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A primary *Helicobacter pylori* infection does not protect against reinfection in children after eradication therapy

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ABSTRACT

Background. *Helicobacter pylori* infection is one of the most common chronic infections in the world, and is acquired mainly during childhood. It is not clear to which extent a primary infection protects the child from reinfection. Our aim was to determine the possible protection conferred by a primary infection against *H. pylori* reinfection in children. **Methods.** A follow-up study with 120 children distributed in two cohorts; the first included 80 children without previous *H. pylori* infection (primo-infection cohort); the second included 40 infected children successfully eradicated (reinfection cohort). Cohorts were monitored during 2 years with urea-breath-test (UBT) at 3, 6, 9, 12, 18 and 24 months for the acquisition of *H. pylori* infection. We compared the rate of reinfection in eradicated children with the rate of infection in children without previous infection. *H. pylori* infection during the follow-up was analyzed and compared between cohorts using χ^2 and survival curves. A questionnaire was performed for the evaluation of possible risk factors for infection in both cohorts. **Results.** No significant differences in rates of primo-infection or reinfection were found; 17 (21.2%) primo-infections and 10 (25%) reinfections were documented. Most of the primo-infections (14/17) occurred in the first year of follow-up. In contrast, reinfection episodes occurred more frequently during the second year (6/10). In both cohorts, most infections were transient. Risk factors were similar for both, primo and reinfection cohorts. **Conclusion.** A primary infection does not protect from reinfection in the population of children studied.

Key words. *Helicobacter pylori*. Childhood. Primo-infection. Reinfection.

La infección primaria por* Helicobacter pylori *en niños no protege contra la infección después del tratamiento de erradicación

RESUMEN

Introducción. *La infección por* Helicobacter pylori *es una de las infecciones crónicas más frecuentes en el mundo, se adquiere principalmente durante la infancia. Hasta el momento no se ha aclarado hasta qué grado la infección primaria protege contra infecciones subsecuentes en niños. Nuestro objetivo fue determinar la posible protección conferida por una infección primaria contra la reinfección por* H. pylori *en niños.* ***Material y métodos****. Estudio de cohortes que incluyó 120 niños distribuidos de la siguiente manera: a) cohorte de primo-infección, 80 niños sin infección previa por* H. pylori*; b) cohorte de reinfección, que incluyó 40 niños infectados por* H. pylori *en los que se erradicó exitosamente la infección. Ambas cohortes se evaluaron a los 3, 6, 9, 12, 18 y 24 meses con la prueba de aliento de la urea (UBT por sus siglas en inglés) para detectar la adquisición de la infección por H. pylori. Se compararon las tasas de primo-infección y reinfección en ambas cohortes mediante la prueba de* χ^2 *y curvas de sobrevida Kaplan Meier. Se elaboró un cuestionario para evaluar los probables factores de riesgo para adquisición de la infección por* H. pylori. ***Resultados.*** *No se encontró una diferencia significativa entre la tasa de primo-infección versus la tasa de reinfección. Durante este periodo de seguimiento se documentaron 17 (21.2%) primo-infecciones y 10 (25%) reinfecciones. La mayoría de las primo-infecciones (14/17) ocurrieron durante el primer año de seguimiento. En contraste, los episodios de reinfección (6/10) ocurrieron más frecuentemente durante el segundo año. En ambas cohortes la mayoría de las infecciones fueron transitorias. Los factores de riesgo fueron similares en ambas cohortes.* ***Conclusión.*** *La infección primaria no protege contra reinfección en la población de niños estudiados.*

Palabras clave. Helicobacter pylori. *Niños. Primo-infección. Reinfección.*

INTRODUCTION

Helicobacter pylori infection is one of the most common chronic infections in the world. The World Health Organization has estimated that over half of the world population is infected, involving 30% of the population living in developed countries and up to 80-90% of the population in developing nations.[1,2] In Mexico the seroprevalence of *H. pylori* infection is high; by age 1 year, 20% of children are infected, whereas after the age 20 yr, 80% of the population is infected.[3] *H. pylori* infection is associated with several gastroduodenal diseases, gastritis, peptic ulcer, MALT lymphoma and gastric cancer.[4,5] Once acquired, and if not treated, the infection usually persists throughout life; however, there is evidence for both transient and persistent infection in children, suggesting that spontaneous eradication is not a rare event in this group of age.[6-9] Eradication of the organism leads to healing of gastritis and peptic ulcer disease.

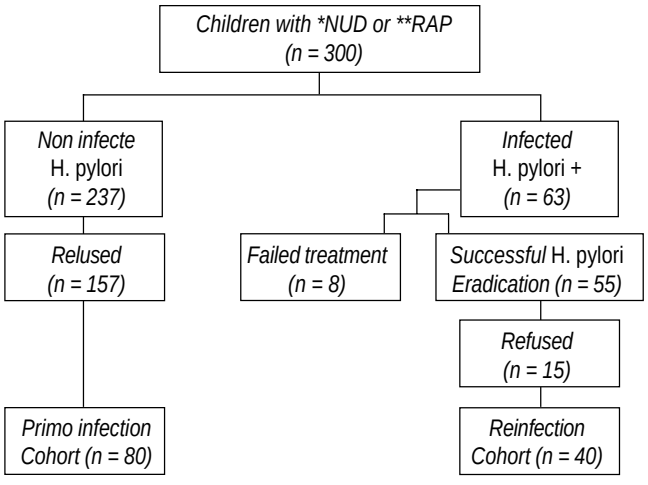
Until now the mechanism by which *H. pylori* is transmitted is not completely clear; although intrafamilial clustering of *H. pylori* infection or increased infection in crowded institutions suggests person-to-person transmission. Early childhood is the critical period for the acquisition of *H. pylori* infection, favoured by risk factors such as low socioeconomic level, poor hygiene, crowding, etc.[3,10-12] Studies in young children have reported widely varying seroprevalence rates, from 1.4 to 15%;[13,14] however, most of these studies have been performed by using stored serum samples and the results are difficult to interpret because commercial serological assays usually fail to diagnose a proportion of infected children aged < 10 years.[15,16] Recent studies have shown that 13-carbon urea breath test (^{13}C-UBT) has a better performance in children and is currently recommended to determine both, the incidence and the eradication of infection after treatment.[17,18] In our population in a study for evaluation of *H. pylori* eradication in young children, ^{13}C-UBT demonstrated a sensitivity of 90.0% and specificity of 91.9%; whereas serology showed sensitivity 77.3% and a specificity of 97.3%.[19]

Several studies have shown the efficacy of different regimen treatments for eradication of *H. pylori* infection in young children;[20,21] however, scarce studies have addressed the issue of protection against reinfection conferred by the primary infection. The aim of this work was to study the possible protection conferred by a primary infection against reinfection, after eradication treatment. To achieve this goal we designed a prospective cohort study to compare the rate of reinfection in infected children after eradication of infection, with the rate of infection in children without previous infection.

MATERIAL AND METHODS

Population

A prospective study of cohorts was conducted from August 1996 to September 1999. We initially studied 300 children referred to the gastroenterology department of the Pediatrics Hospital, Centro Medico Nacional SXXI, Instituto Mexicano del Seguro Social in Mexico City, because of recurrent abdominal pain (RAP) or non ulcer dyspepsia (NUD) (Figure 1). In all these patients endoscopy was indicated as part of the usual diagnostic protocol. Children were classified as *H. pylori* infected or uninfected; the status of infection was evaluated by culture, histology, serological test and ^{13}C-UBT. We consider a patient infected when at least three of the test resulted positive and noninfected when all the tests became negative. All infected patients received treatment for eradication of the infection: omeprazole (0.7 mg/kg/day), amoxicillin (40 mg/kg/day) and clarithromycin (20mg/kg/day) during 14 days. Eradication was studied with a ^{13}C-UBT test four and six weeks after therapy; patients who had negative both ^{13}C-UBT results were considered as *H. pylori* infection eradicated; previous studies



** NUD: Non Ulcer Dyspepsia.*
*** RAP: Recurrent Abdominal Pain.*

Figure 1. *Process of recruitment of children in the two cohorts of the study, for* H. pylori *primo-infection and reinfection.*

Table 1. Characteristics of the children populations in the cohorts studied for *H. pylori* primo-infection and reinfection.

Variables	Primo-infection	Reinfection
Children at risk	80	40
Age, mean ± SD (range)	9.3 ± 3.3 (2-14)	10.9 ± 3.4 (5-17)
Male:Female (ratio)	30:54 (1:1.8)	15:25 (1:1.6)
Person-year of follow-up	117	56
New *H. pylori* infection	17	10
Rate/100 person-year*	14.5	17.8

*RR =1.23, 95% CI 0.56 – 2.70, p= 0.75 for rates of primo-infection *vs.* reinfection.

have shown that a single UBT test performed 4 weeks after end of treatment is not enough to identify patients with successful eradication and a second test increase the possibility to identify cases with treatment failure and with recrudescence.[19,22,23] All children who eradicated the infection and all children who had no previous infection were candidates to participate in the study. In the end, 120 patients were enrolled in the study; the children population was divided in two different cohorts (Table 1). A) Primo-infection cohort included 80 children without previous *H. pylori* infection and B) Reinfection cohort included 40 originally infected children whose infection was successfully eradicated (Figure 1).

Biopsy culture

Three gastric biopsy samples from the antrum and three from the corpus were taken, and suspended in 0.9% saline solution. One biopsy from each region was fixed for histology, and the other two were used for isolation of *H. pylori*. Antrum and corpus biopsies were transported to the laboratory within 2 hrs period, homogenized and inoculated on tripticase soy agar (DIFCO Laboratories, Detroit, MI) supplemented with 5% sheep blood and a cocktail of antibiotics. Agar plates were incubated at 37°C in a CO^2 atmosphere and observed for growth up to 10 days. Suspected colonies were confirmed by urease, catalase and Gram stain.

Histology

Antrum and corpus biopsies were fixed and stained with haematoxylin-eosin and Giemsa. Slides were examined for the presence of *H. pylori* and for inflammation by a pathologist blinded to the diagnosis of the patients.

Serological Test

IgG antibodies against *H. pylori* antigens were determined by an ELISA assay which was previously validated in children, using local strains for the antigen preparation [3]. Microplate wells were coated with 0.5 μg/well of whole cell antigen, followed by serum sample (1:100 dilution) and monoclonal anti-IgG (Southern Biotech, Birmingham, AL) conjugated with alkaline phosphatase at 1:1000 dilution; finally 1 mg/mL of *p*-nitrophenylphosphate (Southern Biotech, Birmingham, AL) was used as substrate. Absorbance was read at 405 nm in a Multiskan analyzer (Labsystems, Helsinki, Finland). Samples with ELISA units > 1.0 were considered positive.

^{13}C-Urea breath test (UBT)

Test was performed after at least 6 hrs fasting; children drank 30 ml of a citric acid solution and 10 min later a baseline breath sample was colleted. Thereafter, children ingested 75 mg of ^{13}C-urea in 50 ml of water; a second breath sample was colleted 30 min later. Samples were colleted by asking the children to blow through a straw into a 10 mL vacuttainer tube, and all breath volume was analyzed in a mass spectrometer (BreathMAT plus, Finnigan, Bremen Germany). The $^{13}CO_2/^{12}CO_2$ ratio was compared with the known isotope ratio of a standard gas. The $^{13}CO_2$ values were corrected using the international PDB-standard. Cut-off was established at 5.0δ. In our children population the assay had a sensitivity of 90.0% and a specificity of 91.9%.[19]

Follow-up of the Cohorts

The two cohorts of children were monitored during two years with ^{13}C-UBT at 3, 6, 9, 12, 18 and 24 months, for either *H. pylori* primo-infection or reinfection. For A) cohort the zero time of the follow-up was considered when the guardian accepted to participate in the study; whereas for the B) cohort zero time was established when eradication was confirmed. An episode of infection or reinfection was documented with a ^{13}C-UBT positive result; these patients were re-tested with a second ^{13}C-UBT 4-6 weeks later. Patients with the first ^{13}C-UBT positive, but the second test negative were considered as having and episode of transient infection, spontaneously eradicated. Patients with the two UBT tests positive were further confirmed with biopsy culture and serology test.

Epidemiologic data

At the time of inclusion to the study, an epidemiologic questionnaire was filled out. Questions included: age, gender, characteristic of housing, number of persons living in housing, number of rooms excluding bathroom, living room and kitchen; parents education level, parents occupation, dietary habits, availability of sewerage, source of water, treatment of water and food for consumption, pets, day-care assistance, breast feeding history, ablactating, and a constructed index of socioeconomic level.

Statistical analysis

Cases with both, transient and with confirmed infections were included in the analyses. The rates of primo-infection or reinfection were calculated as person-time in years; the comparison between both cohorts was performed on the basis of a 95% confidence interval (95% CI) and *p* value. The frequency of primo-infection or reinfection was determined by Kaplan Meier survival curves. To determine the association between *H. pylori* primo-infection or reinfection and the epidemiologic factors, data were analyzed using Squared- Chi test or Fisher test (depending on the size of the sample), OR and 95% CI were estimated. In all cases a $p < 0.05$ was considered to be significant. All analyses were performed with the SPSS software, version 11.0 (SPSS, Chicago, IL).

Ethical considerations

The study was approved by the ethics committee of the Hospital Pediatrico, Centro Medico Nacional SXXI, Instituto Mexicano del Seguro Social, Mexico City. Guardians of each patient were asked to participate in the study and fill out an epidemiologic questionnaire. Before the study, written informed consent was obtained from the guardian of each child who accepted to participate.

RESULTS

Characteristics of the study population

During 1996 to 1999, we studied 300 children attended because of recurrent abdominal pain or non ulcer dyspepsia at the gastroenterology department of the Hospital Pediatrico, CMN SXXI, IMSS, Mexico City. *H. pylori* infection was documented in 63 (21%) of the 300 children, and they all received triple therapy; treatment failed in 8 of them, as documented by a positive ^{13}C-UBT test four weeks after treatment. One hundred twenty of the 300 children were enrolled in the study, 80 without previous infection and 40 infected and successfully eradicated (Figure 1).

A) Primo-infection Cohort. Of the 237 non-infected children 80 were included in the cohort, 50 girls and 30 boys ranging from 2 to 14 years old, mean age 9.3 ± 3.3 years. Follow-up was carrying out during 1,448.8 child-months, with a mean of 17.9 ± 7.7 months. During this period, five cases (6.2%) withdrew the follow-up and 17 infections (21.2%) were documented, 12 in girls and three in boys; the mean age of these children was 7.2 ± 2.5 years; the rate of incidence per 100 person-year of follow-up was 14.5 (Table 1). In most of them the infection was documented as transient (UBT test turn positive at follow up and then became negative weeks after) (12 cases); nine of these 12 transient cases we also documented by seroconversion. Only five of the 17 infections were confirmed with a second UBT test and were considered as permanent infections. These five cases were additionally confirmed by culture, histology or serology. Three of these cases where positive for culture, serology and histology and two for histology and serology. Most of these infections (14/17) occurred in the first year of the follow-up (Figure 2).

B) Reinfection Cohort. Forty children were included in the reinfection cohort; 25 girls and 15

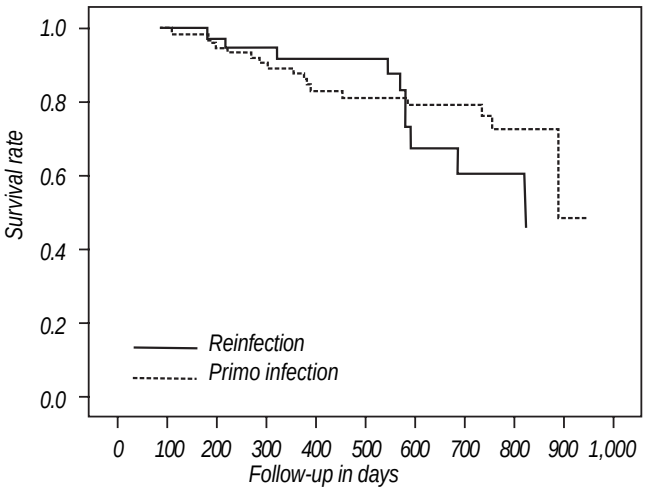


Figure 2. Survival curves for H. pylori *primo-infection and reinfection in children.*

boys ranging from 5 to 17 years old, with a mean age of 10.9 ± 3.4 years. The follow-up of this cohort was during a mean of 16.9 ± 6.5 months with a total 679.6 child-months. During this period, three cases (7.5%) withdrew the follow-up and 10 reinfections (25%) were documented by ^{13}C-UBT. Seven of them occurred in girls and three in boys; the mean age of these children was 8.6 ± 2.7 yr. The rate of incidence during follow-up was 17.8, similar to the primo-infection cohort; with no difference documented (RR 1.23, 95% CI 0.56-2.70, p = 0.75) (Table 1). Seven of 10 reinfections were documented as transient events and only three were confirmed as permanent reinfections. Most of these reinfections (six cases) occurred on the second year of the follow-up (Figure 2).

Demographic variables as risk factors

When we compared variables between both cohorts, there were no major differences. Most of the variables found as risk factors for *H. pylori* infection were the same as for reinfection. Only two variables were exclusively associated with episodes of reinfection and not with cases of primary infection, mothers working out of home, (RR 2.16, 95% IC 0.99 - 4.66, p = 0.05) and ulcer disease in children, (RR 2.49, 95% IC 1.30 - 463.4, p = 0.004) (Table 2).

Discussion

Several epidemiologic studies have shown evidence for acquisition of *H. pylori* primary infection at an early age, mostly in developing countries.[3,8,10,24]

Table 2. Association between epidemiologic factors for *H. pylori* primo-infection or reinfection (univariate analysis).

Variables	Primo-infection Cohort (n = 80)	Reinfection Cohort (n = 40)	OR	95% CI	p value
Age, years					
< 5	13	2	–	–	0.18*
5 – 9	28	14			
≥ 10	29	24			
Gender					
Girls	52	25	0.89	0.41 – 1.96	0.79**
boys	28	15			
Diagnostic					
Ulcer	0	5	2.49	1.30 – 463.4	0.004*
gastritis	80	35			
Crowding‡					
Yes	67	34	1.09	0.39 – 3.04	0.86**
No	13	6			
Worker Mother§					
Yes	30	22	2.16	0.99 – 4.66	0.05**
No	50	17			
Mother Education					
≤ 6 years	13	9	1.50	0.59 – 3.80	0.40**
> 6 years	67	31			
Day-care					
Yes	25	15	1.38	0.62 – 3.03	043*
No	55	24			
Pets					
Yes	23	15	1.49	0.67 – 3.29	0.33**
No	57	25			
Sewerage†					
Yes	77	40	2.61	0.12 – 55.70	0.55*
No	2	0			

*: Fisher test. **: Squared-Chi test. ‡: Crowding was consider >1.5 person per room. §: 6 years is equivalent to elementary school. †: Availability of municipal sewer system.

In Mexico the prevalence of *H. pylori* infection is high (67%), as in other developing countries, and the acquisition of the infection starts at early ages. In Mexico, at 1 year of age 20% of children were already infected and by the age of 10 years, 50% were infected [3]. The mechanisms of *H. pylori* transmission are unknown, but a strong association between *H. pylori* infection and poor living conditions during childhood has been shown. Thus, susceptibility to *H. pylori* infection is increased during childhood, mostly in developing countries;[8,10,12,13,25] these facts make our children population an attractive group to study the frequency of reinfection after a successful eradication and determine to which extent a primary infection protects the child from reinfection.

The aim of the present study was to evaluate the possible protection of a primary infection to a second episode of infection after successful eradication treatment. We documented that the rates of acquisition for primo-infection (21.2%) and for reinfection (25%) were very similar after a follow-up period of two years. In a recent study we reported that in our children population most cases of reinfection are truly episodes of reinfection and not cases of recrudescence, as documented by fingerprints of isolates before treatment and after reinfection; reinfection occurred with both *cagPAI+* and *cagPAI-* strains.[7] These results suggest that in a community were prevalence of infection is high in childhood, a primary infection does not protect against reinfection. The immune response elicited after the first infection at this age group seems to be inefficient to prevent a new episode of reinfection. These results are in accordance with the fact that in previous studies in the same community, we have observed a relatively high frequency of infection with multiple strains, suggesting also that an initial infection does not protect from a second super-infection, leading to multiple infection in several patients.[7, 26] The mechanisms for the lack of protection cannot be explained with this study and we can only suggest that in children, mechanisms for general protection against reinfection are not induced after a primary infection. On the other hand, we can not discard the possibility that in the cohort of primary infection, some of the children might have experienced episodes of transient infection, mainly the oldest children; this represent a limitation to our study and we cannot state by sure that in all children of this cohort the episode of infection we see represent a primary infection.

The study was performed in children requiring diagnostic upper endoscopy because of RAP or NUD. Thus, the population included in this study represents a selected group of patients; still both groups, those without previous infection and those infected and eradicated were outpatients, exposed to the same familiar and community sources of infection as healthy children. In any event, age, symptoms and socioeconomic levels were similar between the two cohorts of children included in this study.

It is interesting to note that most episodes of infection detected in this study represented a transient episode of colonization (60 to 70% of the cases) in both primo-infection and reinfection cohorts. In previous report in Peru it was documented a high rate of transient infections.[7] Perri *et al*., in other study reported a spontaneous eradication of infection in children.[8] Our results together with the previous reports suggest that children are frequently colonized with *H. pylori*, but that colonization is spontaneously eliminated in most cases. Since this transient infection documented in our study occurred similarly in both cohorts of children, it does not seem to be due to a developed immune response. The gastric mucosa of children responds to *H. pylori* infection with a high IL-8 production and a strong infiltration of T lymphocytes;[27-29] this respond may help children to frequently avoid a permanent infection.

There are many reports about the epidemiologic factor associated with acquisition of *H. pylori* infection.[10,12,23,30-32] In previous studies in Mexico, the factors associated to *H. pylori* infection were crowding, socioeconomic index and education level.[3] In this study we looked for differences in risk factors between primo-infection and reinfection and found that most risk factors were the same, except for two variables which were significantly associated to reinfection and not to primary infection, mothers working out of home, and presumably exposing the child to other sources of infection such as day care center; and ulcer disease, suggesting this group of children have had a higher exposition to infection since earlier in life (and thus had time to develop ulcer disease).

In conclusion, despite its limitations, our study presents relevant information regarding the dynamic of *H. pylori* acquisition, loss and reinfection in children. This result suggests that in our population of children a previous *H. pylori* infection does not protect against reinfection after eradication therapy. The study also shows a high frequency of transient *H. pylori* colonization in both primo and reinfection groups, suggesting children have effec-

tive mechanisms to avoid permanent infection. Further studies are needed to help to elucidate the innate mechanisms elicited during *H. pylori* infection in children.

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