

**Environmental Risk Factors associated *Helicobacter pylori*  
Infection Among Dyspeptic Patients**Fatima Fathi Agaeb<sup>(1)</sup>, Mohamed Ibrahim Shakhtor<sup>(2)</sup>, Ali Tumi<sup>(3)</sup>Abdulfattah Fitouri<sup>(3)</sup>, Iman Guma Alamar<sup>(3)</sup>, Abdurrazag Nami.<sup>(4) (5)</sup>

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**المخلص**

تحدث عدوى هيليكوباكتر بيلوري (*H. pylori*) في وقت مبكر من الحياة بتواتر عال في البلدان النامية. وقد أفيد بأن مصدر مياه الشرب هو طريقة محتملة لانتقال العدوى في المناطق الريفية، حيث لا توجد بيانات متاحة بشأن انتشار عدوى الملوية البوابية لدى الأطفال والبالغين المصابين بعسر الهضم، وعوامل الخطر المرتبطة بها في المناطق الريفية في ليبيا. الأهداف: تحديد معدل الانتشار الحالي لعدوى الملوية البوابية بين الأطفال والبالغين الذين يعانون من عسر الهضم الذين يحضرون إلى مستشفى الخمس التعليمي وعوامل الخطر المرتبطة به. المواد والأساليب: عينة دم من (350) مريضاً بعسر الهضم من الأطفال والبالغين (متوسط العمر 29 سنة)، باستخدام طريقة ELISA للكشف عن الأجسام مضادة لبكتيريا المعدة. وتم تعبئة الاستبيان الذي يغطي المتغيرات الديموغرافية الاجتماعية عن طريق المقابلة الشخصية. النتائج: - بشكل عام، كان انتشار الملوية البوابية (41%). حيث كانت الملوية البوابية (23%)، (77%) في الأطفال والبالغين على التوالي. (كان الإصابة في الذكور (56%) وفي الإناث (44%)، وكانت هناك زيادة تدريجية مع تقدم عمر المريض. وكانت أعلى في الأشخاص الذين يستخدمون مياه الشرب الخاصة غير المعالجة في مياه الآبار مقارنة مع المياه التجارية. الاستنتاجات: في منطقة الخمس، يعتبر انتشار الملوية البوابية مرتفعاً في مجموعة المشاركين المصابين بعسر الهضم في هذه الدراسة السكانية، والتي قد تكون مرتبطة بالحالة الاجتماعية والاقتصادية، والظروف المعيشية، والتي تعتبر كعوامل خطر رئيسية لعدوى الإصابة بالملوية البوابية. وتشير بياناتنا إلى أن مصدر مياه الشرب يعتبر آلية مهمة لانتقال الملوية البوابية في المناطق الريفية. ومع ذلك، ينبغي إجراء دراسات أوسع في مناطق أخرى من ليبيا لتأكيد نتائج هذه الدراسة، وذلك باستخدام تقنيات الأحياء الجزيئية للمساعدة في تتبع مسار انتقال العدوى في المستقبل.

**الكلمات المفتاحية:** هيليكوباكتر بيلوري، انتشار، علم الأمصال، عسر الهضم، ليبيا.

**Abstract**

*Helicobacter pylori* (*H. pylori*) infections occur earlier in life with high frequency in developing countries. It has been reported that the source of drinking water as a potential

route of transmission. In Libya, there is no data available regarding the prevalence of *H. pylori* infection in dyspeptic children and adults, and its associated risk factors in rural regions. **Objectives:** To establish the current prevalence of *H. pylori* infection among dyspeptic Children & Adults attending Alkomes Teaching Hospital & its associated risk factors. **Materials and Methods:** A Blood sample of (350) dyspeptic Children and Adults patients (mean age 29 years), using ELISA method to detect anti-*H. pylori* IgG, and questionnaire covering Socio demographic variables were completed by interview. **Results:-** The overall, sero prevalence of *H. pylori* was (41%). However, *H. pylori* was (23%), (77%) in children and Adults respectively. The gastric pathogen was in male (56%) than the female (44%). There was a gradual increase with age. The higher positive infected *H. pylori* in subjects who are using private untreated well-water drinking than those the commercial water supply. **Conclusions:** In Al- Komes region, the prevalence of the gastric *H. pylori* is high in the group of dyspeptic participants of the population study, which might be related to the socioeconomic status, and living conditions, as major risk factors for *H. pylori* infection. Our data indicate that the source of drinking water is an important mechanism for the transmission of *H. pylori* in a rural regions. However, larger studies in other regions of Libya should be conducted to confirm the study finding, using molecular typing techniques to help trace the route of transmission in future.

**Keywords:** Helicobacter pylori, Prevalence, Serology, Dyspeptic, Libya.

#### **Introduction:-**

*Helicobacter pylori* (*H. pylori*) infection remains one of the most common chronic bacterial infections affecting humans. It is helical in shape, gram negative bacteria, microaerophilic, motile. *H. pylori* colonizes the human stomach of over 50% of people worldwide, and it is the major cause of peptic ulcers, gastritis, and gastric cancer ( Narayanan *et al*, 2018).

*H. pylori* infection induces various upper gastro-duodenal diseases, and there is a marked difference in clinical outcomes due to bacterial infection among different regions in the world. The risk of being colonized by *H. pylori* depends on geographic location, socioeconomic status and age of the host (Bakak and Salih 2002).

Most *H. pylori* transmission occurs in childhood, and in some countries up to 90% of children become infected by the age of ten years, with reports of infection as early as the first months of life (Glynn *et al.*, 2002; Salih 2009). As different prevalence rates of *H. pylori* infection have been reported in different regions within the same country. In developing countries, the infection can be almost ubiquitous (Monne *et al.*, 2008; Burucoa and Axon, 2017), whereas in industrialized countries *H. pylori* infects around 30-50 % of adults ( Melese, *et al* 2019).

Detection of the microbial pathogen by the seroepidemiological investigations represent the most rapid and convenient way of obtaining a picture of the prevalence of microbial infection in a study population. A majority of serological studies from developing countries are now conducted with commercial kits which are inexpensive, simple, and available in the local market.

In Libya, a country with the Mediterranean coast and the Sahara Desert, important regional differences are likely to occur. Nevertheless, no local data are available on the epidemiology of *H. pylori* infection in the rural regions of Libya; therefore, the primary aim of this study was to evaluate the incidence of seropositivity *H. pylori* infection among Children and Adults dyspeptic patients in Al-Komes region. The secondary aim to determine the environmental risk factors (age, gender, and source of drinking water which might be associated to the infection with the *H. pylori*.

#### **Materials and Methods: -**

**Study design and participants: -** A cross- sectional study was conducted from December 2020 to November 2021. A group participated in the study consist of three- Hundred and fifty children and adults patients with symptoms of dyspepsia. Based on the questionnaire data, the participant with a history of gastro duodenal ulcer, with current chronic complaints of the upper digestive tract for more than two months (nausea, vomiting, heartburn, indigestion) or those currently using anti-acid or anti- ulcer medications were excluded from the study. After obtained written informed consent from the parents of the child or from the adult participant , a standard questionnaire was completed by direct interview to obtain individual socio-demographic data regarding each individual participant (age, gender, number of family members, source of drinking water, coffee and Tea consumption, family history of gastric diseases). Health status, local of residence and medication taken one month before the interview (particularly proton pump inhibitor and antibiotics) were also recorded.

**The Collection of Blood Samples: -** A Blood sample (5 ml) was obtained from each individual by peripheral venipuncture under aseptic conditions. Samples were refrigerated on ice during transport to the laboratory. After serum separation using (800B Electronic Centrifuge), 250µl serum samples were labeled and frozen at -20°C until testing.

#### **Determination of anti-*H. pylori* IgG:-**

For the diagnosis of infection with *H. pylori*, three Hundred and fifty (350) serum samples from children and adults dyspeptic patients, were collected for the study and tested for evaluation of immunoglobulin G (IgG) antibodies against *H. pylori* by using the commercial enzyme- linked immunosorbent assay (EUROIMMUN Anti-*H. pylori* ELISA (IgG), Germany). The serum concentration of anti-*H. pylori* IgG were expressed in relative units (RU/ ml) as no international standard is available. According to the manufacturer's instructions the sensitivity of the kit was amounted to 100%, and the value of 5 RU/ ml used to discriminate the negative from positive samples.

#### **Statistical analysis:-**

The data obtained were analyzed using SPSS (Statistical Package for Social Science, Version 20.0), chi-square test to determine the prevalence of *H. pylori* infection in the two study group participants, and the difference in the prevalence across the different parameters, and the level of significance was considered when  $p < 0.05$ .

#### **Results:-**

A total od (350 ) dyspeptic children and adults were enrolled in the study ( 171 Male and 179 Females, mean age 29 years), after exclusion of (25) participants who reported taken antibiotics currently or did not complete the questionnaire . Serological technique (ELISA

Test) revealed that 41% (144/ 350) of the dyspeptic were positive for anti- *H pylori* IgG. However, antibodies to *H. pylori* were detected in the serum of 77%, 23% adults and the children respectively (Table 1 &2). No statistical difference between genders. there was a gradual increase with age, where at the age of three, nine, fifteen years old was 5%, 12%, & 17% respectively, however among the adults dyspeptic the age group of 22& 27years old was the higher group infected with *H. pylori* (13%) compared to other group age (Table 3).

**Table 1 :The Prevalence of *H. pylori* infection among Dyspeptic Patients**

Variable	( <i>H. pylori</i> ) Positive	( <i>H. pylori</i> ) Negative	Total (%)
Children	33 (23%)	85 (41%)	118 (34%)
Adults	111 (77%)	121(59%)	232(66%)
<b>Total Dyspeptic Patients</b>	<b>(100%)144</b>	<b>206 (100%)</b>	<b>350(100%)</b>

**Table 2 : The *H. pylori* infection in relation to gender of the Dyspeptic Patients .**

Variable	Female	Male	Total (%)
Children	15(23 %)	18( 23%)	(23%)33
Adults	49( 77%)	62( 77% )	(77%) 111
<b>Total</b>	<b>64( 100%)</b>	<b>80( 100%)</b>	<b>(100%) 144</b>

**Table 3: The *H. pylori* according to gender & Age group of Dyspeptic Patients**

Variable	Category	Frequency	Percentage ( %)
<b>Gender</b>	<b>Male – Female</b>	<b>171 - 179</b>	<b>56 % - 44%</b>
<b>Age group ( Years )</b>	< 3	7 - 9	5%
	4 - 9	20 - 22	12%
	10 - 15	29 - 31	17%
	16 - 21	22 - 26	15%
	22 - 27	26 - 23	13%
	28 - 33	18 - 16	11%
	34 - 40	21 - 19	11%
	41 - 46	16 - 18	10%
	47 - 52	8 - 6	3%
53 - 60	7 - 6	3%	

The association between the life style variables & *H. pylori* infection is presented in (Table 4). There is a statistically significant differences among subjects who drinks tea and those who do not with  $P$  value = 0.07. Subjects who drinks coffee are more likely to develop *H. pylori* infection (47%) as compared to those who don't drink coffee (32%). However, the difference is not statistically significant ( $P = 0.1$ ). From statistical analysis of data, The type of water drunk during childhood could be considered as a risk factor with  $P = 0.10$ . the *H.*

*pylori* positive results were high in subjects who drunk municipality or well water during childhood (30%) while subjects who drunk filtered water during childhood have (24%) *H. pylori* positive results. However, the type of drinking water during adulthood did not influence the outcome of *H. pylori* infection .

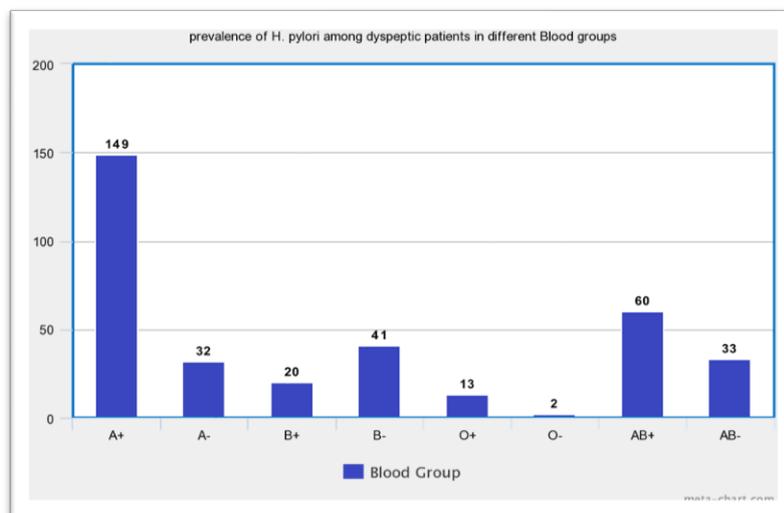
**Table 4 : Seroprvalence of *H. pylori* infection in relation to life Style variables**

Variable	Category	<i>H</i> . <i>p</i> (Positive )	<i>H</i> . <i>p</i> (Negative)	Chi square	<i>P</i> value
Gender	Male	80	91	4.4	.04
	Female	64	115		
Source of drinking water	Safe	58	104	5.4	.06
	Non safe	64	142		
Eating outdoors	Yes	161	108	15.9	< .00006
	No	68	13		
Family History of Peptic Ulcer	Yes	59	143	13.21	.0003
	No	19	129		
Family History of Gastric cancer	Yes	44	98	27.3	. 00001 <
	No	19	189		
Coffee drinking	Yes	108	124	6.62	.010
	No	38	80		
Tea drinking	Yes	168	78	9.43	.07
	No	53	51		

Table 6: Demographic characteristic of the Dyspeptic Patients Participants

Variable	Category	Frequency	Percentage
Hand Washing	Yes	194	55%
	No	100	29%
	Not applicable	56	16%
Family Monthly income	<1000	189	54%
	1000-2000	83	24%
	>2000	78	22%
Educational level	University	158	45%
	Secondary	22	6%
	Primary	18	5%
	Graduated	120	34 %
	Retired	1	1%
	Kindergarten	31	9%

The association of the prevalence of *H. pylori* infection and the life style risk factors such as Eating outdoors, the family history of gastric diseases (Peptic Ulcer Disease & gastric cancer), and social status was also analyzed. There was significant association between presence of *H. pylori* antibodies and Eating outdoors, family history of gastric diseases, as well as the type of water drinking . However, regarding the Blood group, it was found that IgG anti-*H. pylori* was presence in (52%), (17%), (27%), &( 4%) of the dyspeptic patients participants of the A, B, AB, & O blood group respectively, (Figure 1).



**Figure 1: Prevalence of *H. pylori* infection in relation to the Blood Group**

#### **Discussion:-**

In Developing countries, *H. pylori* infection is the most common human pathogen. It becomes important to investigate the epidemiology of *H. pylori* infection in Libya, first because of the high prevalence among the libyan healthy and dyspeptic population (Nami *et al* 2020), secondly, its relation with the upper gastrointestinal diseases including chronic gastritis, Peptic Ulcer Disease & Gastric cancer (Kharel, *et al* 2020). To our knowledge, this is the first study to document the prevalence of *H. pylori* among dyspeptic subjects living in a Al-Komes region.

Colonization with *H. pylori* is not a disease by itself but a condition associated with a number of disorders of the upper gastrointestinal tract (Kusters *et al.*, 2006). The results of the present study demonstrate that the overall prevalence of *H. pylori* infection is (41%) and was (77%, 23%) among the dyspeptic adults & children respectively, which is consider high in Al-Komes city, its surrounding rural residents, which is similar to other reported from several Libyan cities. A study in Benghazi, *H. pylori* infection was detected (82%) of one hundred thirty two patients of 15-83 years of age (average 38) attending the endoscopy unit (Bakka *et al* 2002). Another seroepidemiological study found that the dyspeptic patients who resident in Tripoli, Sabha regions, the overall prevalence of *H. pylori* was 83 % , 37% respectively (Nami *et al* 2019). The results of this study is similar to other developing countries, an Iranian group (Maleki *et al* 2019), conducted a cross-sectional study on 497 individuals ranging 15-65 years of age, in which the prevalence of *H. pylori* infection was 44.5% in the studied population, this prevalence was 41.3% & 47.8% in urban and rural areas, respectively. A study in Kenyan dyspeptic patients detected that the prevalence of *H. pylori* infection in children was 73.3%, & 54.8% in adults (Kimanga *et al* 2010), however , Nine years later, a study in Kenya reported the prevalence of *H. pylori* infection was 40.86%, lower than a previous study conducted at the same hospital, this decreased due to excellent diagnostic modality resulting in earlier treatment, empirical therapy or improved sanitation

(Mwangi, *et al* 2020). A cross sectional study conducted in 306 dyspeptic patients in Kano, Nigeria, *H. pylori* infection was reported as 81.7% ( Bello *et al* 2019). An epidemiological study on Turkish dyspeptic patients found high frequency of *H. pylori* infection 71% of 1605 subjects enrolled to the study (Kosekli 2021).

Regarding the age, *H. pylori* infection may occur in early childhood and most of the infected subjects remain asymptomatic., the results of this study indicate that in dyspeptic subjects with age younger than 22 years, a greater increase in age was associated with an increased prevalence of *H. pylori* infection, reaching a peak at 20 years of age, however in patients subjects with age older than 41 years, the prevalence of *H. pylori* infection did not change significantly with age. The prevalence of *H. pylori* infection in this study increased with increase in age from 5%, 12%, and 17% for children aged less of three years, 4 to 9 years, & 10 to 15 years, respectively, which is similar to what was demonstrated in others studies where infection rates increased with increase in age (Hong, *et al* 2019; Khoder, *et al* 2021).

According to this study, gender was statistically associated with seroprevalence of the *H. pylori*, the bivariate analysis showed a statistical difference ( $P = .04$ ) in *H. pylori* infection between males & females (56%, 44% ) respectively. This gender disparity findings was similar to other studies conducted in neighboring Arab countries (Khoder, *et al* 2021).

The present study assessed various environmental risk factors associated with the acquiring *H. pylori* infection using IgG serology. In terms of living conditions, it was found that dyspeptic participants living in overcrowded conditions of family members ( more than five members), drinking water from unsafe source of water, eating outdoors, and coffee drinking, were statistically more prone to the *H. pylori* infection ( $P < .0001$ ). These findings were in agreement with those of other studies (Kivi *et al* 2003 ; Cheng *et al* 2009; Urita *et al* 2013). Regarding the family history of gastric diseases, dyspeptic participants who had their parents suffering from gastrointestinal diseases such as peptic ulcer disease or gastric cancer, were significantly more exposed to the gastric *H. pylori* infection ( $P < .008$ ).

*H. pylori* infection is usually acquired in childhood from either a parent or a sibling; however the acquisition of *H. pylori* from the environment source (contaminated water of food in the community and endoscopy in the hospital environment) usually only occurs in those countries with a poor public hygiene infrastructure. In this study most of dyspeptic participants 52%% presented *H. pylori* seropositivity using drinking water from unsafe source compare to 48 % who declare that drinking water from safe source. In these study the dyspeptic patients who drinking water from the different commercial places which possibly contaminated with the *H. pylori*, since a sewage network was not exist, and consequently these dyspeptic participants was exposed to the fecal-oral route of *H. pylori* transmission, a condition similar to that observed in other developing countries such as in Brazil where water has been regarded as a source of *H. pylori* infection ( Rocha *et al*. 1994). In Kazakhstan, a study suggest that high prevalence of *H. pylori* among healthy individuals is related to poor sanitation and hygienic practices , and transmission of *H. pylori* can be water borne (Nurgalieva *et al* 2002). Over a one-year sampling period, Boehnke *et al* 2018, detected *H. pylori* in 20.3% of drinking water samples from Lima, Peru using qPCR, which suggests that there is continued contamination of the water supply in Peru.

In Northern Norway, Breckan *et al* 2016, tested 1416 subjects of all age groups in an urban and rural community by stool detection and found *H. pylori* infection in 0.6% of children, 20% of adolescents, and rising to 45% in the highest age group. They concluded that transmission might start not only in childhood, but also in adolescence, where potential transmission routes could be outdoor toilet use, private well water, and farm animals.

In Conclusion, the high prevalence of *H. pylori* seropositivity (41%) among children & adults dyspeptic patients participants attending the Al-Komes teaching hospital, indicates that infection with this gastric gram negative bacterium is still a common health problem in a rural region of Al-Komes and its surrounding area.. However, these study found that the strict relations between *H. pylori* positivity and, the source of water, and living in a crowded conditions, as environmental risk factors in these geographic location of Libya. These data support the finding that personal and environmental conditions do affect *H. pylori* infectivity in young and adult subjects living in such region of the country. Our data indicate that, the detection the viability of *H. pylori* in drinking water sources is substantial, larger studies using molecular methods should be done as a routine test. Establishing such studies would potentially allow for better understanding and estimation of the risk of *H. pylori* infection, furthermore to increase the knowledge about the reservoirs and modes of transmission which could help to explain the high prevalence rates of *H. pylori* in the developing countries.

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