Helicobacter pylori Infection in Infants and Toddlers in South America: Concordance between [13C]Urea Breath Test and Monoclonal H. pylori Stool Antigen Test

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Accurate noninvasive tests for diagnosing Helicobacter pylori infection in very young children are strongly required. We investigated the agreement between the [13C]urea breath test ([13C]UBT) and a monoclonal ELISA (HpSA) for detection of H. pylori antigen in stool. From October 2007 to July 2011, we enrolled 414 infants (123 from Brazil and 291 from Peru) of ages 6 to 30 months. Breath and stool samples were obtained at intervals of at least 3 months from Brazilian (n = 415) and Peruvian (n = 908) infants. [13C]UBT and stool test results concurred with each other in 1,255 (94.86%) cases (kappa coefficient = 0.90; 95% confidence interval [CI] = 0.87 to 0.92). In the H. pylori-positive group, delta-over-baseline (DOB) and optical density (OD) values were positively correlated (r = 0.62; P < 0.001). The positivity of the tests was higher (P < 0.001; odds ratio [OR] = 6.01; 95% CI = 4.50 to 8.04) in Peru (548/878; 62.2%) than in Brazil (81/377; 21.5%) and increased with increasing age in Brazil (P = 0.02), whereas in Peru it decreased with increasing age (P < 0.001). The disagreement between the test results was associated with birth in Brazil and female gender but not with age and diarrhea. Our results suggest that both [13C]UBT and the stool monoclonal test are reliable for diagnosing H. pylori infection in very young children, which will facilitate robust epidemiological studies in infants and toddlers.

Helicobacter pylori infection is acquired primarily in early childhood and is predominantly transmitted within families, infected mother and siblings being the most common familial source of the microorganism (1–5). Infants and toddlers most frequently acquire and lose the infection (3, 6), but there are substantial knowledge gaps in respect to the predictors of initial acquisition, as well as the persistence of the infection, in this age group. In addition, in children, H. pylori infection has been associated with iron deficiency anemia, diarrheic disease, and impairment of growth, weight, and cognitive functions (7, 8). Thus, a simple and reliable noninvasive test to detect H. pylori infection in this age group is required, especially in developing countries, where the prevalence of H. pylori infection is very high.

The noninvasive [13C]urea breath test ([13C]UBT) (9–12) and stool antigen test (13–15) are very reliable for the diagnosis of H. pylori infection in children older than 6 years. The stool test based on monoclonal antibodies has proved to be highly accurate in all age groups (13–15), but the specificity of the [13C]UBT varies from 82% to 100% for young children (9–12). However, studies of this subject are scarce and have not included large enough numbers of infants and toddlers to obtain reliable results. Furthermore, different “gold standard” tests have been used to validate the [13C]UBT, and most studies are in developed countries, where the prevalence of infection is very low (11, 12, 16, 17).

To validate noninvasive tests for diagnosis of H. pylori infection, the indicated “gold standard” includes at least two invasive tests, which is a difficult task with young children due to the current rarity of symptomatic H. pylori infection in this period of life. In addition, as stated by Goodman and Correa, validation using invasive tests has some limitations; recent short-term colonization and patchy distribution of the bacterium in the gastric mucosa may decrease the sensitive of biopsy-based tests in very young children (18). Such problems currently restrict epidemiological investigations of the acquisition of H. pylori infection in infants and toddlers in developing-country settings.

The aim of this study was to investigate whether the two independent noninvasive tests for H. pylori infection diagnosis, the [13C]UBT and monoclonal stool antigen test, have good concordance in young children. For that, we evaluated a cohort of infants and toddlers living in impoverished regions of two developing countries in South America. Our hypothesis was that if the [13C]UBT and monoclonal stool antigen test had good agreement in infants and toddlers, either of the two noninvasive tests could be used for the diagnosis of H. pylori in this age group. We also aimed to investigate causes linked to discordant results between the two tests.

MATERIALS AND METHODS

Children and methods. The study was approved by the Ethics Committees of the participant institutions. The study was also reviewed by the EU

Received 9 July 2013 Returned for modification 7 August 2013 Accepted 19 August 2013 Published ahead of print 4 September 2013

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TABLE 1 Agreement between [13C]UBT and monoclonal stool test results for diagnosis of H. pylori infection in infants and toddlers according to the different [13C]UBT cutoff pointsa

<table>
<thead>
<tr>
<th>Cutoff (%)</th>
<th>No. of discordant results</th>
<th>No. (%) of results in agreement</th>
<th>Kappa coefficient</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>+ UBT/SAT</td>
<td>− UBT/SAT</td>
<td>+ UBT/SAT</td>
<td>− UBT/SAT</td>
<td>+ UBT/SAT</td>
</tr>
<tr>
<td>3.5</td>
<td>37</td>
<td>21</td>
<td>1,225 (92.59)</td>
<td>0.85</td>
</tr>
<tr>
<td>4.0</td>
<td>45</td>
<td>23</td>
<td>1,255 (94.86)</td>
<td>0.90</td>
</tr>
<tr>
<td>5.0</td>
<td>120</td>
<td>18</td>
<td>1,185 (89.57)</td>
<td>0.79</td>
</tr>
<tr>
<td>6.0</td>
<td>191</td>
<td>14</td>
<td>1,118 (84.50)</td>
<td>0.69</td>
</tr>
</tbody>
</table>

a n = 1,323, %, DOB (delta over baseline); + UBT, positive urea breath test; − UBT, negative urea breath test; + SAT, positive stool antigen test; − SAT, negative stool antigen test. Kappa coefficient was calculated according to Cohen’s statistic (18).

TABLE 2 [13C]Urea breath test and stool antigen monoclonal test results for samples from Brazilian and Peruvian infants and toddlers

<table>
<thead>
<tr>
<th>Result categorya</th>
<th>No. of results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Brazil</td>
</tr>
<tr>
<td>All</td>
<td>415</td>
</tr>
<tr>
<td>Concordant</td>
<td></td>
</tr>
<tr>
<td>HP+</td>
<td>81</td>
</tr>
<tr>
<td>HP−</td>
<td>296</td>
</tr>
<tr>
<td>Total</td>
<td>377</td>
</tr>
<tr>
<td>Discordant</td>
<td></td>
</tr>
<tr>
<td>HP+−</td>
<td>14</td>
</tr>
<tr>
<td>HPSA− UB+</td>
<td>24</td>
</tr>
<tr>
<td>Total</td>
<td>38</td>
</tr>
</tbody>
</table>

a HP+, H. pylori positive; HP−, H. pylori negative; HPSA, H. pylori stool antigen assay. The DOB (delta over baseline) of 4‰ was adopted.

RESULTS

A total of 1,323 breath and stool samples were tested. For 28 (2.1%) children, only one sample was tested. The mean of samples tested per child was 3.9. No difference in the gender balance (P = 0.41; OR = 1.1; 95% CI = 0.87 to 1.40) was observed between Peru (453/908; 49.9% girls) and Brazil (197/415; 47.5% girls), but, the mean age (± standard deviation [SD]) in months of the Peruvian children (17.84 ± 6.14) was lower (P = 0.001) than that of the Brazilian children (19.86 ± 12.16).

Concordance between [13C]UBT and stool antigen test. Because the best concordance, even in each country separately, was obtained by adopting the cutoff point of 4‰, all analyses were done with this cutoff value (Table 1). The [13C]UBT and stool test results concurred with each other for 1,255 (94.9%) breath/stool samples with a kappa coefficient of 0.90 (95% CI = 0.87 to 0.92). [13C]UBT results concurred with stool test results for 878/908 (96.7%) of the Peruvian samples (kappa coefficient of 0.93; 95% CI = 0.91 to 0.96) and for 377/415 (90.8%) of the Brazilian samples (kappa coefficient of 0.75; 95% CI = 0.68 to 0.83), being significantly higher in the former (P < 0.001; OR = 2.95; 95% CI = 1.75 to 4.97) (Table 2).

The concordance between the tests did not differ (P = 0.43; OR = 2.25; 95% CI = 0.53 to 13.50) when unformed/watery stool samples (80 of 82, concordance of 97.6%) were compared with normal stool samples (1,175 of 1,241, concordance of 94.7%) and when boys were compared with girls (P = 0.62; OR = 0.86; 95% CI = 0.51 to 1.43). The mean age (± SD) in months (18.47 ± 8.47 and 19.21 ± 9.87; P = 0.48) and the mean time interval (days)
between breath and stool sample collection (13.97 ± 10.35 and 14.16 ± 12.09; \(P = 0.88\)) did not differ between concordant and discordant results, respectively.

The positivity of the tests was higher (\(P < 0.001\); OR = 6.01; 95% CI = 4.50 to 8.04) for samples from Peru (546/878; 62.2%) than for those from Brazil, (81/377; 21.5%). In Brazil, no difference was observed between the genders (41/138 girls versus 40/158 boys; \(P = 0.61\); OR = 1.17; 95% CI = 0.70 to 1.98), whereas in Peru, samples from girls (299/437; 68.4%) were more frequently positive than those from boys (247/441; 56.0%) (\(P < 0.001\); OR = 1.70; 95% CI = 1.28 to 2.26), even after Bonferroni adjustment for age. A greater number of positive samples that became negative by the two tests from the first year to the second year of life (Fig. 2B). Significantly higher DOB values were observed for Brazilian than for Peruvian children (Table 3). No differences in the mean DOB (\(P = 0.02\)) and OD (\(P = 0.02\); OR = 1.17; 95% CI = 1.02 to 1.39) were observed for Peruvian females (84.5 to 62.9%; \(P = 0.001\) but not for males (67.0 to 59.6%; \(P = 0.02\); OR = 1.2; 95% CI = 1.02 to 1.39). The prevalence of the infection increased with increasing age in Brazil (\(P = 0.02\)), whereas in Peru it decreased with increasing age (\(P < 0.001\)) (Fig. 1).

In the group of \(H. pylori\)-positive (\(r = 0.62\); \(P < 0.001\)) but not \(H. pylori\)-negative (\(r = 0.02\); \(P = 0.67\)) children, DOB and OD values were correlated. In the positive group, both the DOB and OD values increased with increasing age (Fig. 2A and C). In the negative group, a significant increase in DOB values occurred from the first to the second year of life (Fig. 2B). Significantly higher DOB values were observed for Brazilian than for Peruvian positive children (Table 3). No differences in the mean DOB (\(P = 0.80\)) and OD (\(P = 0.58\)) values were observed between watery (20.19 ± 25.55 and 0.72 ± 1.09, respectively) and normal (19.63 ± 24.09 and 0.76 ± 1.04, respectively) positive stool samples. No other difference in respect to DOB and OD values was observed.

Discrepant results between \([^{13}\text{C}]\text{UBT}\) and stool antigen test and associated factors. The results of the tests were discrepant in 68/1,323 (5.1%) samples (23 [33.8%] negative; \([^{13}\text{C}]\text{UBT}\) and positive-stool-test samples and 45 [66.2%] positive-\([^{13}\text{C}]\text{UBT}\) and negative-stool-test samples) (Table 2).

Only birth in Brazil (\(P < 0.001\); OR = 3.14; 95% CI = 1.92 to 5.16) was associated with the disagreement between the tests. We then constructed two other models of analysis. First, the positive-\([^{13}\text{C}]\text{UBT/negative-stool-test}\) group was compared with the concordant group, and only the country of birth remained associated in the multivariate analysis (Table 4). Second, the negative-\([^{13}\text{C}]\text{UBT/positive-stool-test}\) group was compared with the concordant group. Female gender, increased time interval between stool and breath sample collection, and birth in Brazil remained directly and independently associated (Table 4).

Effect of temperature on performance of stool antigen test. When stool samples with initial OD values of 1.449 and 1.620 maintained at 25°C were retested, the OD values dropped to 1.305 and 1.356, respectively, after 6 h and to 0.944 and 0.921, respectively, after 48 h. The decrease of the OD values was more pronounced when the samples were maintained at 37°C, dropping to 1.225 and 1.210, respectively, in the first 6 h and to 0.159 and 0.173, respectively, after 48 h. Remarkably, when the stool samples with OD values of 0.149 and 0.183 were retested after 6 h of incubation at 37°C, the OD dropped to values below the cutoff (0.125 and 0.120, respectively).

**DISCUSSION**

Although there are studies demonstrating high accuracy of the monoclonal stool antigen test (13, 14) for the diagnosis of \(H. pylori\) infection in young children, there is not concordance among studies evaluating another noninvasive test, the \([^{13}\text{C}]\text{UBT}\) (9, 11, 12). Furthermore, since most validation studies to date have been undertaken in developed countries, where the prevalence of \(H. pylori\) infection is low, only a small number of young infected children have been included. Concomitant \(H. pylori\) infection and diarrheal disease might also influence the performance of the noninvasive diagnostic tests in developing countries, also indicating the need to test the reliability of the \([^{13}\text{C}]\text{UBT}\) and stool antigen test in developing countries.

Notably, by evaluating a large number of samples obtained from infants and toddlers in two developing countries, we demonstrated that the results of the \([^{13}\text{C}]\text{UBT}\) were highly in agreement with those of the monoclonal stool antigen test, which points to a good accuracy of each test for the diagnosis of \(H. pylori\) infection in very young children. Reinforcing this hypothesis, the positive correlation observed between \([^{13}\text{C}]\text{UBT}\) DOB and stool test OD values in the group of \(H. pylori\)-positive children is in agreement with our previous report for older children (20).

In the \(H. pylori\)-positive infants and toddlers, the DOB values increased with age, which is in contrast to observations of older children by others (21, 22) and our group (20). In the present study, the OD values in the stool antigen assay also increased with age. No association between OD values and age has been observed in older children (14, 20). Taken together, these results suggest that DOB and OD values increase with age only in very young children and represent a progressive increase in the gastric bacterial load at this age. In \([^{13}\text{C}]\text{UBT}\)-negative children, however, the DOB values increase only from the first to the second year of life.

Clear differences between the two studied populations in South America were observed. First, \(H. pylori\) positivity increased with increasing age in Fortaleza but decreased in Lima. Spontaneous clearance of the infection as observed in Lima has been questioned. Some authors argue that the DOB values are higher in \(H. pylori\)-negative infants, which could lead to \([^{13}\text{C}]\text{UBT}\) false positivity at this age (21). However, in the \([^{13}\text{C}]\text{UBT}\)-negative Lima population, the lowest DOB values were observed in the first year.

**FIG 1** \(H. pylori\) prevalences based on concomitant positive results of \([^{13}\text{C}]\text{UBT}\) and the monoclonal stool antigen test according to age range in Peru (\(P < 0.001\)) and Brazil (\(P = 0.001\)). The numbers at the bottom refer to the number of samples tested in each age group (total number = 1,255). Statistical analysis was done using the chi-square test.
of life, in parallel with the highest \textit{H. pylori} positivity, also observed in the first year of life. Furthermore, we could not demonstrate any cause of false-positive results in both tests in Peruvian children. Differences in respect to the prevalence of the infection, which was higher in Lima than in Fortaleza, were also observed. One explanation is that Peruvian children live in an area of very prevalent \textit{H. pylori} infection, being highly exposed to the bacterium (23), which contributes to very early infection. Ethnic differ-

\begin{table}
\centering
\caption{Mean values of \textsuperscript{13}C\textsubscript{15N2} UBT DOBs and HpSA Plus ODs for Brazilian and Peruvian children who had concordant results between the two tests\textsuperscript{d}}
\begin{tabular}{llllll}
\hline
\textbf{Result} & \textbf{Country} & \textbf{Peru} & \textbf{Brazil} & \textbf{P value} & \textbf{Gender} & \textbf{Boys} & \textbf{Girls} & \textbf{P value} \\
\hline
\textbf{UBT DOB} & & & & & & & \\
Negative & 1.37 ± 1.63 & 1.36 ± 1.30 & 0.38 & 1.30 ± 1.36 & 1.48 ± 1.67 & 0.23 \\
Positive & 18.87 ± 23.36 & 25.20 ± 28.24 & 0.05 & 18.37 ± 21.44 & 19.45 ± 25.38 & 0.52 \\
\textbf{HpSA OD} & & & & & & & \\
Negative & 0.07 ± 0.02 & 0.07 ± 0.03 & 0.35 & 0.07 ± 0.03 & 0.07 ± 0.02 & 0.54 \\
Positive & 0.76 ± 0.99 & 0.78 ± 0.93 & 0.26 & 0.77 ± 1.06 & 0.76 ± 1.03 & 0.85 \\
\hline
\end{tabular}
\textsuperscript{d} DOB, delta over baseline, mean ± SD (‰); OD, optical density, mean ± SD; cutoff values adopted were those recommended by the manufacturers (\textit{n} = 1,255). Data were analyzed by using Student’s \textit{t} test after natural log transformation.
\end{table}
A large time interval between stool and breath sample collection, a diarrheic stool sample, and a positive stool test. In young children, the infection may be cleared from the gastric mucosa in the interval between the collection of stool and breath samples.

Variables associated with discrepant results between [13C]UBT and HpSA Plus for Brazilian and Peruvian children

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariate analysis, P value</th>
<th>Multivariate analysis OR 95% CI P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive UBT/negative HpSA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Increasing age</td>
<td>0.53</td>
<td></td>
</tr>
<tr>
<td>Female gender</td>
<td>0.60</td>
<td></td>
</tr>
<tr>
<td>Unformed/watery stools</td>
<td>0.65</td>
<td></td>
</tr>
<tr>
<td>Time interval&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.15</td>
<td>0.99</td>
</tr>
<tr>
<td>Country of birth</td>
<td>&lt;0.001</td>
<td>2.81</td>
</tr>
<tr>
<td>Negative UBT/positive HpSA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unformed/watery stools</td>
<td>0.75</td>
<td></td>
</tr>
<tr>
<td>Increasing age</td>
<td>0.89</td>
<td></td>
</tr>
<tr>
<td>Female gender</td>
<td>0.02</td>
<td>3.12</td>
</tr>
<tr>
<td>Time interval</td>
<td>0.03</td>
<td>1.03</td>
</tr>
<tr>
<td>Country of birth</td>
<td>0.001</td>
<td>3.70</td>
</tr>
</tbody>
</table>

<sup>a</sup> A Hosmer-Lemeshow test showed good fitness of the model (8 degrees of freedom, P ≥ 0.48, and 10 steps). OR, odds ratio; CI, confidence interval.

<sup>b</sup> Time interval, interval of time in days between breath and stool sample collection (≤30 days).

girls, because the elimination of *H. pylori* antigens in the stool may take longer.

Importantly, having a diarrheic stool sample was not associated with test result disagreement. High concordance between the two tests was observed for children both with and without diarrhea. One might speculate that the concentration of *H. pylori* antigens would be lower in watery stools than in normal stools. To avoid this possibility, we adopted the manufacturer’s recommendations using a high volume of stool. A false-positive stool test result, due to cross-reactivity between antigens of *H. pylori*-related bacteria, has also been suggested (12). Against this possibility, we used a highly specific monoclonal HpSA Plus enzyme immunoassay (EIA) for detection of *H. pylori* antigens in stool samples. Reinforcing the reliability of our results, we observed positive results of the stool antigen test for 98.3% of the diarrheic children with positive-[13C]UBT. Furthermore, we are unaware of causes of false-positive [13C]UBT results for diarrheic children.

In conclusion, this study showed excellent agreement between the results of the [13C]UBT and the stool antigen test for infants and toddlers in both high- and moderate-*H. pylori* prevalence developing countries in South America. This indicates that both noninvasive tests are reliable methods for the diagnosis of *H. pylori* infection in very young children, which will facilitate robust epidemiological studies in infants and toddlers in developing countries.

**ACKNOWLEDGMENTS**

This work was supported by the Sixth Framework Programme of the European Union, Project CONTENT (grant number INCO-DEV-3-032136), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq/Brazil), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES/Brazil), and Instituto de Biomedicina do Semi-Arido (INCT-IBISAB/Brazil).

We thank the CONTENT external advisor Guillermo Perez-Perez for his expert advice at the commencement of the project, the members of the communities of the Pampas de San Juan and Parque Universitário for their collaboration, Lilà Cabrera for her assistance in field work in Lima, and Maria Luiza Scarabelli for her assistance in reading results of [13C]UBT assays of this study.

**REFERENCES**