

The source of water and High prevalence of *Helicobacter pylori* Infection in Asymptomatic Children and Adults. How is Important.

Abdurrazag Nami^{(1) (2)}, Reem Algalal⁽³⁾, Abdulfattah Fitouri⁽⁴⁾, Goma Huwiage⁽⁵⁾

1-Biology Dept. Faculty of Arts & Science, Qasr Khair, Al-Mergib University, Libya.

2-Libyan Helicobacter Research Laboratory, Tripoli-Libya.

3-College of Graduate Studies, Al-Mergib University, Alkomes - Libya.

4-Department of Medicine, Tripoli Central Hospital, Tripoli- Libya.

5-High Institute of Medical Sciences & Technology, Algarabolli, Libya.

Corresponding Author: abdnami@gmail.com

الملخص: -تصيب بكتيريا المعدة الحلزونية الانسان في مرحلة مبكرة من العمر، وتعتبر مشكلة صحية عامة بسبب الانتشار المرتفع في الدول النامية. وقد أكدت العديد من الدراسات العلاقة بين مصدر مياه الشرب والاصابة بهذه البكتيريا الحلزونية. في ليبيا لا توجد بيانات بشأن انتشار عدوى البكتيريا لدى الاطفال والبالغين القاطنين بالمناطق الزراعية. الأهداف: - لمعرفة معدل انتشار عدوى بكتيريا المعدة وعوامل الخطورة المصاحبة لها عند طلبة الجامعة والمدارس الثانوية الاصحاء بمدينة قصر الاخير. المواد والطرق: - جمعت عينات دم من طلبة بالمدارس الثانوية عددهم (125) ومجموعة طلبة من (100) يدرسون بكلية الاداب والعلوم، وكلا المجموعتين لا يعانون من أعراض مرضية، وتم اجراء الاختبار المصلى (اليزاء) للتحري على وجود الأجسام المضادة ضد بكتيريا المعدة وتمت تعبئة استبيان يغطي المتغيرات الاجتماعية والديموغرافية. النتائج: - بشكل عام كان معدل الأجسام المضادة (85%) وأظهرت بين الطلبة الجامعيين عدم وجود فرق بين الذكور والاناث، بينما كانت عند طلبة المدارس الثانوية الإناث أعلى انتشارًا (81%) من الذكور (65%). في كلتا المجموعتين، كانت هناك زيادة تدريجية بمعدل الاصابة مع التقدم في العمر وكان معدل الانتشار أعلى بالنسبة لأولئك الذين يشربون الماء من الابار الجوفية الغير معالجة مقارنة مع أولئك الذين يشربون الماء من المحلات التجارية. أظهرت النتائج بأن عوامل الخطورة الأخرى مثل فصيلة الدم، غسل اليدين، آلام البطن، وشرب الشاي أو القهوة لم يختلف معدل انتشار البكتيريا بشكل كبير بين المجموعتين من الطلبة الاصحاء. الاستنتاجات: - أكدت الدراسة أن بكتيريا المعدة ذات معدل عالي بين الطلبة الاصحاء بالمدارس الثانوية وبكلية الاداب والعلوم المقيمين بمدينة قصر الاخير والتي قد تكون مرتبطة بالحالة الاجتماعية والاقتصادية وظروف المعيشة والعادات الغذائية وأسلوب الحياة، كعوامل خطورة رئيسية لعدوى الاصابة ببكتيريا المعدة. كذلك مصدر مياه الشرب من الابار الجوفية كعامل خطورة لنقل الاصابة ببكتيريا المعدة ومع ذلك، ينبغي إجراء التحاليل الدقيقة لمياه الشرب باستعمال تقنية الحمض النووي مع المزيد من الدراسات التي تشمل على عدد عينات أكبر، وفي مدن أخرى من ليبيا للتأكد من النتائج المرصودة في هذه الدراسة.

Abstract: -*Helicobacter 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. There is no information available regarding the prevalence of *H. pylori* infection in asymptomatic children and adults, and its associated risk factors in rural

regions. **Objectives:** To establish the current prevalence of *H. pylori* infection among asymptomatic Secondary- School and university students in Kasr Khiar city and its associated risk factors. **Materials and Methods:** A Blood sample of (125) healthy students of two secondary schools, and (100) healthy adult students of Faculty of Arts & Sciences, Al- Merghib University in Kasr Khiar city, using immunochromatographic rapid method to detect anti-*H. pylori* IgG, and questionnaire covering Sociodemographic variables were completed by interview. **Results:** overall, seroprevalence of *H. pylori* was 85% in the healthy university students, there was a gradual increase with age, and no statistical difference between genders. However, *H. pylori* was 65%, 81% in the secondary school boys' children, secondary school girls' children respectively. The highest percentage of positive infected *H. pylori* in subjects who are using private untreated well-water drinking than those the commercial water supply. **Conclusions:** The prevalence of *H. pylori* infection is high among the secondary school healthy students, as well as the university adult students in Kasr Khiar region, 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 region. 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, Asymptomatic, Libya.

Introduction

Helicobacter pylori (*H. pylori*) is a gastric pathogen that chronically infects more than half the world's population. *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 area, 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). 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 (The EUROGAST Study Group, 1993).

Seroepidemiological investigations represent the most rapid and convenient way of obtaining a picture of the prevalence of *H. pylori* infection in a 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 of huge size, 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 asymptomatic university and secondary school students in Kasr Khiar region. The secondary aims were to determine the risk factors (age, gender, and source of drinking water which might be related to the infection with the *H. pylori*.

Materials and Methods: -**Study design and participants: -**

A cross-sectional descriptive study was conducted from December 2018 to March 2019 on two students' groups: one hundred healthy students of the Faculty of Arts and Sciences, Al-Mergib University, and another group which consist of one-hundred and twenty-five (125) healthy secondary school students. Based on the questionnaire data, students with a history of gastro duodenal ulcer, with 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 study. After we obtained written informed consent from the family head of the secondary school students and from the university students, a standard questionnaire was completed by direct interview to obtain individual socio-demographic data regarding each student participant (age, gender, number of family members, smoking, source of drinking water, coffee and Tea consumption, family history of gastric ulcer or gastric cancer). 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 student by peripheral venipuncture under aseptic conditions. Samples were refrigerated on ice during transport to the laboratory (Mira Laboratory, Qasr Khair). After serum separation using (800B Electronic Centrifuge), 250µl serum samples were labeled and frozen at -20°C until testing. Detection of anti-*H. pylori* IgG using immunochromatographic rapid method (*H. pylori* Antibody Rapid Test Cassette Right Sign- China,) was performed according to the manufacturer instructions. The linear tendency of the proportions of positivity of anti-*H. pylori* antibodies in different parameters of exposure was analyzed by the chi-square test for trends. The level of significance was set at $p < 0.05$.

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 study subjects, and the difference in the prevalence across the different parameters, the level of significance was considered when $p < 0.05$.

Results: -

After exclusion of (45) students from the secondary schools and Faculty of Arts and Sciences who reported taken antibiotics currently or did not complete the questionnaire, 225 asymptomatic individuals were enrolled in the study (100 adult university students, 13 Male and 87 Females, mean age 23 years, and 125 young secondary school students, 40 boys and 85 girls, mean age 17 years). Serological testing revealed that 85% (85/ 100) of adult university individuals were positive for anti- *H. pylori* IgG; there was a gradual increase with age, and no statistical difference between genders. However, antibodies to *H. pylori* were detected in the serum of 65%, 81% the secondary school boys and girls respectively. The association between the source of water drinking and *H. pylori* infection is presented in (Table 1). Among the university students, 51(60%) presented *H. pylori* seropositivity using private untreated well water as a source of drinking water, compared to 34 (40%) who brought water from the commercial supermarket. For the

secondary school boys' and girls' students, the association of *H. pylori* positivity and drinking water from the private well was 18(69.3%), 59(72.8%) respectively, when compared with 8 (30.7%), 12(17.4%) for those who bought drinking water from the commercial sector.

Table1. *H. pylori* infection in relation to source of water drinking for the university and Secondary Students school boys & girls in Kasr Khair city.

Water source	University Students (100)	Secondary School Boys (40)	Secondary School Girls (85)			
	<i>H.p</i> Positive	<i>H.p</i> Negative	<i>H.p</i> Positive	<i>H.p</i> Negative	<i>H.p</i> Positive	<i>H.p</i> Negative
Private well	51(60%)	8(53.3%)	18(69.3%)	8(57.1%)	57(82.6%)	9(56.2)
Supermarket	34(40%)	7(46.7%)	8 (30.7%)	6 (42.9%)	12 (17.4%)	7(43.7%)
Total	85 (85%)	15(100%)	26(65%)	14(35%)	69(81.1%)	16(18.8%)

There was no significant association between *H. pylori* seropositivity and the associated risk factors such as Blood group, smoking, parents' education level, family history of stomach diseases.

Discussion:—

To our knowledge, this is the first study to document the prevalence of *H. pylori* among healthy subjects living in a rural western region of Libya. 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 serological testing for *H. pylori* antibody helps in early detection of “silent” peptic ulcer (Vaira *et al.*, 1994). The results of the present study demonstrate that the prevalence of (85%) of asymptomatic university students and (65%, 81%) among the secondary school boys and girls respectively was high in Kasr Khair city and the area surround it, which is similar to other reported from several Libyan cities e.g. Benghazi, where the authors found in healthy individuals (71.4%) infected with *H. pylori* (Mohammad *et al.*, 2011). However, five years later other study from Benghazi found (56.5%) (Almehdawi and Ali, 2016), the reason for the decrease might be the use of antibiotics during the last few years. In Al-Komes region, a study found that (65%) of asymptomatic persons were infected with the gastric pathogen (Lragaa *et al.*, 2014, Nami *et al.*, 2019), our results is similar to other developing countries in which 69% to 82% of adults and children who are infected by 10 years of age. In a rural area from Brazil the antibodies to *H.pylori* were detected in the serum of 77.5% children & teenagers, and in 84.7 % adults (Souto *et al.*, 1998). In Kosovo, the seropositivity of *H.pylori* is moderately high (56.9%) among healthy blood donors (Zhubi *et al.*, 2011). In Iraq, a study conclude that *H. pylori* are highly prevalent (55.8%) among university students in Erbil region, higher prevalence found in older students and those from low social class (Hussen *et al.*, 2013).

Transmission of *H. pylori* is still not entirely clarified, but human- to -human spread through oral-oral or fecal-oral route is thought to be most plausible (Goh *et al.*, 2011). *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 our study most of the students of the university(60%) and the secondary schools(69.3% Boys),(82.6% Girls) used untreated drinking water which possibly contaminated with the *H.pylori*, since a sewage network was not exist, and consequently our study population was exposed to the fecal-oral route of bacterial transmission, a condition similar to that observed other developing countries such as in Brazil where water has been regarded as a source of infection(Rocha *et al.*, 1994).In Kazakhstan, a study suggests 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). Our results highlighted the association between the source of drinking water and *H. pylori* infection. Prevalence of *H. pylori* was higher in participants consuming from the private well non-filtered or non-boiled water compared with participants using water filters or boiling the water before consumption (who bought drinking water from the commercial sector). Our findings are in line with previous research (Rodrigues *et al.*, 2004; Mhaskar *et al.*,2013; Amaral *et al.*, 2017).

A Norwegian group tested 1416 subjects of all age groups in an urban and rural community in Northern Norway 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 (Breckan *et al.*, 2016). *Helicobacter* species have been detected in 142 vertebrate species, including animals from every continent and all four nonfish vertebrate taxonomic classes (Schrenzel *et al.*, 2010). In a rural community, the prevalence of enteric *Helicobacter* species was investigated in domestic and free -living birds (Robino *et al.*, 2010). *Helicobacter pullorum* was detected in 68.6% of intensively farmed poultry and 21.7% of poultry raised in the rural farms. *Helicobacter canadensis* was detected in intensively reared Guinea fowl and for the first time in pheasants from rural farms. (Zanoni *et al.*, 2010). Furthermore, previous findings show that *H. pylori* is capable of survival in different types of aquatic environments under an array of physical variables, the bacterium, unlike other pathogens, is unusually tolerant to pH fluctuations (West *et al.*, 1992).

An interesting research study, concluded that the presence of *H.pylori* in the wells correlated with clinical infection in the consumers, and with the presence of *Escherichia coli*, indicating fecal contamination, and consumption of untreated well water should be considered a risk factor for *H.pylori* infection (Baker and Hegarty, 2001). In Libya, for the difficulty to culture *H. pylori* which required selective medium and Microaerophilic condition, therefore, analysis the private well water from different regions of Libya, using molecular techniques such as Polymerase Chain Reaction (PCR) to confirm the presence of *H. pylori* or other *Helicobacter* species.

In Conclusion, the high prevalence of *H. pylori* positivity (85%) that we obtained in adult university students, and 65%, 81% among the secondary school boys and girls respectively, indicates that infection with this gastric gram-negative bacterium is still a common health problem among our young population. However, we find that the strict relations between *H. pylori* seropositivity and, the source of water, as important risk factor

in our study. These data support the finding that personal and environmental conditions do affect *H. pylori* infectivity in young subjects living in rural region of Libya.

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Notice: The result of this study is part of a project title of the seroprevalence of *Helicobacter pylori* Infection among Libyan Population.