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Study to determine the prevalence of *Helicobacter pylori* infection among the Sirte population (Libya) using an Antibody Rapid Test Cassette method.

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Highlights

- ***Helicobacter pylori* infection is bacteria that infect the gastrointestinal tract causing chronic inflammation (infection) in the stomach and duodenum.**
- **The *H. pylori* Antibody Rapid Test Cassette method is rapid chromatographic immunoassays for the qualitative detection of antibodies to H. pylori in serum.**
- **Antibody Rapid Test cassette method could be used for detection and diagnosis of *H. pylori* infection.**
- **Study estimated prevalence of *H. pylori* infection among adult patients in Sirte, Libya.**

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ABSTRACT

Helicobacter pylori (H. pylori) is a major pathogen in the context of gastroduodenal ulceration, gastric carcinoma, and other types of gastric and extra gastric disease. *H. pylori* infection can be diagnosed by different methods including invasive techniques such as endoscopy and biopsy, and non-invasive methods such as urea breath and serology tests for the detection of antibody and antigens. This required expensive laboratory equipment compared with the Antibody Rapid Test Cassette method for detection and diagnosis of *H. pylori*.

Objective: This study aimed to determine the prevalence of *H. pylori* infection among the Sirte population (Libya) using an Antibody Rapid Test Cassette method. The Antibody Rapid Test Cassette method is a rapid, cheap, and simple test that utilizes a combination of *H. pylori* antigen and anti-human IgG to qualitatively and selectively detect *H. pylori* antibodies in serum.

Methods: A total of 60 patients (30 males and 30 females) having *H. pylori* symptoms, were included in the study, Blood samples were collected from these 60 patients from different clinical laboratories in Sirte. All the samples were tested for *H. pylori* antibody using an (Antibody Rapid Test Cassette method).

Results: Results showed that a positive antibody was detected in 50 samples of the 60 samples (the infection rate was 83%). There was a high level of antibody against *H. pylori* in 35/50 of the positive samples. 20 of the patients with higher levels were Male and 15 were female. 15/50 were low or medium positive reaction, P≤0.1099. Whilst 10/60 (16.66%) of the samples were negative, 3 of these 3 were from male patients, and 7 were from female patients.

Conclusion: Serologic tests are widely available, noninvasive, inexpensive, and appropriate for screening in large epidemiologic studies**.** The occurrence of *H. pylori* infection reported in our study was high and therefore calls for health education about behavioral changes and adequate sanitation; Population screening and diagnosis using multiple tests are required to reduce *H. pyloric* infections.

1. Introduction

Helicobacter pylori (*H. pylori*) is a spiral-shaped, Gram-negative, Oxidase, Catalase, and Urease positive, micro-aerobic human pathogen. *H. pylori* play a prominent role in the pathogenesis of many gastrointestinal diseases, varying from chronic active gastritis, peptic ulcer diseases, atrophic gastritis, mucosa-associated lymphoid tissue (MALT) lymphoma, and non-cardia gastric cancer (Yoa-Kuand Wang *et al*., 2015). *H. pylori* is a bacterium that is present in the digestive tract as part of normal flora. All gastric species of the genus *Helicobacter* are highly motile because of their flagella (Goh *et al*., 2011). The flagella help the movement towards the more neutral pH of the gastric mucosa, and their urease positivity allows the bacteria to live for a short duration of time in the highly acid gastric lumen. These two factors contribute to their high abundance in the gastric mucosa.

In developed countries, the prevalence of H pyloric in the normal floral of the gastrointestinal tract remains below 40% and is lower in children and adolescents than in adults and aged people (Salih BA., 2009). The *H. pylori* prevalence rates in developing countries rise rapidly in the first decade of life and then remain a plateau and go up thereafter. A rapid decline in the prevalence of *H. pylori* has been seen of late in developed countries, whereas it has remained relatively constant in developing countries (Mitchell

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et al., 1992). Good living conditions, improved sanitation, and antimicrobial treatment likely contribute to reduced prevalence. The exact mechanisms of how *H. pylori are* acquired are not clear. But thought to occur as a consequence of direct human-to-human contact, via oral-oral or faecal-oral route or both. *H. pylori* have been found in saliva, vomitus, gastric refluxate, and faeces. (Kusters *et al*., 2006; Gold *et al*., 2014; Tsongo *et al*., 2015).

H. pylori infection in children may lead to decreases in the relative height and weight gain compared to their peers, with evidence of IgG antibodies remaining for several months and possibly years (Gold *et al*., 2014). In children, *H. pylori* is associated with several extragastric diseases, including growth reduction, iron-deficiency anaemia, and idiopathic thrombocytopenic purpura (Baggett *et al*., 2006; Gold *et al*., 2014). School going children in developing countries are more prone to these infections due to poor living conditions, low socioeconomic status, poor quality of drinking water, overcrowding living environment, poor personal and environmental hygiene, and food contamination (Darko *et al*., 2015; Tsongo *et al*., 2015). *H. pylori* is a common chronic bacterial infection worldwide, and it has been seen that 50% of the population is infected (Bravo *et al*., 2018). The occurrence of *H. pylori* infection varies greatly worldwide, with infection rates of more than 80% in some developing countries and below 20% in some developed countries. *H. pylori* cause peptic ulcers in 10% to 15% and stomach cancer in another 1% to 2% of those infected (Venerito *et al*, 2018). Since the turn of the century, the prevalence of *H. pylori* infection has declined in industrialized countries and plateaued in developing and newly industrialized countries (Lin *et al*., 2007). Patients colonized with *H. pylori* elicit a specific antibody response which is used as a diagnostic aid and for monitoring the disease state during a treatment (Pronovost *et al*., 1994). Several treatment modalities using combination antibiotics, bismuth compounds, and PPI have shown to be effective in treating active *H. pylori* infection. Until now, the best therapeutic approach remains unknown (Zullo *et al*., 2007), but Bismuth quadruple therapy and salvage treatment regimens are the preferred options (Chey *et al*., 2017).

Methods for the detection of *H. pylori* can be classified into invasive and non-invasive tests (Cutler *et al*., 1995). The serology test is commonly used in routine practice. If the patient demonstrates a high titre, it is suggestive of an active infection, whereas low titre suggests previous exposure (Yaxley and Chakravarty., 2014). The noninvasive tests, like serology and urea breath test (UBT), are sensitive and specific. The objective of this study was to determine the prevalence of *H. pylori* infection among symptomatic patients in Sirte population (Libya) using an Antibody Rapid Test Cassette method.

2. Materials and methods

2.1 Samples collection

This study was carried out from January 2019 to June 2019. A total of 60 patients in different age groups were selected for the study (both males and females). Blood was drawn from 60 patients with clinical symptoms of H. pylori infection (30 males and 30 females) from different clinical laboratories in Sirte city. All of the samples were tested for *H. pylori* antibody using an Antibody Rapid Test Cassette in the microbiology laboratory at the Department of Microbiology, Faculty of Medicine, Sirte University.

2.2. Serological tests

The collected samples were transported to the laboratory, and then sera were separated by centrifugation at 15000 rpm for 10 min, separated serum was used for serological tests.

2.3 Screening of H. pylori IgG Antibodies

The IgG anti-*H. pylori* antibody in serum was detected using *H. pylori* Antibody Rapid Test Cassette (Right sign, Biotest, Indonesia). The *H. pylori* Antibody Rapid Test Cassette (Serum/Plasma) is a qualitative membrane-based immunoassay for the detection of *H. pylori* antibodies in serum or plasma. After removing the "Test Device" from its foil wrapper, a drop of patient's serum was added on each specimen well and left for the 30s until liquid absorbed completely, after which two drops of diluent Buffer from the vial provided were added after 10 min the result was read. If there was a rose-pink colour band in the control region (marked with a "C"), and a rose-pink colour band in the test region (marked with a "T"), *H. pylori* antibodies are present and the specimen was considered positive. The absence of a colour band in the test region next to the letter "T" indicated the absence of any detectable *H. pylori* antibodies. Levels of Antibody against *H. pylori* were subdivided into high, medium, and low based on antibody titres.

3. Results

The positive antibody was detected in 50 samples whereas 10 samples were negative (the infection rate was 83%) (Table 1 and Fig. 1). There was a high level of infection in 35/50 of the positive samples. Of these 20 samples were from male patients and 15 were from female patients. For 15 of the 50 positive samples, low or medium positive reactions were observed (Table 2 and Fig. 2). 10 of the 60 (16.66%) samples were negative (3 from male patients, and 7 from female patients).

Table 1

Seroprevalence according to gender

Test	Male	Female	Total	*P*-value
Positive (+)	33	17	50	P≤0.1099
Negative (–)	3	7	10	(P<0.05, significant)
Total	36	24	60	

Table 2

Level of infection according to gender

Positive reaction	Male	Female	Total
High infection	20	15	35
Low and medium	13	2	15
Total	33	17	50

3. Discussion

Around 50% of children are infected with *H. pylori* once during childhood, remains in the gastric mucosa, and present at some point of life. (Rajindrajith *et al*., 2009; Yamaoka, 2012). The prevalence of *H. pylori* infection varies greatly worldwide, with infection rates of more than 80% in some developing countries and below 20% in some developed countries (Kusters *et al*., 2006; Kim N., 2016; Sjomina *et al*., 2018; Venerito *et al*., 2018).

In this study, 60 serum samples were collected from 60 symptomatic patients tested for *H. pylori* Antibody, (30) were male and (30) were female in different age groups. Results showed that positive antibody was detected in 50 of 60 samples indicating an infection rate of 83% as shown in Table 1 and Fig. 1. Compared to other studies in Libya this rate is higher than the results obtained by Khaled and Ramadan (2016). A study carried out in Benghazi (Libya), which found that the occurrence of *H. pylori* infection was 56.5%. Also, Ibrahim *et al*., (2015) in their study in Tripoli (Libya) found that the infection rate was 36%.

The occurrence of *H. pylori* infection varies according to topography, age, gender, ethnicity, and socioeconomic status (Trindadea *et al*., 2017; Genta *et al*., 2017). A Seroprevalence study gives us information on the clinical occurrence of *H. pylori* infection in geographically and demographically diverse populations. The annual rate of seroconversion in adult populations in developed countries appears to be small, about 0.2-1.0% (Brown, 2000). It was also found to be higher under unusual circumstances, such as desert storms, war; when the seroconversion rates were 6.4 and 7.3% respectively (Xia and Talley, 1997; Richard *et al*., 1998).

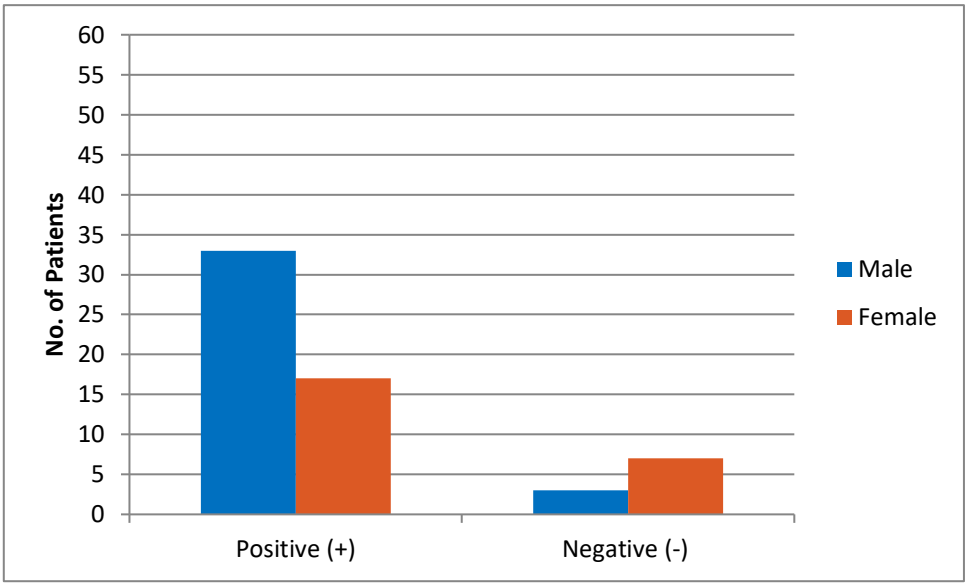

Fig. 1. Seroprevalence according to gender.

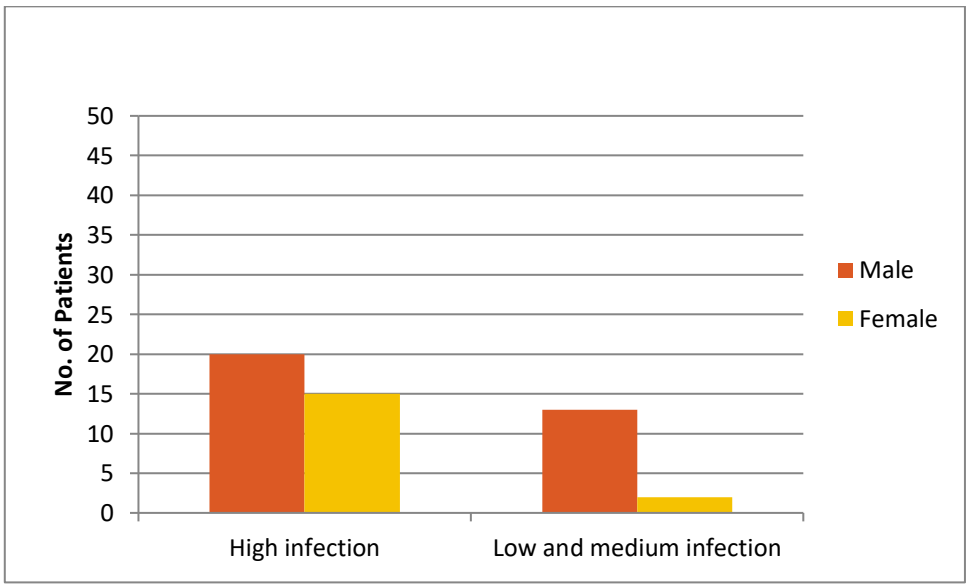

Fig. 2. Level of infection according to gender.

The study found male prevalence rate is 66% with *H. pylori* infection, which is higher than Female rates 34% as shown in Table 2 and Fig. 2. The gender effect on the prevalence of *H. pylori* infection in many populations is varied. Woodward and colleagues reported a higher prevalence in men than women (Woodward *et al.*, 2000), whiles others reported no difference. These differences in *H. pylori* prevalence by race, ethnicity may reflect differences in social or/and hygiene factors or the use of antimicrobials for the treatment of other common infections. Although some studies have reported an excess of *H. pylori* in one gender than the other, no noteworthy gender differences exist (Replogle *et al*; 1995; Smith *et al.*, 2019). The comparison of prevalence rates by age suggests that the acquisition of *H. pylori* is declining in recent cohorts. This finding is mostly seen in the developed countries and is due to the improved hygiene practices (Brown 2000).

In the present study, it was seen that males had higher *H. pylori* seroprevalence than females, (57.14% Male and 42.85% were female). Also, Khaled and Ramadan (2016) in their study in Benghazi (Libya) found that The *H. pylori* IgG was seen in (41.6%) males compared to (58.4%) of females. Ibrahim *et al.* (2015) in their study in Tripoli (Libya) concluded that the rate of incidence was most similar between males and females which were 35.9% and 36% respectively and there was no major difference in the occurrence of *H. pylori* with respect to gender.

Some studies have shown a high incidence among men (Fawcett *et al.*, 1996). Other studies have reported no gender variations for *H. pylori*. (Graham *et al.*, 1991; De Martel and Parsonnet, 2006). While the eradication rate of *H. pylori* is similar in male and female, it seems very difficult to eradicate *H. pylori* infection because the bacteria live beneath the gastric mucus, where antimicrobial drugs have restricted access to this area (Masoodi *et al.*, 2013).

A major advantage of this serologic test is that it enables large numbers of subjects to be screened quickly and it is relatively inexpensive; thus, it is a good test to use in epidemiologic studies. There are certain limitations to the use of serology:

- Since no single antigen is recognized by sera, antigen reagent preparations should contain multiple strains of *H. pylori*.
- It is difficult to define the cut off value from positive to negative subjects.
- The test is sensitive to changes in reagents and laboratory conditions
- Serology is not a test indicated immediately following treatment of *H. pylori*.

4. Conclusion

Serologic tests are widely available, noninvasive, inexpensive, and appropriate for screening in large epidemiologic studies**.** The

high seroprevalence of *H. pylori* that we reported in our study warrants the need to call for immediate intervention measures that could reduce transmission and lessen the clinical consequences of infection.

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