Clinico-Epidemiological Correlates and Prevalence of Helicobacter Pylori Infection [via HpsAgT*] in Pediatric Acid-Related Disorder in Baguio and La Trinidad

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Abstract

Background. Helicobacter pylori (Hp) infection increases the risk for gastric cancer. Early detection and treatment are preventive. Hp has no pathognomonic symptoms but is associated with several Acid Related Disorder (ARDs).

Objectives. a) Determine prevalence of Hp infection in pediatric ARD patients in Baguio City and La Trinidad, and b) determine if the known predisposing, protective and preventive factors are applicable to this population.

Methods. Cross sectional prospective study of Hp infection in ARD patients via HpsAgT. Medical history, diagnosis and medications, dietary/feeding habits, handwashing practices, congestion index and family history were obtained with an interviewer-assisted questionnaire.

Results. Stools of 117 ARD children (2-19yrs) were analyzed. Hp positivity was 6%. It was not correlated with older age (p=0.96), gender (p=0.77) or family history of ARD (p=0.56), but it was weakly correlated with ethnicity (p=0.066, OR 6.37, 95% CI 0.9-151.8). No single clinical diagnosis effectively predicts a positive Hp stool antigen test (HpsAgT). Among ARD symptoms, abdominal pain had the best correlation (p=0.03, OR: 6.25, 95% CI 1.2-35.9).

Conclusion. There is a low prevalence of Hp via HpsAgT in this population compared to other local studies. Most of the known predisposing/protective factors do not apply.

Key Words: Helicobacter pylori, acid related disorder, H pylori stool antigen test, prevalence

INTRODUCTION

Helicobacter pylori (Hp) is one of the most common bacterial infections and is present in more than 50% of the population worldwide. (1) The prevalence can vary from 8.9% in developed nations to 72.8% in developing nations.(2) Infection rate is 2%-3% among children in developed countries, to as high as 16% among children in developing countries.(3-16) Local Philippine studies cite 10.53% Hp positive in pediatric dyspepsia (Cebu) to as high as 57.8% in children with GI symptoms (Manila).(11, 17) There has not been any determination (published or otherwise) regarding the statistics on patients with ARD in the Baguio City and La Trinidad area and the rates of Hp infection.

Hp is now known to be the cause of the overwhelming majority of peptic ulcer disease (PUD), and its eradication leads to cure of the disease.(18, 19) Hp was classified in 1994 as a class I carcinogen and risk for gastric cancer is

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increased by a factor of 2-3 in Hp infected individuals. (20, 21) Gastric cancers are rare during the first two decades of life, and Hp-associated gastric cancer has not been reported in children, but Hp colonization and infection does occur in the first two years of life in high risk populations. (22) In Europe and North America, the rates of Hp infection in children have decreased with the improvement of economy and education. The infection is still very common though, and rising in many developing countries. In these resource depleted areas, early Hp control will make the most impact.

In untreated individuals, infection is lifelong but most are asymptomatic. Hp has no absolute and pathognomonic clinical case definition that allows physicians to be able to immediately diagnose it with certainty in pediatric patients. Its known clinical presentations usually overlap with several illnesses classified under ARD. ARD is an umbrella term that includes conditions whose pathophysiology is believed to be the result of damage from acid and pepsin activity in the gastric secretions. The most common ARDs are gastroesophageal reflux disease (GERD) and PUD, others are esophagitis, hyperacidity, gastritis and dyspepsia.

*Hp pylori stool antigen test* (HpSAgT). Identification of Hp is done conventionally by culture, histology or detection of its chemical properties (oxidase, catalase, and urease activity) or bacteriologic components. Many of the noninvasive tests do require patient cooperation, making it difficult to perform in infants, toddlers or physically challenged children.

Between urea breath test (UBT) and HpSAgT which are advocated in the 'Test and Treat' strategy, the latter has been favored because it is less uncomfortable and distressing, substantially cheaper and more practical than trial therapy with acid suppression therapy. (23) It has even been suggested that HpSAgT replace the UBT for detection of Hp infection in children with comparable reliability and accuracy with a sensitivity of 91.6%, and specificity of 98.6%. (24, 25) Furthermore, the performance of HpSag in comparison to the other diagnostic modalities overall (UBT, culture, biopsy, urease test, histology, serology and PCR) is also comparable with a sensitivity of 92.6 and specificity of up to 100%. Similar detection rates in pediatric populations have also been reported [Sensitivity 82-92%, Specificity 82-98%]. (26, 27) It offers additional advantage as its results are also unaffected by proton pump inhibitor (PPI) use. (25) The SD Bioline® (Kyonggi, Korea) Rapid Stool Antigen Test claims 100% sensitivity and 100% specificity vis-à-vis Campylobacter-like Organism (CLO) Test and Respiratory Test as benchmarks. (28) Furthermore, many studies in various regions of the world have validated these sensitivities (91-100%) and specificities [83-100%]. (24, 29-38)

HpSAgT is part of the recommendations for management of Hp infection in adults. (23, 39-41) However, this strategy is not yet recommended in pediatrics. (42) In the Philippines, despite many recent convincing evidence that the HpSAgT provides an economical, fast and non invasive option with a high specificity and sensitivity, only a few pediatricians and family doctors use it.

**OBJECTIVES**

**General Objective:**

To determine the prevalence of Hp infection in children with ARD in Baguio and La Trinidad via Monoclonal HpsAgT, and establish clinico-epidemiological correlates.

**Specific objectives:**

1. To determine the prevalence of Hp infection in children with ARD
   a. Pretreatment diagnosis/Clinical Assessment
   b. Age
   c. ethnocultural origin
   d. Congestion Index
   e. Breast/formula feeding of infant patients
   f. Thumbsucking and pacifier use
   g. Dietary habits (intake of spicy, sour and/or caffeinated beverages)
   h. Hand washing
   i. Probiotic intake
   j. Family History of ARD
METHODOLOGY

Research Subjects
This research was a cross sectional prospective study of pediatric ARD patients from Baguio and La Trinidad, who were recruited from major private/government hospitals (SLU HSH, BGH MC, BeGH), private clinics and Walk in Satellite Collection Centers. (Fig. 1)

Inclusion Criteria:
1. Pediatric patients age: 2yr 0 months to 18 yrs 11 months
2. Must have at least one of the following Key Symptoms recurring at least once a week for 2 weeks or longer:
   1. Nausea/vomiting,
   2. Reflux/heartburn,
   3. Epigastric or other abdominal pain,
   4. Indigestion/fullness/frequent belching, OR
3. Physician-diagnosed:
   1. ARD
   2. Hyperacidity
   3. Gastritis
   4. GER/D
   5. Dyspepsia
   6. PUD

Exclusion Criteria:
1. The above Key Symptoms must NOT be caused by a coexisting medical condition
   a. Gastroenteritis/Colitis
   b. UTI
   c. Post-tussive emesis (in Respiratory Infections or Asthma)
   d. Pregnancy
   e. Migraine or recurrent headache
   f. Caustic or alcohol ingestion
   g. medication with NSAIDS, Steroids
   h. Blunt trauma to the abdomen
   i. Constipation
   j. Lactose Intolerance/Lactase deficiency
2. Has already completed or is taking (triple/quadruple) combination therapy for Hp
3. Presence of alarm signs that require immediate intervention/hospitalization (anemia, melena, hematochezia, shock, bowel perforation)

Conduct of Interview and Data Collection Questionnaire
Questionnaire validation for content and structure was done by a panel consisting of professionals and physicians with background in research. This was also screened and evaluated by the SLU HSH Ethics Committee and BGH MC ERC. This was subsequently ran on a pilot sample and revised further. The final questionnaire was administered by an interviewer to the participants or caregivers of participants.

Stool Collection and Testing.
The participants were given the instructions for proper stool collection. Stool collection was done at patient’s home, laboratory, hospital or clinic. Screening of stool sample for proper collection procedure, timing and adequacy was done by a medical technologist. The stool sample was numbered corresponding to the questionnaire number. Another medical technologist blinded to the clinical status of patient performed the HpSAg test.

Follow-up was through SMS every week until the scheduled end of data collection. A social networking site (SNS) was maintained for information and reminders regarding specimen collection and satellite collection centers. Specimen of admitted patients were collected by study personnel. Participants were informed of test results.

HpSAgT. The monoclonal One Step H pylori Antigen Rapid Test in Human Fecal Specimen Pack (SD Bioline, Standard Diagnostics, Inc., Korea) was used according to the manufacturer’s instructions.

Active Ingredients in the test strip include Mouse monoclonal anti-Hp – gold conjugate (0.12 ±0.24mg), Mouse monoclonal anti-Hp – gold conjugate (0.64 ±0.128mg), and Goat anti-Mouse IgG (0.64 ±0.128mg). The assay buffer components include phosphate buffer
(20mM), Bovine serum albumin (1%), sodium azide (0.01%), sodium chloride (0.1M), Tween 20 (0.1%).

Stool specimens were tested as soon as they were received. Refrigerated and frozen specimens were allowed to sit at room temperature before the procedure was done. A portion (about 50 mg) of the stool sample was taken using the supplied sterile swab. The swab was inserted into the sample collection tube containing the assay diluent. The swab was swirled at least 10 times until the sample has been dissolved. The swab was squeezed against the wall of the tube, and discarded. Tube was allowed to settle for 5 minutes. Three (3) drops (about 100mL) were placed into the sample well of the test device. Results were interpreted after 10-15 minutes.

Sample size computation

Sample size was determined using Open Source Epidemiologic Statistics for Public Health (Open Epi; Version 2.3.1, September 2010). At 90% confidence interval, the minimum number of participants required was 102. Computation was based on the Hp positivity of 10.53% in 38 children with dyspepsia in a study by Uy in Cebu City, Philippines (11).

The formula used was: Sample size n = [DEFF*NP(1-p)]/ [(ε/2Z21-α/2*(N-1)+p*(1-p)]. With population size (n): 1,000,000; hypothesized % frequency of outcome factor in the population (p): 10.53%+/-.5; confidence limits as % of 100 (absolute +/- %) (d): 5%; and design effect (for cluster surveys - DEFF): 1.

Data Processing and Analysis

Data were encoded and analyzed using SPSS Statistics Release 17.0.0 (August 23, 2008). Analyses were verified statistically using Epi Info™ Statistical Software (Centers for Disease Control & Prevention, USA and World Health Organization, Geneva, Switzerland; Version 7.1.0.6, August 2012).

To determine associations between Hp infection status and study variables, t-test was used for continuous data, and chi-square test (corrected for 2 x 2 tables using Yates method) for categorical data, except when the expected value of a cell was ≤ 5; thus, results from Mid-P Exact Test were obtained instead. Odds ratios were then calculated using Open Epi™ to determine the strength of associations reported at 95% confidence interval (p-value, 2-tail < 0.05). When a potentially clinically important effect was observed with a p-value above 0.05, an arbitrary judgment was done to claim evidence of an effect; hence, borderline p-value (p=0.05 – 0.10) was considered nearly significant (59).

Ethical/Biosafety Clearance

A Patient Information and Informed Consent Form was given to the subject/ parents/ guardian describing the purpose and conduct of the study, precautions for proper collection, confidentiality and voluntary participation. This was approved by the Ethics Committee of SLU HSH and BGH MC.

RESULTS

Data collection transpired from June 1 to December 15, 2012. Recruitment of subjects is summarized below.

The mean age of the population was 9.62 ± 4.52 years, with the youngest participant being 2.13 yrs, and the eldest being 18.7. When grouped by age, majority belonged to the older age group (Table 1). More than half the population (n=62, 53%) were of school age. There was almost an equal distribution of males and females. About 50% were Cordilleran, and the remaining half were either non-indigenous or had no response.
**Hp Prevalence and Demographics.**
This pediatric study population was found to have a 6% prevalence (7 HpsAgT(+)s among 117 participants). This study's youngest HpsAgT(+) was 2.17yrs. The difference in Hp infection status did not seem to be affected by mean age (p=0.80), age group (0.96), or gender (0.77). However, there appeared to be a borderline significant difference (p = 0.07) in the Hp infection status between Cordillerans and non-Cordillerans. More than 85% of HpsAgT(+)s were Cordilleran. If this were considered indeed significant, it would seem that Cordillerans were at ~6 times greater risk of having Hp infection than non-Cordillerans (OR: 6.368, 95% CI: 0.902, 151.800).

**Table 1 Baseline Characteristics of study participants according to Hp infection status**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>HpsAgT Status</th>
<th>OR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Over-all Mean</td>
<td>Positive N=7 6%</td>
<td>Negative N=110 94%</td>
<td>9.65 ± 6.452</td>
</tr>
<tr>
<td>(SD) Range</td>
<td>2.17 - 15.65</td>
<td>2.03 - 18.74</td>
<td></td>
</tr>
<tr>
<td>Age groups</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infant/toddler</td>
<td>1 (14.3)</td>
<td>12 (10.9)</td>
<td>1.43</td>
</tr>
<tr>
<td>(2.0 - 3.9 yrs)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preschool</td>
<td>1 (14.3)</td>
<td>12 (10.9)</td>
<td>1.43</td>
</tr>
<tr>
<td>(4.0-5.9 yrs)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>School age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6.0-12.9 yrs</td>
<td>3 (42.9)</td>
<td>59 (53.6)</td>
<td>1.283</td>
</tr>
<tr>
<td>Adolescent</td>
<td>2 (28.6)</td>
<td>27 (24.5)</td>
<td></td>
</tr>
<tr>
<td>(13.0 - 18.9 yrs)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>4 (57.1)</td>
<td>56 (50.9)</td>
<td>6.37</td>
</tr>
<tr>
<td>Female</td>
<td>3 (42.9)</td>
<td>54 (49.1)</td>
<td></td>
</tr>
<tr>
<td>Ethnic Origin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cordilleran</td>
<td>6 (85.71)</td>
<td>53 (48.18)</td>
<td></td>
</tr>
<tr>
<td>Non-Cordilleran</td>
<td>1 (14.29)</td>
<td>57 (51.82)</td>
<td></td>
</tr>
<tr>
<td>Non-Indigenous/Not</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Symptomatology and Hp infection.**
The most common presenting symptoms of ARD patients were abdominal &/or epigastric pain, 116 out of 117 (99.15%) (Table 2). Among those who presented with abdominal pain &/or epigastric pain, 109 (94%) tested negative for Hp. Although only 7 (6%) among them tested positive, it should also be noted that all of those who tested positive indeed presented with abdominal &/or epigastric pain (7 out of 7, 100%). Nevertheless, it seemed that abdominal &/or epigastric pain could be significantly associated (borderline: p=0.06) with Hp infection status. Based on the reciprocal of the odds ratio, those who presented with abdominal &/or epigastric pain had 7.8 times greater chances of testing positive for Hp via HpsAgT than those who did not present with the symptoms (OR: 0.13, 95% CI: 0.004, 4.17). Except for abdominal pain per se, all the other symptoms did not seem to significantly affect Hp infection status (p=0.03). It seemed that patients who complained of abdominal pain were 6.2 times more likely to test positive for Hp than those without abdominal pain (OR: 6.24, 95% CI: 1.20, 35.90).

**Table 2 Symptoms reported by Pediatric ARD patients according to Hp infection status (N=117)**

<table>
<thead>
<tr>
<th>Symptoms N (%)</th>
<th>HpsAgT Status</th>
<th>OR at 95% CI (Lower &amp; Upper Limits)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abdominal pain &amp;/ or epigastric pain: 116 (99.2)</td>
<td>7</td>
<td>7</td>
<td>0.128 (0.004, 4.17)</td>
</tr>
<tr>
<td>Abdominal pain alone</td>
<td>4</td>
<td>4</td>
<td>6.24 (1.20, 35.90)</td>
</tr>
<tr>
<td>Epigastric pain alone</td>
<td>6</td>
<td>6</td>
<td>0.48 (0.06, 12.20)</td>
</tr>
<tr>
<td>Nausea &amp;/or vomiting: 84 (71.79)</td>
<td>3</td>
<td>3</td>
<td>0.27 (0.06, 1.27)</td>
</tr>
<tr>
<td>Indigestion/ fullness: 39 (33.33)</td>
<td>4</td>
<td>4</td>
<td>2.83 (0.55, 15.89)</td>
</tr>
<tr>
<td>Flatulence: 38 (32.48)</td>
<td>2</td>
<td>2</td>
<td>0.82 (0.11, 4.39)</td>
</tr>
<tr>
<td>Frequent belching: 27 (23.08)</td>
<td>2</td>
<td>2</td>
<td>1.36 (0.17, 7.33)</td>
</tr>
<tr>
<td>Reflux: 17 (14.53)</td>
<td>2</td>
<td>2</td>
<td>2.51 (0.31, 13.99)</td>
</tr>
</tbody>
</table>
**Working diagnosis.** The most common diagnosis prompting referral to the study were: (1) gastritis [39 (33.3%)], (2) ARD [26 (22.2%)], and (3) hyperacidity [5 (4.3%)]. All of the participants who were diagnosed to have gastritis turned out to be negative for *Hp*. However, no significant association was found between each ARD diagnosis and *Hp* infection status.

**Food habits/ sanitary practices.** All of the participants practiced handwashing. About 94% took probiotics and 73% shared dipping sauces. Among the younger group, only 8 (6.8%) had a history of either thumbsucking or pacifier use. No significant association was found between food habits and acquiring *Hp* infection.

**Correlation with drink/food preference.** Almost 4 out of every 5 participants drank coffee, tea, &/or carbonated beverages. However, drinking these beverages did not seem to influence the risk of acquiring *Hp* infection (p=0.59). Neither did eating spicy/sour food (p=0.48) nor drinking alcoholic beverages (p=0.16).

**Correlation with family history of ARD.** Seventy participants (60%) had a family history of any ARD. Among the many ARDs, PUD was most common (36.7%). Only 4 (3.4%) had a family member with dyspepsia. None among these 4 patients were actually HpSAgT(+). Parents and grandparents were the most common infected family members. Among the 21 participants who had a mother positive for ARD, 95% tested negative. The associations between family history for ARD and actual *Hp* infection were not statistically significant (p=0.56).

**Correlation with living conditions and *Hp* infection status.** Congestion index (p=0.54), number of siblings (p=0.90), and parents’ employment status (p=0.80) did not seem to influence *Hp* infection status either.

**DISCUSSION**

It appears that the prevalence of *Hp* infection in these patients with ARD is less than that reported in local and international studies. In our study, the prevalence was 6%. *Hp* infection in this study was defined solely as a positive HpSAgT.

ARD is a common diagnosis in pediatrics and the challenge lies in detecting the subset of patients who will benefit from early anti-*Hp* treatment. It was difficult to predict which ARD patients will test positive for *Hp*, based on a physician-made diagnosis, or from the symptoms that a pediatric patient feels and relates to his guardian then to the health care provider. There were no pathognomonic signs of an *Hp* infection. While there have been associations of *Hp* infection with ARD, the traditionally perceived and most common signs of epigastric pain and vomiting showed non-significant correlation with *Hp* positivity. Abdominal pain, which in itself is a nonspecific finding, showed the greatest correlation in this population. Since it is also not a wise practice to screen all ARD patients with abdominal pain for *Hp* infection, it is still important to combine history, family history, physical examination, response to initial treatment and laboratory tests to narrow down the differentials before considering anti-*Hp* therapy.

Various studies associate *Hp* infections with socioeconomically deprived populations and older age groups. The interaction of different dietary, environmental and familial factors are also thought to heavily influence infection rates. (15-16, 46-47) Strongly associated with *Hp* infection are low socioeconomic status, increased number of sibling, crowded living conditions in childhood and poor hygienic practices. (15-16, 46-48) This was not replicated in our study. Food habits, sanitary practices, drink/food preference, living conditions and family history of ARD did not correlate to *Hp* positivity. *Hp* infection has been known to correlate directly with increasing age(7-8), family history of PUD(8,14), and gastric cancer especially if the father or the mother is affected.
They share genetic and environmental factors and also the same bacterial strain and pathogenic properties. *Hp* therefore tends to be confined to families(44), communities and racial groups. Transmission of infection not only requires both close contact between family members or members of the community but also long periods of contact starting from childhood.(45)

The Philippines is a developing country, but rural centers with heightened health awareness and medical care is accessible. The population studied is representative of mixed urban and rural social strata equipped with tertiary referral hospitals and numerous private clinics and physicians. We did not find significant differences in risk factors in the infected and non-infected ARD patients; and it is also inconclusive as to what the protective factors are. We believe that this heterogeneity is caused by the fact that Baguio and La Trinidad are cultural melting pots that have many immigrants and mixed ethnic lineages. Additionally, most children are handled by various caregivers, have mobile lifestyles and the classic maternal child transmission as described in previous studies may no longer apply.

CONCLUSION

The *Hp* prevalence via HpSAGT in pediatric ARD patients in Baguio-La Trinidad is 6%. *Hp*(+) cases that present as ARD may be missed or their diagnosis and treatment delayed, since the clinical assessment and symptoms are varied. Abdominal pain appears to be the only typical clinical manifestation that is at the least, significantly predictive of *Hp* infection. Despite the known predisposing factors in literature for *Hp* infection, none of these were replicated in this study including older age, overcrowding, thumb sucking, pacifier use, preference for sour or spicy food, or ARD family history. There was only a weak correlation with ethnic origin. Neither were there observed protective effects from handwashing, probiotic intake, and breastfeeding. In the absence of typical indicators from patient history and examination, the *HpSAgT* is an important diagnostic adjunct to allow the physician to confirm *Hp* suspicion.

LIMITATIONS AND RECOMMENDATIONS

Further studies are recommended on:

1. Larger study population.
2. Correlating other environmental, epidemiologic factors and dietary practices not traditionally known to predispose or protect against *Hp*; explore regional variation intergroup variation among Cordilleran groups.
3. Screening other relatives and housemates of the *HpSAgT*(+) patients for better correlation of environmental transmission and genetic predisposition.
4. Testing for sensitivity and specificity of this and other brands of *HpSAgT* in the local market.
5. Comparison of *HpSAgT* with endoscopy (histology and biochemical testing).
6. Consideration of the mobile nature of most families and history of frequent transfers of residence and caretakers and how this affects or predicts *Hp* infection.
7. Alternative stool collection techniques for patients unable to collect stool (i.e., rectal swab) to increase specimen yield.

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