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• *BRIEF REPORTS* •

Lewis blood genotypes of peptic ulcer and gastric cancer patients in Taiwan

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Abstract

AIM: The Lewis b (Le^b) antigen has been implicated as a possible binding site for attachment of *Helicobacter pylori* (*H pylori*) to gastric mucosa. However, studies both supporting and denying this association have been reported in the literature. Differences in secretor (Se) genotype have been suggested as a possible reason for previous discrepancies. Therefore, we investigated the relationship between Le and Se genotypes and *H pylori* infection rates in people with peptic ulcer or gastric cancer.

METHODS: Peripheral blood samples were obtained from 347 patients with endoscopic evidence of peptic ulcer disease (235 cases of duodenal ulcer, 62 of gastric ulcer, and 50 of combined duodenal ulcer/ gastric ulcer) and 51 patients with gastric cancer on endoscopy. Peripheral blood specimens from 101 unrelated normal volunteers were used as controls. Lewis phenotype was determined using an antibody method, whereas Le and Se genotypes were determined by DNA amplification and restriction enzyme analysis. Gastric or duodenal biopsies taken from patients with endoscopic evidence of peptic ulcer or gastric cancer were cultured for *H pylori*. Isolates were identified as *H pylori* by morphology and production of urease and catalase. The *H pylori* infection status was also evaluated by rapid urease test (CLO test), and urea breath test (^{13}C-UBT). Results of studies were analyzed by chi-square test (taken as significant).

RESULTS: *H pylori* was isolated from 83.7% (303/347) of patients with peptic ulcer disease. Statistical analysis did not show any significant difference in Lewis phenotype or genotype between patients with and without *H pylori* infection. No significant association was found between Lewis genotype and peptic ulcer or gastric cancer.

CONCLUSION: Lewis blood genotype or phenotype may not play a role in the pathogenesis of *H pylori* infection. However, bacterial strain differences and the presence of more than one attachment mechanism may limit the value of epidemiological studies in elucidating this matter.

Key words: Lewis histoblood group; *Helicobacter pylori*; Peptic ulcer; Gastric cancer

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INTRODUCTION

The Lewis blood group determinants are structurally related to the antigens of the ABO and H/h blood group systems. They are made by sequential addition of specific monosaccharides onto terminal saccharide precursor chains on glycolipids or glycoproteins. The glycolipids on which they reside on the erythrocyte surface are not synthesized in erythroid tissues, but are acquired by erythrocyte membranes from other tissues through circulating soluble forms that are bound to lipoproteins[1].

The synthesis of the epitopes is dependent on the interaction of two different fucosyltransferases: alpha(1,2) fucosyltransferase encoded by the *FUT2* or secretor (Se) locus of the H/h blood group system, and alpha(1,3;1,4) fucosyltransferase (FUT3) encoded by the *FUT3* locus. Fucosylation by FUT3 gives rise to the Le^a epitope, whereas the action of both enzymes results in Le^b.

If *FUT3* is not expressed, the phenotype is Le^{a-b-} regardless of whether *FUT2* (Se) is expressed or not. If *FUT3* and *FUT2* are both expressed, the phenotype is Le^{a-b+}. If *FUT3,* but not *FUT2* is expressed, the phenotype is Le^{a+b-}. A Le^{a+b+} phenotype may occur, if there is reduced expression of *FUT2*.

The Le^b glycan has been reported to mediate the attachment of *Helicobacter pylori* (*H pylori*) to human gastric mucosa[2,3]. However, the clinical significance of this reported association remains a topic of debate. It has also been reported that people who do not secrete soluble Lewis b antigen are more susceptible to *H pylori* infection than people with secretor phenotypes[4].

This study aimed to explore the association among Lewis antigen phenotypes and genotypes, infection with *H pylori,*

and consequent development of peptic ulcer or gastric cancer.

MATERIALS AND METHODS

Peripheral blood samples were collected as previously described[4] from 347 patients with endoscopic evidence of peptic ulcer disease (235 cases of duodenal ulcer, 62 cases of gastric ulcer, 50 cases of duodenal ulcer and gastric ulcer) and 51 patients diagnosed with gastric cancer on endoscopy. Peripheral blood specimens from 101 unrelated healthy volunteers were used as normal controls. Subjects were enrolled from the Division of Gastroenterology, Department of Internal Medicine, Taipei Municipal Jen-Ai Hospital from August 1998 to December 2002.

The erythrocyte Lewis phenotype was determined by a tube method using monoclonal antibody (Gamma-CloneR, anti-Le^a, anti-Le^b Gamma Biologicals, Inc. Houston, TX, USA) at Taipei Municipal Jen-Ai Hospital.

Total genomic DNA was isolated from peripheral blood leukocytes as described previously[5], and Le and Se genotypes were determined by DNA amplification (polymerase chain reaction, PCR) and restriction enzyme analysis[6,7]. Oligonucleotide primer design and restriction enzyme analysis were carried out as previously described[5]. The primer sequences and restriction enzymes used in this study are shown in Table 1. The amplified products were digested with appropriate restriction enzymes, followed by electrophoresis on 1.5-4% agarose gels. Direct sequencing of the PCR products in selected cases provided a check on the validity of the procedure.

Gastric or duodenal biopsies were taken from all patients with endoscopic evidence of peptic ulcer or gastric cancer and cultured for *H pylori*. Bacterial isolates were identified as *H pylori* by morphology and production of urease and catalase[8]. The *H pylori* infection status was also evaluated by rapid urease test (CLO test), and urea breath test (^{13}C-UBT). *H pylori* infection was defined as positive results by culture or two positive test results on histology, rapid urease test, and ^{13}C-urea breath test[9].

Results were analyzed by the chi-square test, $P<0.05$ was considered statistically significant.

RESULTS

H pylori was isolated from 83.7% (303/347) of patients with peptic ulcer disease. Statistical analysis did not show a significant difference in Lewis phenotype (Table 2) or Lewis genotype (Table 3) between peptic ulcer patients with *H pylori* infection and those without infection. Among the 51 gastric cancer patients, *H pylori* infection prevalence in the different Lewis phenotypes was Le^{a-b+}, 30/33 (90.9%); Le^{a-b-}, 7/8 (87.5%); Le^{a+b-}, 3/3 (100%); and Le^{a+b+}, 6/7 (85.7%).

H pylori was detected in 109/124 (87.9%) of peptic ulcer patients with Se/Se genotype, 148/174 (85%) of those with Se/se genotype, and 53/58 (91.3%) of those with se/se genotype. The difference in proportion of infected patients was not significant ($P = 0.436$).

Similarly, no significant difference ($P = 0.440$) was found for presence of *H pylori* infection in peptic ulcer patients with Le/Le (86/97, 88.6%), Le/le (182/215, 84.6%) or le/le (31/34, 91.3%) genotypes.

No significant association was found between Lewis genotype and presence of peptic ulcer disease or gastric cancer (Table 4). Again, an analysis by Se genotype showed no significant difference between patients with peptic ulcer disease and normal controls ($P = 0.915$) or between patients with gastric cancer and normal controls ($P = 0.741$), whereas analysis by Le genotype gave similar, non-significant results

Table 1 Primer sequences and restriction enzymes of mutation in Se and Le genes

Mutation	Primer sequence	Enzyme
Se gene		
A385T	UP[1]: GATGGAGGAGGAATACCGCCTC	Ear I
	DP[2]: GATCTCCTGGCGGAGGTGGTGGTAGAAGATC	
G428A	Identical to A358T primer pairs	Bgl II
C571T	UP: AGGAGATCCTCCAGGAGTTCA	Dde I
	DP: AGAAGGAGAAAAGGTCTCAAAGG	
G849A	Identical to C571T primer pairs	Dde I
C628T	UP: AGTGTGGAAGGGGGTGGTGCCC	Bgl I
	DP: CCACTCTGGCAGGAAGGC	
Fusion gene	UP: CTGCCTCCTGACCATGTCC	Pst I
	DP: identical to reverse primer of C628T	
Le genes		
T202C and C314T	UP: CCACCCTCCTGATCCTGCTC	Msp I (T202C)
	DP: GATATCCCAGTGGTGCACGATGATGATC	Bcl I (C314T)
C445A	UP: identical to UP of T202 C and C314 T	BstN I
	DP: GAGATTGAAGTATCTGTCCAAGGC	
G508A	UP: TCAACTTGGAGCCACACCCT	Alu I
	DP: AGTTGGACACCGCCCAGGCCACCAG	
A1007C	UP: GCTCCTTCCGCTGGGCACTAG	Alu I
	DP: TGGCCACAAAGGACTCCAGC	
T1067A	UP: GTACCAGACGGTGCGATGCA	Nsi I
	DP: identical to DP of A1007 C	

[1]UP: upstream primer; [2]DP: downstream primer; The mutated base is underlined.

Table 2 Correlation between Lewis phenotype and *H pylori* infection in peptic ulcer disease

Phenotypes	Le^{a-b+} (n = 230)	Le^{a-b-} (n = 59)	Le^{a+b-} (n = 14)	Le^{a+b+} (n = 44)	P
H pylori status					0.5662
Positive	195	54	12	39	
Negative	35	5	2	5	
Prevalence %	84.7	91.5	85.7	88.5	

Table 3 Correlation between Lewis genotype and *H pylori* infection in peptic ulcer disease

Genotypes	SeLe/SeLe (n = 33)	SeLe/seLe (n = 65)	SeLe/Sele (n = 42)	SeLe/Sele (n = 90)	SeLe/Sele (n = 3)	SeLe/Sele (n = 19)	SeLe/Sele (n = 37)	SeLe/Sele (n = 46)	SeLe/Sele (n = 12)	P
H pylori status										0.446
Positive	28	59	36	72	2	17	33	41	12	
Negative	5	6	6	18	1	2	4	5	0	
Prevalence %	84.8	90.7	85.7	80	66.6	89.4	89.1	89.1	100	

Table 4 Lewis genotypes in peptic ulcer and gastric cancer

Genotypes	Peptic ulcer n = 347	Gastric cancer n = 51	Normal n = 101
SeLe/SeLe	33	2	7
SeLe/seLe	65	11	25
SeLe/Sele	42	4	10
SeLe/sele	90	15	21
Sele/Sele	3	3	5
Sele/sele	19	4	7
seLe/seLe	37	3	12
seLe/sele	46	5	9
sele/sele	12	4	5
P	0.129[1]	0.881[2]	

[1]P = 0.129 peptic ulcer *vs* normal controls; [2]P = 0.881 gastric cancer *vs* normal controls.

(P = 0.067 and P = 0.344 respectively).

Finally, no significant correlation was obtained between ABO blood group type and *H pylori* infection (Table 5).

DISCUSSION

H pylori is the main causative agent of gastric and duodenal ulcers[10] and gastric adenocarcinoma[11]. Attachment is a prerequisite for microbial colonization of epithelial surfaces and is mediated through interaction of adhesins on the bacterial surface and proteins or glycoconjugates on the surface of the epithelial cells[12,13]. Borén *et al.*[2], reported that the attachment of *H pylori* to gastric mucosa is mediated by the Lewis^b (Le^b) antigen and that the availability of receptors might therefore be reduced in individuals of blood groups A and B compared to people with blood group O.

Carneiro *et al.*[14], found that there is a significant relationship between ABO blood group in combination with Lewis phenotype on the one hand and *H pylori* infection on the other. *H pylori* is present in 100% of those with Le^{b+}/O phenotype but in only 57% of Le^{b-}/A or B phenotype. However, infection is also present in 92% of Le^{b-}/O individuals and 86% of Le^{b+}/A or B individuals. Nonetheless, the Carneiro group challenged the finding of Niv *et al.*[15], that positivity for *H pylori* is not associated with blood group O and their conclusion that their observations do not support the contention that the receptor for *H pylori* in the gastric mucosa is the Le^b antigen.

However, Clyne and Drumm[16] found that adherence of *H pylori* to isolated human gastric cells is not dependent on Lewis antigen expression on the cells, and Umlauft *et al.*[17], could not demonstrate any *in vivo* correlation between *H pylori* infection or disease and Le^b antigen. Taylor *et al.*[18], also found that there is no correlation between Lewis antigen expression by *H pylori* and gastric epithelial cells in infected patients.

More recently, Keller *et al.*[19], found that there is no significant association between secretor status or specific ABO blood group and *H pylori* infection or occurrence of gastro-duodenal ulcer. Aguiar[20] also found that there is no significant association between the presence of *H pylori* and ABO, Lewis or secretor phenotype, while Nogueira *et al.*[21], actually found that Le^b expression is nearly twice as common among children without *H pylori* (15/23, 65%) as in those with *H pylori* (16/47, 34%).

However, Yang *et al.*[22], found that there are significant

Table 5 ABO blood types and susceptibility to *H pylori* infection (n = 230)

Blood types	A (n = 44)	B (n = 54)	O (n = 122)	AB (n = 10)	P
H pylori status					0.255
Positive	34	49	106	8	
Negative	10	5	16	2	
Prevalence %	77.2	90.7	86.8	80	

relationships between Lewis phenotype and *H pylori* infection. Expression of Le^a antigen (whether Le^{a+b-} or Le^{a+b+}) is associated with a higher infection rate but a lower bacterial density, a lower severity of chronic inflammation, and a lower frequency of lymphoid follicles in the gastric cardia. To complicate things even further, *H pylori*-infected patients expressing the Le^b antigen have a lower rate of gastro-duodenal ulcers but a higher bacterial density and inflammation severity in the gastric cardia. It is difficult to know how to interpret these results.

In vitro studies by Lindén *et al*.[23], and Van de Bovenkamp *et al*.[24], showed that binding of *H pylori* to human gastric MUC5AC mucin is blood group antigen-binding adhesin dependent and mediated by the Le^b structure in the mucin. However, it has also been shown that *H pylori* bacteria that do not express Lewis antigens but do express other complex carbohydrates that may still have the ability to form long-term colonies in the stomach[25,26].

It may be that differences in reported results are due to strain differences in *H pylori*. Hennig *et al*.[27], found that there is considerable heterogeneity among *H pylori* isolates in expression of the BabA adhesion, which is thought to bind to Le^b antigen present on the surface of gastric epithelial cells. Sheu *et al*.[28], in a study of 188 dyspeptic patients with *H pylori* infection in Taiwan, found that all isolates have a positive babA2 genotype and that, among 139 patients with Le^b expression, *H pylori* density increases with Le^b intensity. In the 49 patients without gastric Le^b expression, *H pylori* density is positively correlated with Le^x and Le^a expression.

In conclusion, Lewis blood genotype or phenotype may not play a role in the pathogenesis of *H pylori* infection. However, bacterial strain differences and the presence of more than one attachment mechanism may limit the contribution of epidemiological studies toward elucidating this matter.

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