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• *BRIEF REPORTS* •

Association of *Helicobacter pylori* infection and giardiasis: Results from a study of surrogate markers for fecal exposure among children

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Abstract

AIM: To investigate whether *Helicobacter pylori* (*H pylori*) infection is associated with hepatitis A virus (HAV) infection, presence of enteroparasites, and other surrogates of fecal exposure.

METHODS: We conducted a cross-sectional study in 121 children consecutively admitted at a pediatric hospital in Salvador, Brazil. *H pylori* and HAV infection were identified by the presence of serum antibodies. Stool specimens were examined for the presence of ova and parasites. A structured questionnaire inquiring about sanitary conditions and life style was applied to each subject.

RESULTS: Fifty-one of the 121 children (42.1%) were found to be seropositive for *H pylori*, and 45 (37.2%) for HAV. The seroprevalence of *H pylori* and HAV both increased significantly with age. Cross-tabulation of data showed that 26 (21.5%) were seropositive and 51 (42.1%) were negative for both *H pylori* and HAV antibodies (χ^2 = 7.18, OR = 2.8, CI 1.30-5.97). The age adjusted OR for an HAV-infected child being *H pylori* positive was 2.3 (CI 1.02-5.03). The agreement between *H pylori* and HAV seropositivity was fair (κ = 0.24). After controlling for possible confounding, the variables remaining independently associated with seropositivity to *H pylori* were age, presence of *Giardia lamblia* in feces (OR = 3.2, 95%CI, 1.1-9.5) and poor garbage disposal quality (OR = 2.4, 95%CI, 1.1-5.1).

CONCLUSION: Our data suggest that *H pylori* infection is associated with surrogate markers of fecal exposure. Thus, we conclude that the fecal-oral route is relevant in the transmission of HP among children in an urban setting of a developing country. The association observed between *G. lamblia* and *H pylori* infection may have several explanations. Further studies to investigate this relationship are warranted.

Key words: *H pylori*; Hepatitis A virus; Epidemiology; Transmission; Enteroparasites; Brazil

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INTRODUCTION

Helicobacter pylori (*H pylori*) is now thought to be one of the most important factors in the pathogenesis of upper gastroduodenal diseases such as gastritis and peptic ulcer disease[1,2]. Several mechanisms of *H pylori* transmission involving the fecal-oral or oral-oral route have been proposed[3-5]. Yet the design of prevention measures is hampered by uncertainties about the exact mode and route of *H pylori* transmission. Earlier studies have indicated that person-to-person transmission is a major mode of spread of *H pylori* [6-8]. However, even when *H pylori* is transmitted from person to person, it is still controversial whether the transmission route is via an oral-oral, fecal-oral or some other route[9,10].

The isolation of *H pylori* from feces has been used to argue for the fecal-oral route of transmission[11]. The consumption of uncooked vegetables contaminated by raw sewage in the water supply has also been suggested to be a key factor in the transmission of *H pylori*[12]. Despite such evidence supporting fecal-oral transmission, *H pylori* has also been isolated from dental plaque and saliva[4,13,14], suggesting that the bacterium may be acquired by the oral-oral route. Thus, as an attempt to clarify this issue, we directly compared the seroprevalence of *H pylori* with the prevalence of intestinal parasites known to be acquired by the fecal-oral route, and with the seroprevalence of antibody to hepatitis

A virus (HAV) also known to be a sensitive marker of fecal-oral exposure[15], in a population of children admitted to a pediatric hospital in Salvador, Brazil.

MATERIALS AND METHODS

This study was conducted in a non-profit, 90-bed pediatric hospital (Hospital da Criança), which serves uninsured children of low socioeconomic status in the city of Salvador, in northeastern Brazil. Salvador has an ethnically diverse population of 2.3 million, and is the third largest city in Brazil.

Subject selection

We enrolled 121 out of 132 eligible children consecutively admitted at the Hospital da Criança over a 6-month period (from August 2001 to February 2002). Thus, the response rate was 92%. Children with life-threatening diseases, living outside the metropolitan area of Salvador or who had been vaccinated against hepatitis A virus (HAV) were excluded from the study.

Data collection

A structured questionnaire was administered in person by trained and certified interviewers to either parent of each child. On an average, each interview lasted 30-45 min. Neither the interviewers nor the children's parents were aware of the *H pylori* infection status of the study subject at the time the interview took place. The protocol was approved by the Hospital Ethical Committee, and written informed consent was obtained from the parents of all children, before enrollment in the study.

The data collection instrument contained approximately 88 items, including questions on demographic and household characteristics. A crowding index was calculated by dividing the number of household members by the number of rooms. Garbage disposal system was classified as good or poor. Good systems included scheduled garbage collection and absence of garbage in children's playgrounds (household vicinity). Also, poor systems included non-scheduled garbage collection or presence of garbage in children's playgrounds. Household excreta disposal system was also classified as good or poor. Good systems included either the municipal sewer or a septic tank with leach field, and poor systems included cesspools and portables.

Serology

Serum was separated, frozen, and stored at -20 ℃ until assayed. *H pylori* status was determined by the presence of anti-*H pylori* IgG antibodies in the ELISA using a commercially available kit (Enzignost® Anti-*H pylori* II/ IgG; Dade Behring, Marburg, Germany). All testing was performed according to the manufacturer's instructions. ELISA test uses inactivated *H pylori* antigen (detergent extract from a CagA- and VacA positive strain isolate) absorbed to reaction wells. A sample was considered positive for IgG antibodies to *H pylori* if the corrected absorbance reading of the serum sample was 0.250 or more (cut-off). The specificity and sensitivity are estimated to be 98.8% and 93.4%, respectively (Dade Behring, data on file). It has been shown that the ELISA test and other serological tests distinguish those infected with *H pylori* from those who have not been infected[16]. All sera were tested blindly.

Serum Hepatitis A antibodies were analyzed by a commercially available microparticle enzyme immunoassay (AxSYM HAVAB 2.0, Abbott Laboratories, Abbott Park, IL) on an AxZYM analyzer. Serum samples with absorbance levels ranging from 0 to 1 *A* were considered reactive for hepatitis A antibodies. The sensitivity of this enzyme immunoassay is 98.9%, and the specificity is 99.7% (data provided by Abbott Laboratories, Abbott Park, IL).

Stool examination

Stool specimens were examined using the spontaneous sedimentation method. Fifty grams of feces were mixed with about 100 mL of tap water and sieved through a 2 mL mesh sieve, washed with 50 mL of water and pressed with a spatula to recover as much of the water as possible. After 40 min, the supernatant was decanted until 50 mL remained. The beaker was refilled to 200 mL with tap water and the suspension allowed standing. After 40 min, the supernatant was decanted until 30 mL remained, and 1 mL thereof was examined (in about 200 μL aliquots) microscopically at 100× magnification for the presence of ova and parasites.

Statistical analysis

The seroprevalence of *H pylori* infection and HAV was separately analyzed in relation to all considered variables. Odds ratio (OR) with 95% CI for *H pylori* or HAV infection, given the presence of a particular characteristic, was used as the measure of association. In addition, because we wished to study the unconfounded effects of the variables examined, we entered these terms into a model and used logistic regression in order to estimate OR and CI adjusted for selected covariates. In this multivariate analysis, full models were fitted, and the nonsignificant ($P>0.1$) variables were eliminated in a stepwise, backward elimination algorithm, least significant first, to determine the final model. P values less than 0.05 were considered statistically significant. The relation between seroprevalence of *H pylori* infection and HAV was evaluated by means of the χ^2 test with continuity correction, OR, and κ statistical analysis (calculated according to Landis and Koch)[17]. The κ statistic, a measure of the agreement between two observers or tests, ranges from -1 to 1 with 1 indicating perfect agreement, 0 indicating the agreement expected on the basis of chance alone, and values between 0 and 0.4 a poor to fair agreement [18]. All analyses were conducted with the SPSS statistical program (SPSS, Inc., Chicago, Illinois, USA).

RESULTS

Table 1 presents selected sociodemographic characteristics, sanitary conditions and the prevalence of intestinal parasites in the 121 patients. The sample comprised mainly of Black/mixed children with a mean age of 4.8 years, and more than two thirds of family supporters who had attended less than middle school. The presence of intestinal parasites was common: 76 of 121 patients (63%) had at least one parasite.

The frequency distribution of parasites detected is shown in Table 1. The most common and chief complaints on hospital admission included respiratory tract diseases (33. 9%), urinary tract diseases (12.4%), skin/muscle infection (10.7%) and gastrointestinal tract diseases (7.4%).

Of the 121 patients, 51 (42.1%) children were found to be seropositive for *H pylori*, and 45 (37.2%) for HAV. The seroprevalence of *H pylori* and HAV both increased significantly with age (Table 2) and did not differ according to gender (data not shown). Cross-tabulation of data showed that 26 (21.5%) were seropositive, and 51 (42.1%) were negative for both *H pylori* and HAV antibodies, whereas 25 (20.7%) and 19 (15.7%) were seropositive only for *H pylori* or HAV, respectively ($\chi^2 = 7.18$, OR = 2.8, CI = 1.30-5.97). The age adjusted OR for an HAV-infected child being *H pylori* positive was 2.3 (CI 1.02-5.03). The agreement between *H pylori* and HAV seropositivity, as measured by κ statistic, was fair ($\kappa = 0.24$).

The associations between selected characteristics and *H pylori* or HAV infection are depicted in Table 3. Gender and ethnicity were not significantly associated with either *H pylori* or HAV infection. Children at the highest category of the crowding index had an increased prevalence of HAV infection. Having a poor garbage disposal system significantly increased the odds of both *H pylori* and HAV infection by 2.5 and 2.3, respectively. Our results also suggested that having a poor household excreta disposal system increased the likelihood of *H pylori* and HAV seropositivity, but only the latter association was statistically significant. Patients living in homes with a restroom outside the household were found to have significantly higher prevalences of *H pylori* and HAV infection (Table 3). In regard to enteroparasites, the presence of *G lamblia* significantly increased the odds of *H pylori* and HAV infection by 3.6 and 4.3, respectively. There was no significant association between the other intestinal parasites investigated and either *H pylori* or HAV infection (Table 3).

Table 1 Selected characteristics of 121 children admitted to a public hospital in Salvador, Brazil, 2001-2002

	N (%)
Age group (in yr)	
1–3	43 (35.5)
4–6	50 (41.3)
7–9	16 (13.2)
10–12	12 (9.9)
Gender	
Male	66 (54.5)
Female	55 (45.5)
Ethnicity	
Black	56 (46.3)
Mixed	59 (48.8)
White	6 (5.0)
Educational attainment of family supporter	
Less than middle school	87 (71.9)
Complete middle and less than high school	21 (17.4)
High school or more	13 (10.7)
Household monthly income[1]	
Less than $US 100.00	38 (31.4)
$US 100.00 to $US 190.00	63 (52.1)
More than $US 190.00	20 (16.5)
Crowding index	
≤1.0	50 (41.3)
1.1–2.0	45 (37.2)
2.1–3.0	15 (12.4)
>3.0	11 (9.1)
Presence of enteroparasites	
Endolimax nana	61 (50.4)
Ascaris lumbricoides	48 (39.7)
Trichiurus trichiura	23 (19.0)
Giardia lamblia	19 (15.7)
Entamoeba histolytica	6 (5.0)
Iodamoeba butschlii	6 (5.0)

[1]In $US.

Table 2 Seropositivity for *H pylori* and HAV in the study population, Salvador, Brazil, 2001-2002

Age group (in yr)	Number of subjects	Seropositive for *H pylori n* (%)	Seropositive for HAV *n* (%)
1–3	43	12 (27.9)	7 (16.3)
4–6	50	25 (50.0)	19 (38.0)
7–9	16	7 (43.8)	12 (75.0)
10–12	12	7 (58.3)	7 (58.3)
Total	121	51 (42.2)	45 (37.2)

χ^2 for linear trend: 4.01 ($P = 0.045$) and 16.07 ($P<10^{-4}$) for age in the *H pylori* and HAV groups, respectively.

Table 3 Age-adjusted OR for the association between *H pylori* or hepatitis A infection (HAV) and selected characteristics in 121 children, Salvador, Brazil, 2001-2002

Sociodemographic characteristics	Seropositivity for *H pylori* OR (95% CI)[1]	Seropositivity for HAV OR (95% CI)[1]
Gender		
Male	1 (referent)	1 (referent)
Female	1.0 (0.5–2.0)	0.8 (0.4–1.7)
Ethnicity		
Non-black	1 (referent)	1 (referent)
Black	2.1 (0.9–4.5)	0.7 (0.3–1.6)
Crowding index		
≤1.0	1 (referent)	1 (referent)
1.1–2.0	0.8 (0.4–1.6)	1.0 (0.5–2.2)
2.1–3.0	1.0 (0.3–3.2)	1.0 (0.3–3.4)
>3.0	3.3 (0.9–12.4)	9.6 (2.0–46.1)[b]
Sanitary conditions		
Poor garbage disposal quality (*vs* good)	2.5 (1.1–5.3)[a]	2.3 (1.0–5.2)[a]
Poor household excreta disposal system (*vs* good)	1.7 (0.8–3.6)	2.8 (1.2–6.1)[a]
Home restroom outside the household (*vs* inside)	2.6 (1.0–6.3)[a]	3.8 (1.4–9.9)[b]
Presence of intestinal parasites		
None	1 (referent)	1 (referent)
Ascaris lumbricoides	1.3 (0.6–3.1)	2.1 (0.8–5.4)
Trichiurus trichiura	0.9 (0.3–2.7)	1.4 (0.4–4.7)
G lamblia	3.6 (1.1–11.8)[a]	4.3 (1.3–13.9)[a]
Endolimax nana	1.7 (0.8–3.8)	2.1 (0.9–4.9)
Entamoeba histolytica	0.7 (0.1–4.6)	0.3 (0.0–3.4)
Iodamoeba butschlii	1.5 (0.3–8.6)	0.4 (0.0–3.5)

[1]OR (95% CI); [a]$P<0.05$, [b]$P<0.01$ other groups.

Multivariate analysis

After adjusting for selected covariates using logistic regression, the variables that remained independently associated with seropositivity for both *H pylori* and HAV were age and the presence of *G lamblia* in feces. Poor garbage disposal quality remained significantly associated with *H pylori* infection only, as shown in Table 4.

Table 4 Results of the multivariate analysis of selected variables and their association with *H pylori* or HAV infection in 121 children, Salvador, Brazil, 2001-2002

	Seropositivity for *H pylori*	Seropositivity for HAV
	OR (95% CI)[1]	OR (95% CI)[1]
Age group (in yr)		
1–3	1 (referent)	1 (referent)
4–9	2.6 (1.1–6.1)[a]	5.1 (1.9–13.9)[b]
10–12	4.2 (1.0–16.7)[a]	9.0 (2.0–39.5)[b]
Sanitary conditions		
Poor garbage disposal quality (*vs* good)	2.4 (1.1–5.1)[a]	2.2 (0.9–4.9)
Presence of intestinal parasites		
G. lamblia	3.2 (1.1–9.5)[a]	3.7 (1.2–11.7)[b]

[1]OR (95% CI); [a]$P<0.05$, [b]$P<0.01$ *vs* others.

DISCUSSION

The sources and routes of transmission of *H pylori* infection are still a topic of debate. This study was designed to investigate putative risk factors for *H pylori* infection, focusing on those operative in childhood. HAV is a fecally transmitted agent with a high incidence of infection in areas with poor sanitation practices. If the fecal-oral route is significant in the transmission of *H pylori*, we would expect the seroprevalence status of this bacterium to correlate with that of HAV (allowing for differences in infectivity). Primary analysis of our data showed that there is a significant correlation between the serostatus of *H pylori* and HAV, with an overall concordance rate of seropositive and seronegative results of 63.6% ($\chi^2 = 7.18$). However, the χ^2 statistic is not a good measure of the degree of association, particularly when dealing with highly prevalent variables. The κ statistic is a more suitable method for ascertaining the strength of agreement in such situations, and it revealed only a fair association between these two infections. These results, thus, offer only a weak support to the hypothesis of a common mode of transmission of those two infections. They agree with previous studies that found the correlation between *H pylori* and HAV to be only a result of the high age-related seroprevalence of these two infections[19-21].

In our data, poor garbage disposal was associated with both *H pylori* and HAV infection. It has been shown that houseflies have the potential for mechanical transmission of *H pylori*[22]. The accumulation of garbage with organic matter may result in larger numbers of this vector and increase the likelihood of food contamination by flies. The same can be argued for HAV infection. There was also a strong association of poor household excreta disposal system with HAV infection, but not with *H pylori* infection; even though our estimates suggested an association in the same direction. Lastly, having a restroom outside the household was significantly associated with both *H pylori* and HAV infection. In these communities, restrooms outside the household have no sink or faucet, and typically lack toilet paper. Therefore, subjects using such facilities are more likely to have poor hygiene practices and to be at increased risk for acquiring infections transmitted by the fecal-oral route. The variables we used as surrogates for the fecal-oral mode of transmission were correlated with *H pylori* infection and have been cross validated with HAV infection. Thus, suggesting the fecal-oral route may play a significant role in the transmission of *H pylori*.

We found an association between *H pylori* infection and the presence of *G. lamblia* in feces. It is well known that *G. lamblia* is transmitted by the fecal-oral route, and thus, this association may indicate that both microorganisms share this common route of transmission. Consistently with that, *G. lamblia* infection was also correlated to HAV infection, a sensitive marker of fecal exposure. One may also argue that gastric giardiasis increases the susceptibility to *H pylori* infection. In a study in Italy, Doglioni *et al*[23], found *H pylori* infection in 37 of 41 (90.2%) patients with gastric giardiasis. Alternatively, *H pylori*-induced chronic gastritis may increase the susceptibility to *G. lamblia* infection. In both scenarios, the association observed between *H pylori* and *G. lamblia* infection could occur independently of a common mode of transmission.

Methodological merits and limitations

In this study, a consecutive sample of subjects with a high participation rate has made it unlikely that selection bias might have occurred. Parents and interviewers were both blinded to the serostatus of the children, thus preventing information and observational bias, respectively. A limitation of our study is related to the difficulties in measuring variables reflecting sanitary and environmental conditions. As we had to rely on self-report when assessing these characteristics, this might have posed an additional source of inaccuracy to our data, which is likely to result in nondifferential classification error and attenuation of the associations measured.

In conclusion, Our results suggest that *H pylori* infection is associated with some surrogate markers of fecal exposure which were cross-validated by HAV infection, a known indicator of such exposure. Therefore, we conclude that the fecal-oral route may be relevant in the transmission of HP among children in an urban setting of a developing country. The association between *G. lamblia* and *H pylori* infection also described here can have several explanations. Further studies to investigate this relationship in more detail are warranted.

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