

Original article

Infection by CagA Positive *Helicobacter pylori* among Dyspeptic, and Healthy Blood Donors in Tripoli city

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ARTICLE INFO

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Received: 28-07-2023

Accepted: 18-08-2023

Published: 21-08-2023

Keywords. *Helicobacter pylori*, Prevalence, Dyspeptic, Cag A, Libya.

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ABSTRACT

Background and aims. *Helicobacter pylori* (*H. pylori*) infections occur earlier in life with high on geographic area, socioeconomic status, and age of the host infected. Virulence factors of *H. pylori* such as *cag* pathogenicity island (*cagPAI*) have been demonstrated to be predictors of gastric diseases. In Libya, there is no data available regarding the prevalence of *H. pylori* infection in adults dyspeptic, & healthy blood donors. This study was conducted to determine the current prevalence of *H. pylori* and its relationship with age, gender, CagA status among healthy blood donors & adult dyspeptic, patients attending Teaching Hospitals in Tripoli. **Methods.** A Blood sample of (125) dyspeptic & (175) Healthy Blood Donors participants, anti-*H. pylori* IgG & anti-CagA IgG seroprevalence were determined with ELISA method (Biotech USA). Questionnaire covering sociodemographic variables were completed by interview. **Results.** The overall, seroprevalence of *H. pylori* was (83%), & (85%) in dyspeptic, & healthy blood donors respectively. There was a gradual increase with age. No statistical difference between genders. However, *cagA* status in *H. pylori* strains was 28%, 29.5% in dyspeptic & healthy blood donors respectively. **Conclusions.** The detection of the *H. pylori* pathogenicity marker (CagA) is crucial for reducing severe gastrointestinal disease in Libya. Larger studies and molecular techniques are needed to determine the genotype of *H. pylori* strains.

Cite this article. Abdelgawad A, Nami A, Tumi A, Fitouri A, Ellafi A. Infection by CagA positive *Helicobacter pylori* Among Dyspeptic, and Healthy Blood Donors in Tripoli city. *Alq J. Med App Sci.* 2023;6(2):482-490. <https://doi.org/10.5281/zenodo.8267528>

INTRODUCTION

Helicobacter pylori is a pathogenic Gram-negative bacterium that colonizes over 50% of the world's human population. Colonization with *H. pylori* are linked to numerous gastric disorders including gastritis, peptic ulcer disease and gastric adenocarcinoma, although gastric cancer occurs in fewer than 1% of people colonized by *H. pylori*, it is still the second-most common cause of cancer mortality worldwide, and more than 50% of gastric adenocarcinomas can be attributed to infection with *H. pylori*, most people infected with *H. pylori*, however, do not develop gastric cancer and the molecular mechanisms underlying this disparity have yet to be fully elucidated (1). Several authors have emphasized the role of factors such as age, socio-economic status, poor hygiene/deficient sanitation, density/ crowded living conditions, smoking, use of a nonsteroidal anti-inflammatory drug (NSAID), blood group O, high body mass index and family history of gastric disease in the acquisition and transmission of *H. pylori*

(2). Techniques utilized to detect *H. pylori* infection are grouped as invasive and noninvasive tests and include the rapid urease test (RUT), microbiological culture, histology, and polymerase chain reaction (PCR), in which esophagogastroduodenoscopy (OGDS) is required to obtain the stomach biopsy. Noninvasive methods consist of the stool antigen test (SAT), urea breath test (UBT), and Blood test for detection of *H. pylori* antigens or anti *H. pylori* antibody (3). Many studies have proven that *H. pylori* is the most common cause of chronic or atrophic gastritis, gastric and duodenal ulcers, gastric adenocarcinoma, and mucosa-associated lymphoid tissue (MALT) lymphoma. Colonization and pathogenicity is known to be mediated by four main virulence factors: urease activity ensuring survival in the acidic stomach; flagella-mediated motility toward epithelial cells; adhesin-mediated attachment to host cells; and, in more virulent strains, host tissue damage by cytotoxins such as the vacuolating cytotoxin A (VacA), and the oncogenic CagA protein, encoded by a gene on the pathogenic island (PAI). The presence of certain CagA variants is associated with a higher risk of developing gastric adenocarcinoma due to altered host cell signaling (4). In Libya, a country of huge size, important regional differences are to occur. Nevertheless, no local data are available on the epidemiology of *H. pylori* infection; therefore, the primary aim of this study was to determine the incidence of seropositivity *H. pylori* infection among asymptomatic Blood donors. The secondary aim is to correlate the risk factors among voluntary healthy Blood donors and Dyspeptic patients in the Tripoli region and its surroundings.

METHODS

Study design and Data collection

A cross-sectional seroprevalence study was conducted from August 2016 to May 2017 among two different groups: healthy Blood volunteers and Dyspeptic patients in Tripoli city attending the Central Blood Bank and Tripoli Central Hospital. Three hundred individual samples (221 males and 79 females) were included in the study. The individual subjects were divided into two groups according to their health status, namely a Dyspeptic group and a healthy Blood donor group. A dyspeptic group is defined as a group of patients with chronic or recurrent pain or discomfort centered in the upper abdomen. The healthy Blood donors' group is defined as an asymptomatic Blood donor that displays No signs or symptoms. Based on the questionnaire data, Blood donors' individuals with a history of gastroduodenal ulcer, current chronic complaints of the upper digestive tract for more than two months (nausea, vomiting, heartburn, pyrosis, or indigestion), or those currently using anti-acid or anti-ulcer medications were excluded from the 3 Special Issue for The 5th Annual Conference on Theories and Applications of Basic and Biosciences, September 4th, 2021 study. After all subjects were examined by a physician and the purpose and procedures of the study were explained, we obtained written informed consent from each participant. A standard questionnaire was completed by direct interview to obtain individual socio-demographic data regarding each Blood donor participant (age, gender, number of family members, Blood group, smoking, source of drinking water, coffee and Tea consumption, monthly family income, family history of gastric ulcer or gastric cancer, etc.). Health status, place of residence, and medication taken one month before the interview (particularly proton pump inhibitors and antibiotics) were also recorded. A Blood sample (5 ml) was collected from each participant by peripheral venipuncture in the blood bank laboratory. Following separation, 250l serum samples were labeled and stored at -20oC until further analysis.

Determination of H. pylori

For the diagnosis of infection with *H. pylori*, three hundred (79 females and 221 Males) serum samples were collected for the study and tested for the evaluation of immunoglobulin G (IgG) antibodies against *H. pylori* by using the commercial enzyme-linked immunosorbent assay (EUROIMMUN Anti-Helicobacter pylori ELISA (IgG), Germany). The serum concentration of anti-*H. pylori* IgG was expressed in relative units (RU/ml) as no international standard is available. According to the manufacturer's instructions, the sensitivity of the kit was 100%, and a value of 5 RU/ml was used to discriminate the negative from the positive sample.

Procedure Statistical analysis

Data analysis was made by using SPSS version (24) software, Chi-square test for present of association between prevalence of *H. pylori* and different parameters, and standard of living P. values < 0.05 over considered to show significant difference.

RESULTS

Three hundred individual samples, with a mean age of 32 years, were included in the study. The overall seroprevalence of *H. pylori* among the Healthy Blood donors group was 85.1% (table 1) and was 83.2% among the Dyspeptic patients (Fig. 1).

The distribution of *H. pylori* positivity according to gender was found among healthy Blood donors (90.5% male and 64.8% female), as shown in Fig. 2. (33.6% male and 66.3% female) among dyspeptic patients, as shown in table 2.

Table 1. Prevalence of *H. pylori* infection by IgG in healthy blood donors

IgG Antibody detection	Result of <i>H. pylori</i> IgG Test in No.	Result of <i>H. pylori</i> IgG test in %
Positive	149	85.1%
Negative	26	14.9%
Total No.	175	100%

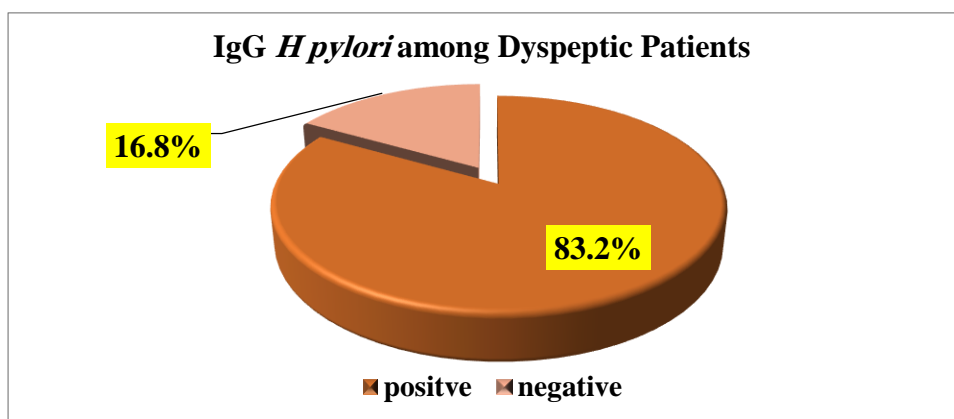


Figure 1. Overall seroprevalence of *H. pylori* among the Dyspeptic Patients

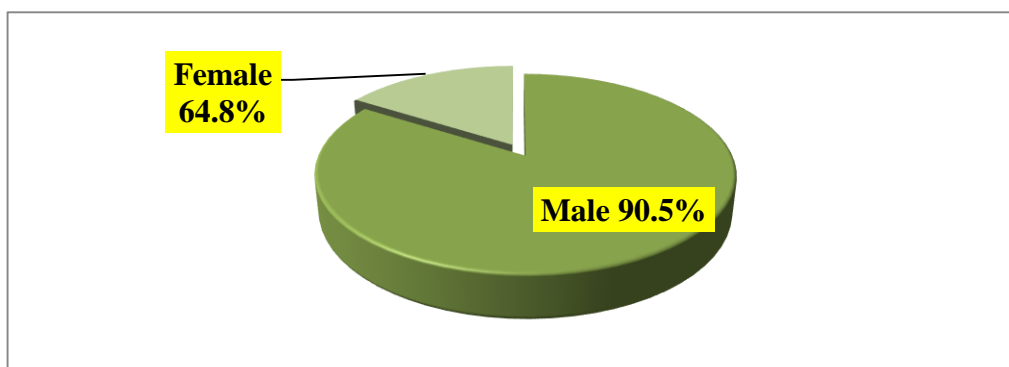


Figure 2. Seroprevalence of *H. pylori* among the Healthy Blood donors according to gender.

In our study, no significant association was found between *H. pylori* infection and, gender as well as in age group in the two study groups, asymptomatic adults and Dyspeptic patients with P value 0.30 as shown in table 2.

Moreover, the distribution of participants according to the age group was shown as follows: the highest age group was (≥ 40) and the lowest group was (20–24) in the healthy blood donors (Fig. 3), while in dyspeptic patients (67/104), 64.4% were highest in the age group ≥ 40 (Fig. 4).

Table 2. Distribution of *H. pylori* positive and negative in Dyspeptic Patients according to gender.

Gender				P_Value
	Positive	Negative	Total	
Male	35 (83.1%)	7 (16.9%)	42 (33.6%)	0.304111
Female	69 (83.3%)	14 (16.9%)	83 (66.4%)	
Total	104 (83.2%)	21 (16.8%)	125 (100.0%)	

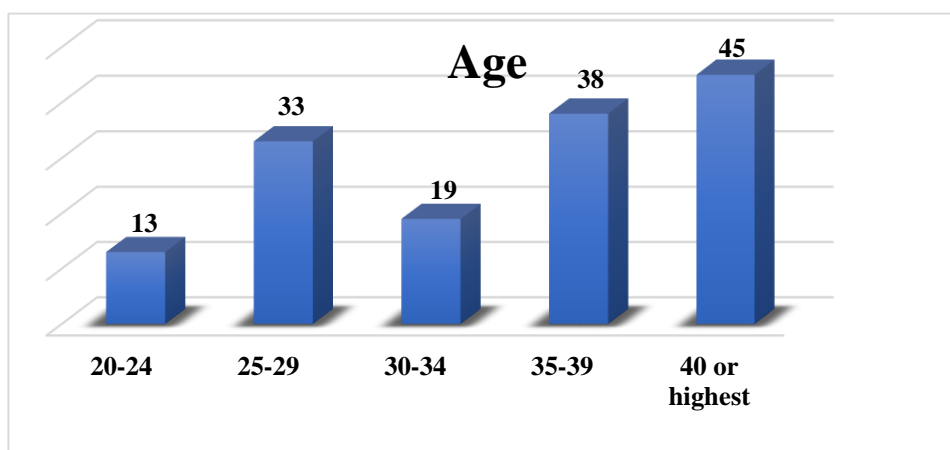


Figure 3. Prevalence of *H. pylori* in Healthy blood donors in different age groups

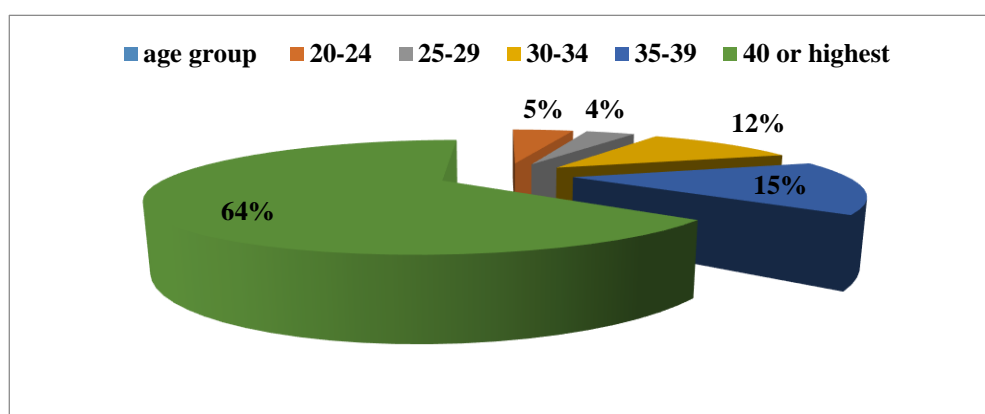


Figure 4. Prevalence of *H. pylori* in Dyspeptic Patients in different age groups.

The prevalence of CagA of *H. pylori* positive Healthy Blood donors and Dyspeptic patients:

The prevalence of the IgG CagA in the positive *H. pylori* healthy Blood donors was (29.5%, 44/149), and 70.5%) of the sample had negative IgG Cag A, as in (Fig.5), while there was (27.9%, 29/104) positive IgG cag A among Dyspeptic patients, as in Fig. (6). The prevalence of the IgG CagA according gender was 11.4% female from the total sample, compared to 88.6% male from the total sample among positive *H. pylori* healthy blood donors (Fig. 7), while in positive *H. pylori* Dyspeptic patients (31.4% female) and (20.6% male) as shown in table 3, the P value was not significant. The overall seroprevalence of *H. pylori* positive CagA among healthy Blood donors was high in the 40 or highest age group (Fig. 8), similar to the age group 40 or highest for *H. pylori* positive CagA in Dyspeptic Patients (Fig. 9).

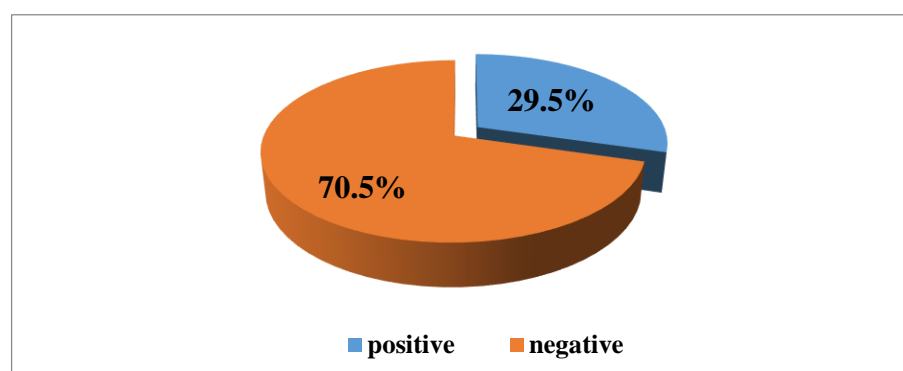


Figure 5. IgG CagA among *H. pylori* positive Healthy Blood donors.

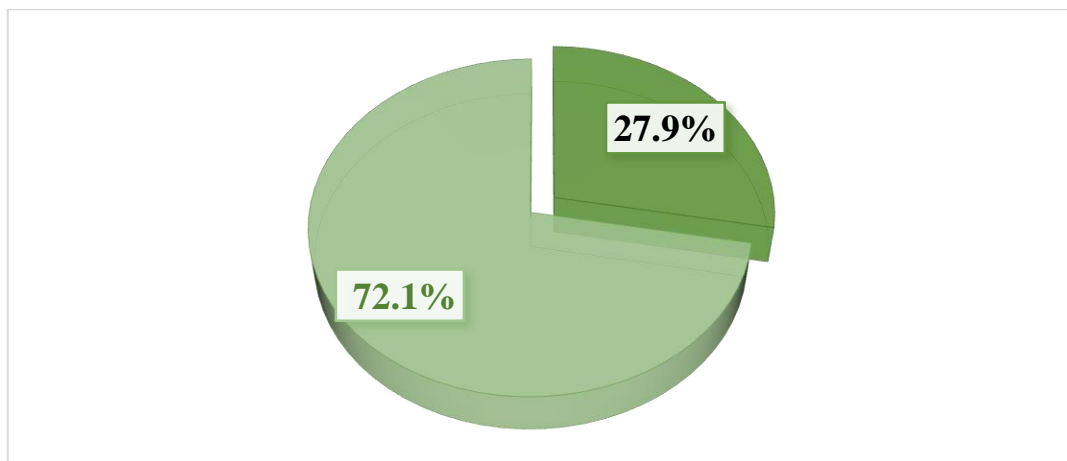


Figure 6. Prevalence of IgG CagA among *H. pylori*-positive dyspeptic patients

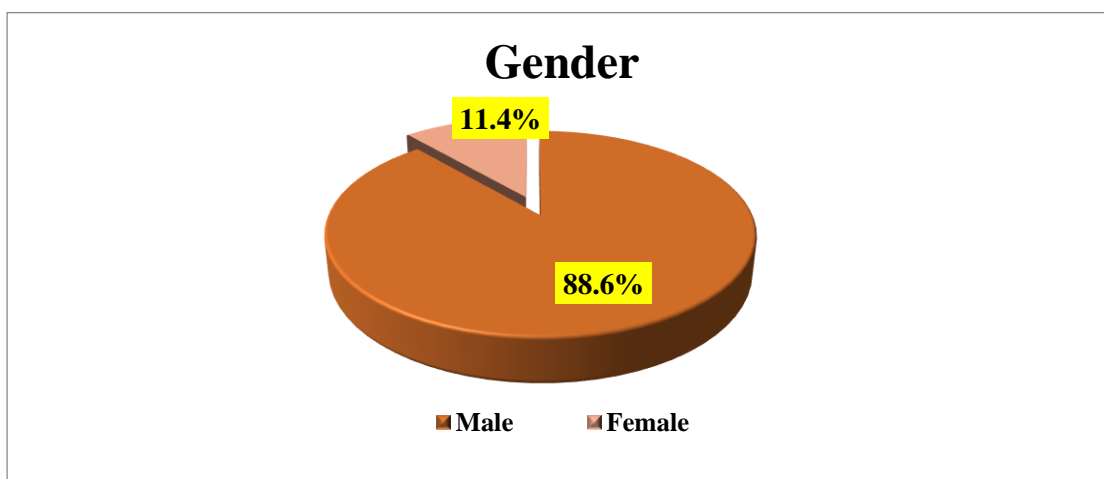


Figure 7. The relation of IgG CagA to the gender of Healthy Blood *H. pylori* positive.

Table 3. Demonstrate of IgG CagA in Dyspeptic Males and Females of *H. pylori* positive.

Gender	Positive	Negative	Total	P_Value
Male	7 (20.6%)	27 (79.4%)	34 (32.7%)	0.247506
Female	22 (31.4%)	48 (68.6%)	70 (67.3%)	
Total	29 (27.9%)	75 (72.1%)	104 (100.0%)	

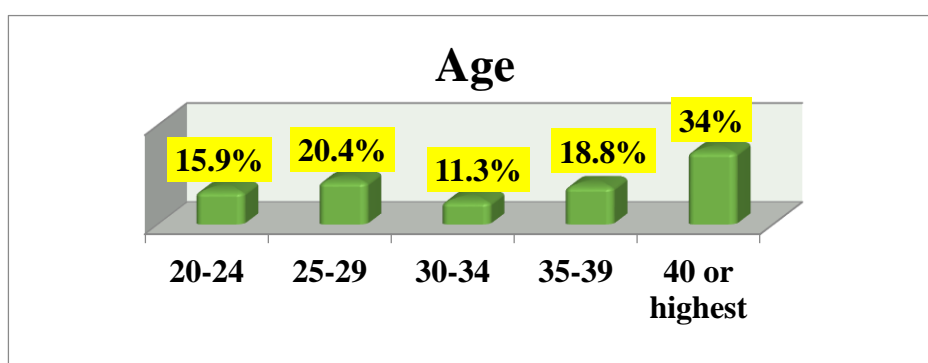


Figure 8. Prevalence of *H. pylori* CagA in Healthy Blood donors in different age groups.

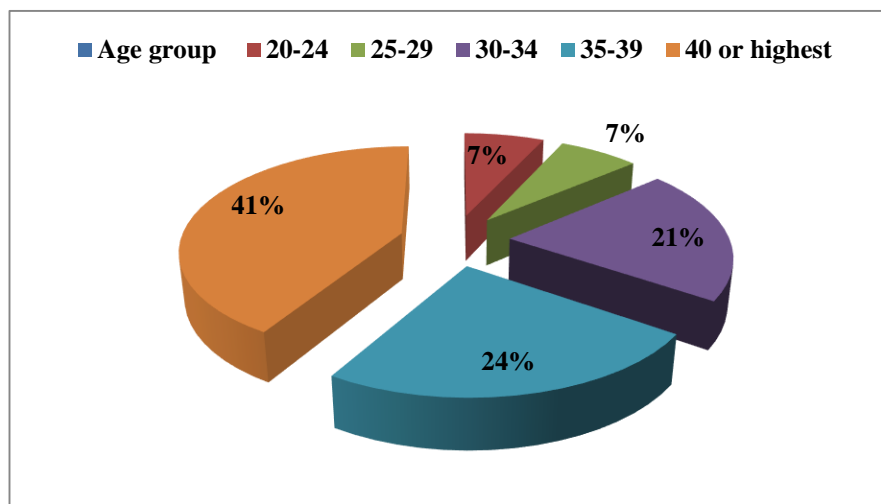


Figure 9. Prevalence of IgG cagA *H. pylori* according to Dyspeptic Patients age group.

DISCUSSION

Infection with *H. pylori* is not a disease by itself but a condition associated with a number of disorders of the upper gastrointestinal tract [5]. The serological testing for *H. pylori* antibodies helps in the early detection of "silent" peptic ulcers [6]. The present study was the first to report the seroprevalence of *H. pylori* infection in adult healthy Blood donors in the western region of Libya. The results of the present study demonstrate that the prevalence of asymptomatic individuals (85.1%) was high in Tripoli city and the area surrounding it, which is similar to other reports from several Libyan cities, e.g., Benghazi, where the authors found healthy individuals (71.4%) infected with *H. pylori* [7]. However, five years later, another study from Benghazi found (56.5%) the reason for the decrease [8]. might be the use of antibiotics during the last few years. In the Al-Komes region, an epidemiological study found that 65% of asymptomatic persons were infected with the gastric pathogen *H. pylori* [9,10]. Our results are similar to those of other developing countries in which 69 percent to 82 percent of adults and children are infected by 10 years of age. In a rural area of Brazil, antibodies to *H. pylori* were detected in the serum of 77.5 percent of children and teenagers and 84.7 percent of adults [11]. In Kosovo, the seropositivity of *H. pylori* is moderately high (56.9%) among healthy Blood donors [12]. In Kenya, 93% of the 14 asymptomatic volunteers were found to have *H. pylori* infections [13]. A study in Iraq concluded that *H. pylori* is highly prevalent (55.8%) among university students in the Erbil region, with a higher prevalence found in older students and those from lower socioeconomic status [14].

Our results indicate that the seroprevalence of *H. pylori* is increasing with age, which is similar to other studies, such as those showing that among the Algerian children, 43% were seropositive for the bacterium, and the prevalence rose steadily with age, reaching a peak of 92% between the ages of 40 and 49 years. An age-specific increase in the prevalence of *H. pylori* infection was observed in the Ivory Coast population, where the seroprevalence of the gastric bacteria *H. pylori* in children was 54%, rising gradually to a plateau of 70%–80% throughout adulthood [13]. In Ghana (15). Our results showed that the prevalence of *H. pylori* antibodies was significantly higher in males compared to females (88.6% vs. 11.4%, respectively), although results similar to ours were reported from other countries such as Iran [16], Italy [17], New Zealand [18]. However, the anti-*H. pylori* antibodies in the dyspeptic patients had a higher prevalence in females (66.3%) compared to males (33.7%). However, in some studies, no significant statistical difference was observed between the sexes [19].

The causal relationship between the bacterial pathogen *H. pylori* and various forms of gastric disease, including gastric cancer (GC), has been well known. Pathogenicity of the bacteria is significantly related mainly to cytotoxin-associated protein A (CagA), encoded by the frequently regarded to represent virulence marker CagA gene, located within the pathogenicity island, *cag* (*cagPAI*), and vacuolating cytotoxin (*VacA*), encoded outside the *cag* PAI (20,21). The two genes are present in the genomes of around 60% of strains [5]. *H. pylori* can be divided into type One and type Two strains based upon the presence of CagA and the secretion of *vacA*. Type One strains are CagA positive and secrete *vacA*, while type Two strains are CagA negative and do not secrete *vacA*. However, the totality of *cag* PAI is important, since if the type two secretion system is not functional (due to deletion of *cag* PAI), then CagA will not be injected into the host cell. This feature is important, as the ability to induce IL-8 production in cells is correlated with the presence of a complete Cag PAI; then, the presence of an intact Cag PAI is correlated with the

development of more severe pathology [22]. In our study, the overall prevalence rate of the CagA gene was 29.5% and 27.9% by using the ELISA method in healthy Blood donors and dyspeptic patients, respectively.

The result of the present study showed that 29.5% of healthy Blood donors and 27.9% of dyspeptic patients had antibodies to CagA. The *cagA* gene has been identified as a possible marker of the virulence of *H. pylori* [23]. Since the cytotoxin-associated gene product (CagA, 120 to 140 kDa) encoded by CagA is immunodominant, serum IgG antibodies to the CagA antigen may be a reliable marker of carriage of a CagA⁺ *H. pylori* strain [19]. The seroprevalence of anti-CagA antibodies varies geographically. In other studies, the seroprevalence of anti-CagA antibodies in *H. pylori*-infected asymptomatic adults was reported to be 60% in Iranian healthy subjects [24]. In our study, it seems that the prevalence and magnitude of the IgG response to CagA are higher in adults of the age group of 40 years or older (34% than in those with an age of 25–29 years (20.4%), of the healthy blood donors. Accordingly, the age of the subject may influence the seropositivity rate and the titer of the cluti-CagA antibody. However, in dyspeptic patients, the anti-CagA antibodies were higher in the sum age groups under age 39 years (58.6%) compared to the other age group ≥ 40 was 41.3%.

In a Sudanese study to examine the prevalence of antibodies to CagA both in 82 African patients with Dyspepsia who are undergoing endoscopy and in 89 asymptomatic healthy African volunteers, found a high prevalence of CagA seropositivity in dyspeptic 84.5% and 87.7% of the healthy volunteers studied [25]. In our study, similarly, the prevalence of CagA immunoreactivity in an asymptomatic control population (29.5%) was not significantly different from that in dyspeptic patients with or without gastroduodenal disease (27.9%). Thus, although our study is the first in Libya to determine the prevalence of CagA, we suggest that in our population, the presence of endoscopic gastroduodenal disease is associated with CagA seropositivity. The prevalence of antibodies to CagA in *H. pylori*-positive healthy volunteers questions the importance of this potential virulence marker in gastroduodenal disease, at least in our Libyan population. Our results showed that the prevalence of anti-*H. pylori* antibodies were significantly higher in males compared to females (88.6% vs. 11.4%, respectively), although results similar to ours were reported from other countries such as Iran [16], Italy [17], and New Zealand [18]. However, in the dyspeptic patients, anti-*H. pylori* antibodies had a higher prevalence in females (66.3%) compared to males (33.7%). However, in some studies, no significant statistical difference was observed between the sexes [19]. Our results showed for the first time that the prevalence of anti-CagA was markedly higher in males compared to females in both populations of our study; similar results were obtained in other studies [16,24]. Accordingly, it seems that the male gender is more susceptible to infection and colonization by CagA-positive strains of *H. pylori*.

CONCLUSION

This study is the first to detect *H. pylori* infection utilizing an ELISA test for Dyspeptic patients in Tripoli, Libya, and could be considered an attempt to determine possible risk factors associated with the acquisition of the microorganism as well as to compare two different population groups. The overall finding of the present study shows that *H. pylori* infection is a serious health problem in the Tripoli area, with the rate of *H. pylori* infection among 125 adult Dyspeptic subjects undergoing an ELISA test being relatively high (83.2%). Therefore, ELISA tests are relatively inexpensive, noninvasive, simple, and accurate tests for detecting *H. pylori* in blood samples, useful for primary diagnosis as well as for the assessment of *H. pylori* among young populations.

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الإصابة ببكتيريا هيليكوباكتر بيلوري إيجابية CagA بين المصابين بعسر الهضم والمتبرعين بالدم الأصحاء في مدينة طرابلس

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المستخلص

الخلفية والأهداف. تحدث عدوى هيليكوباكتر بيلوري (*H. pylori*) في وقت مبكر من الحياة مع ارتفاع في المنطقة الجغرافية والوضع الاجتماعي والاقتصادي وعمر المضيف المصاب. وقد ثبت أن عوامل الفوعة لبكتيريا الحلزونية البوابية مثل جزيرة cag المرصدة (cagPAI) برهنت على أنها تنبؤات لأمراض المعدة. في ليبيا ، لا توجد بيانات متاحة فيما يتعلق بانتشار عدوى *H. pylori* لدى البالغين المصابين بعسر الهضم والمتبرعين بالدم الأصحاء. أجريت هذه الدراسة لتحديد معدل الانتشار الحالي لجرثومة الملوية البوابية وعلاقته بالعمر والجنس وحالة CagA بين المتبرعين بالدم الأصحاء والبالغين المصابين بعسر الهضم الذين يترددون على المستشفيات التعليمية في طرابلس. **طرق الدراسة.** عينة دم قوامها (125) مشاركاً مصاباً بعسر الهضم و (175) متبرعاً بالدم من الأصحاء ، تم تحديد الانتشار المصلي لـ *anti-H. Pylori IgG & anti-CagA IgG* باستخدام طريقة (Biotech USA) ELISA ، استكمل الاستبيان الذي يغطي المتغيرات الاجتماعية والديموغرافية عن طريق المقابلة. **النتائج.** كان معدل الانتشار المصلي الإجمالي لجرثومة الملوية البوابية (83%) ، و (85%) في المصابين بعسر الهضم والمتبرعين بالدم الأصحاء على التوالي. كانت هناك زيادة تدريجية مع العمر. لا يوجد فرق إحصائي بين الجنسين. ومع ذلك ، كانت حالة CagA في سلالات الحلزونية البوابية 28% ، 29.5% المصابين بعسر الهضم والمتبرعين بالدم الأصحاء على التوالي. **الاستنتاجات.** يعد الكشف عن العلامة المرضية للحلزونية البوابية (CagA) أمراً بالغ الأهمية للحد من أمراض الجهاز الهضمي الشديدة في ليبيا. هناك حاجة لدراسات وتقنيات جزيئية أكبر لتحديد النمط الجيني لسلالات الحلزونية البوابية.

الكلمات الدالة. هيليكوباكتر بيلوري ، انتشار ، المصاب بعسر الهضم ، CagA ، ليبيا