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Full Length Research Paper

Current Concepts in the Immunological Diagnosis of *H. pylori* in Basrah Pediatric Oncology Unit

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Objective*: The concentration of Immunological markers (IgG, IgM, IgA, C3 and C4) were determined for children with cancer in Basrah Pediatric Oncology Unit during 2009.*
Methods*: A prospective comparative case-control study was carried out over 7 months from 12th of March 2009 to 26th of September 2009, the study included 29 children (20 male and 9 female) with different types of malignancies. Investigations were done for H.pylori and one step diagnostic test (which detect the antigen for H. pylori) and determine the concentration of (IgG, IgM, IgA, C3, C4).*
Results: *There is statistically significant higher percentage of patient group having H.pylori comparing to control group with P < 0.05. Regarding H.pylori in relation to immunological status of studied patients, there was a significant association between H.pylori and high IgG with a mean (715.01) for H.pylori positive patient and mean (553.20) for H.pylori negative patient, P< 0.05 which is statistically significant, this was the same regarding IgA when there was a significant association between H.pylori and high IgA with a mean (94.29) for H.pylori positive patient and mean (58.54) for H.pylori negative patient P< 0.05 which is statistically significant, also the same regarding C4 , there was a significant association between H.pylori and low C4 with a mean (47.31) for H.pylori positive patient and mean (79.57) for H.pylori negative patient and P< 0.05 which is statistically significant.*
Conclusion: *This study shows evidence of strong relationships between frequency of H.pylori and immunocompromised children according to immunological investigation.*

Keywords: *H. pylori*, immunology, cancer, children.

INTRODUCTION

Helicobacter pylori, or *H. pylori*, is a spiral-shaped bacterium that is able to grow in the human stomach. Normally, the acidic stomach environment prevents the survival of viruses, bacteria, and other microorganisms[1]. However, *H. pylori* has evolved to be uniquely suited to thrive in the harsh stomach environment. *H. pylori* bacteria secrete urease, a special enzyme that converts urea to ammonia. Ammonia reduces the acidity of the stomach, making it a more hospitable home for *H. pylori*[2,3]. The ability to survive in the stomach provides *H. pylori* with a useful hiding place. White blood cells that would normally recognize and attack invading bacteria are unable to cross from blood vessels into the stomach lining. Instead, the ineffective white blood cells continue to respond to the site of infection, where they die and release nutrients that feed *H. pylori* [3,4]. *H. pylori* has co-existed with humans for thousands of years. However, because scientists believed the stomach was

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a sterile organ, this bacterium was not discovered until the 1980s. Some other gut bacteria actually aid their human hosts in the absorption of nutrients and defence against other more dangerous microbes[5]. Because *H. pylori* is relatively newly discovered, the complex interactions between this microbe and humans, including its risks and benefits, are still being discovered[6]. More than 50% of the world's population harbor *H. pylori* in their upper gastrointestinal tract. Infection is more prevalent in developing countries, and the incidence is decreasing in western countries [7].

The route of transmission is unknown, although it is known that individuals typically become infected in childhood [6,8]. Diagnosis of infection is usually made by checking for dyspeptic symptoms and by other tests which can indicate *H. pylori* infection. One can test noninvasively for *H. pylori* infection with a blood antibody test, stool antigen test, or with the carbon urea breath test (in which the patient drinks ^{14}C- or ^{13}C-labelled urea, which the bacterium metabolizes, producing labelled carbon dioxide that can be detected in the breath) [9]. However, the most reliable method for detecting *H. pylori* infection is a biopsy check during endoscopy with a rapid urease test, histological examination, and microbial culture. There is also a urine ELISA test with a 96% sensitivity and 79% specificity. None of the test methods is completely failsafe. Even biopsy is dependent on the location of the biopsy. Blood antibody tests, for example, range from 76% to 84% sensitivity. Some drugs can affect *H. pylori* urease activity and give false negatives with the urea-based tests [3,4,10]. *H. pylori* colonize the stomach and induce chronic gastritis, a long-lasting inflammation of the stomach.

The bacterium persists in the stomach for decades in most people [8]. Most individuals infected with *H. pylori* will never experience clinical symptoms despite having chronic gastritis. Approximately 10-20% of those colonized by *H. pylori* will ultimately develop gastric and duodenal ulcers. *H. pylori* infection is also associated with a 1-2% lifetime risk of stomach cancer and a less than 1% risk of gastric MALT lymphoma [9,11]. It is widely believed that in the absence of treatment, *H. pylori* infection—once established in its gastric niche—persists for life [3]. In the elderly, however, it is likely that infection can disappear as the stomach's mucosa becomes increasingly atrophic and inhospitable to colonization. The proportion of acute infections that persist is not known, but several studies that followed the natural history in populations have reported apparent spontaneous elimination [5,6]. While *H. pylori* has been disappearing from the stomach of humans, the incidence of the related disorders, acid reflux disease, Barrett's esophagus, and esophageal cancer have been rising dramatically[12]. In 1996, Martin J. Blaser advanced the hypothesis that *H. pylori* has a beneficial effect: by regulating the acidity of the stomach contents, it lowers the impact of regurgitation of gastric acid into the esophagus [13].

The hypothesis is not universally accepted as several randomized controlled trials failed to demonstrate worsening of acid reflux disease symptoms following eradication of *H. pylori* [14]. Nevertheless, Blaser has refined his view to assert that *H. pylori* is a member of the normal flora of the stomach. He postulates that the changes in gastric physiology caused by the loss of *H. pylori* account for the recent increase in incidence of several diseases, including type 2 diabetes, obesity, and asthma. His group has recently shown that *H. pylori* colonization is associated with a lower incidence of childhood asthma [9,11,14]. Colonization of the stomach by *H. pylori* results in chronic gastritis, an inflammation of the stomach lining. The severity of the inflammation is likely to underlie *H. pylori*-related diseases [15]. Duodenal and stomach ulcers result when the consequences of inflammation allow the acid and pepsin in the stomach lumen to overwhelm the mechanisms that protect the stomach and duodenal mucosa from these caustic substances [12]. The type of ulcer that develops depends on the location of chronic gastritis, which occurs at the site of *H. pylori* colonization [10].

The acidity within the stomach lumen affects the colonization pattern of *H. pylori* and therefore ultimately determines whether a duodenal or gastric ulcer will form. In people producing large amounts of acid, *H. pylori* colonizes the antrum of the stomach to avoid the acid-secreting parietal cells located in the corpus (main body) of the stomach [8]. The inflammatory response to the bacteria induces G cells in the antrum to secrete the hormone gastrin, which travels through the bloodstream to the corpus. Gastrin stimulates the parietal cells in the corpus to secrete even more acid into the stomach lumen [15]. Chronically increased gastrin levels eventually cause the number of parietal cells to also increase, further escalating the amount of acid secreted. The increased acid load damages the duodenum, and ulceration may eventually result. In contrast, gastric ulcers are often associated with normal or reduced gastric acid production, suggesting that the mechanisms that protect the gastric mucosa are defective [16]. In these patients, *H. pylori* can also colonize the corpus of the stomach, where the acid-secreting parietal cells are located [10]. However, chronic inflammation induced by the bacteria causes further reduction of acid production and, eventually, atrophy of the stomach lining, which may lead to gastric ulcer and increases the risk for stomach cancer[17].

Two related mechanisms by which *H. pylori* could promote cancer are under investigation. One mechanism involves the enhanced production of free radicals near *H. pylori* and an increased rate of host cell mutation [18]. The other proposed mechanism has been called a "perigenetic pathway" and involves enhancement of the transformed host cell phenotype by means of alterations in cell proteins such as adhesion proteins [3,11]. It has been proposed that *H. pylori* induces inflammation and locally high levels of TNF-α and/or interleukin 6 [8]. According to the proposed perigenetic mechanism, inflammation-associated signaling molecules such as TNF-α can alter gastric epithelial cell adhesion and lead to the dispersion and migration of mutated epithelial cells without the need for additional mutations in tumor suppressor genes such as genes that code for cell adhesion proteins [15,19].

The aims of the present study are to study the frequency of *H. pylori* among patients with cancer and to determine the immunological parameters of these patients in comparison with the control group.

MATERIAL AND METHODS

A prospective comparative case-control study was carried out over 7 months from 12th of March 2009 to 26th of September 2009, the study included 29 children (20 male and 9 female) with different types of malignancies who were admitted to the pediatric oncology unit for treatment at the Basrah Maternity and Children's hospital and their age ranged between 18 months to 11 years, and regarded as patient group.

A total of 22 children (12 male and 10 female) who were admitted to Basrah Maternity and Children's Hospital for chest infection and after stabilization, critically ill children were excluded from the study, they were matched for sex and age with patient group and randomly selected as a control group .

A special questionnaire was designed for the purpose of the study (Appendix I). The following information was taken:

1. Name, age (in month), and sex.
2. Date of admission.
3. Residence of patients.
4. Type of cancer (ALL, AML, solid tumours), risk group of patient with leukemia.
5. Clinical features including symptoms and signs: epigastric pain, dyspepsia, abdominal pain and Vomiting.
6. Family history of peptic ulcer or endoscopy or recurrent abdominal pain. - consanguinity.
7. Social history including father and mother smoking.
8. Father and mother education.

Investigations were done for *H.pylori* and one step diagnostic test (which detect the antigen for *H.pylori*) and determine the concentration of (IgG, IgM, IgA, C3, C4).

One milliliter of serum was taken from the patient for the purpose of immunological study and one step diagnostic test. One step diagnostic test detects the antibodies to *H.pylori* with 95.9% sensitivity and 89.6% specificity, but it can be positive in other campylobacters (designed by human company, Belgium). The limitations of this test attributed to that the test is used as qualitative rather than quantitative and does not indicate the titer of the antibody in the specimen.

A kits of radial immunodifusion plate (Bussero Co.,Millano, Italy) were used to determine the concentration of immunoglobulins; IgG and IgM, components of complement C3 and C4 as follows:

- Agarose gel containing monoclonal IgG antisera.
- Agarose gel containing monoclonal IgM antisera.
- Agarose gel containing monoclonal C3 antisera.
- Agarose gel containing monoclonal C4 antisera.

The wells were filled with 5 µl of sample (patient's serum) and allowed to be completely adsorbed of the test sample after 5 minutes. The plates were incubated for 72 hrs in an incubator at 37 °C. Plates of IgG, C3 and C4 were read after 18 hrs, while IgM plates after 72 hrs. The end point of diffusion was indicated by a sharp precipitating ring, which was achieved when the incubation time was finished. Readings were done at this time. The diameters of each ring were measured directly by using a magnifying lens with micrometers scale. The diameter of the ring was related to antigen concentration and the results were evaluated by using reference standard table (WHO reading, mg/dl) that is packaged with the kit instruction method supplied (Bussero (Millano) Italy.

Statistical analysis was done using SPSS program, data were expressed and comparison of proportions was performed using chi-square test. P value of less than 0.05 was considered as statistically significant, P value of less than 0.01 as highly significant and P value of less than 0.001 as extremely significant.

RESULTS

Distribution of patients and control, according to age and sex is shown in table(1)

Regarding age group: 51.7% of patients were >5 years in comparison to 54.5% of control were >5 years, so there was no significant difference regarding the age group between patient and control. Regarding sex groups, 29 children were patient group and the majority of them were male with male:female ratio equal to 2.2, while 22 children were control group with male:female ratio equal to 1.2, with a p value >0.05, so there was no significant difference between the two groups regarding sex. Results of *H.pylori* antibody testing among patient and control are illustrated in table (2).

Among 51 children included in the study, 29 children were patients (of them 79.3% having positive one step diagnostic test), while 22 children were controlled (of them 54.5 having positive one step diagnostic test). There is statistically a significant higher percentage of patient group having H. pylori compared to the control group with a p value < 0.05.

Table 3 shows that there is a significant association between H.pylori and high IgG, IgA and low C4 but there is no significant association between *H.pylori* IgM and C3 among patient group p> 0.05.

Table(4) shows that there is no significant association between H.pylori and the type of cancer with a p value > 0.05.

DISCUSSION

Helicobacter pylori represents one of the most common and medically prominent infection worldwide, many researches have been done regarding H.pylori but only a small number of researches were made about the relation of H.pylori and childhood cancer including acute lymphocytic leukemia and solid tumor.[20]

Regarding H.pylori in relation to immunological status of studied patients, there was a significant association between H.pylori and high IgG with a mean (715.01) for H.pylori positive patient and mean (553.20) for H.pylori negative patient and p value less than 0.05 which is statistically significant, this was the same regarding IgA when there was a significant association between H.pylori and high IgA with a mean (94.29) for H.pylori positive patient and mean (58.54) for H.pylori negative patient and p value less than 0.05 which is statistically significant, also the same regarding C4 , there was a significant association between H.pylori and low C4 with a mean (47.31) for H.pylori positive patient and mean (79.57) for H.pylori negative patient and p value less than 0.05 which is statistically significant.

Seroprevalence of H.pylori is low in patients receiving organ transplants, possibly due to the use of antibiotics[21], suggesting that systemic administration of antibiotics eradicates H.pylori, and most of the patients in Basrah hospital had received antibiotics in their course of treatment as well, so the false negatives occur in immunocompromised patients. Nonetheless, the sensitivity of serological assays is poor in children. The strains in Asia are different from those that are circulating in the rest of the world[22]. The mean antibody levels in young children are significantly lower than in older children and adults and these age-related standard values have not been established for children[23].

This supports the hypothesis that in developing countries the acquisition of H. pylori infection can occur in early childhood [24].

Table 1: Distribution of patients and control according to age and sex:

Age / gender		Patient group No.	%	Control group	%	P value
Age	>5years	15	51.7%	12	54.5%	>0.05
	<5years	14	48.3%	10	45.5%	
Total		29	100%	22	100%	
Sex	Male	20	69%	12	54.5%	>0.05
	Female	9	31%	10	45.5%	
Total		29	100%	22	100%	

Table 2: Results of H.pylori antibody testing among patient and control

One step diagnostic test	Patient	Control	Total	P value
H.pylori + ve	23 79.3%	12 54.5%	35 68.6%	< 0.05
H.pylori - ve	6 20.7%	12 54.5%	16 31.4%	
Total	29 100%	22 100%	51 100%	

Table 3: concentration of immunoglobulins among immunocompromised children with H.pylori

Immunoglobulins	*H.pylori* +ve (no. 13)	*H.pylori* -ve (no. 16)	P value
IgG	715.01±151.38	553.20±265.47	< 0.05
IgM	58.06±15.67	52.88± 17.83	> 0.05
IgA	94.29±22.81	58.54±28.61	< 0.05
C3	101.33±38.92	138.22±60.68	> 0.05
C4	47.33±11.05	79.57±16.78	< 0.05

Table 4: Relation between types of H.pylori and types of malignancy

Types of *H. pylori*	Acute lymphocytic leukem	Solid tumor	Total	P value
H.pylori + ve	10 76.9%	3 31.8%	13 100%	>0.05
H.pylori - ve	13 81.3%	3 18.7%	16 100%	

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