Detection of *H.pylori* in a Group of Iraqi Patients with Colonic Cancer (Histological and Molecular Study)

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ABSTRACT:

BACKGROUND:

H. pylori is a one of the gastrointestinal organism which infects more than half the population of the world. H.pylori had been recognized as a class I human carcinogen by the International Agency for Research on Cancer. Recently *H. pylori* were detected in the mucosa of normal colon. Certain studies suggested a possible relationship between CagA (Cytotoxin Associated Gene A) positive H.pylori and Colonic Cancer while other studies did not find causal relationship between CagA positive H.pylori and Colonic Cancer.

OBJECTIVE:

We studied and isolated *H.pylori* from at or near by the site of lesion of Colonic Cancer and it is found to assess a possible relationship between CagA positive *H.pylori* and Colonic Cancer.

PATIENTS AND METHODS:

Study involved 30 patients of colonoscopically and Histopathologicaly determined Colonic Cancer (CC group) compared with patients with normal colon (n=90) as a control group (NC group). Patients divided into <20 years age group, 20-40 years age group and >40 years age group. Endoscopic specimens were tested for Biopsy Urease Test (BUT) and Hematoxylin & Eosin (H&E) method for detection of H. pylori also the detection of CagA mRNA using *In Situ Hybridization* technique with a biotin labeled probe.

RESULTS:

There was 66.7% H.pylori positive in patients with Colonic Cancer (by BUT) and 60% by (H&E) compared to 33.3% (by BUT) and 26.7% (H&E) in patients with normal colon (Control). A comparison between NC&CC groups regarding H.pylori infection using H&E method revealed significant difference (P=0.001).Using In Situ Hybridization technique, there was 66.7% CagA positive H.pylori in CC group in correspondence to 25% in NC group. CagA positive H.pylori infection between CC and NC groups revealed significant difference (P=0.008)

CONCLUSION:

H.pylori detected at or near by the site of lesion of Colonic Cancer. In addition it was found that there is a statistical relationship between CagA positive H.pylori infection and Colonic Cancer which suggested a possible role of CagA positive H.pylori in Colonic Cancer.

KEYWORDS: H.pylori, colonic cancer, caga, biopsy urease test, in situ hybridization

INTRODUCTION:

H.pylori considered as one of the commonest bacterial pathogens in human. [1]

H. pylori is a ubiquitous gastrointestinal organism which infects more than half the population of the world. However, in developing countries, H. pylori infects most of the population, so that most people in Eastern Europe, Asia, Africa and South America are carriers. [2] Pathogenesis depends upon strain virulence, host genetic susceptibility and environmental cofactors [3].

H. pylori produces a number of virulence factors, including vacuolating cytotoxin (VacA) [4] in addition to many virulent factors had been identified in H.pylori that contribute to its pathogenesis, most important among is the "128 – Kda cytotoxin- associated gene A"(Cag A) [5].

H.pylori strains which have CagA gene create a stronger inflammatory response [6]. CagA injection in the gastric epithelial cells triggers proinflammatory and anti-apoptotic responses which are detrimental for the human host in the

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long-term and favor the development of ulcer and cancer [7].

Diagnosis of the infection is based on both non-invasive and invasive techniques, which require endoscopyand biopsy.[8]) However, it should be stressed that, as no optimal technique has yet become available which would allow the bacteria to be unequivocally identified; only a combination of several techniques is held to yield satisfactory diagnostic results [9].

Studies using molecular biological techniques are of particular significance, since they enable a rapid and precise diagnosis to be made [10].

In situ hybridization is the method involving hybridization of labeled total genomic DNA, used as a probe, to spread or sectioned denatured chromosome preparations in situ, followed by detection and visualization of the sites of hybridization [11]. The application of these probes to tissue sections allows DNA or RNA to be localized within tissue regions and cell types. [12]

Previous studies demonstrated that *H. pylori* detected in the normal colon (13-15). Furthermore Many previous studies found higher rate of H.pylori infection in Colonic Cancer than control which suggested causal relationship (14,16-18) while other studies concluded that there was no association between H.pylori infection and Colonic Cancer (15,19,20) i.e. there is controversy.

PATIENTS AND METHODS:

Patients were selected between December 2009 and January 2011 from those attending "the Endoscopic Unit" in the Baghdad Teaching Hospital. There were 30 patients with Colonic Cancer (CC patients) and 90 patients with normal colon (NC patients). 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 18 to 65 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.

Selected patients were with different lower gastrointestinal complaints like bleeding per rectum, bloody diarrhea and/or c

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.

Patients were subjected to colonoscopy to obtain colonic biopsies. One biopsy specimen was used for biopsy urease test (BUT) to detect H.pylori in tissue sample.

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 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 Windows software SPSS 16. Differences among groups were evaluated by using Chi-square test and Fisher's exact test (2 x 2 Table).

RESULTS:

In CC group, the percentage of H.pylori infection in different age groups using BUT and H&E methods was demonstrated respectively. (Tables 1&2)

Chi-square test showed there was no significant difference (P>0.05) between age and *H.pylori* infection.

The same results was found in NC group, (Tables 3&4)

A comparison was done between CC & NC patients according to H.pylori infection using H&E method. Fisher Exact Test revealed that there was significant difference between the two groups (P=0.001). (Table 5)

Another comparison was done between CC & NC patients according to CagA positive H.pylori infection using In Situ Hybridization method. Fisher Exact Test revealed that there was significant difference between the two groups (P=0.008). (Table 6)

Table 1: H.pylori distribution in sections of Colonic Cancer using BUT.

age	CC	
	Positive (%)	Negative (%)
<20	1(50)	1(50)
20-40	8(57.1)	6(42.9)
>40	11(78.6)	3(21.4)
Total	20(66.7)	10(33.3)
P value	>0.05	

No significant difference (P>0.05)

Table 2: H.pylori distribution in sections of Colonic Cancer using H&E

age	CC	
	Positive (%)	Negative (%)
<20	1(50)	1(50)
20-40	7(50)	7(50)
>40	10(71.4)	4(28.6)
Total	18(60)	12(40)
P value	>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 method.

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: H.pylori infection in NC and CC groups using H&E method.

Group	H.pylori infection		Total
	Positive (%)	Negative (%)	
NC	24(26.7)	66(73.3)	90
CC	18 (60)	12 (40)	30

P=0.001 significant difference

Table 6: CagA positive H.pylori infection between CC and NC groups.

Group	CagA status		Total
	Positive (%)	Negative (%)	
NC	6(25)	18(75)	24
CC	12(66.7)	6 (33.3)	18

P=0.008 significant difference

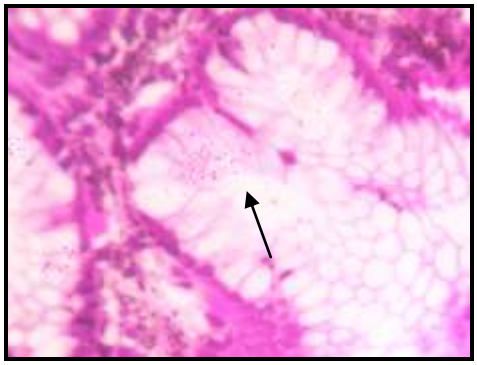

Figure 1: Detection of H.pylori in the mucosa of normal colon in 31 years old patient. (Haematoxylin and Eosin, 400X).

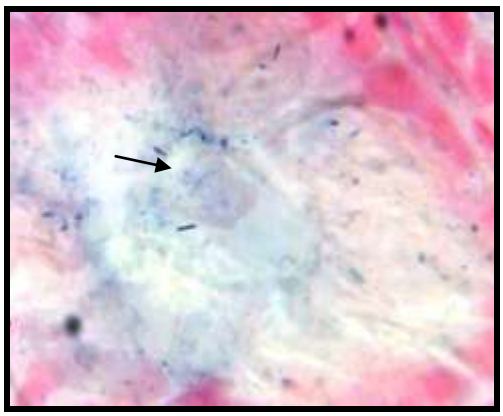

Figure 2: CagA positive expression by BCIP/NBT (bluish purple) and counter stained by NFR (1000x). The biopsy was taken from a 54 years old patient with normal colon.

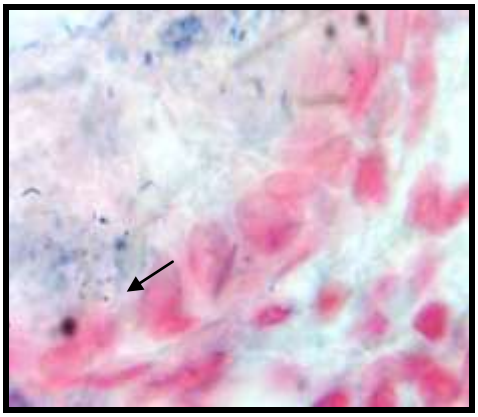

Figure 3: CagA positive expression by BCIP/NBT (bluish purple) and counter stained by NFR (1000x). The biopsy was taken from a 61 years old patient with Colonic Cancer.

DISCUSSION:

Infection with *H. pylori* may be the most common human infection and in many populations, infection rates of 80–90% are not unusual [21].

With regards to malignant disease, H.pylori had been recognized as a class I human carcinogen by the "International Agency for Research on Cancer" [22], primarily because of extensive epidemiological data revealing an association between H.pylori seropositivity and increased gastric cancer risk. The exact mechanisms that lead to gastric cancer induction are not clear, but the study of the bacterial factors (CagA positive H.pylori) important for colonization in addition to the host responses to the infection (NFkB activation) that are starting to yield important clues.

Previous studies (23-26) depended on serological methods in which they found significant increase in serology of patients with Colonic Cancer compared to control. Although these studies agreed with our results in which the rate of H.pylori infection is higher in CC group than control but still the percentage of H.pylori infection in these studies higher than our findings due to the dependence on serology rather than the detection of H.Pylori at the site of lesion.

Other studies (18,27-29) isolated H.pylori at the site of lesion using different methods including Immunohistochemistry and/or PCR.

Several causes are suggested about the mechanism of H.pylori action in the pathogenesis of CRT, these are:

1- Stimulation of circulating growth factors or other local, more site-specific mechanisms. [30]

2- H.pylori moves through intestinal tract and makes contact with colonic mucosa, then locally activates colonic carcinogenesis through production of virulent factors (CagA and Vac) in addition to the possibility of activation of certain host factors (like Nuclear Factor kappa B) [28]

3-H.pylori carriage can affect normal gastrointestinal flora as a result of progressive chronic gastritis with glandular atrophy and decreased acid production, which might further influence colorectal carcinogenesis. [28, 30]

4-Persistant H.pylori exposure induces hypergastrinemia [31]. Gastrin has been shown to be mitogenic for colonic cancer cells in vitro [32], and stimulate growth of experimental colonic tumors in rodents. [33,34]

On the contrary to the above mentioned Studies, certain studies concluded that there was no association between H.pylori infection and Colonic Cancer [15,19,20,35,36]. The reason for the inconsistent findings reported to date is unclear, but it might be at least partly explained by the methodological issue. These include selection bias, small sample sizes and an inadequate consideration of potential confounding variables in the data analysis.

Salihi et al. [18] demonstrated 60% CagA positive H.pylori in Colonic Cancer patients using PCR as a detection tool. These results are close to our findings which is 66.7%.

Salehi et al. [18] concluded that there is a statistically significant strong association between CagA positive H.pylori infection and Colonic Cancer Our study is in agreement with these findings (P=0.008)

CONCLUSION:

H.pylori isolated at or nearby the site of lesion in 66% of patients with Colonic Cancer BUT showed results close to H&E method in detection of H.pylori infection. CagA positive H.pylori had a

statistical relationship with patients with Colonic Cancer (i.e. possible etiological link). This point requires further study in future.

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