Seroprevalence of *Helicobacter Pylori* in Secondary Immunocompromised Children

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Abstract: Background: *Helicobacter pylori (H. pylori)* infection is a worldwide problem, more than half of the world's population in both developed and developing countries are infected with this organism. Studies which estimated *H. pylori* infection in immunocompromised children have been done in developed countries. Objective: Evaluate the prevalence of *H. pylori* infection in secondary immunocompromised children, admitted to the Pediatric Hematology and Oncology and Nephrology Units of Zagazig University Hospitals, during the year 2010, using a non-invasive test. Study design: Sixty newly diagnosed secondary immunocompromised children (25 acute lymphoblastic leukemia-ALL, 15 nephrotic syndrome, 10 acute myeloblastic leukemia-AML, 5 T-cell lymphoma and 5 rhabdomyosarcoma), of ages ranging from 3 to 12 years (mean ± standard deviation, X ±SD = 7.51 ± 2.6 years), who received corticosteroids, immuno-suppressive drugs, or both were enrolled consecutively into the study. In addition, thirty age-and sex-matched healthy children served as a control group. Written consents were obtained from the parent (s) of all children. All children were subjected to history-taking, clinical examination, and routine investigations (CBC, liver and kidney function tests), as well as, serum ELISA for the detection of immunoglobulin G (IgG) antibodies specific for *H.pylori* antigens. Results: The prevalence of *H.pylori* seropositivity accounted for 60% in secondary immunocompromised children versus 37% in control children, with significant difference. In patients, the seroprevalence of anti-*H. pylori* antibody was non-significantly higher among children with malignancy than that in children with nephrotic syndrome. The prevalence rates of seropositivity were significantly higher in females than in males and in neutropenic children than in non-neutropenics, with nonsignificant differences in various age groups. The sensitivity and specificity of seropositivity, in all studied children accounted for 77% and 100%, respectively. Conclusion: 1) *Helicobacter pyloi* infection is common among patients suffering secondary immunodeficiency, and 2) testing of immunocompromised children, consuming the non-invasive ELISA serologic assay is a good negative test (specificity 100%) of *Helicobacter pylori* infection. Recommendations:1) routine serologic assay is essential for follow-up of these patients, 2) a large –scale study with more selection criteria of immunocompromised children is advised.

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1. INTRODUCTION

Helicobacter pylori (*H .pylori*) are spiral-shaped gram negative bacteria, first cultured and identified as *Campylobacter pylori* in 1982[(1)]. By 1989, it was renamed as *H. pylori* and recognized to be associated closely with antral gastritis (gastric and duodenal ulcers in adults and children). By the early-to-mid 1990s, further evidence supported a link between chronic gastritis of *H. pylori* infection in adults and malignancy, specifically gastric lymphoma and adenocarcinoma [(2)]. However, reports on the association between *H. pylori* infection and extra-gastrointestinal diseases have been increasing, especially in immuno-compromised subjects [(3)].

Most children examined in the office because of frequent infections are immunologically healthy [(4)]. On other hand, most hospitalized children have some immunocomprimising conditions [(5)]. Secondary or acquired immunodeficiency is more common than primary immunodeficiency [(6)].

There are two types of diagnostic tests used to detect *H. pylori* infection; non-invasive and invasive. Non-invasive tests include the urea breath test, stool test for the detection of *H. pylori* antigens, and serological tests to detect *H. pylori* antibodies (IgG, IgM and IgA) in serum. Tests of serum IgA or IgM antibodies are unreliable to detect gastric colonization and, therefore, only IgG antibodies against *H. pylori* denote active infection which continues throughout life unless a course of eradication therapy is instituted [(7)].

2. Subjects and Methods

This cross-sectional study was carried out in the Pediatric Department of Zagazig University Hospitals, on 60 cases of secondary immunocompromised children who received corticosteroids, immuno-suppressive drugs or both (Table 1). In addition, 30 age-and sex-matched healthy children served as a control group.

Exclusion criteria

- No recent administration of live attenuated vaccine.
- Patients who had taken proton pump inhibitors and antimicrobial drugs, 2 weeks before study.
- History of contact to a family member with *H. pylori* infection.
- Children who have heart failure.

Table (1): Distribution of 60 study cases according to the primary diagnosis, presented as number (n) and percent (%).

Primary diagnosis	n (%)
ALL	25 (41.7)
Nephrotic syndrome	15 (25)
AML	10 (16.7)
T-cell lymphoma	5 (8.3)
Rhabdomyosarcoma	5 (8.3)

ALL: acute lymphoblastic leukemia.
AML: acute myeloblastic leukemia.

Methods:

All children were subjected to the following:

- Full history-taking and clinical examination.
- Laboratory investigations, including:

A) Routine investigations: CBC with differential white blood cell count, liver and kidney function tests.

B) Serum enzyme linked immuno-sorbent assay (ELISA) for the detection of IgG antibodies specific for *H. pylori* antigen[8].

A 5-ml sample of blood was collected and kept at room temperature for 1 hour, followed by centrifugation at 1500 rpm, for 10 minutes. The serum was aliquoted into cryovials and stored at 70ºC, until tested.

Serum samples were tested for the presence of anti-*H. pylori* antibodies by using the HM-CAP ELISA kit (EZ-EM, Inc, West Burg, New York). According to manufacture directions, samples with an ELISA value of < 1.8 ELISA units were considered negative, and samples with an ELISA value of >2.2 ELISA units were considered positive. Samples with values between 1.8 and 2.2 ELISA units were considered intermediate and were retested. A sample that was still intermediate after the repeat test was recorded as negative.

Statistical Analysis:

A statistical analysis program (SPSS version 14; SPSS, Inc), was used to analyze the data. Standard methods were used to calculate sensitivity, and specificity for positive and negative results of ELISA for the detection of IgG antibodies specific for *H. pylori* antigen, against the gold standard (stool antigens of *H. pylori*) defined in previous studies[9]. Quantitative data were expressed as means with standard deviation. Differences between means were evaluated using paired t-tests. Qualitative data were expressed as number and percentage. One way analysis of variance was used to compare between groups.

Previous studies have suggested that the age of the patient may affect the sensitivity and specificity for *H. Pylori* testing. Therefore, test characteristics were determined on the group as a whole as well as after stratification to children ≤ 6 years of age or > 6 years of age [9].

3. Results

The seroprevalence of anti-*H. pylori* antibodies was 60% (36/60) in patients with secondary immunodeficiency versus 37% (11/30) in control children, with statistically significant difference, p = 0.02. The prevalence rate of positive *H. pylori* IgG antibodies was nonsignificantly higher among children with malignancy than that among children with nephrotic syndrome (Table 2).

Among two patients and control age groups (≤ 6 years and > 6 years), the prevalence rates of seropositivity were non-significantly different. The seroprevalence rate, in patients group is significantly higher in females than in males, p=0.02. On other hand, the seroprevalence rate, in control group is significantly higher (p= 0.024)in males than in females (Table 3). Table (4) shows that the seroprevalence of *H. pylori* antibody test is significantly higher (P <0.01) in neutropenic patients than that in non-neutropenic ones (89% vs 11%).

The sensitivity and specificity values of serum *H. pylori* antibody test, in all studied children, accounted for 77% and 100%, respectively (Table 5).

Table (2): *H. pylori* serum antibody test in 60 imunocompromised children veruses 30 control children, presented as number (n) and percent (%)

	Positive *H. pylori* serum antibody test	χ^2	P-value
Control (n = 30)	11 (37%)	4.86	0.02 (S)
All patients (n = 60)	36 (60%)		
- Malignancy (n = 45)	26 (72.2%)	0.41	0.51 (NS)
- Nephrotic syndrome (n = 15)	10 (66.7%)		

S: significant NS: nonsignificant

Table (3): Prevalence of *H. pylori* antibodies according to age and sex, in 60 immunocompromised children versus 30 control children, presented as number (n) and percent (%).

	Positive patients (n=36)			Positive control (n = 11)		
	n (%)	χ^2	P	n (%)	χ^2	P
Age (years) ≤ 6 > 6	17 (47.2) 19 (52.8)	2.39	0.3 (NS)	5 (45.5) 6 (55.5)	0.14	0.93 (NS)
Gender Males Females	16 (44.4) 20 (55.6)	4.92	0.036 (S)	7 (63.6) 4 (36.4)	4.98	0.028 (S)

NS: nonsignificant S: significant

Table (4): Positive seroprevalence of *H. pylori* antibodies among neutropenic patients versus non-neutropenic patients

	Positive cases		χ^2	P-value
	n	%		
Neutropenics, n = 45	32	71.1	9.07	0.01 (S)
Non-neutropenics, n = 15	4	26.7		

S: significant

Table (5): Sensitivity and specificity values of serum *H. pylori* antibody test, among 90 study children (60 patients and 30 control).

True positive (+ve)	True negative (-ve)	False (-ve)	False (+ve)
47	43	14	0

Sensitivity (ability of the test to detect positive cases) :

$$= \frac{\text{True (+ve)}}{\text{True (+ve) + false (-ve)}} = \frac{47}{47 + 14} = 77\%$$

Specificity (ability of the test to detect negative cases) :

$$= \frac{\text{True (-ve)}}{\text{True (-ve) + false (+ve)}} = \frac{43}{43 + 0} = 100\%$$

4. Discussion

H. pylori have been classified as a carcinogenic pathogen. Its prevalence is high in developing countries. In addition, impaired host immunity should be associated with a high prevalence of this infection, although a definitive relationship has not been established [3].

Once *H. pylori* colonize the stomach of an individual, it probably remains present for many years. However, many colonized people remain asymptomatic suggesting that the host factors are important for progression to *H. pylori* – mediated diseases [10].

Serology with IgG (as a non – invasive test to detect *H. pylori* infection) is widely used in Europe. Unfortunately, serology does not provide any data as to whether there is an active or past infection [11].

In this study, the seroprevalence of anti-*H. pylori* antibodies was significantly higher in secondary immunocompromised children than that in control subjects. Similar results were obtained by Sullivan *et al.*, in immunocompromised children, with chronic diarrhea and malnutrition [12] and by Masukawa *et al.*, in acute and chronic leukemia [13], and by Amal *et al.*, among healthy children in Assiut Governorate, Upper Egypt [14].

As regard age groups, of both patients and control children, we didn't find a significant relationship of age with *H. pylori* seroprevalence. This result is similar to that obtained by other studies [14,15]. On other hand, other studies reported that age of children is positively associated with *H. pylori* seropostivity, i.e., the higher the age, the higher the infection rate [16,17].

In this study, there was a significant difference between sex distribution and *H. pylori* seropositivty among patients group, with higher proportion of seropositivity among females than males. However the reverse is true among control children. This is in contrast to that obtained by Nguyen *et al.*, who concluded that *H. pylori* seropositivity is not related to many variable including gender [16].

The incidence of *H. pylori* infection was higher in neutropenic immuno- compromised children than that in those who had normal neutrophil count, with statistically significant difference, P < 0.01. Similar result was obtained by Papadaki *et al.* [18].

The sensitivity and specificity, of *H. pylori* IgG antibody test in all children (n= 90) accounted for 77% and 100%, respectively. Studies have reported sensitivities ranging from 54% to 94% and specificities between 59% and 97%. However, in children, serologic assay cutoff values (i.e., differentiating between positive and negative serology) may be very different compared with that in adults [19].

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