Study of H.Pylori in a Group of Iraqi Patients with Inflammatory Bowel Disease (Histological and Molecular Study)

Sinan S. Farhan , Khalid A. Al-Khazraji ,Fadhil A. Al-Khafaji, Huda M. Al-Khateeb, Zahraa Q. Al-Kassam

ABSTRACT:

BACKGROUND:

Helicobacter pylori is one of the commonest bacterial pathogens in human. The organism is associated with development of peptic ulcer diseases, lymphoproliferative disorders and gastric cancer.

Helicobacter pylori could be isolated from patients with inflammatory bowel disease (IBD) but still the possibility of etiological link need further study therefore

OBJECTIVE:

Is to assess the possible causal factor of H.pylori in development of inflammatory bowel disease, in addition to assess Cytotoxic associated gene A(CagA) gene status in *H.pylori* positive samples.

PATIENTS AND METHODS:

Study involved 120 patients of colonoscopically determined normal colon (n=90) & patients with IBD (n=30) including both Ulcerative colitis(n=16) and Crohn's disease(n=14). Those patients is further divided into three age groups including <20 years group, 20-40 years group and >40 years group of Iraqi patients. Endoscopic specimens after histopathological confirmation of diagnosis will be tested for Biopsy Urease Test (BUT) and Hematoxylin & Eosin (H&E) methods (for detection of H. pylori) also detection of CagA mRNA using *In Situ Hybridization* technique with a biotin labeled probe (to specify pathogenic *H. pylori*)

RESULTS:

H.pylori detected in 36.7% (using BUT) and 30% (using H&E) in the colon of IBD patients and 33.3% (using BUT) and 26.7% (using H&E) in patients with normal colon (NC patients) as a control. Among our *H.pylori* positive patients, there was significant difference (P=0.036) regarding CagA status in which 25% were CagA positive of NC patients and 66.7% CagA positive H.pylori in IBD patients using *In Situ Hybridization* technique.

CONCLUSION:

H.pylori was isolated from nearby or the site of lesion of patients with inflammatory bowel disease although there was no statistical relationship between H.pylori and IBD. In addition CagA genes were more prominent in *H.pylori* that isolated from IBD patients than H.pylori of normal colon. Also there was no possible relationship between age and infection rate of *H.pylori* in both IBD & NC patients.

***KEYWORDS*: *h.pylori*,** inflammatory bowel disease, biopsy urease test, in situ hybridization

INTRODUCTION:

More than 50% of the world's population harbor *H. pylori* in their upper gastrointestinal tract. Infection is more prevalent in developing countries, and incidence is decreasing in western countries. it is known individuals typically become infected in childhood [(1)].

H. pylori's helix shape (from which the generic name is derived) is thought to have evolved to penetrate the mucoid lining of the stomach [(2)].

Most references mentioned that *H. pylori* exclusively colonizes gastric epithelium and only found in first part of duodenum [(3,4)]. However, *H. pylori* was recently detected in the normal and certain pathological colonic mucosa [(5)].

The vast majority of colonized people remain healthy and asymptomatic and only 15% develop disease.

bowel disease (IBD) varies widely between populations (10).Ulcerative colitis and Crohn's disease are had a protracted relapsing and remitting course, usually extending over years. The diseases

College of Medicine Baghdad University .

have many similarities and it is sometimes impossible to differentiate between them. A crucial distinction is that ulcerative colitis only involves the colon, while Crohn's disease can involve any part of the gastrointestinal tract from mouth to anus. The incidence of inflammatory

Pathogenesis depends upon strain virulence, host genetic susceptibility and environmental cofactors [6].

Cytotoxin- associated gene A"(Cag A)which was identified as an immune dominant antigen, located on the surface of the *H.pylori* [7]. The type IV secretion apparatus also injects the cag PAI-encoded protein CagA into the stomach's epithelial cells, where it disrupts the cytoskeleton, adherence to adjacent cells, intracellular signaling, cell polarity and other cellular activities [8].

Inflammatory bowel disease (IBD) is a group of inflammatory conditions of the colon and small intestine. The major types of IBD are Crohn's disease and ulcerative colitis [9].

Certain articles stated that *H. pylori* detected in the normal colon [5,11,12] while other studies did not find *H.pylori* in colon [13, 14], Previous studies had a controversy on the possible relationship between *H.pylori* and IBD in which certain studies suggested that there was no relationship between infection with *H.pylori* and development of IBD [11,12,15] while other studies [14,16] suggested a possible relationship between them, therefore and from this controversy, we got the aim of this study.

PATIENTS AND METHODS:

Patients were selected between December 2009 and June 2010 from those attending "the Endoscopic Unit" in the Baghdad Teaching Hospital including 90 patients with normal colon (NC patients) and 30 patients with IBD including both Ulcerative colitis(n=16) and Crohn's disease(n=14). The diagnoses of patients were confirmed through histopathological examination of biopsies. Patients' slides were assessed by two examiners for the double blind assessment. The range of patient's age was 15 to 62 years. The patients were divided according to their age into three groups <20 years group, 20-40 years group and >40 years group of Iraqi patients.

All selected patients should not use any antibiotic, proton pump inhibitor (PPI) therapy or H2 blocker for at least 4 weeks. Also they should not have taken bismuth treatment and NSAIDs for at least 3 weeks.

One day before the colonoscopy, the patients submitted to a special dietary regimen and received laxative in order to evacuate the colon. Intestinal biopsy samples were taken, during colonoscopy (Olympus instrument and a multibite forceps) from the, cecum, ascending, transverse and descending colon, sigmoid, of each patient. Following endoscopic diagnosis, 2-4 mucosal punch biopsy specimens were taken. One biopsy specimen was used for biopsy urease test (BUT) to detect *H.pylori* in tissue sample. The Urease reaction obtained from *H. pylori* in Rapid Urease Medium occurs more quickly than that seen by other Urea splitting organisms. As a result, it is an effective test for the detection of *H.pylori* with the development of a pink-red or red-violet color. Negative outcome tests were kept up to 20 hours. Other biopsy specimens were fixed with 10% buffered neutral formalin for the preparation of paraffin embedded tissue blocks.

Sections of 5µm in thickness were mounted on ordinary slides for H&E stain and on charged slides for In Situ Hybridization procedure to detect CagA mRNA using Biotinylated DNA probe together with in situ hybridization detection kit (Maxim Biotech/USA).

The kit contains a house keeping gene probe as a positive control. The procedure was done according to manufacturer instructions including deproteinization using Proteinase K , hybridization of denatured biotinylated probe to the sequence of target mRNA in tissue section. The hybridized probe was then detected by Streptavedin-Alkaline phosphatase (Streptavedin-AP) conjugate. Upon addition of substrate solution 5-bromo, 4-chloro 3,indolylphosphate/ nitro blue tetrazolium (BCIP/NBT), an intense blue signal appeared at the specific site of hybridized probe in addition to use of nuclear fast red (NFR) as a counter stain.

Statistical analysis was done using software SPSS 16 for Windows. Differences among groups were evaluated by using Chi-square test and Fisher's exact test (2 x 2 Table). Differences were considered to be statistically significant at P value less than (0.05).

RESULTS:

In tables 1&2, they revealed the percentage of *H.pylori* infection in different age groups of patients with IBD using BUT and H&E methods (Figure 1) respectively. Chi-square test showed there was no significant difference (P>0.05) therefore, there was no statistical relationship between age and *H.pylori* infection.

In tables 3&4, also Chi square test revealed that there was no statistical relationship between age and *H.pylori* infection in NC patients using both BUT and H&E methods respectively.

In table 5&6 assessment of the relation between age and status of CagA gene in *H.pylori* among IBD & NC patients was done (figure 2&3). Chi square test revealed that there was no statistical relationship between age and CagA gene in *H.pylori* in both NG & NC patients.

In table 7, a comparison was done between IBD & NC patients according to *H.pylori* infection using BUT. Fisher Exact Test revealed that there was no significant difference between the two groups (P>0.05), therefore, there was no statistical relationship between IBD patients and *H.pylori* infection.

In table 8, the same result was found between the two groups using H&E method (P>0.05).

In table 9, CagA positive *H.pylori* were more in IBD patients (66.7%) than patients of NC group (25%) Fisher Exact Test revealed that there was significant difference between the two groups (P=0.036).

Odd ratio revealed that *H.pylori* in IBD patients was six times more to be CagA positive than *H.pylori* in NC patients.

Table 1: H.pylori prevalence among different age groups of IBD patients using BUT.

Age Group	*H.pylori*	
	Positive (%)	Negative (%)
<20	1(50)	1(50)
20-40	5(38.5)	8(61.5)
>40	5(33.3)	10(66.7)
Total	11(36.7)	19(63.3)
P value	>0.05	

No significant difference (P>0.05)

Table 2 : **H.pylori prevalence among different age groups of IBD patients using H&E.**

Age Group	*H.pylori*	
	Positive (%)	Negative (%)
<20	0	2
20-40	4(30.8)	9(69.2)
>40	5(33.3)	10(66.7)
Total	9(30)	21(70)
P value	>0.05	

No significant difference (P>0.05)

Table 3: *H.pylori* distribution in sections of normal colon using BUT

Age Groups	*H.Pylori* infection using BUT		Total
	Positive	Negative	
<20	6(28.6)	15(71.4)	21
20-40	9(39.1)	14(60.9)	23
>40	15(32.6)	31(67.4)	46
Total	30(33.3)	60 (66.7)	90

No significant difference (P>0.05)

Table 4: *H.pylori* distribution in sections of normal colon using H&E stain.

Age Group	*H.Pylori* infection using H&E		
	Positive (%)	Negative (%)	Total
<20	5(23.8)	16(76.2)	21
20-40	7(30.4)	16(69.6)	23
>40	12(26.1)	34(73.9)	46
Total	24(26.7)	66(73.3)	90

No significant difference (P>0.05)

Table 5: CagA gene expression among positive *H.pylori* patients with IBD.

Age Group	CagA gene expression	
	Positive (%)	Negative (%)
<20	0	0
20-40	3(75)	1(25)
>40	3(60)	2(40)
Total	6(66.7)	3(33.3)

No significant difference (P>0.05)

Table 6: CagA gene expression among positive *H.pylori* patients with normal colon.

.Age Groups	CagA gene expression		Total
	Positive (%)	Negative (%)	
<20 y	1(20)	4(80)	5
20-40	1(14.3)	6(85.7)	7
>40	4(33.3)	8(66.7)	12
Total	6(25)	18(75)	24

No significant difference (P>0.05)

Table 7: (2x2) table showing *H.pylori* distribution among IBD and NC groups. (BUT assessed)

Group	*H.Pylori*		
	Positive (%)	Negative (%)	Total
IBD	11(36.7)	19(63.3)	30
NC	30(33.3)	60 (66.7)	90
Total	77(51.3)	73(48.7)	150

No significant difference (P>0.05)

Table 8: (2x2) table showing *H.pylori* distribution among IBD and NC groups. (H&E assessed)

Group	*H.Pylori*		
	Positive	Negative	Total
IBD	9 (30)	21(70)	30
NC	24(26.7)	66(73.3)	90
Total	66(44)	84(56)	150

No significant difference (P>0.05)

Table 9: (2x2) table disclosing the distribution of CagA gene between IBD and NC groups.

Group	CagA		
	Positive	Negative	Total
IBD	6(66.7)	3(33.3)	9
NC	6(25)	18(75)	24
Total	13(19.7)	53(80.3)	66

significant difference (P=0.036)

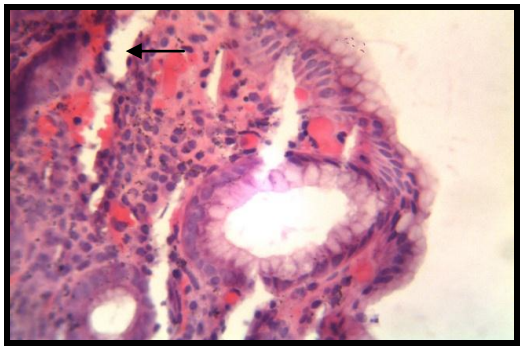

Figure 1: Detection of *H.pylori* in the colon of 31 years old patient with IBD. (Haematoxylin and Eosin, 400X).

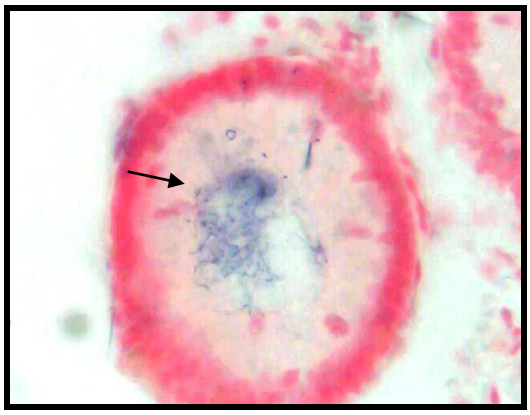

Figure 2: CagA positive expression by BCIP/NBT (bluish purple) and counter stained by NFR (400x). The biopsy was taken from a 30 years old patient with normal colon.

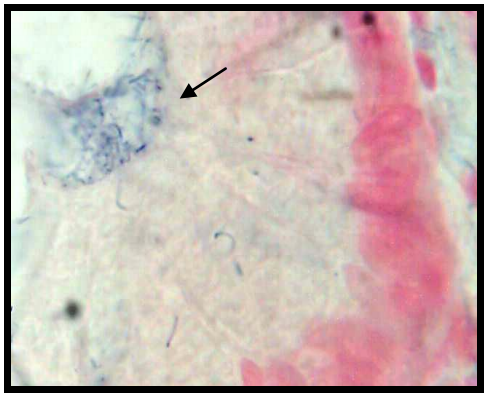

Figure 3: CagA positive expression by BCIP/NBT (bluish purple) and counter stained by NFR (1000x). The biopsy was taken from a 42 years old patient with IBD.

DISCUSSION:

Infection with *H. pylori* may be the most common human infection and in many populations, infection rates of 80–90% are not unusual [17].

The prevalence of *H. pylori* infection also varies widely by geographic area, age, race, and socioeconomic status. [4] However, only few of those colonized people developed diseases related to *H.pylori*.

Regarding IBD, there are many evidences supporting a multifactorial genesis comprising a combination of genetic predisposition, immune response, and environment, most notably the bacterial gut microbes or luminal antigens [11].

Inflammatory bowel disease is affected by cigarette smoking, sanitation and hygiene [18].

Certain studies detected *H.pylori* infection among IBD patients, so they suggested a possible role of *H.pylori* in IBD although other studies suggested no possible role of *H.pylori* in IBD so the role of *H.pylori* in IBD is still controversial [19,20].

Our study disclosed presence of *H.pylori* in the colon in 36.7% (using BUT) and 30% (using H&E) of IBD compared to 33.3% (BUT) and 26.7% (H&E) in NC group (Control). A comparison was done between *H.pylori* infection in both NC & IBD groups which revealed no significant difference (P>0.05)

Halme et al. [15] elicited 15% *H.pylori* positive cases among IBD cases (using seroprevalence and urea breath test).

Moreover, Pearce et al. [21] reported *H.pylori* positive in 21.6% of ulcerative colitis and 11.9% of patients with Crohn's disease (using serological and urea breath tests).

Vare et al. [22] reported 24% *H.pylori* positive cases in IBD and 37% in their controls using serological test.

The above studies depended on Serological detection of *H.pylori* which is not precise in detection of previous from recent infection [23] while our results depend on direct isolation of *H.pylori* from site of lesion which could be more possible to have a role in IBD occurrence.

Oliviera et al. [11] elicited 30% *H.pylori* positive in Crohn's disease cases (Identification in intestinal mucosa using PCR) which is similar to our findings.

H.pylori had been investigated owing to the *possible* causality and pathogenesis of IBD. Most investigations concluded that *H.pylori* is unlikely to play a role in the pathogenesis of IBD, as they found a negative association between *H.pylori* infection and inflammatory bowel disease. Their study employed different parameters as Urea Breath Test, Immunohistochemistry, culture of *H.pylori* from stool, serological tests and PCR [11,12,15].On the contrary, other authors speculated that *H.pylori* may act as ulcerative pathogens and contribute to the pathogenesis of Crohn's disease. [14,16]

Our results suggested that there is no possible etiological role of *H.pylori* in IBD but still the detection of *H.pylori* in site of lesion of IBD need further study in both (Crohn's and Ulcertive colitis) and with a relative large sample to assess their possible role.

Our work demonstrated 66.7% CagA positive *H.pylori* in IBD patients compared to 25% were CagA positive in NC patients. According to our

knowledge, this is the first Iraqi molecular study survey results (MOH records and Internet reviewed).

Generally, it was stated that about 50-70% of *H.pylori* strains in western countries carry CagA gene (24).

Crabtree and Farmey, [25] reported 51% CagA positive *H.pylori* in IBS cases and 19.6% CagA positive *H.pylori* in normal people. The above results were relatively close results to our findings.

In addition to above, our results demonstrated that *H.pylori* in IBD patients was six times more liable to have CagA gene than *H.pylori* in NC patients (i.e. more virulent). This topic according to our knowledge is the first to be recorded (MOH, Internet and Medline reviewed) and require further investigation in future.

CONCLUSION:

H.pylori was isolated from the site of lesion of patients with inflammatory bowel disease.BUT showed results close to H&E method in detection of *H.pylori* infection. CagA positive *H.pylori* in IBD patients was higher than NC patients.

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