Journal of Biomedical Science (2008) 15:29–36
DOI 10.1007/s11373-007-9202-2

Helicobacter pylori infection can change the intensity of gastric Lewis antigen expressions differently between adults and children

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Received 3 May 2007; accepted 1 August 2007

Key words: Lewis antigens, child, adult, bacterial load, *H. pylori*

Abstract

This study tested whether there were different expressions of gastric Lewis antigens between children and adults with *Helicobacter pylori* infection, and whether the difference was related to the infection outcome. About 68 dyspeptic children and 110 dyspeptic adults were enrolled to check *H. pylori* infection, its colonization density, and the related histology. Gastric Lewis antigens b (Le^b), x (Le^x), and sialyl-Lewis x (sialyl-Le^x) were immunohistochemically stained and scored for the intensity. The *H. pylori*-infected adults, but not the children, had a lower Le^b intensity over the antrum ($p = 0.019$) but higher Le^b intensity over the corpus ($p = 0.001$) than the non-infected ones. Over the antrum, both the *H. pylori*-infected children and adults had a lower Le^x and higher sialyl-Le^x intensity than those non-infected ones ($p < 0.05$). The *H. pylori*-infected adults had a higher bacterial density ($p = 0.004$) and Le^b intensity ($p = 0.016$) over the corpus than the *H. pylori*-infected children. For the *H. pylori*-infected adults, but not children, the corpus had a higher Le^b ($p = 0.038$) and lower Le^x ($p = 0.005$) intensity than the antrum. Furthermore, the *H. pylori*-infected adults expressed a higher Le^b and had a higher bacterial density than those with weak Le^b (antrum, $p < 0.001$; corpus, $p = 0.001$). In conclusion, *H. pylori* infection is associated with the intensity change of Lewis antigen expressions in the stomach. The changes of gastric Lewis antigen expressions are different between adults and children with *H. pylori* infection, which may exert different *H. pylori* colonization over the corpus between adults and children.

Introduction

Helicobacter pylori play a major role in chronic gastritis and peptic ulcer disease in both children and adults [1–3]. The prevalence of *H. pylori* infection of children ranges from 20 to 61% worldwide, but such prevalence is usually lower than that of adults in the same area [4–6]. This lower prevalence of *H. pylori* in children may account for the lower incidence of *H. pylori*-related peptic ulcer diseases in children than in adults [4–6]. In previous reports only 33–62% of children with duodenal ulcers had *H. pylori* infection, which was in great contrast to the more than 90% of duodenal ulcers with infection in adults [4–7]. This suggests that there should be certain factors other than *H. pylori* infection to induce peptic ulcer disease in children. Moreover, *H. pylori*-related peptic ulcer diseases in children

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with abdominal pain were less frequent (1.1–1.3%) than in dyspeptic adults (17.7–72.7%) [4–8].

Both in children and adults, infection with *cagA*-positive *H. pylori* isolates increases the risk of peptic ulcer and gastric cancer [9, 10]. A blood group binding adhesin (BabA) expressed by *H. pylori* selectively binds to the fucosylated Lewis antigen (Le^b) of the human gastric epithelium. This adhesin is correlated with the presence of severe *H. pylori*-related diseases [11–13]. *Helicobacter pylori* strains isolated from Taiwanese were uniformly *cag*A-positive and *babA2*-positive [14, 15]. Therefore, the two common bacterial virulence factors should have played a very limited role in inducing the different clinico-histological severity of *H. pylori* infection between children and adults.

The intensity of the Le^b expression on the gastric epithelium of the infected host is positively related to the density of *H. pylori* colonization [15]. In addition, a sialic acid-binding adhesin (SabA) encoded by the *sabA* gene of *H. pylori* was found to interact with the sialylated Le^x antigen (sialyl-Le^x), which was expressed after chronic inflammation induced by the *H. pylori* flora adherent with BabA [16]. As an increased density of *H. pylori* on the gastric mucosa has been reported to be related to more severe gastritis and an increased incidence of peptic ulcers [17–21], it is rational to test whether children have different Lewis antigen expression patterns on gastric epithelium and thus harbor fewer bacteria and a lower risk of developing severe pathologies than adults.

In children, the expression of the Le^x antigen, but not the Le^b superficial expression, was associated with duodenal ulcers with *H. pylori* [22]. Based on an *in vitro* study, a known Le^b-binding *H. pylori* revealed significantly lower binding to gastric tissues from children and adolescents than to tissues from adults [23]. Nevertheless, there is no available data to compare the gastric Lewis antigen expression and its role in determining the *H. pylori*-related histology and colonization density between adults and children with dyspepsia. We thus conducted this prospective study to test whether the expression of gastric Lewis antigens could be different between children and adults, and to elucidate whether any such differences could be related to the different *H. pylori* colonization and infection outcomes.

Materials and methods

Patients and diagnosis of H. pylori infection

After obtaining informed consent, 68 dyspeptic children and 110 dyspeptic adult patients were prospectively enrolled for panendoscopy to collect gastric biopsies for histology. None of the participating patients included had taken antibiotics or acid-secretion inhibitors for 4 weeks prior to the panendoscopy and had had no previous *H. pylori* checkup before enrollment. The presence of *H. pylori* infection was defined by a positive result of culture or positive results of both rapid urease test and histology review. In each patient, two biopsy samples from the antrum for culture and two samples from both the antrum and corpus for formalin embedded were taken at examination.

Analysis of gastric histology

The same pathologist, unaware of the endoscopic and culture results, analyzed the gastric histology. The *H. pylori* density (HPD) for each specimen used the scale as published before [15, 24]: score 0, no bacteria; score 1, one or two small clusters with less than 10 bacteria; score 2, less than half the superficial crypt area with less than 10 bacteria in each crypt; score 3, less than half the area but with more than 10 bacteria, or more than half the area with less than 10 bacteria in each crypt; score 4, >10 bacteria in foveola with some free area; and score 5, >10 bacteria without a free area. The acute inflammatory score (AIS, range 0–3), chronic inflammation score (CIS, range 0–3), atrophic change (AT, range 0–3), and intestinal metaplasia (IM, range 0–3) were graded using the Updated Sydney system [25].

Immunochemical staining and scoring for gastric Lewis expression

Immunostaining of the biopsy specimens for the Le^b, and Le^x antigens was performed using the standard avidin-biotin-peroxidase technique in the formalin-fixed and paraffin-embedded tissue sections, including both antrum and corpus samples from each patient. Slides were washed with distilled water and then put in 1× PBS for 5 min. Incubation with 3% hydrogen peroxide for 3 min was done to block the endogenous peroxidase

activities of these sections. After incubation with 2% BSA for 2 h and washing with PBS, the primary monoclonal antibodies (anti-Lewis structure Le^b, and Le^x, Signet Laboratories, Inc., Dedham, MA) to detect gastric Lewis antigens were used. The incubation time for the primary monoclonal antibodies (anti-Le^b, and Le^x) was 3 h at 25 °C. These slides were again washed with PBS and incubated for 2 h at 25 °C with the secondary antibody to achieve a 1:2000 dilution of anti-mouse IgG + IgM conjugated to horseradish peroxidase (Chemicon International Inc., Temecula, California, USA). These slides were finally washed with PBS, and the AEC kit (Sigma, St Louis, USA) was used for the staining [15].

Besides Le^b and Le^x antigen staining, the monoclonal antibody against sialyl-Le^x (mouse IgM MAB2096, Chemicon International, Inc., Temecula, CA) was selected to stain the same specimens of each enrolled patient [26]. The HRP polymer detection method (PicTure-Plus Kit [Mouse – DAB]; Zymed Laboratories, Inc., South San Francisco, CA) was used. Nonspecific binding sites were saturated with 0.3% bovine serum albumin. Tissue sections were treated with primary antibody against sialyl-Le^x at a dilution of 1:50 at 4 °C overnight. HRP/Fab polymer conjugate was adapted for staining. Following the manufacturer's instructions, 3-amino-9-ethylcarbazole was selected as the chromogen. Sections were counter-stained with haematoxylin. Non-immune mouse IgG was used as a negative control.

For each gastric site, the intensity of Le^b, sialyl-Le^x and Le^x, was scored from 0 to 3 or 4 (Figure 1). The scoring grades were different between Le^b, sialyl-Le^x and Le^x according to the stratified expression of each antigen. The Le^b and sialyl-Le^x expressed mostly at superficial epithelium and extended to the deep glands; the Le^x expression decreased from deep glands to superficial epithelium.

Statistical analysis

The Student's t test was used for the parametric differences. The Pearson's χ^2 test was used to test the difference of ulcer rate between adults and children. The histological staining on Lewis antigens were compared with the Mann-Whitney U test between the *H. pylori* positive and negative patients in both adults and children. Within the same subject, the difference of Lewis antigen expression between different locations was analyzed by the Wilcoxon signed ranks test. The

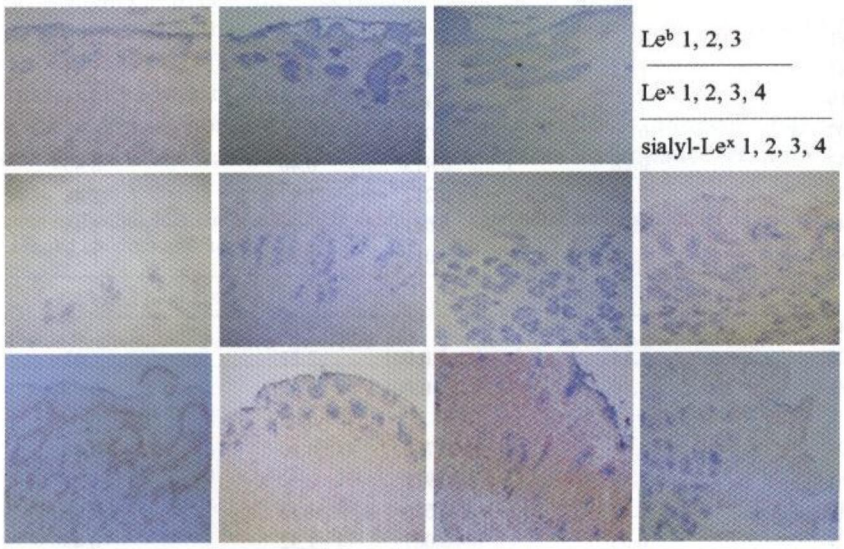

Figure 1. Immunohistochemical (IHC) staining and grading of Lewis b (Le^b), Lewis x (Le^x), and sialyl-Lewis x (sialyl-Le^x) antigens from human gastric biopsies (200X). The IHC staining showed negative for three Lewis antigens referred to as score 0. For Le^b, score 1: stain only on superficial surface; score 2: superficial epithelium and pit surface; score 3: superficial epithelium and deep glands. For Le^x, score 1: scant staining (< 5%) in deep glands; score 2: deep glands 5–50%; score 3: deep glands > 50%; score 4: deep glands with superficial epithelium. For sialyl-Le^x, score 1: only surface mucus positive; score 2: Mucus and upper epithelium; score 3: upper epithelium and deep glands (chief cells < 50%); score 4: upper epithelium and deep glands (chief cells ≥50%).

correlation between the intensity of Lewis antigens and *H. pylori* density was tested by the Spearman rank correlation test. All tests were two-tailed with a p value less than 0.05 taken as statistically significant.

Results

Enrolled subjects and their H. pylori infection status

A total of 68 dyspeptic children (M/F = 1.0, median age 12.1 years, range 4.2–18) and 110 dyspeptic adults (M/F = 0.93, median age 42.0 years, range 20–72) were studied. A total 28 of the children and 50 of the adults had *H. pylori* infection. Peptic (duodenal or gastric) ulcer diseases were disclosed in 19 children (12 with and seven without *H. pylori* infection) and 28 adults (20 with and eight without *H. pylori* infection). For those *H. pylori*-infected subjects, nine children and 16 adults developed duodenal ulcers, three children and four adults had gastric ulcers, and the others had gastritis. The relative risk of peptic ulcers through the presence of *H. pylori* infection was higher in the adults (OR, [95% CI]: 4.3, [1.7–11.0]; p = 0.001) than in the children (OR, [95% CI]: 3.5, [1.2–10.7]; p = 0.02).

Gastric histological features and Lewis antigens of the enrolled subjects

In Table 1, the histological features of the enrolled patients with and without *H. pylori* infection are listed. For both children and adults, *H. pylori* infection led to a higher AIS and CIS in both antrum and corpus biopsies ($p < 0.01$). However, there were no differences in AIS, CIS, AT, and IM over the antrum and corpus between the *H. pylori*-infected children and adults ($p > 0.05$). No children had IM over the corpus, despite having *H. pylori* infection. In the children without

Table 1. The intensity of gastric Lewis antigen expression and other histological findings between children and adults with and without *H. pylori* infection.

Mean	Children			Adults		
	HP (+) (n = 28)	HP (−) (n = 40)	p value	HP (+) (n = 50)	HP (−) (n = 60)	p value
Age (y)	13.1	11.1	0.016	42.8	39.7	0.161
Antrum						
HPD	2.81	0	–	2.94	0	–
CIS	2.81	1.23	< 0.001	2.86	1.32	< 0.001
AIS	1.19	0	< 0.001	1.38	0.03	< 0.001
IM	0.07	0	0.083	0.16	0.03	0.022
AT	0.37	0.28	0.413	0.40	0.45	0.599
*Le^b	2.00	2.24	0.212	1.97	2.48	0.019
[a]*Le^x	2.46	3.00	0.047	1.79	3.48	< 0.001
Sialyl-Le^x	1.89	1.08	0.013	2.20	1.28	< 0.001
Corpus						
[a]HPD	2.44			3.28		
CIS	2.26	1.15	< 0.001	2.27	1.02	< 0.001
AIS	0.81	0.10	< 0.001	0.67	0	< 0.001
IM	0	0	–	0.06	0	0.268
AT	0.04	0.05	0.786	0.10	0.07	0.506
[c]*Le^b	1.88	1.89	0.922	2.32	1.93	0.001
[b]*Le^x	2.19	2.37	0.520	1.33	2.72	< 0.001
Sialyl-Le^x	1.29	0.63	0.007	1.32	0.45	< 0.001

HP, *H. pylori*; CIS, chronic inflammation score; AIS, acute inflammation score; HPD, *H. pylori* density; IM, intestinal metaplasia; AT, gastric atrophy. [a]p value = 0.004, [b]p value < 0.001, [c]p value = 0.016 between *H. pylori*-infected children and adults. *For analyzing the intensities of Le^b and Le^x, the cases with both negative stains over the antrum and corpus were excluded. Accordingly, there were 26 HP(+) children, 38 HP(−) children, 39 HP(+) adults, and 60 HP(−) adults were included for Le^x analysis. As well, there were 25 HP(+) children, 38 HP(−) children, 38 HP(+) adults, and 58 HP(−) adults included for Le^b analysis.

H. pylori infection, none of them had IM over either the antrum or corpus. For *H. pylori*-infected subjects, the mean HPD was significantly higher in the corpus biopsies of the adults than that of the children (3.28 vs. 2.44; $p = 0.004$).

For the *H. pylori*-negative children ($n = 40$) and the adults ($n = 60$), the positive stains (either antrum or corpus) of Lewis antigens Le^b, Le^x, and sialyl-Le^x were 95, 95, and 47.5% in the children and 96.7, 100, and 68.3% in the adults, respectively. However, for the *H. pylori*-infected children ($n = 28$) and adults ($n = 50$), the positive rate of Le^b, Le^x, and sialyl-Le^x were 89.3, 92.8, and 82.1% in the children and 76, 78, and 90% in the adults, respectively. Only the intensity of Le^x but not Le^b or sialyl-Le^x was positively correlated to the increment of age over the antrum (R square = 0.11, p value = 0.001) and corpus (R square = 0.06, p value = 0.014) in *H. pylori*-negative subjects.

For reducing the bias of patients without Lewis antigens (Le^b and Le^x) expressions, we excluded the patients with negative stains on both antrum and corpus for Le^b or Le^x in Table 1. The intensity of the gastric Le^x expression over the antrum and corpus were significantly lower in the *H. pylori*-infected adults than in the non-infected ones ($p < 0.001$). However, the gastric Le^b of *H. pylori*-infected adults was significantly lower over the antrum ($p = 0.019$), but higher over the corpus ($p = 0.001$), as compared with that of the non-infected adults (Table 1). The Le^b and Le^x expressions were both marginally lower in the *H. pylori*-infected children than those non-infected over antrum biopsies, whereas there was no difference in Le^b or Le^x over the corpus. In contrast to the Le^b and Le^x, the sialyl-Le^x expression was significantly higher in both the *H. pylori*-infected children and adults over the antrum (children, $p = 0.013$; adult, $p < 0.001$) and corpus (children, $p = 0.007$; adult, $p < 0.001$) than those non-infected ones.

For the *H. pylori*-infected subjects, the intensity of Le^x expression, but not sialyl-Le^x expression, was less in adults than in children over the antrum (1.40 vs. 2.29, $p = 0.004$) and corpus (1.04 vs. 2.04, $p < 0.001$). In contrast to a lower intensity of Le^x, the intensity of Le^b expression was significantly higher in adults than in children over the corpus (2.32 vs. 1.88, $p = 0.016$) but not antrum (1.97 vs. 2.00, $p = 0.911$).

Histology and Lewis antigens between the H. pylori-infected children and adults

As shown in Table 2, for both the *H. pylori*-infected children and adults, the CIS, AT and sialyl-Le^x were significantly different between the antrum and corpus ($p < 0.05$). Furthermore, the *H. pylori*-infected adults, but not children, were disclosed to have a lower AIS ($p = 0.005$) and Le^x ($p = 0.005$) in the corpus than in the antrum. In contrast, the *H. pylori*-infected adults had a significantly higher Le^b in the corpus than in the antrum ($p = 0.038$).

Correlation of Lewis antigens to HPD and peptic ulcers

Because Lewis antigens may serve as putative receptors to enhance *H. pylori* colonization, we

Table 2. The differences of histological features and Lewis antigen expressions between antrum and corpus biopsies of the same patient in *H. pylori*-infected children and adults.

Corpus-antrum n	Children ($n = 28$)				Adults ($n = 50$)			
	Negative	Positive	Ties	[a]p value	Negative	Positive	Ties	[a]p value
HPD	15	7	6	0.395	11	21	18	0.161
CIS	12	1	15	0.003	23	1	26	<0.001
AIS	10	4	14	0.118	18	1	31	<0.001
IM	2	0	26	0.157	8	1	41	0.083
AT	9	0	19	0.003	18	3	29	0.001
Le^b	7	4	17	0.366	3	10	37	0.038
Le^x	9	7	12	0.287	16	5	29	0.005
Sialyl-Le^x	12	4	12	0.029	29	5	16	<0.001

HPD, *H. pylori* density; CIS, chronic inflammation score; AIS, acute inflammation score; IM, intestinal metaplasia; AT, gastric atrophy; Negative, corpus is higher than antrum; Positive, corpus is lower than antrum; Ties, corpus is equal to antrum. [a]Analyzed by Wilcoxon Signed Ranks test.

thus tested whether there was any Lewis antigen correlation to the higher HPD over the corpus in the adults than in the children. There were similar HPD values between the adults with high and low intensity of Le^x or sialyl-Le^x ($p > 0.05$). However, the HPD over the antrum (3.8 vs. 2.2, $p < 0.001$) and corpus (3.6 vs. 2.8, $p = 0.01$) were significantly higher in the *H. pylori*-infected adults with high Le^b (≥2) expression than in those with low Le^b (<2) expression. Nevertheless, the rate of peptic ulcers was not correlated to the intensity of Le^b expression over the antrum (52% vs. 30%, $p = 0.11$) and corpus (46% vs. 32%, $p = 0.30$) in the *H. pylori*-infected adults.

As the HPD over the corpus of the adults was higher in those with high Le^b, we thus determined whether the role of the Le^b antigen enhances the HPD in the children. There was no difference of HPD over the antrum and corpus between the *H. pylori*-infected children with high Le^b (≥2) expression than in those with low Le^b (<2) expression. However, the *H. pylori*-infected children expressed high Le^b over the corpus and had a higher rate of peptic ulcers than those with low Le^b (60% vs. 0, $p = 0.008$, odd ratio: 2.5, 95% CI: 1.5–4.3).

For both the *H. pylori*-infected children and adults, there was no correlation between Le^x or sialyl-Le^x intensity and HPD over the antrum and corpus. The Spearman rank correlation test confirmed again that the intensity of the Le^b antigen over the antrum ($p < 0.001$) and corpus ($p = 0.001$) were positively correlated to the HPD in the *H. pylori*-infected adults but not in the children.

Discussion

Helicobacter pylori infection has been proven to have a close relationship to duodenal and gastric ulcer disease [1, 3]. In this study, as the presence of *H. pylori*-associated peptic ulcers was higher in adults than in children (Relative risk: 4.3 vs. 3.5), there could be certain novel or chronicity-related responses to *H. pylori* infection that cause more ulcers in adults than in children.

In Table 1, the *H. pylori* colonization density, represented by HPD, on the corpus is significantly higher in the adults than that in the children (3.28 vs. 2.22; $p = 0.004$). As HPD was mediated by the expression of gastric Lewis antigens serving as bacterial receptors [11, 12, 15, 16], it is of interest to determine whether a different Lewis antigen expression exists between adults and children. As shown in Table 1, the intensity of Le^x (antrum and corpus) and Le^b (corpus) antigens shows a significant difference between the adults and the children with *H. pylori* infection. Such data imply that there were different Lewis antigen expressions between the adults and children after *H. pylori* infection, and this may possibly account for the different HPD between the two groups.

Our study, as shown in Table 1, revealed that *H. pylori* infection can be associated with changes of gastric Lewis antigens expressions, such as decreased Le^b and Le^x and increased sialyl-Le^x expressions, in both children and adults. Moreover, the *H. pylori*-infected subjects had different topographic features of the histology and Lewis antigen expressions between the antrum and corpus. Besides the CIS, AT, and sialyl-Le^x expressions having topographic differences between the antrum and corpus in both the *H. pylori*-infected adults and children, the *H. pylori*-infected adults had a higher Le^b ($p = 0.038$) and lower Le^x ($p = 0.005$) in the corpus than that in the antrum. This result further supports that there really are topographic differences in histology features and Lewis expressions between adults and children after *H. pylori* infection.

Based on the interaction between BabA and Le^b remaining the dominant pathway for *H. pylori* colonization [15, 26], it is prudent to determine whether the higher Le^b expression after *H. pylori* infection is related to higher HPD in adults and children. We found that adult patients with a high Le^b expression (score ≥2) had significantly higher HPD than those with a low Le^b expression (score <2) over the antrum (3.8 vs. 2.2, $p < 0.001$) and corpus (3.6 vs. 2.8, $p = 0.01$). Furthermore, the Spearman rank correlation test confirmed that the higher expression of Le^b correlates with a higher HPD in the *H. pylori*-infected adults but not in the children.

In addition, we tested whether the expression of Lewis antigens can be correlated with ulcer outcome in *H. pylori*-infected patients. In contrast to the adults, the *H. pylori*-infected children with a high Le^b expression over the corpus was significantly associated with a higher rate of peptic ulcers than those with a low Le^b expression (60% vs. 0, $p = 0.008$). This finding thus indicates that

although Le^b, does not mediate a higher HPD in children, it is related to the increased ulcer rate in children. The reason why a higher Le^b expression leads to an increased ulcer rate in children could be independently connected to a more heavy colonization of *H. pylori*, but should be possibly mediated with the immunologic responses to mediate the ulcer formation. Further studies are thus promising to determine the exact role of Le^b or its associated process during ulcerogenesis in *H. pylori*-infected children.

In conclusion, *H. pylori* infection changes the gastric Lewis antigen expressions in both adults and children. The intensity of Le^b expression is correlated to the *H. pylori* density over gastric antrum and corpus in adults, and increases the ulcer risk in children. Accordingly, interventions to control the Le^b–mediated pathway are thus promising for the control of *H. pylori* infection.

Acknowledgements

The study was supported by grants NSC93–2314-B-006-064, NSC94-2314-B-006-001, NSC94-2314-B-006-013 and NSC94-2314-B-006-062 from the National Science Council, and NCKUH 95-064 from National Cheng Kung University Hospital, Taiwan.

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