Prevalence of *Helicobacter pylori* Infection and associated risk Factors among Healthy Blood donors.

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Background:- *Helicobacter pylori* (*H. pylori*) is one of the most frequent bacterial gastric pathogen in different parts of the world. In developing countries the infection can be almost ubiquitous, whereas in industrialized countries *H. pylori* infects around 30-50 % of adults. The risk of being colonized by *H.pylori* depends on geographic area, socioeconomic status and age of the host. Now, *H. pylori* has been associated with the most of gastro duodenal diseases. **Aims**:- To find out the prevalence of (IgG) antibodies of the gastric bacteria *H.pylori*. To correlate with the risk factors in voluntary asymptomatic healthy blood donors attending Central Blood Bank in Tripoli city. **Materials & Methods**:- A Blood sample of (175) adult healthy blood donors (50 Females, 125 Males mean age 35 years), anti-*H.pylori* IgG seroprevalence were determined with the ELISA method (Biotech USA), questionnaire covering Sociodemographic variables were completed by interview.

Results:- The overall prevalence of *H.pylori* was 85.1% in the healthy Blood donors, there was a gradual increase with age, and no statistical difference between genders.

Conclusion:- In Tripoli region, *H.pylori* detection in those adult healthy blood donors was high of aged 25-40 years, which might be related to the socioeconomic status, domestic crowding and the source of drinking water as a major risk factors for *H.pylori* infection. Also we confirm that as a non- invasive method, the serologic test such as (ELISA) is a useful technique to detect *H.pylori* infection among healthy population. However, larger studies in other regions of Libya should be conducted to confirm our study findings.

Key words: *H.pylori – cagA –* serology – Blood donors.

Introduction

Helicobacter pylori (*H.pylori*) is a major human gastric pathogen that infects more than half the world's population. It is closely related with a wide range of gastrointestinal diseases. Among individuals infected with *H.pylori*, the host, environmental, and the virulence factors of the infecting strain have been demonstrated to be predictors of gastric pathologies (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 10 years, with reports of infection as early as the first months of life (Bardhan, 1997, Glynn *et al* 2002).

Seroepidemiological investigations represent the most rapid and convenient way of obtaining a picture of the prevalence of *H.pylori* infection in a population (Apan *et al*, 2008). A majority of serological studies from developing countries are now conducted with commercial kits which are inexpensive, simple, and available in the local market (The EUROGAST Study Group, 1993)

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; therefore, the primary aim of this study was to determine the incidence of seropositivity *H.pylori* infection among asymptomatic Blood donors. The secondary aim to correlate with the risk factors in voluntary healthy Blood donors in Tripoli region and its surrounding.

Materials and Methods

A cross- sectional seroprevalence study was conducted from September 2016 to May 2017 among healthy subjects in Tripoli city attending Central Blood bank. Based on the questionnaire data, Blood donors individuals 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 all subjects 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, local of residence and medication taken one month before the interview (particularly proton pump inhibitor and antibiotics) were also recorded. A Blood sample (5 ml) was collected from each participant by peripheral venipuncture under aseptic conditions. Samples were refrigerated on ice during transport to the Blood bank laboratory. After separation, 250µl serum samples were labeled and frozen at -20°C until analysis.

Determination of anti-*H.pylori* IgG

For the diagnosis of infection with *H.pylori*, one Hundred and seventy –five (175) serum samples 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-Helicobacter 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 healthy subjects, and the difference in the prevalence across the different parameters, the level of significance were considered when p < 0.05.

Results

After exclusion of (125) healthy Blood donors, who reported current taken antibiotics or did not complete the questionnaire, (175) asymptomatic individuals were enrolled in the study (125 Male and 50 Females, mean age 23 years). Serological testing revealed that 85% (149/ 175) of adult healthy individuals were positive for anti- *H pylori* IgG, and a statistical significant difference was found in H.pylori IgG between genders. (Table 1); there was a gradual increase with age, (Table 2).

The association between the source of water drinking and *H.pylori* infection is presented in (Figure 1). Among the healthy blood donors, 111 (74.4%) presented *H.pylori* seropositivity using water from the commercial supermarket compare to 20 (13.4%) and 18(12%) who using private untreated well water and tape water as a source of drinking water.

Table 1. Prevalence of *H.pylori* infection in relation to sex.

	H.pylori Positive	H.pylori Negative	Total	P-Value
Male	125 (90.6%)	13 (9.4%)	138 (78.9%)	
Female	24 (64.9%)	13 (35.1%)	37 (21.1%)	0.000346
Total	149 (85.1%)	26 (14.9%)	175 (100.0%)	

Table 2. *H.pylori* infection in relation to age group of the asymptomatic blood donors.

	H.pylori positive	H.pylori negative	Total	P_Value
20-24	13 (68.4%)	6 (31.6%)	19 (10.9%)	0.038803
25-29	33 (94.3%)	2 (5.7%)	35 (20.1%)	
30-34	19 (76.0%)	6 (24.0%)	25 (14.4%)	
35-39	38 (92.7%)	3 (7.3%)	41 (23.6%)	
40 or highest	45 (83.3%)	9 (16.7%)	54 (31.0%)	
Total	148 (85.1%)	26 (14.9%)	174 (100.0%)	

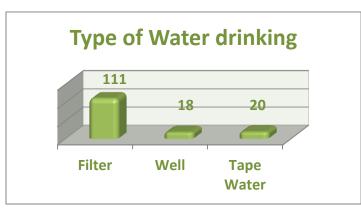


Figure 1. Helicobacter pylori infection rates in relation to the source of drinking water.

The possible of the prevalence of *H.pylori* infection and the risk factors associated such as smoking, members of family, education level, the monthly income, family history of stomach diseases, type of work, and social status was also analyzed. There was no significant association between presence of *H.pylori* antibodies and smoking, family monthly income, the social status as well as the type of work. However, regarding the blood group, it was found that IgG anti-*H.pylori* was presence in 54 (36%), 27 (18%), 6 (4%), and 62(42%) of the healthy blood donors of the A, B, AB, and O blood group respectively, (Figure 2)

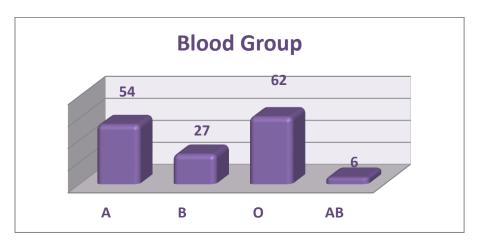


Figure 2. Seroprevalence of anti-H.pylori IgG in asymptomatic subjects according to Blood group.

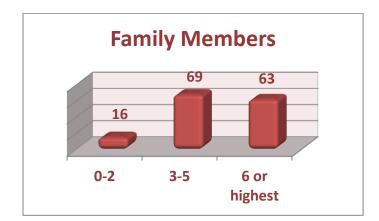


Figure 3. Seroprevalence of anti-H.pylori IgG in healthy blood donors in relation to family members.

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 (Kusters *et al* 2006). The serological testing for *H.pylori* antibody helps in early detection of "silent" peptic ulcer (Vaira *et al*, 1994). The present study was the first seroprevalence of *H.pylori* infection in a adult healthy blood donors in western region of Libya. The results of the present study demonstrate that the

prevalence of (85%) of asymptomatic individuals was high in Tripoli 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, an epidemiological studies found that (65%) of asymptomatic persons were infected with the gastric pathogen *H.pylori* (Lragaa et al 2014, Nami et al 2017), 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.pvlori* 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 Kenya 93% of the (14) asymptomatic volunteers were found to have *H.pvlori* infection (Lachlan et al 1989). 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). Our results indicate that the seroprevalence of *H.pvlori* is increasing with the age, which is similar to other study such as among the Algerian children the seropositive of the bacterium were 43%, and the prevalence rose steadily with age, reaching a peak of 92% between the ages of 40 and 49 years (Megraud et al 1989). An age specific increase in the prevalence of H.pvlori infection was observed in 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(Lachlan *et al* 1989). In Ghana (Awuku.*et al* 2017)

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 healthy blood donors74.4% presented *H.pylori* seropositivity using water from the commercial supermarket compare to 13.4% and 12% who using private untreated well water and tape water as a source of drinking water respectively. Drinking filter water from the commercial places 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 in 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 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). A study concluded that the presence of *H.pvlori* in the wells correlated with clinical infection in the consumers, and with the presence of Escherichia coli, indicating fecal contamination, and consumption of contaminated water should be considered a risk factor for *H.pylori* infection (Baker and Hegarty, 2001).

In Conclusion, the high prevalence of *H.pylori* positivity (85%) that we obtained in adult healthy blood donors, 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, and living in a crowded conditions, as risk factors in our study. These data support the finding that personal and environmental conditions do affect *H.pylori* infectivity in young subjects living in western region of Libya. Our data indicate that, the detection of *H.pylori* using molecular

methods should be done as a routine test, and knowledge about the reservoirs and modes of transmission could help to explain the high prevalence rates found for *H.pylori* in the developing countries.

References

Almehdawi, K.A., and Ali, R.H., 2016 "The prevalence of *Helicobacter pylori* Infection in Benghazi, Libya", IOSR Journal of Dental and Medical Sciences, 15, 73-77.

Apan, T.Z., Iseri, L., Aksoy, A., Guliter, S., 2008. The antibody response to *Helicobacter pylori* in the sera from a rural population in the central Anatolia region of Turkey. The Journal of Health Sciences, 54:671=674.

Awuku.Y, A, Simpong,D.L, Alhassan,I.K, Tuoyire,D.A, Afaa.T, and Adu,P. Prevalence of helicobacter pylori infection among children living in a rural setting in Sub-Saharan Africa.BMC Public Health (2017) 17:360-365.

Bardhan, P.K., 1997, "Epidemiological features of *Helicobacter pylori* infection in developing countries", Clinical Infectious Diseases, 25, 973-979.

Bakak, A.S., Salih, B.A., 2002, "Prevalence of *Helicobacter pylori* in asymptomatic subjects in Libya", Diagnostic Microbiology and Infectious Disease, 43, 265-268.

Baker, K., and Hegarty, J.P., 2001, "Presence of *Helicobacter pylori* in drinking Water is Associated with Clinical Infection", Scandinavian Journal of Infectious Diseases, 33, 744-746.

Burucoa, C., and Axon, A, 2017"Epidemiology of *Helicobacter pylori* infection", Helicobacter, 22 (Supplement 1), 1-5.

Glynn M.K, Friedman C. R, Gold B. D, Khanna B, Hutwagner L, Lihoshi N, Revollo C, Quick R. 2002, "Seroincidence of *Helicobacter pylori* Infection in a Cohort of Rural Bolivian Children: Acquisition and Analysis of Possible Risk Factors", Clinical Infectious Diseases, 35, 1059-1065.

Hussen, B.M., Qader, S.S., Ahmed, H.F., Ahmed, S.H. 2013, "The Prevalence of Helicobacter pylori among University Students in Iraq", Indian Journal of Science and Technology, 6,5019-5023.

Kusters, L., van Vliet, A., Kuipers, E.J., 2006, "Pathogenesis of *Helicobacter pylori* Infection", Clinical Microbiology Reviews, 19, 449-490.

Lachlan GW, Gilmour HM, Jass JJ. Campylobacter pylori in Central Africa. British Medical Journal 1988;296:66-70

Lragaa, K., Dedeh, A., Nami, A., 2014, "Frequency and risk factors of *Helicobacter pylori* among diabetic Patients in Al-komes Central Hospital, Libya; Preliminary results, 11thInternational Workshop on Pathogenesis and Host Response in Helicobacter Infections, Helsingor, Denmark, 2-5 July[Abstract].

Megraud F, Brassens-Rabbe M P, DenisF, Belbouri A, Hoa D Q. Seroepidemiology of Campylobacter pylori infection in various populations. Journal of Clinical Microbiology 1989;27:1870–1873

Mohammad, M.A., Altayar, M., Toboli, A.B., Bakka, A. 2011, "Characteristics of *Helicobacter pylori* infection in Libyan Healthy peoples in two teaching Hospitals in Benghazi", Medical Journal of Islamic World Academy of Sciences, 19:1,27-32.

Monne, R., Volpe, A., Basho, M., Fumarola, L., Trerotoli, P., Kondili, L.A., Bino, S., Schinaia, N., Dentico, P., 2008," *Helicobacter pylori* seroprevalence in selected groups of Albanian volunteers", Infection, 36,345-350.

Nami, A., Alagaali, M., Abushnag, D., Bader, R., Algalal, R., Qendeela, J. 2017, "Prevalence of *Helicobacter pylori* infection in asymptomatic Libyan children and adults", Helicobacter, 22 (Supplement 1) 98. [Abstract].

Nurgalieva, Z.Z., Malaty, H.M., Graham, D.Y., Almuchambetova, R., Machmudova, A., Kapsultanova, D., Osato, M.S., Hollinger, F.B, and Zhangabylov, A. 2002," *Helicobacter pylori* infection in Kazakhstan: effect of water source and Household hygiene" American Journal of tropical Medicine and Hygiene, 67, 201-206.

Rocha, G.A., Oliveira, A.M.R., Queiroz, D.M.M., Moura, S.B., Mendes, E.N., 1994, "Prevalence of *Helicobacter pylori* infection in two different populations from Minas Gerais, Brazil", American Journal of Gastroenterology, 89, 1313(Abstract 111).

Souto, F.J.D., Fontes, C.J.F., Rocha, G.A., Oliveira, A.M.R., Mendes, E.N., Queiroz, D.M.M. 1998, "Prevalence of *Helicobacter pylori* Infection in a rural Area of the State of Mato Grosso, Brazil", Mem. Institute of Oswaldo Cruz, Rio de Janaeiro, 93(2), 171-174.

The EUROGAST Study Group, 1993, "Epidemiology of, and risk factors for, *Helicobacter pylori* infection among 3194 asymptomatic subjects in 17 populations", Gut, 34, 1672-1676.

Vaira, D., Miglioli, M., Mule, P., Holton, J., Menegatti, M., Vergura, M., et al 1994, "Prevalence of peptic ulcer in Helicobacter pylori positive blood donors, Gut, 35, 309-312.

Zhubi, B., Baruti-Gafurri, Z., Mekaj, Y., Zhubi, M., Merovci, I., Bunjaku, L., Topciu, V., Devoli-Disha, E, 2011, "*Helicobacter pylori* infection according to ABO blood group among blood donors in Kosovo", Journal of Health Sciences, 1(2), 83-89.