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The seroprevalence of *Helicobacter pylori* and its relationship to malaria in Ugandan children

Vinay Gupta[a,*], Guillermo I. Perez-Perez[b], Grant Dorsey[c], Philip J. Rosenthal[c], Martin J. Blaser[b]

[a] *Columbia University, College of Physicians & Surgeons, 630 W. 168th Street, New York, New York 10032, USA*
[b] *New York University School of Medicine, 6027 W VA Medical Center, 423 E. 23rd Street, New York, New York 10010, USA*
[c] *University of California, San Francisco, Box 0811, San Francisco, CA 94143, USA*

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ABSTRACT

Helicobacter pylori epidemiology in sub-Saharan Africa, particularly among children, has been little investigated. A secondary endpoint of our study was to examine for associations between the seroprevalence of *H. pylori* and the incidence of malaria. We explored *H. pylori* prevalence by measuring serum IgG antibodies to *H. pylori* whole cell and cytotoxin-associated gene A (CagA) antigens by ELISA in a longitudinal cohort of 200 Ugandan children, aged 1–10 years at enrollment, in whom malaria incidence was followed over 572 person-years. First-sample seroprevalence for *H. pylori* -specific IgG (63%) and for the *H. pylori* protein CagA (78.5%) were both high, and they were positively associated with advancing age (per each 1-year age increase, OR (95% CI): 1.60 (1.39–1.85), $P < 0.001$). We observed nearly universal prevalence of CagA+ *H. pylori* by the age of 10 years in Kampala and found no evidence that *H. pylori*–positivity is protective against malaria.

1. Introduction

Gastric colonization by *Helicobacter pylori*, a curved, microaerophilic, gram-negative bacterium, increases risk in adults for peptic ulcer disease and gastric cancer. *Helicobacter pylori* strains containing the cytotoxin-associated gene A (CagA) are associated with higher rates of gastric inflammation and malignancy than CagA-negative strains.[1] *Helicobacter pylori* prevalence is 20–50% among 60 year old persons in western countries,[2] whereas rates among persons of similar age in the developing world are 90–100%.[3]

Much less is known about *H. pylori* epidemiology among children, particularly in sub-Saharan Africa. Some studies in the developing world have linked childhood acquisition of *H. pylori* to failure to thrive,[4] frequent diarrhea, stunted growth,[5] and other co-morbidities, suggesting that *H. pylori* could be an important and preventable cause of illness in underserved populations. Alternatively, a growing body of evidence suggests that *H. pylori* could protect against childhood-onset asthma,[6] and esophageal diseases[7] both of which are increasing in areas where *H. pylori* is disappearing.[8]

Within this framework, we hypothesized that if *H. pylori* could confer partial protection from a commonly lethal infection, *falciparum* malaria, there may have been positive selection for its presence, explaining its ubiquity until recently. A putative mechanism for this interaction is centered around the previously characterized finding in animals[9] and humans[10] that iron-deficiency anemia, itself associated with *H. pylori* gastric colonization,[11] has a protective effect against malaria.[12] The primary objective of this study was to explore *H. pylori* seroepidemiology among children in Uganda, a region of Africa that has been little characterized in this regard. We also conducted

* Corresponding author. Tel.: +609 306 1764; fax: +415 648 8425.
E-mail address: vg2153@columbia.edu (V. Gupta).

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a preliminary analysis of the relationship between *H. pylori* and malaria incidence within this cohort, despite the need for future studies to further investigate this question.

2. Materials and methods

2.1. Study site and recruitment of study participants

Children enrolled in our *H. pylori* survey study comprised a nested cohort of a much larger cohort designed to study malaria in Uganda. Subjects for this malaria cohort were recruited from the Mulago III parish of Kampala, Uganda, a densely populated urban slum with an area of approximately one square kilometer.[13] This cohort was established to compare the efficacies of combination therapies to treat *Falciparum* malaria in an urban African setting over several years of follow-up.[13]

Malaria in Kampala is mesoendemic, with two annual peaks that follow the rainy seasons in March–May and September–November.[14] Prior to cohort recruitment, a census was conducted.[13] All households and potential mosquito breeding sites were mapped using Pathfinder Pocket Global Positioning System (GPS) receivers (Trimble, 935 Stewart Drive, Sunnyvale California, 94085) connected to personal digital assistants (PDAs) using TerraSync software (version 2.40; Trimble). From November 2004 through April 2005, 601 children from 322 households randomly selected from the census survey were enrolled in a longitudinal trial of antimalarial therapy if they met the following eligibility criteria: (1) age 1–10 years, (2) agreement to come to the study clinic for any febrile episode or illness, (3) agreement to avoid medications administered outside the study, (4) agreement to remain in Kampala during the study period, (5) no known adverse reactions to the study medications, (6) weight $\geq$10 kg, (7) no severe malnutrition or known serious chronic disease, (8) no laboratory screening results indicating a life-threatening condition and (9) written informed consent from the child's parent or guardian. The criterion of weight >10Kg was included since at the time of the antimalarial efficacy study, artemether-lumefantrine was only approved for children above this weight.

Of the 601 study children, 20 per birth year (from each age between 1 and 10 years) were selected by random number sampling to comprise a cohort of 200 children for this sub-study. For the *H. pylori* sub-study, each child had two serum samples obtained at least 2 years apart and no known serious illnesses, including sickle cell anemia, on enrollment. Children had not been screened for HIV on enrollment, but received a mid-study screen that was done by two rapid tests, using a third as a tie-breaker.

2.2. Baseline assessment and malaria incidence data

At enrollment, blood was collected by venipuncture for laboratory screening tests, including examination for sickle hemoglobin by hemoglobin electrophoresis and glucose-6-phosphate dehydrogenase (G-6-PD) activity using a spectrophotometric kit (Randox Laboratories, Crumlin, Co. Antrim, UK); subjects were categorized as G-6-PD-deficient if activity was <110 mU/10^9 erythrocytes.[15] In addition, a measure of socioeconomic status, designated as wealth index, was determined for each child as described.[14] A household survey based on standardized instruments was conducted within two weeks of study enrollment to obtain demographic information about the participants and their households, as described.[16] Participants were asked to attend a designated study clinic open seven days a week for their healthcare needs. Routine field visits were performed every 30 days to ensure compliance with study protocols. Subjects who presented to the clinic with history of fever in the previous 24 h or measured fever (tympanic temperature $\geq$38.0 °C) had blood obtained by finger stick for a thick blood smear. If the thick blood smear was positive for parasites, the participant was diagnosed and treated for malaria regardless of parasite density, as described.[14]

2.3. Malaria diagnosis

Thick and thin blood smears were stained with 2% Giemsa for 30 minutes. Parasite density was estimated by counting the number of asexual parasites per 200 white blood cells and calculating parasites per microliter, assuming white blood cell counts of 8000/μL. A smear was judged to be negative if no parasites were seen after review of 100 high-powered fields. The diagnosis and management of malaria was based on initial readings of blood smears. Final blood smear microscopy results were based on a rigorous quality-control system that included rereading by a second microscopist and resolution of any discrepancies by a third microscopist. No patient diagnosed with uncomplicated malaria based on the initial blood smear reading was found to be negative after the quality control readings.

2.4. Serologic assays for Helicobacter pylori

Assays for *H. pylori*-specific immunoglobulin (Ig) G were performed on two serum samples collected at least two years apart from each of the 200 children (Figure 1) using an ELISA, as described.[17] An optical density ratio (ODR) value >1.0 was considered seropositive. To correct for plate-to-plate variation, results were expressed as ODR in relation to standard sera, as described.[18] Serological assays were performed in duplicate on at least two different days. An ELISA to detect anti-CagA IgG was performed using purified recombinant CagA antigen and CagA ODR values calculated in relation to reference sera, with values $\geq$0.350 considered seropositive, as described.[1]

2.5. Statistical analysis

Data were double-entered in Access (Microsoft Corporation, Redmond, WA, USA) and statistical analysis was performed using Stata version 10 (Stata, College Station, TX, USA). Confidence intervals for seroconversion and seroreversion rates were calculated using exact Poisson 95% confidence intervals. Associations between risk factors and *H. pylori* seropositivity were estimated using logistic regression, and the resulting odds ratios (ORs) with significance levels are reported. Associations of *H. pylori* serology