RESEARCH STUDY

KCMJ 2013; 9(2): 17-20

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# Serodiffusion of Helicobacter Pylori and HAV in Patients Attending Two Centers in Baghdad

ARTICLE INFORMATION

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***Article history:***
Received; September, 8, 2012.
Revised form; December, 11, 2012.
Accepted; December, 18, 2012.

***Keywords:***
Helicobacter pylori
HAV
ELISA
Endoscope
Gene A

***Background:*** Helicobacters are motile curved, oxidase and catalase positive, gram negative rods similar in morphology to vibrios. The cells have polar flagella and are often attached at their ends given pairs "S" shapes or seagull appearance.

***Objectives:*** The present study was undertaken to estimate the serodiffusion of H. pylori and hepatitis A virus (HAV) in 300 patients attending two centers in Baghdad.

***Methods:*** HAV was determined by the detection of HAV-IgM in the serum samples. Detection of H. pylori was by ELISA and endoscopic examination.

***Results:*** The serodiffusion of H. pylori was 40% (n=120). Out of the 120 patients infected with H. pylori, 50(41 .6%) patients were diagnosed clinically to have gastric antraum, the remaining 70 (58.4%) patients were apparently free of any disease. The present findings showed that the serodiffusion of anti hepatitis A virus (HAV) antibody was 30 % (n=90).

***Conclusions:*** This study shows relatively high serodiffusion of H. pylori and HAV in the studied group. None of the cases had co-infection with both H. pylori and HAV; this may be due to the small size number of the patients under study.

## Introduction:

Helicobacters (H) are motile curved, oxidase and catalase positive, gram negative rods similar in morphology to vibrios. The cells have polar flagella and are often attached at their ends given pairs "S" shapes or seagull appearance [(1)].

H. pylori grows optimally at a pH of 6.0-7.0 and would be killed or not grow at the pH within the gastric lumen. Gastric mucus is relatively impermeable to acid and has a strong buffering capacity.On the lumen side of the mucus, the pH is low (1.0-2.0) while on the epithelial side the pH is about 7.4.H pylori is found deep in the mucous layer near the epithelial surface where physiologic pH is present. H. pylori also produce a protease that modifies the gastric mucus and further reduces the ability of acid to diffuse through the mucus. H. pylori produce potent urease activity, which yields production of ammonia and further buffering of acid. H. pylori are quite motile, even in mucus, and are able to find its way to the epithelial surface. H. pylori overlies gastric-type but not intestinal-type epithelial cells [(1,2)].

Helicobacter pylori infection is a major cause of chronic antral gastritis, which is associated with duodenal or gastric ulcer and probably gastric adenocarcinoma. Although the precise mechanism of transmission is not yet known, there is strong evidence for person -to- person transmission of H. pylori via the oral-fecal route. Overcrowding and poor sanitary conditions are risk factors for acquisition of H. pylori [(3,4)].

The human pathogen H. pylori colonizes the stomachs of more than 50% of the world's population.The molecular interaction between H. pylori and cells of the gastric epithelium is thought to be the major factor inducing inflammatory responses of the infected host, which can result in the development of the malignant diseases gastric cancer or lymphoma of the MALT (mucosa-associated lymphoid tissue) system [(2)].The pathogenesis of H. pylori mainly depends on the exposure of several bacterial factors, including cytotoxin-associated gene A (Cag A), the type IV secretion system(T4SS), vacuolating cytotoxin A (Vac A), outer inflammatory protein A (Oip A) and several adherence factors, to the host [(5-7)].

Hepatitis A virus (HAV) is a distinct member of the picornavirus family. HAV is a27 to 32nm spherical particle with cubic symmetry, containing a linear single-stranded RNA genome with a size of 7.5 kb. Only one serotype is known. There is no antigenic cross-reactivity with HBV or with the other hepatitis viruses. Genomic sequence analysis of a variable region involving the junction of the 1D and 2A genes divided HAV isolates into seven genotypes [(8)].

HAV is stable to treatment with 20% ether, acid (pH 1.0 for 2 hours), and heat (60$^{o}$C for 1 hour), and its infectivity can be preserved for at least 1 month after being dried and stored at 25$^{o}$C and 42% relative humidity or for years at 20$^{o}$C. The relative resistance of HAV to disinfection procedures emphasizes the need for extra precautions in dealing with hepatitis patients and their products [(1,8)].

Hepatitis A virus is the cause of what formerly termed infectious hepatitis or short-incubation hepatitis. This virus is spread by the fecal- oral route, and out-breaks may be associated with contaminated food or water [(9)].

The prevalence of exposure to HAV increases with increasing age; decreases with increasing socioeconomic class; is independent of sex and race; varies in different parts of the world as a function of hygienic, developmental, and unrecognized geographic factors; and is not affected by immune deficiency or immaturity [10]. Transmission of type A hepatitis is enhanced by poor personal hygiene such as that seen in institutions for the mentally retarded. No epidemiologic evidence has confirmed the existence of viremic or intestinal carriers of HAV, and the virus is rarely, if ever, spread by parenteral mechanisms [11]. Finally, HAV appears to play no role in chronic liver disease and a very minor role in fulminant hepatitis; however, HAV is responsible for a sizable proportion (approximately 20%-40%) of sporadic hepatitis among urban adults [8]. Several former studies showed that the emerging epidemiologic pattern of H. pylori infection seems very similar to that of HAV [12,13].

The aim of this study was to investigate the serodiffusion of HAV and H. pylori infection in patients attending two centers in Baghdad.

## Methods:

Study population and serum samples collection: Serum samples were obtained from 300 patients, their ages range between 10-60 years with mean age 35 years (120 females and 180 males), attended the Medical City Teaching Hospital, and Gastrology and Hepatology Center in Baghdad, between April 2008 and December 2008 .Ten milliliters of blood were taken and the serum was separated at -20Ċ for serological processes.

Detection of hepatitis A virus HAV: Hepatitis A virus infection in patients of the studied group was determined by the detection of HAV-IgM in the serum samples. IgM anti HAV was measured by enzyme linked immunoassorbant assay (ELISA) (DRG international anti HAV IgM kit [14].

Detection of Helicobacter pylori:

1- By (ELISA): serum antibodies against H. pylori were analyzed by means of enzyme immunosorbent assay (ELISA) (Biohit plc. REF 601 040.01IVD) [15].
2- By endoscopic examination: All subjects gave informal consent for the collection of biopsy tissue. Patients who had taken (antibiotics, $H_2$ blocker, colloidal Bismuth or omeprazol, one to two months prior to endoscopy were excluded from below [16]. Endoscopy was performed under injection diazepam 10mg IV as premedication, from each patient six biopsies were taken from the gastric antrum, the biopsy specimens were subjected to the following test procedure:
   a. One minute Endoscopy Room Test (OMERT): In this test, two biopsy specimens were put in 1ml of 10X w/v freshly prepared urea solution in deionized water (pH 6.8) at room temperature, two drops of 1% phenol red were added to above solution as an indicator. A change in color from yellow to pink observed 1-5 minutes after addition of indicator was taken as positive test (i.e. H. pylori present), whereas absence of such a color change or change of color after 5 minutes was taken as negative test [17].
   b. Histology: Two antral biopsy specimens from each patient were fixed in 10% buffered formalin and were processed routinely [18,19], paraffin wax sections were cut and stained with giemsa stain. Every specimen was examined by the same experimental histopathologist without knowledge of the result of the other tests.
   c. Microbiology analysis: Two biopsy specimens from antrum were rubbed on a dry slide (separate glass slide used for separate site) heat fixed and then stained for the presence of H. pylori under light microscopy [19].

## Results:

In the present study we conducted the serodiffusion of H. pylori IgG antibody and HAV antibody in300out and in patients attending two centers in Baghdad (Table1).

Table1: Serodiffusion test of H. pylori and HAV.

| Serodiffusion test | Patients with positive test | |
|---|---|---|
| | No. | (%) |
| H. pylori IgG | 120 | 40 |
| HAV IgG | 90 | 30 |

The serodiffusion of H. pylori was 40% (n=120). Of those 120 patients infected with H. pylori 50 patients (41.6%) were diagnosed by the clinician to have gastric antraum, the remaining 70 patients (58.4%) were apparently free of any disease, those patients how were H. pylori IgG positive and apparently healthy were considered as controls .

H. pylori positivity among gastric antraum cases were 23 (46%) by histology, 17 (34%) by gram's staining and 10 (20%) by OMERT (Table 2).

Table 2: H.pylori positivity among Gastric antrum patients.

| Diagnostic test | Patients with positive test | |
|---|---|---|
| | No. | (%) |
| Histology | 23 | 46 |
| Gram's staining | 17 | 34 |
| OMERT | 10 | 20 |

Prevalence of H. pylori in controls was 35 (50%) by histology, 20 (28.6%) by gram's staining and 15 (21.4%) by OMERT (Table 3).

Table 2: H.pylori positivity among Controls.

| Diagnostic test | Patients with positive test | |
|---|---|---|
| | No. | (%) |
| Histology | 35 | 50 |
| Gram's staining | 20 | 28.6 |
| OMERT | 15 | 21.4 |

## Discussion:

Currently many different diagnostic tests exist for detecting H. pylori infection .Each test has its own merits and demerits in terms of indication, sensitivity, specificity, cost and time [16,20]. In our study, we observed that

histology had the highest detection rate, compared to other two tests. Thus, histology is superior to other test procedures and could be considered to confirm H. pylori diagnoses following IgG test by ELISA. All positive cases in gastric antrum patients were positive by ELISA. While all controls (n=70) were positive by ELISA, 11 of them were negative by Histology, gram's staining and OMERT. Association between H. pylori infection and gastric ulcers has been supported by many previous reports [21,22].

The serodiffusion of H. pylori infection varies both between and within populations, with the rateof acquisition being generally higher in underdeveloped than in industrialized countries [23]. Cross-sectional studies have consistently shown a gradual increase in H. pylori serodiffusion with age, which has been interpreted as a birth cohort effect reflecting a decrease in the rate of acquisition in successive generations of children as sanitation improved and standards of living increased [24].

Helicobacter pylori infection is a common bacterial infection among humans. Current knowledge implies that acquisition of H. pylori seems to occur predominantly in childhood and a major role of intra-familial spread is now beyond controversy. H. pylori has been classified by the World Health Organization as a type I carcinogen. Nearly 50% of the world's population is estimated to be infected with H. pylori. The prevalence of H. pylori infection continues to vary markedly between developing countries and developed countries, and according to ethnicity, place of birth and socioeconomic factors among people living in the same country [25]. According to the nation-wide serodiffusion study for 5,732 asymptomatic Korean population, the serodiffusion of H. pylori infection was 46.6% [26]. Garza et.al [24], found H. pylori in 49.3% of their studied group. Naja et.al [27], reported 10% seropositivity to H. pylori in Canadian patients.

Communicable diseases are still one of the main heath problems throughout the world [28]. Present findings showed that the serodiffusion of anti-hepatitis A virus antibody was 30% (n=90). Many studies have been carried out to investigate the serodiffusion of HAV in different countries. Takahashi et.al [28], found HAV antibodies in 27% of their studied group. In addition Normann et.al, reported 25% of HAV antibody in turkey patients [29].

Former study carried out by Yu Lin et.al [30], registered much lower serodiffusion of HAV which was 1.4%. Kocuipchyk et.al [31], also reported much lower serodiffusion of HAV (4.3%) in Canadian patients. Significant relationship has been found between HAV seroprevlaence and source of water supply and socioeconomic state [32].

Hepatitis A virus (HAV) is prominent in many areas of the world [33]. In North America, infection rates have declined with better hygiene practice and public sanitation but remain heterogeneous across geographic and socioeconomic state [34]. Further decline is possible with HAV vaccines, which provide consistent long-lasting protection and have been available since mid-1990s [33]. In the United States, universal vaccination of children and youth has been in place for about 6 years in high endemicity areas leading to historically low rates nationally in recent years. In Canada, the current national immunization guide recommends HAV vaccines for individuals at increased risk of infection or its complications [35].

## Conclusions:

In conclusion, this study shows relatively high serodiffusion of H. pylori and HAV in the studied group. None of the cases had confection with both H. pylori and HAV, this may be due to the small size number of the patients under study.

## Recommendations:

For our knowledge there is no previous study in Iraq accounting the serodiffusion of H. pylori and HAV in general population, and determine the most important unusual risk factors for H. pylori infection acquisition. The epidemiologic association between H. pylori and HAV also should be evaluated in a detailed study.

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