevalence with positive *H. pylori* infection was noted in age group 20-30 years in both genders. This finding regularly was decreased with age up to an age of 70 years. This result is on contradictory to the findings of [26, 27, 45]. The most affected ages were between 20-50 and fewer than 60 in Benghazi [17], and in Tripoli (46).

Total cases were tested for comparison between level of IgG and IgM antibodies against *H. pylori* from stool and serum samples. According to this study, the

highest percentage of level of IgG/ IgM antibodies against *H. pylori* was noted with serum followed by stool for both gender. This is consistent with study in Zeliten City [27]. However, epidemiological surveys usually use serological tests for high sensitivity and specificity which will not limit the accuracy of prevalence estimates [47]. Most of the data available on the prevalence of *H. pylori* are unsatisfactory. Ag test were considered the gold standard method for diagnosis of *H. pylori* infection. Stool Ag test is attributed high sensitivity and specificity (up to 97%) [48] and its excellent positive and negative predictive values regardless of *H. pylori* prevalence [49]. Serological tests are also useful non-invasive methods for the diagnosis of *H. pylori* infection. They are acceptable by patients because of their non-invasiveness; quick results, less liability to be affected by antibiotics [50]. It is not also affected by those local changes in the stomach that could lead to a low bacterial load and to false negative results [51].

The RBC (parameters) levels were significantly lower in cases compared to controls for both male and female subjects. Similar observations were reported elsewhere [52]. Previous studies suggested a possible pathogenic mechanism of anemia and explained it by blood loss secondary to chronic erosive gastritis and decreased iron absorption secondary to chronic gastritis and hypochlorhydria [53, 54]. Valiyaveettil *et al.*, conducted [55] conducted a randomized controlled trial with 52 anemic adult patients undergoing upper gastrointestinal endoscopy; findings were suggestive of *H. pylori* eradication improving iron status. Four meta-analyses of randomized control trials have supported this association and also suggested a role of *H. pylori* in iron absorption [56-59]

When related to *H. pylori*, mean values of total WBC and percentage number of neutrophils were found significantly higher in positive cases compared to negative cases. Leukocytosis was reported to be high in H. pylori infected patients [60-62]. The elevation of WBC in *H. pylori* observed in infected cases may be attributed to increase production of inflammatory cytokines from epithelial cells in the gastric mucosa [63]. Number of neutrophils is considered to be associated with formation, complexity, and activation of atheromatous plaque [64]. Consistent with the literature, the present study showed that the number of neutrophils increased in *H. pylori* infected subjects; moreover, the number of neutrophils increased as symptoms of H, pylori increased [60]. The decreasing in the percentage number of lymphocytes was found in this study. That was considered to be consistent with more increase in the number of neutrophils compared to that of lymphocytes and in the conditions associated with inflammation [60].

Results of platelets parameters were decreased without significant deference in both female and male subjects with *H. pylori* infection. In contrast of another study found that the rate of complete platelets response was low in *H. pylori*-positive patient [65-67]. *H. pylori* infection is a well-known cause of secondary ITP. The prevalence of *H. pylori* infection in patients with ITP is higher than age and gender matched healthy individuals [68]. Platelet count responses may occur independently of *H. pylori* infection as a result of the immune modulating effects of macrolide antimicrobials or the removal of other commensal bacteria [66].

In the current study, the highest prevalence was found among individuals with blood group A, B and O. Previous studies shown that a strong association between the O blood group and infection caused by *H. pylori* [69-71]. However, result from another study found no association between ABO blood group and presence of *H. pylori* infection [72]. Moreover, controversy exists in the literature in relation to the association of the non-secretor state and blood groups with *H. pylori* related peptic ulcer disease, with many studies measuring antibodies to *H. pylori* [73-78].

The mean levels of liver and kidney enzymes in the serum were found in normal values in *H. pylori* infection subjects. In agreement to our results, a recent study showed that all previous parameters were in normal range in *H. pylori* positive subjects [79]. However, these results disagree with study that reported there was significant difference between

both groups regarding AST and ALT between those with or without *H. pylori* infection [80, 81].

In summary, over the past year, the knowledge of *H. pylori* pathogenesis and disease development has been improved by the studies focusing on investigation of bacterial factors. Application of large-scale screening methods should have broad relevance to understanding *H. pylori* infection-mediated carcinogenesis.

Disclaimer

The article has not been previously presented or published.

Conflict of Interest

There are no financial, personal, or professional conflicts of interest to declare.

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