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Original Article

Helicobacter Pylori in Egyptian Pediatric Patients With Nephrotic Syndrome

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ABSTRACT:

Background: Helicobacter pylori (H. pylori) is a known cause of gastritis and peptic ulcer in children especially among immunocompromised patients as those with nephrotic syndrome (NS). The infection is acquired via droplet or fecal-oral routes. Eradication of the organism prevents the development of peptic ulcer disease.

Objectives: Assessment of the prevalence of H. pylori infection by various diagnostic modalities among nephrotic patients. Also, to evaluate the effect of specific treatment on clinical and laboratory findings.

Methods: This study was conducted at the Pediatric Nephrology Clinic of Ain Shams University Children's Hospital. It included 50 patients with NS subdivided according to response to steroid therapy into dependent, resistant and responsive in remission. They were 34 males and 16 females with a mean age of 9.5 ± 7.2 years, in addition to 24 healthy children as control group. H pylori infection was detected by several methods including serum anti H. pylori IgA antibodies, urease test, culture and PCR of a gastric aspirate. Patients who proved positive by one of the last three methods were given triple therapy (amoxycillin, omeprazole and clarithromycin) for one week and then reevaluated after one month by the same tests.

Results: Abdominal complaints were present in 27 out of 50 patients (54%). Serum specific IgA was positive in 22 patients (44%). Urease test was positive in 14 patients (28%) while culture for H. pylori revealed positive results in only two patients (4%). PCR for H. pylori was positive in 10 patients (20%). Thus, the most sensitive method was serum specific IgA (80%) while the most specific method was culture (100%) then urease test (75%). After treatment, both IgA and PCR positivity were significantly reduced and urease positive patients turned negative.

Conclusions: Culture of gastric aspirate proved to be the most specific of the methods used while serum specific IgA was the most sensitive compared to PCR of gastric aspirate as a standard test for detection of H. pylori infection. Triple therapy was very effective in eradicating H. pylori infection and improving abdominal complaints in children with NS.

INTRODUCTION

Infection has been recognized as an important cause of morbidity and mortality in children with nephrotic syndrome. Hypo-gammaglobulinemia and renal insufficiency are additional risk factors for bacterial infection in patients with nephrotic syndrome[1].

Helicobacter pylori (H. pylori) is believed to be one cause of recurrent abdominal pain in children[2]. Moreover, 95% of duodenal ulcer patients are infected with H. pylori and eradication of H. pylori results in cure of duodenal ulcer disease[3]. H. pylori appears as a short, curved gram negative bacteria, with one to two turns to the spiral. This gives the organism its characteristic "s". In culture, the cells may

tend to round up and form what have been described as "coccoid forms". The rate at which population acquires H. pylori infection is greater in developing than in industrialized countries. Genetic predisposition, socioeconomic status (including overcrowding especially during childhood) and environmental factors may play a role in its transmission. The prevalence of infection was 40% in Saudi Arabia (between 4-10 years of age) and it reached 60% in India. Person to person transmission of H. pylori was implicated to be the most likely mode of disease spread[4].

AIM OF THE WORK

The aim of this work was to assess the prevalence of H. pylori infection by various diagnostic modalities among pediatric nephrotic patients. Also, to evaluate the effect of specific treatment on clinical and laboratory findings.

SUBJECTS AND METHODS

Subjects:

This study was conducted at the Pediatric Nephrology Clinic, Children's Hospital, Ain Shams University. It included 74 pediatric subjects who were divided into 2 groups:

Group I: included 50 patients with nephrotic syndrome. They were 34 males (68%) and 16 females (32%). Their ages ranged between 3-15 years (mean 9.5 ± 7.24 years). Patients were classified according to their response to corticosteroids as defined by Barratt and Clark (1994)[5] into the following subgroups:

Group IA: included 21 patients who were steroid dependent (42%).

Group IB: included 20 patients who were steroid sensitive (40%) in remission.

Group IC: included 9 patients who were steroid resistant (18%) (2 were on cyclophosphamide, 3 were on cyclosporine A and 4 were on levamisol).

Group II (control group): included 24 healthy non-relative, non-household subjects. They were 11 males (45.8%) and 13 females (54.2%). Their ages ranged between 6-12 years (mean 8.41 ± 1.93 years).

Methods:

Both patients and control were subjected to:

A. Clinical evaluation including full medical history laying stress upon gastrointestinal symptoms related to H. pylori infection as abdominal pain, distension, vomiting and dyspepsia. Also, thorough clinical examination for body systems, blood pressure measurement, weight, height and edema.

B. Laboratory evaluation included:

1. Estimation of serum specific IgA against H. pylori by ELISA technique for both groups (H. pylori IgA, Biotest, Dreieich-Germany)[6].
2. For patients only, the following investigations were done:
   - Complete stool examination laying stress upon parasitic infestations.
   - Occult blood in stools[7].
   - Aspiration of a gastric juice sample using a Ryle tube of suitable size after an overnight fast. This sample was used for performing:
     – Urease test[8].

- Culture on Skirrow's medium for H. pylori[9].
- PCR for detection of DNA of H. pylori. DNA was extracted from the gastric juice using Wizard Genomic DNA Kit (promega, USA). Each sample was subjected to a consensus primer for seminested PCR method. Negative control used in this work was sterile nuclease free water biotechnology grade, supplied by (Amresco, USA). Amplification of DNA was performed with the thermal cycler (Perkin-Elmer Cetus, USA). Detection of the amplified DNA was done by agarose gel electrophoresis. The gel was examined under ultra-violet light as ethidium bromide intercalate between the bases of the DNA and will fluoresce[10].

- Routine chemical work-up including serum total proteins, albumin and cholesterol on Synchron CX5 Autoanalyzer.

Twenty patients showed positive results for H. pylori infection either by urease test, culture or PCR of gastric aspirate, eighteen of them received treatment[11] for one week in the form of:

- Amoxycillin (E-Mox by EIPICO) at a dose of 50 mg/kg/day, with a maximum dose of 2 gm/day.
- Clarithromycin (Klacid by Glaxo-Well-come Egypt) at a dose of 7.5 mg/kg/day.
- Omeprazole (proton pump inhibitor, Eprazole by EIPICO) at a dose of 20 mg/ day.

All treated patients were re-evaluated after the end of their course of treatment by one month by doing specific serum IgA against H. pylori, urease test, culture on Skirrow's medium and PCR of gastric aspirate.

Statistical analysis of the obtained data was done using student t, paired t, Chi square and multiple regression tests. Also analysis of variance and covariance for different tests to detect H. pylori was done with evaluation of its sensitivity, specificity, positive and negative predictive values as well as efficacy[12]. These tests were performed via standard computer programs. Graphic presentations were used to display results.

RESULTS

The results of various methods for detection of H. pylori used in this work are shown in Table 1.

Among the 27 cases presenting with GI complaint 9 were urease positive (33.3%), 9 were IgA positive (33.33%), 2 were culture positive for H. pylori and 8 were PCR positive for H. pylori, with no statistical relation between any of these tests and abdominal complaints ($p > 0.05$). In addition, H. pylori infection could not be correlated to the duration of illness, age, intake of NSAID, total proteins or serum cholesterol.

The diagnostic validity of urease, culture and serum specific IgA against H. pylori in relation to the standard test PCR in gastric aspirate are shown in Table 2.

The response to treatment after one month of the end of therapy by various tests used in this study is shown in Table 3.

Table 1: Count and percent of different tests in studied patient groups

Test	Steroid dependent (IA) (n = 21)	Steroid sensitive (IB) (n = 20)	Steroid resistant (IC) (n = 9)	Total (n = 50)
Positive urease	6 (28.5%)	6 (30%)	2 (22.2%)	14 (28%)
Positive	2 (9.5%)	0	0	2 (4%)
Positive IgA	11 (52.4%)	7 (35%)	4 (44.4%)	22 (44%)
Positive PCR	5 (23.8%)	3 (15%)	2 (22.2%)	10 (20%)

Table 2: Diagnostic validity in relation to PCR

PCR	Sensitivity (%)	Specificity (%)	Negative predictive value (%)	Positive predictive value (%)	Efficacy (%)
Urease	40	75	83	29	68
Culture	20	100	83	100	84
IgA	80	68	93	62	70

Table 3: Response to treatment by various tests used (n = 18)

Test	Before Treatment	After Treatment Count (%)
Positive urease	14	1 (7.14%)
Positive culture	2	0 (0%)
Positive IgA	9	1 (11.11%)
Positive PCR	10	2 (20%)

DISCUSSION

It is now proved that H. pylori plays a significant role in chronic gastritis and peptic ulceration in children and adults[13]. It has also been proposed as a possible etiologic factor of non-ulcer dyspepsia[14], and recurrent abdominal pain in children[15].

The infection has a worldwide distribution and is significantly more frequent in developing than industrialized countries[16]. Moreover, many gastric symptoms induced by steroids may be aggravated by H. pylori infection. To our knowledge, this is the first study done to detect the prevalence of H. pylori infection among nephrotic children with various available methods using non-invasive techniques.

Children included in the control group were selected to be non-relatives, non household contacts to the patients to avoid acquisition of H. pylori infection by close contact with the infected individuals in early childhood[17]. The absence of statistical relation between abdominal complaints and positive results for H. pylori detection by various methods used in this study justifies performing screening tests for all nephrotic pediatric patients and not to rely on the presence of such complaints to think about the possibility of H. pylori as a cause of these complaints. Furthermore, the presence of abdominal complaints may have several causes in nephrotic patients such as steroid therapy and should not be ignored.

In our study urease test was positive in 14 patients (28%). These results were in contrast with Abd El-Fattah (1997)[18], who reported positive urease in 50% of children with chronic renal failure. This may be explained by the fact that the present work used more sensitive methods for H. pylori detection as the PCR and culture. So, the efficacy of urease test was much lower than what he found. Urease test showed no significant statistical difference among different groups of patients (steroid dependent, sensitive or resistant).

The immunoglobulin evaluated in this study was of the IgA variety because the immunoglobulin lost in urine in nephrotic patients is the IgG type. Therefore IgA against H. pylori was found to be more reliable in the assessment of these children regarding H. pylori infection[19]. In the present study IgA against H. pylori was positive in 22 patients (44%) out of 50 with a statistically significant association with PCR ($p < 0.01$). This was supported by a high sensitivity percentage (80%). Thus, IgA against H. pylori may be considered an ideal screening test for H. pylori infection being highly sensitive and non-invasive.

The present study revealed positive culture of gastric aspirate for H. pylori in two cases only (4%), with a highly significant statistical correlation ($p < 0.001$) between culture and PCR for H. pylori. This explains the 100% specificity of culture in our study. In other words, when culture is positive for H. pylori, there is no need to do PCR, as it will be positive also. However the technical difficulties of culture and the delay of growth up to 10 days render this procedure difficult as a screening test for H. pylori infection.

PCR for H. pylori was considered the gold standard test in the present study, to which other tests for detection of H. pylori were compared. Ten out of 50 patients (20%) were PCR positive by seminested

DNA primers for detection of H. pylori in the gastric juice. The advantage of using PCR upon gastric juice versus biopsy specimen (from antrum or pylorus) is that the organisms are known to be distributed all over the gastric juice, but in the biopsy specimen, it may be hidden in the submucosa. So, if the biopsy forceps included only the mucosa, H. pylori will not be detected[10]. Moreover, using the biopsy technique for PCR, the biopsy forceps will need extensive cleaning and disinfection to avoid DNA contamination[20].

Our results showed significant correlation ($p < 0.01$) between PCR positivity for H. pylori and the gastrointestinal complaint, eight out of the 10 patients (80%) who were PCR positive had abdominal complaints. PCR was the only test for detection of H. pylori which showed this correlation demonstrating its unique specificity, sensitivity, predictive values and efficacy. However, PCR was not significantly different among different groups of patients (steroid dependent, sensitive and resistant).

In this work, 18 patients who were urease positive, culture positive or PCR positive for H. pylori were given triple therapy (omeprazole + amoxycillin + clarithromycin) for one week. All patients were re-evaluated 4 weeks after the end of therapy because eradication is defined as the presence of negative tests for H. pylori 4 weeks or longer after the end of antimicrobial therapy[21].

One month after treatment, the mean serum specific IgA against H. pylori was significantly reduced ($p < 0.01$), urease test was still positive in 7.14% of patients and culture became negative in all treated subjects, while PCR positivity significantly dropped ($p < 0.01$) although it was still positive in 20% of patients. These results showed the efficacy of this triple therapy and the sensitivity of PCR in detection of H. pylori infection post-treatment with great accuracy. The short duration of this protocol of treatment, compared to other protocols, made the compliance of treated patients very good. Also, side effects with this treatment were not detected for several months of follow up after treatment. These results were in parallel with the results of other authors who used the same treatment both in adults and in children. Lind et al. (1996) [22] showed 95-96% efficacy in adults as detected by urea breath test, while Ramadan et al. (1998)[11] showed 100% efficacy in eradicating symptoms among children.

In conclusion, the prevalence of H. pylori is not uncommon among nephrotic patients. Screening for this organism should be done in all nephrotic patients regardless duration of the disease or type of treatment of nephrotic syndrome. This is better done by serum specific IgA against H. pylori which had the highest sensitivity and is non-invasive. Positive IgA justifies further investigations using PCR for H. pylori being the most accurate of the methods studied. It has the highest efficacy. PCR is recommended to be done upon the gastric aspirate, not upon biopsy specimen. This prevents misinterpretation of results by contamination of biopsy forceps or embedding of H. pylori in the submucosa. Eradication of H. pylori infection even in the asymptomatic patients is possible using triple therapy (amoxycillin + clarithromycin

+ omeprazole) for one week and prevents the development of gastritis or peptic ulcer especially in the immunocompromized group.

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