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The synergistic effect of *Blastocystis hominis* and *H. pylori* in Iraqi colorectal cancer patients

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Abstract

Blastocystes hominis is one of the parasites that cause problems in the digestive system of the human body. *H. pylori* are dangerous bacteria that cause gastric ulcer and may even lead to cancer. This study was done during the period from1st February 2018 to 15th June 2018. One hundred sixteen stool samples were collected from patients with *Blastocystes hominis* and *H. pylori* infection. Fifteen tissue samples of colorectal cancer were taken from 15 suspected patients out of 116 infected cases and with possibility of a hereditary mutation. The findings showed the distribution of *B. hominis* and *H. pylori* infections among different age groups. Regarding *B. hominis*, the highest rate was among the age group 31-50 years (35.5%) followed by 10-30 years (33.3%). The lowest rate was among the age group <10 years (11.1%). Samples with mixed infection *Blastocystis hominis* and *Helicobacter pylori* infections were found in 27(67.2%) a result that shows a highly significant difference ($p<0.01$). Fifteen samples had mutations of enter exons in 6 samples of *KRAS* number of mutation, 2(1.94) in positions c34 (G > C) and (G > T) and c.37, 4(1.94) (T > A), 11(10.98), (G > C), 12(11.65) (G > T), 5(4.85), (G > C), and c38, 1(.097), (G > C). While in 9 samples of *TP53* genes in positions c318, the number of mutation 1(0.97), (G > C), c273, 8(0.97), (C > G), c440, 1(0.97) (T > G), c511, 1(0.97), (T > G), c302 8(1.94), (G>A), c742, 3(2.92), (C > T), c761, 1(0.97), (T > G), 52, 4(4.85), (G>A), and c248, 7(0.97), (C>T) in colorectal cancerous tissues.

Key word: synergism *Blastocystis hominis*, *H. pylori*, colorectal cancer

INTRODUCTION

The presence of these two microscopic bodies, *B. hominis* and *H. pylori*, is controversial, since there is a cooperation between them that has made them be in the same place which is the intestine [1]. The parasite analyzes the protein by the enzyme protease and the bacteria change the medium to alkaline [2]. These bacteria may be migrated from the stomach to the intestine for this reason [3]. The presence of these two microorganisms in the site of injury, especially the site of alteration the colon tissue is really serious [4]. *H. pylori* bacteria are the most dangerous microorganisms that infect humans and cause to them a disease that may be excessive [5]. The migration of these bacteria to different parts of the human body made the attention drawn to them, where there are found in the heart and lungs [6]. The movement toward epithelium cells by flagella-mediated motility; attachment to host cells by adhesions/receptors interaction, causes tissue damage by toxin release. [7]. Molecular investigation is used for identifying the roles of novel virulence factors and their association with different disease outcomes, especially the bacterial adhesions, *cag* pathogen city island, and vacillating cytotoxin. Recently, the development of large-scale screening methods, including proteomic, and transcriptomic tools, has been used to determine the complex gene regulatory networks in *H. pylori*. In addition, a more available complete genomic database of *H. pylori* strains isolated from patients with different gastrointestinal diseases worldwide is helpful to characterize this bacterium [8]. *B. hominis* is an unsatisfactory parasite, but analyzes the protein inside the host's body [9]. Perhaps this parasite helps bacteria migrate to the colon [10]. TFF1 may play a role in locating *H. pylori* in gastric mucus. TFF1 bound almost exclusively to human gastric mucins and dose not interact with human colonic mucins. There was a strong correlation between binding of TFF1 and H. pylori to human gastric mucins, and between binding of both TFF1 and *H. pylori* to gastric mucins with that of Griffonia simplicifolia lectin-II, which is specific for terminal non-reducing α- or β-linked N-acetyl-D-glucosamine. These results suggest that TFF1 may help to locate *H. pylori* in a discrete layer of gastric mucus and hence restrain their interactions with epithelial cells [11]. Colorectal malignancy includes generation of reactive metabolites and carcinogens, alterations in host carbohydrate expression and induction of chronic mucosal inflammation [12] Long-term colonization of the colon by rogue commensal bacteria capable of inducing chronic DNA damage could contribute to sporadic CRC developement, thereby suggesting sporadic CRC as an infectious disease. *Helicobacter pylori* (*Hp*), a curved spiral gram-negative bacterium found in the gastric mucosa of a large proportion of humans worldwide (>50%), has been evaluated as a possible etiologic agent for CRC and recent data indicate that there is a serological association between *Hp* infection (*Hp*-I) and the risk of CRC, especially for left-sided and early-stage cancers [13].

MATERIAL AND METHOD

One hundred and sixteen stool samples were collected from each person in a sterile disposable container. Each sample was divided into two parts: the first part was kept in the cold box for detection of *H. pylori*, while the second part was mixed with (2-3 ml) potassium dichromate (2.5 %) to preserve the samples till examination of *Blastocystis hominis* in the laboratory. Upon arrival to the laboratory, each sample was prepared for examination. Stool samples were examined under the microscope for detection of any

parasitic forms like cyst, trophozoites, ova, helminths, bacteria, yeast and undigested food. The microscopic examination included direct wet preparation (direct wet mount), [14]. *H. pylori* were detected by Rapid *H. pylori* Antigen Test: (immune-chromatography, ATG Biotech Co. Germany). It used high-resolution melting analysis (HRM) and direct DNA sequencing to characterize the mutations CRC-related pathways. Gene Scanning Software Version 1.5, compared with the clinic-pathological data of CRC patients with the driver gene mutation status [15].

Distribution of *B. hominis* and *H. pylori* positive cases according to age group:

Table (1) shows the distribution of *B. hominis* and *H. pylori* among different age groups. Regarding *B. hominis*, the highest rate was among the age group 31-50 years (35.5%) followed by 10-30 years (33.3%). The lowest rate was among the age group <10 years (11.1%). Statistically, there was no significant difference according to age groups (P>0.05). While with *H. pylori*, the highest rate was among the age group 10-30 years (39.0%) followed by 31-50 years (36.5%). The lowest rate was among the age group <10 years (4.8%). Statistically, there was a highly significant difference according to age groups (P<0.01).

Table (1): Distribution of *B. hominis* and *H. pylori* positive cases according to age groups

Age groups (Year)	No. examined (116)	*B. hominis*		*H. pylori*	
		NO.	%	NO.	%
< 10	11	5	11.1	2	4.8
10 – 30	31	15	33.3	16	39.0
31 – 50	51	16	35.5	15	36.5
51 – 70	23	9	20	8	19.5
χ^2 test (P-value)		P> 0.05 (NS)		P< 0.01 (HS)	

Distribution of *B. hominis* and *H. pylori* Positive Cases According to Gender:

The rate of *B. hominis* among females 29 (46.9 %) was higher than males 22(43.1%), and the rate of *H. pylori* among females 40(65.5%) was higher than males 25 (38.5%), but statistically there was no significant difference according to gender (P>0.05) as shown in table (2).

Table (2): Distribution of *B. hominis* and *H. pylori* positive cases according to gender

Gender	No. examined (116)	*B. hominis*		*H. pylori*	
		NO.	%	NO.	%
Male	51	22	43.1	29	46.9
Female	65	25	38.5	40	65.5
χ^2 test (P-value)		P > 0.05 (NS)		P> 0.05 (NS)	

Mixed infections with both *H. pylori* and *B. hominis:*

Table (3) shows that out of 116 subjects, mixed infection with *B. hominis* and *H. pylori* was 27 (23.32%), while the rate of infection with *B. hominis* alone was 18(15.5%) and with *H. pylori* alone 14(12.02%). Statistically, there was a highly significant relationship between the two infections (p<0.01).

Table (3): Mixed Infections of *H. pylori* and *B. hominis* Among Infected Patients

B. hominis		*H. pylori*		Total	χ^2 test (P-value)
		Positive	Negative		
Positive	N	27	18	45	P=0.00 Highly sign. (P<0.01)
	%	23.32%	15.5 %	38.83 %	
Negative	N	14	57	71	
	%	12.02 %	49.11 %	61.13 %	
Total	N	41	75	116	
	%	35.34 %	64.66 %	100 %	

The sequences of primers used for amplification of axon 8 of *TP53* gene

EXON 8	**F:GGTAATCTACTGGGACGGAAC R: GCTTCTTGTCCTGCTTGCTT**	**158 bp**

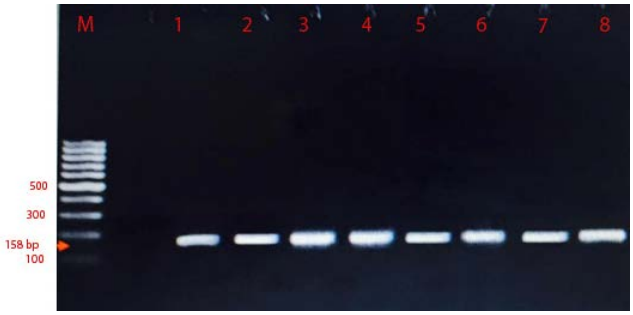

Figure 1: PCR products amplified by Taq Polymerase on agarose gel electrophoresis, it is obvious the exon 8 segment is substantially amplified by Taq polymerase is used in PCR reaction in 158 bp..

KRAS and TP53 gene mutation

In the total series, MSI was found in 18% of the samples, ranging from 13% in the <50 age group to 20% in the 70 age group. MSI was significantly associated with the localized disease, as shown table 4. The genes were mutated within the expected range of frequencies. Mutation frequencies for each gene independently, and frequencies of combinations of mutations in the genes tested, were equally distributed between all stages. Differences attributed to age at onset are pointed out below.

Table 4: The pathological mutations of KRAS and TP53 genes detected in colorectal cancer.

Gene	Mutation	Number of mutations, *n* (%)	Minor allele frequency in cancer
KRAS	c.34G>C	2 (1.94)	0.97%
KRAS	c.34G>T	2 (1.94)	0.97%
KRAS	c.35G>A	2 (1.94)	0.97%
KRAS	c.35G>C	11 (10.98)	5.34%
KRAS	c.37A>T	4 (11.65)	5.83%
KRAS	c.38G>C	4 (4.85)	2.43%
TP53	c.302A>G	8(0.97)	0.49%
TP53	c.273C>T	8 (0.97)	0.49%
TP53	c.440T>G	1 (0.97)	0.49%
TP53	c.511G>T	1 (0.97)	0.49%
TP53	c.734G>A	2 (1.94)	0.97%
TP53	c.742C>T	3 (2.91)	1.46%
TP53	c.761T>G	1 (0.97)	0.49%
TP53	c.52G>A	5 (4.85)	2.43%
TP53	c.248T>C	7 (0.97)	0.49%

TP53 Gene	TP53 Gene Mutation	Normal Sequence	Mutation Sequence
TP53 Reference gene	c 37 (T-A)	CCGTCCCAA	CCGACCCAA
	c 38 (A-G)	TCCCAAGCA	TCCCAGGCA
	c 52 (G-A)	GAACAATGG	GAACAATGT
	c 37 (T-A)	CCGTCCCAA	CCGACCCAA
	c 52 (G-A)	GAACAATGG	GAACAATGT
	C 273 (T-A)	GTGCGTGTT	GTGCGTGTA
	c 38 (A-G)	TCCCAAGCA	TCCCAGGCA
	C 248 (C-T)	AACCGGAAG	AACTGGAAG
	C 302 (G-A)	CCAAGGGAGC	CCAAGAGAGC
	C 273 (T-A)	GTGCGTGTT	GTGCGTGTA
	C 248 (C-T)	AACCGGAAG	AACTGGAAG
	C 302 (G-A)	CCAAGGGAGC	CCAAGAGAGC

Figure 2: TP53 mutation characterized with colorectal carcinoma

Statistical analysis

For the Statistical analysis of the data, mean ± SEM (standard error of mean) and chi –square (χ^2 test) were applied by using SPSS Microsoft Office Excel program.

DISCUSSION

Colorectal cancer is a difficult condition to treat or cure due to genetic mutations that occur in the cells of the intestinal tissue. There are several causes that may be caused by viruses, bacteria and even intestinal parasites [14]. In this study, two microorganisms, one of which is known to cause gastrointestinal cancers, were identified, but the second is the parasite, *B. hominis* and may be co-operative agent in the occurrence of colon cancer. These findings agreed with Abdurahaman Seid, *et al.,* 2018, [10] who found that Besides *H. pylori* infection, *G. lamblia* has been found to trigger symptoms of gastrointestinal disorders with dyspepsia reported to be the main symptoms of gastric giardiasis. The involvement of this parasite may be a suitable medium for bacterial growth and efficacy. Fifteen cases of genetic mutations were identified on *KRAS* and *TP53* genes of parasite infected with *H. pylori* and *B. hominis* together, and the results were in a harmony with Palacio-Rúa, *et al.,* 2014 [14], who reported the total mutation frequency in the 59 samples analyzed. In addition, a high frequency of polymorphisms was found in the 3 genes, identifying a total of 7 different polymorphisms in the 59 samples; the most frequent was the rs41115 (c.4479G>A, p.Thr1493Thr) polymorphism, located in exon 15 of the *APC* gene. All the polymorphisms identified in this study are reported in the SNP database [15]. The virulence factor in the *H. pylori* causes tissue damage to the colorectum. The findings agreed with Zhang Y, *et al.,* 2017 [14], who reported that the chronic inflammation is one of the major risks of CRC. Patients with IBD, including ulcerative colitis and Crohn's disease, have a higher risk to develop colitis-associated CRC compared to the general population Recently, a study on 44,278 individuals showed an association between a higher dietary inflammatory index, which is developed to evaluate the inflammatory potential of an individual, and an increased prevalence of colorectal adenomas, which are caued by bacteria such as *H. pylori* [16]. The mutation occurrence in 15 positions and different exons of 2(1.94) in positions c34 (G > C) and (G > T) and c.37, 4(1.94) (T > A), 11(10.98), (G > C), 12(11.65) (G > T), 5(4.85), (G > C), and c38, 1(.097), (G > C). While in 9 samples of *TP53* genes in positions c318, the number of mutation occurred in 1(0.97), (G > C), c273, 8(0.97), (C > G), c440, 1(0.97) (T > G), c511, 1(0.97), (T > G), c302 8(1.94), (G>A), c742, 3(2.92), (C > T), c761, 1(0.97), (T > G), 52, 4(4.85), (G>A), and c248, 7(0.97), (C>T), [17]. These changes have occurred in these genetic sequences due to the damage caused to the bacteria and their toxic substances, and these findings correspond with Dunne, C. *et al.,* 2018 [11], who reported that alternatively, there may be a link between the microbe and the host, and the microbe allows their long-term co-existence and optimizes their interaction outcomes. Selection of microbes in made to be able for the evasion of the host's immune response, for the efficient use of the host resources and for the limitation of interaction toxicity [18]. Conversely the host may possess mechanisms for tissue injury minimizing or for more virulent organisms exclusion specially [11]. It is observe the carriage the gastric *H. pylori* is related to the elevated risk for peptic ulcer and noncardia gastric adenocarcinoma and that the *H. pylori* removal affects the natural history peptic ulcer [19]. More recently, it has also been clear that individuals without *H. pylori* are at great risk to develop gastroesophaeas reflux disease and it's sequels become evident that individuals without *H. pylori* are at greater risk for gastroesophageal and its sequelae, Barrett's esophagus and esophageal adenocarcinoma. There for the pathogenic us, from clinical and epidemiological studies, the pathogenic significance of *H. pylori* is mixed from epidemiological and clinical studies [19].

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