CHAPTER V

RESULTS

1. Culture and identification

Gastric biopsy specimens were obtained from 360 patients between August 2003 to June 2004. Colonies grown on Columbia agar were examined for small, translucent and grey to yellow appearance. Suspicious colonies were gram-stained to look for curved or S-shaped Gram negative rods. The presumptive phenotypic characteristics (urease test, oxidase and catalase test) were tested with suspected bacterial isolates. A total of 62 isolates were cultured and identified as the *Helicobacter pylori* based on the presumptive phenotypic characteristics. They were all positive for catalase test, oxidase test and rapid urease reaction.

Out of 62 *H. pylori* clinical isolates, 20 were from patients with gastric ulcer, 4 were from patients with duodenal ulcer and 38 were from patients with non-ulcer dyspepsia. In addition to clinical specimens, 18 *H. pylori* clinical isolates maintained at -70°C were included in this study. Fifteen were from patients with gastric ulcer, two were from patients with NUD and one was from patients with duodenal ulcer.

Distribution of clinical data of 80 *H. pylori* infected patients was as follows : 40 patients (age rage, 23 – 79 years; mean 48.83 ± 15.53 SD years, male/female : 13/27) had non-ulcer dyspepsia and 40 patients (age rage, 25-91 years ; means 57.90 ± 15.44 SD years, male/female : 27/13) had peptic ulcer disease, of whom 35 patients had gastric ulcers and 5 patients had duodenal ulcers underwent upper gastrointestinal endoscopy. Location stayed in childhood during ten years in patients peptic ulcer diseases almost stayed at Bangkok(50%) and Central region (32.5%). Like in patients non-ulcer dyspepsia almost stayed at Bangkok (57.5%) and Central region (17.5%) (Table 8).

Table 8. Demographic data of patients with PUD and NUD

Variables	PUD (n = 40) (35 GU + 5 DU)	NUD (n = 40)	*P* value[b]
Age range (years)	25-91	23-79	0.007[a]
Mean± SD	57.90 ± 15.44	48.83 ± 15.53	
Sex (M/F)	27/13	13/27	0.004
Location			
Central	13(32.5%)	7(17.5%)	0.20
BKK	20(50%)	23(57.5%)	0.65
N	1(2.5%)	1(2.5%)	0.75
NE	3(7.5%)	5(12.5%)	0.72
S	0	2(5%)	0.25
W	1(2.5%)	1(2.5%)	0.75
E	2(5%)	1(2.5%)	0.50

[a] Analysed by t-test

[b] Analysed by Epi Info version 6, Centers for Disease Control and Prevention (CDC), 1994

PUD: Peptic Ulcer Disease

NUD: Non-Ulcer Dyspepsia

Bkk: Bangkok

N: Northern

NE: Northeast

E: Eastern

S: Southern

W: Western

2. Polymerase Chain Reaction (PCR) analysis of genes *cagA, vacA* and *iceA* in *H. pylori*

2.1 Detection of *cagA* genotype

For detection of the *cagA* genotype, primers CAGAF and CAGAR which yield a fragment of 349 bp from the middle conservative region of the *cagA* genotype were used. Amplicons were obtained for all the of 80 *H. pylori* isolates examined (Figure 8).

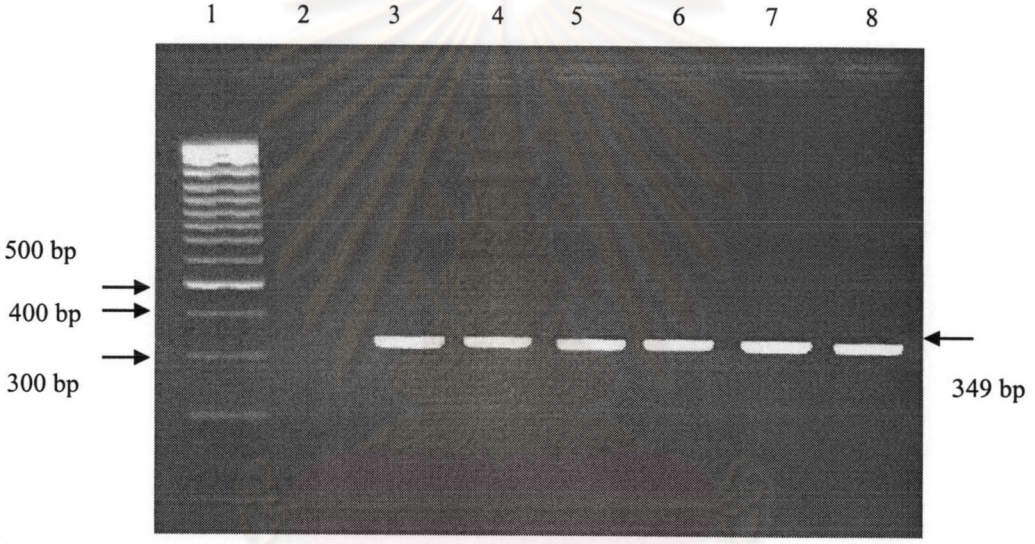

Figure 8. PCR analysis of *cagA* genotype. Lanes : 1, 100 bp DNA ladder; 2, negative control; 3, *cagA* genotype – positive control; 4 – 8, *H. pylori* DNA from clinical isolates.

2.1.1 Detection of cag PAI empty site genotype

For detection of cag PAI empty site, primers Luni 1 and R5280 which yield a fragment of 550 bp used for confirm *cagA* negative. The expected 550 bp products was not detected in all the *cagA* type of *H. pylori*.

2.2 Detection of *vacA* genotype

vacA genotype is composed of a hypervariable s-and m-region allele. The *vacA* subtypes are determined by the combination of s genotype (s1a, s1b, s1c and s2) and m genotype (m1a, m1b and m2).

2.2.1 Detection of *vacA* s region

For analysis of the *vacA* s region, primers VA1-F and VA1-R yielded a fragment of 259 bp for allele type s1 and a fragment of 286 bp for allele type s2 (Figure 9). Amplification products of type s1 were obtained from 78 of 80 *H. pylori* isolates and those of type s2 were obtained from 2 of 80 *H. pylori* isolates.

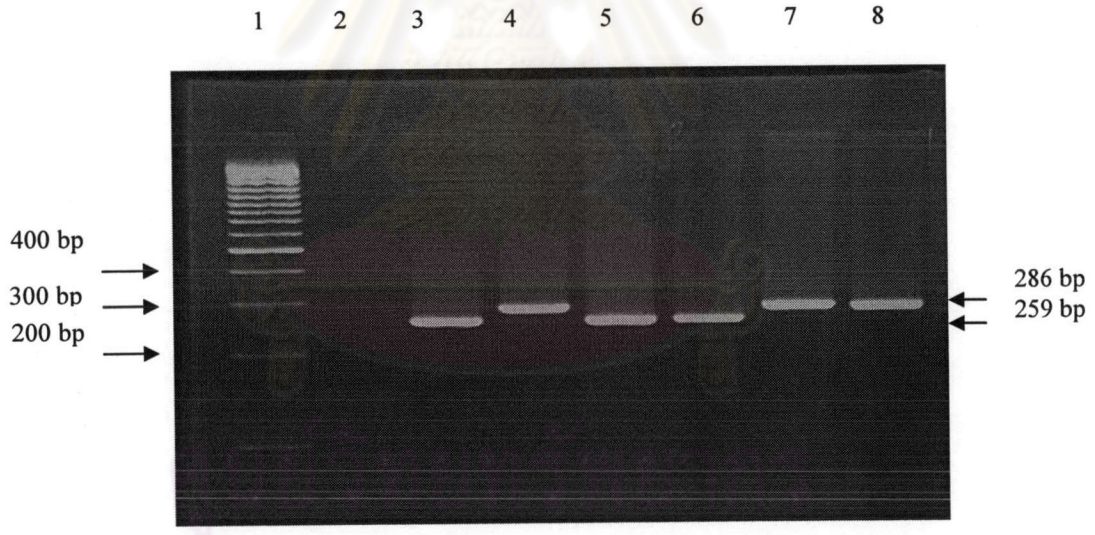

Figure 9. PCR analysis of *vacA* s region. Lanes : 1, 100 bp DNA ladder; 2, negative control; 3, allele type s1- positive control; 4, allele type s2- positive control; 5 – 8, *H. pylori* DNA from clinical isolates.

2.2.2 Detection of *vacA* s1a subtype

For detection of the *vacA* s1a subtype, primers S1A-F and VA1-R yielded a fragment of 212 bp (Figure10). Amplification products of *vacA* s1a subtype were obtained from 76 of 78 *vacA* s1 type *H. pylori*.

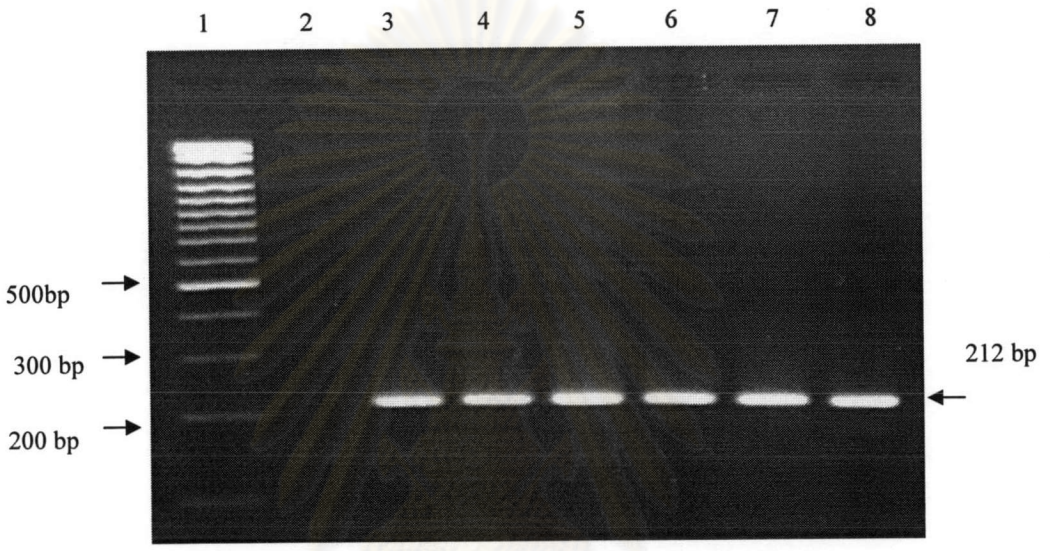

Figure 10. PCR analysis of *vacA* s1a subtype. Lanes : 1, 100 bp DNA ladder; 2, negative control; 3, *vacA* s1a genotye - positive control; 4-8, *H. pylori* DNA from clinical isolates.

2.2.3 Detection of *vacA* s1b subtype

vacA s1b subtype was determined by PCR amplification using primers SS3-F and VA1-R . The expected 187 bp product was not detected in any of 78 *vacA* s1 type *H. pylori*.

2.2.4 Detection of *vacA* s1c subtype

For detection of the *vacA* s1c subtype, primers S1C-F and VA1-R yielded a fragment of 213 bp (Figure 11). Amplification products of *vacA* s1c subtype were obtained from 2 of 78 *vacA* s1 type *H. pylori* .

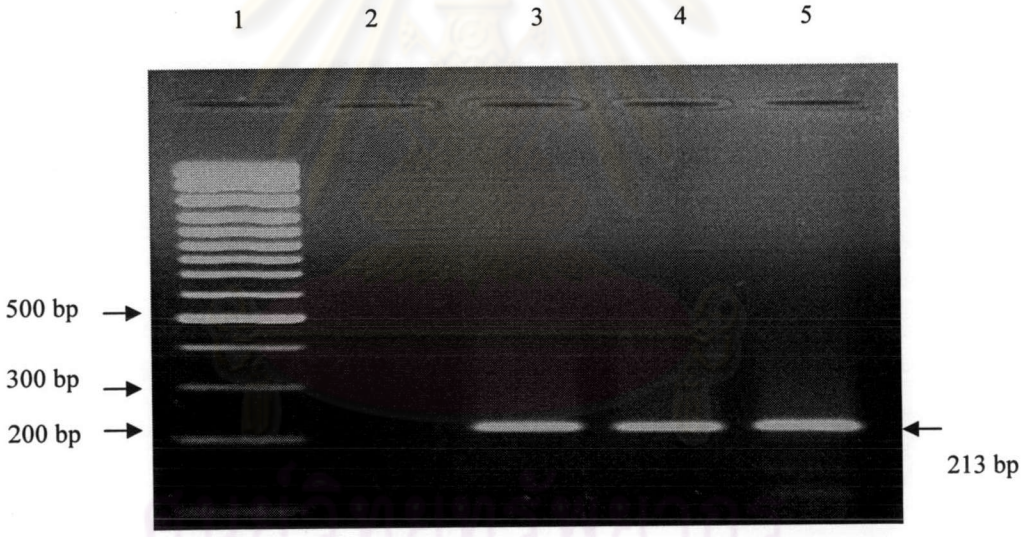

Figure 11. PCR analysis of *vacA* s1c subtype. Lanes : 1, 100 bp DNA ladder; 2, negative control; 3, *vacA* s1c subtype - positive control; 4-5, *H. pylori* DNA from clinical isolates.

2.2.5 Detection of *vacA* m region

For analysis of the *vacA* m region, primers VAG-F and VAG-R yielded a fragment of 570 bp for allele type m1 and a fragment of 645 bp for allele type m2 (Figure 12). Amplification products of type m1 were obtained from 32 of 80 *H. pylori* isolates and those of type m2 were obtained from 48 of 80 *H. pylori* isolates.

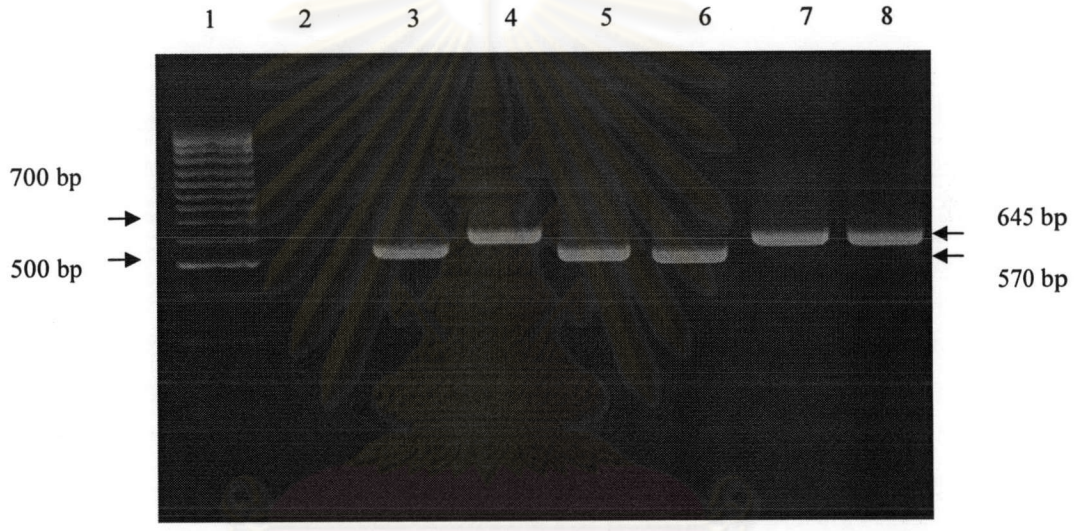

Figure 12. PCR analysis of *vacA* m region. Lanes : 1, 100 bp DNA ladder; 2, negative control; 3, *vacA* m1 allele type - positive control; 4, *vacA* m2 allele type - positive control; 5–8, *H. pylori* DNA from clinical isolates.

2.2.6 Detection of *vacA* m1a subtype

vacA m1a subtype was determined by PCR amplification using primers VA3-F and VA3-R. The expected 290 bp product was not detected in any of 32 *vacA* m1 type *H. pylori*.

2.2.7 Detection of *vacA* m1b subtype

For detection of the *vacA* m1b subtype, primers VAm-F3 and VAm-R3 yielded a fragment of 295 bp (Figure 13). Amplification products of *vacA* m1b subtype were obtained from all *vacA* m1 type *H. pylori*.

Figure 13. PCR analysis of *vacA* m1b subtype. Lanes : 1, 100 bp DNA ladder; 2, negative control; 3, *vacA* m1b subtype - positive control; 4 – 8, *H. pylori* DNA from clinical isolates.

A complete *vacA* genotype was obtained in all isolates. The majority (78 of 80; 97.5%) of isolates contained the s1 allele, and of these most (76 of 78 ; 97.4%) were subtype s1a, whereas 2 of 78 (2.6%) were subtype s1c and subtype s1b was not found. Only 2 of 80 (2.5%) isolates contained the s2 allele. Within the m alleles, m1 and m1b occurred in 32 (40%), whereas subtype m1a was not found. The majority (48 of 80 ; 60%) of isolates contained m2 allele. All m1 allele type was identified to be m1b subtype. Based on analysis of *vacA* s-and m-region, five diffent genotypes can be recognized. Multiple *vacA* genotypes were not found. The prevalence of each of these five genotype in 80 isolates among ulcer and non-ulcer patients was shown in Table 9.

2.3 Detection of *iceA* genotype

There are two main allelic variants of the genes: *iceA1* and *iceA2*.

2.3.1 Detection of *iceA1* genotype

For analysis of the *iceA1* genotype, primers *iceA*1F and *iceA*1R yielded a fragment of 247 bp (Figure 14). Amplification products of the *iceA1* genotype were obtained from 5 of 80 *H. pylori* isolates.

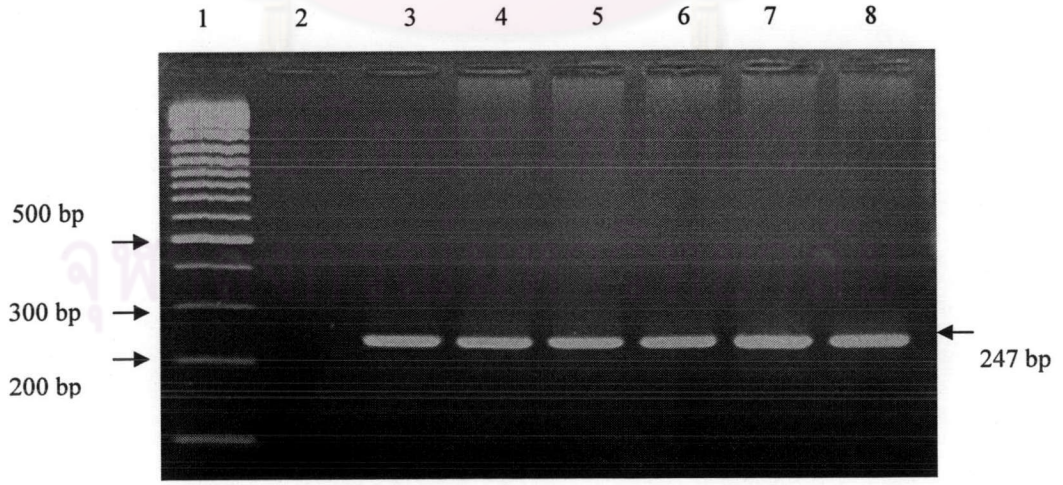

Figure 14. PCR analysis of *iceA1* genotype. Lanes : 1, 100 bp DNA ladder; 2, negative control; 3, *iceA1* genotype - positive control; 4-8, *H. pylori* DNA from clinical isolates.

2.3.2 Detection of *iceA2* genotype

For analysis of the *iceA2* genotype, primers *iceA*2F and *iceA*2R yielded a fragment of 229 bp (Figure 15). *iceA2* genotype has only one type of 229 bp according to PCR product. Amplification products of the *iceA2* genotype were obtained from 30 of 80 *H. pylori* isolates.

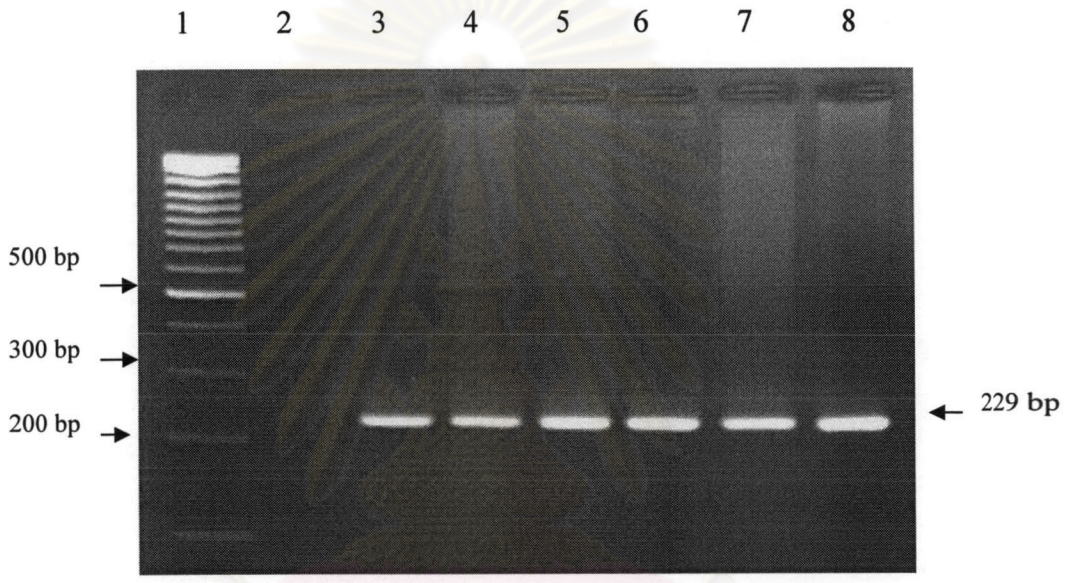

Figure 15. PCR analysis of *iceA2* genotype. Lanes : 1, 100 bp DNA ladder; 2, negative control; 3, *iceA2* genotype - positive control; 4-8, *H. pylori* DNA from clinical isolates.

2.3.3 Detection of mixed *iceA* genotype

Isolates with both the *iceA1* and *iceA2* allele were demonstrated by the yield of 247 bp and 229 bp fragments. Amplification products of the mixed *iceA* genotype were obtained from 39 of 80 *H. pylori* isolates.

The prevalence of *iceA* genotype in *H. pylori* isolates among ulcer and non ulcer patients was shown in Table 9. Out of 5 isolates with *iceA1* genotype, 2 (5.7%) were obtained from patients with GU and 3 (7.5%) were obtained from patients with NUD. Out of 30 isolates with *iceA*2 genotype, 11 (31.4 %) were from patients with GU and 19 (47.5 %) were from patients with NUD. Thirty-nine isolates (48.75%) were positive for both *iceA1* and *iceA2* and none of them contained multiple *vacA* genotypes, suggesting the presence of multiple *iceA* genotypes in these isolates instead of mixed infection. Three isolates in patients with NUD (7.5%), 1 isolates (20%) in patients with DU and 2 isolates (5.7%) in patients with GU did not yield any PCR product for *iceA* .

Table 9. *cagA*, *vacA* and *iceA* genotypes of *H. pylori* isolates from 80 patients

Genotype		Clinical outcome			Total (n[a]=80)
		Peptic ulcer		Non- ulcer dyspepsia (n=40)	
		Gastric ulcer (n=35)	Duodenal ulcer (n=5)		
cagA	*cagA* $^{+}$	35.0	5.0	40.0	80(100%)
	cagA $^{-}$	0.0	0.0	0.0	0.0
vacA	s1a/m1b	10.0	4.0	17.0	31(38.75%)
	s1a/m2	24.0	1.0	20.0	45(56.25%)
	s1c/m1b	0.0	0.0	1.0	1(1.25%)
	s1c/m2	0.0	0.0	1.0	1(1.25%)
	s2/m2	1.0	0.0	1.0	2(2.5%)
iceA	*iceA1*	2.0	0.0	3.0	5(6.25%)
	iceA2	11.0	0.0	19.0	30(37.5%)
	mixed *iceA*	20.0	4.0	15.0	39(48.75%)
	iceA $^{-}$	2.0	1.0	3.0	6(7.5%)

[a] number of isolates (%)

3. Prevalence of the *cagA*, *vacA* and *iceA* genes in 80 *H. pylori* isolates

The *cagA* was present in all of the 80 (100 %) isolates. The *vacA* s1 allele was present in 78 (97.5 %) isolates, out of which 76 (95.0 %) had subtype s1a and two (2.5 %) had subtype s1c. The *vacA* s2 allele was present in 2 (2.5 %) isolates. Thirty-two (40 %) of 80 isolates had m1 allele and all was m1b subtype, while 48 (60 %) isolates had m2 allele. The *vacA* subtype s1b or m1a was not found in any of these Thai isolates. The *iceA1* genotype was detected in 5 (6.25 %) isolates, *iceA2* genotype was found in 30 (37.5 %) isolates. While mixed *iceA* genotype was found in 39 (48.75 %) isolates (Table 10).

Table 10. Prevalence of genes *cagA, vacA* and *iceA* in *H. pylori* isolated from patients in Thai setting

Virulence Gene		Prevalence (n = 80)	No. %
cagA		80	100
vacA	*vacA* s1	78	97.5
	vacA s1a	76	95
	vacA s1c	2	2.5
	vacA s2	2	2.5
	vacA m1	32	40
	vacA m1b	32	40
	vacA m2	48	60
iceA	*iceA1*	5	6.25
	iceA2	30	37.5
	mixed *iceA*	39	48.75

4. Association between genes *cagA, vacA* and *iceA* and peptic ulcer disease

The association between individual genotypes and peptic ulcer disease was assessed by the Fisher's exact test or the chi-squre test and multiple logistic regression. A multiple logistic regression was used to relate the different combinations of *vacA* and *iceA* genotypes of *H. pylori* to peptic ulcer disease.

4.1 Univariate analysis of the association between genes *cagA, vacA* and *iceA* and clinical outcome

The *cagA* gene was present in 100% of *H. pylori* isolates obtained from patients with PUD and NUD. Thirty-nine of 40 patients with PUD (97.5 %), 39 of 40 patients with NUD (97.5%) were *vacA* s1 allele positive ($P = 1.00$). The presence of *vacA* s1 allele in the patients with PUD and NUD was not statistically significant ($P > 0.05$). Thirty-nine of 40 patients with PUD (97.5 %), 37 of 40 in patients with NUD (92.5%) were *vacA* s1 allele a positive ($P = 0.62$). The presence of *vacA* s1a in the patients with PUD and NUD was not statistically significant ($P > 0.05$). Two of 40 patients with NUD (5 %) were *vacA* s1c subtype positive ($P = 0.49$). One of 40 patients with PUD and NUD (2.5 %) were *vacA* s2 positive ($P = 1.00$). Fourteen of 40 patients with PUD (35 %) and 18 of 40 patients with NUD (45%) were *vacA* m1 allele and m1b subtype positive ($P = 0.49$). The presence of *vacA* m1 and m1b was not statistically significant in these two groups of patients ($P = 0.05$). Twenty-six of 40 patients with PUD (65 %) and 22 of 40 patients with NUD (55%) were *vacA* m2 positive ($P = 0.49$). Three of 40 patients with NUD (7.5 %) and 2 of 40 patients with PUD (5%) were *iceA1* positive ($P = 1.00$). Eleven of 40 patients with PUD (27.5 %), 19 of 40 patients with NUD (47.5 %) were *iceA2* positive ($P = 0.11$). Twenty-four of 40 patients with PUD (60 %), 15 of 40 patients with NUD (37.5%) were mixed *iceA* positive ($P = 0.07$). The results of univariate analysis showed that the presence of genes *cagA, vacA* and *iceA* in ulcer and non-ulcer patients was not statistically significant ($P > 0.05$). Therefore, there was no association between the presence of these genes and the clinical outcome (Table 11).

Table 11. Univariate analysis of the association between genes *cagA*, *vacA* and *iceA* and clinical outcome

Virulence Gene		Patient Group		
		PUD (n=40) No. %	NUD (n=40) No. %	*P* value[a]
cagA		40 (100.0)	40 (100.0)	-
vac A s region	*vacA* s1	39 (97.5)	39 (97.5)	1.00
	vacA s1a	39 (97.5)	37 (92.5)	0.62
	vacA s1c	0 (0.0)	2 (5.0)	0.49
	vacA s2	1 (2.5)	1 (2.5)	1.00
vacA m region	*vacA* m1	14 (35.0)	18 (45.0)	0.49
	vacA m1b	14 (35.0)	18 (45.0)	0.49
	vacA m2	26(65.0)	22(55.0)	0.49
iceA	*iceA1*	2 (5.0)	3 (7.5)	1.00
	iceA2	11 (27.5)	19 (47.5)	0.11
	mixed *ice A*	24 (60.0)	15 (37.5)	0.07

[a] Logistic regression analysis by SigmaStat for Windows version 3.1

PUD: Peptic Ulcer Disease

NUD: Non-Ulcer Dyspepsia

4.2 Multivariate analysis of the association between genes *cagA, vacA* and *iceA* and clinical outcome

4.2.1 The presence of genes *cagA, vacA* and *iceA* in clinical outcome

The *cagA* gene was present in 100% of *H. pylori* isolates obtained from patients with PUD and NUD. Thirty-nine of 40 patients with PUD (97.5 %), 39 of 40 patients with NUD (97.5 %), were allele *vacA* s1 positive (P = 0.87). The presence of allele *vacA* s1 in the patients with PUD and NUD was not statistically significant (P >0.05). Thirty-nine of 40 patients with PUD (97.5 %), 37 of 40 patients with NUD (92.5%) were allele *vacA* s1 positive (P = 0.16). The presence of *vacA* s1a subtype in the patients with PUD and NUD was not significant (P>0.05). Two of 40 the patients with NUD (5 %) were *vacA* s1c positive (P = 1.00). One of 40 patients with PUD and NUD (2.5 %) were *vacA* s2 positive. Fourteen of 40 patients with PUD(35 %) and 18 of 40 patients with NUD(45%) were *vacA* m1 and m1b positive (P = 0.33). The presence of *vacA* m1 and m1b was not statistically significant. Twenty-six of 40 patients with PUD (65 %) and 22 of 40 patients with NUD (55%) were *vacA* m2 positive (P = 0.33). Three of 40 patients with NUD (7.5 %) and 2 of 40 patients with PUD (5%) was *iceA1* positive (P = 0.32). Eleven of 40 patients with PUD (27.5 %), 19 of 40 patients with NUD (47.5 %) was *iceA2* positive (P = 0.15). Twenty-four of 40 patients with PUD (60 %), 15 of 40 patients with NUD was mixed *iceA* positive (P = 0.09). The presence of mixed *iceA* was higher in the patient with PUD than in the patients with NUD but this difference between the groups was not statistically significant (P>0.05). Therefore, there was no association between genes *cagA, vacA* and *iceA* of *H. pylori* and peptic ulcer disease (Table 12.)

Table 12. Multivariate analysis of the association between genes *cagA*, *vacA* and *iceA* and clinical outcome

Virulence Gene		Patient Group		
		PUD (n=40) No. %	NUD (n=40) No. %	*P* value[a]
cag A		40 (100.0)	40 (100.0)	-
vac A s region	*vac A* s1	39 (97.5)	39 (97.5)	0.87
	vac A s1a	39 (97.5)	37 (92.5)	0.16
	vac A s1c	0 (0.0)	2 (5.0)	1.00
	vac A s2	1 (2.5)	1 (2.5)	0.87
vac A m region	*vac A* m1	14 (35.0)	18 (45.0)	0.33
	vac A m1b	14 (35.0)	18 (45.0)	0.33
	vacA m2	26(65.0)	22(55.0)	0.33
ice A	*ice A1*	2 (5.0)	3 (7.5)	0.32
	ice A2	11 (27.5)	19 (47.5)	0.15
	mixed *ice A*	24 (60.0)	15 (37.5)	0.09

[a] Logistic regression analysis by SigmaStat for Windows version 3.1

PUD: Peptic Ulcer Disease

NUD: Non-Ulcer Dyspepsia

4.2.2 Multivariate analysis to relate genes *vacA*, other allelic variants and *iceA* in *H. pylori* with clinical outcome by comparison between diseases

Logistic regression analysis was used to relate genes *vacA* and allelic variants in *H. pylori* with clinical outcome by comparison between diseases. The patients were classified to three groups as having peptic ulcers (n = 40), gastric ulcers (n = 35) and duodenal ulcers (n = 5). Each group was compared with non-ulcer dyspepsia (n = 40). The result showed no difference in the presence of gene *vacA* s region (s1and s2) in the patients with PUD vs NUD ($P = 0.87$) and in patients with GU vs NUD ($P = 0.92$). The presence of genes *vacA* s1and s2 in the patients with DU vs NUD was not significant ($P>0.05$). The presence of gene *vacA* subtype a was not different in patients with PUD vs NUD ($P = 0.16$), in patients with GU vs NUD ($P = 0.20$)and the presence of gene *vacA* s1a in the patients with DU vs NUD was not significant ($P>0.05$) . The presence of gene *vacA* subtype c in patients with PUD vs NUD, GU vs NUD and DU vs NUD was not significant ($P>0.05$). The presence of gene *vacA* m1, m1b and m2 in patients with PUD vs NUD ($P = 0.33$), in patients with GU vs NUD ($P = 0.23$) and in the patients with DU vs NUD was not significant ($P=0.27$). There was no association between the presence of *vacA* allelic variants and the clinical outcomes. The result showed no difference in the presence of gene *iceA1* in the patients with PUD vs NUD ($P = 0.32$), in patients with GU vs NUD ($P = 0.85$) and in the patients with DU vs NUD ($P = 1.00$). The presence of gene *iceA2* was not different in patients with PUD vs NUD ($P = 0.15$), in patients with GU vs NUD ($P = 0.24$) and in the patients with DU vs NUD ($P = 1.00$). The presence of gene mixed *iceA* was not different in patients with PUD vs NUD ($P = 0.09$), in patients with GU vs NUD ($P = 0.13$) and in the patients with DU vs NUD ($P = 0.21$). Therefore, In conclusion the *iceA* genotype and clinical outcome were not associated (Table 13).

Table 13. Multivariate analysis of genes *vacA*, other allelic variants and *iceA* in *H. pylori* and clinical outcome by comparison between diseases

Virulence Gene	*P* value[a]		
	PUD vs NUD		
	GU vs NUD	DU vs NUD	Total PUD vs NUD
vacA s region			
vacA s1	0.92	1.00	0.87
vacA s1a	0.20	1.00	0.16
vacA s1c	1.00	1.00	1.00
vacA s2	0.92	1.00	0.87
vacA m region			
vacA m1	0.23	0.27	0.33
vacA m1b	0.23	0.27	0.33
vacA m2	0.23	0.27	0.33
iceA1	0.85	1.00	0.32
iceA2	0.24	1.00	0.15
mixed *iceA*	0.13	0.21	0.09

[a] Logistic regression analysis by SigmaStat for Windows version 3.1

PUD: Peptic Ulcer Disease (n =40)

NUD: Non-Ulcer Dyspepsia (n =40)

DU: Duodenal Ulcer (n =5)

GU: Gastric Ulcer (n =35)

4.2.4 The interaction of genes *vacA* s1, *vacA* m and *iceA* subtype in *H. pylori* and clinical outcome

Analysis of *vacA* s-region type (s1), *vacA* m-region and subtype (m1, m2 m1a, m1b) and the *iceA* type (*iceA1, iceA2* and mixed *iceA*) with a single combined genotype was shown in Table 14. The patients were classified to three groups as having peptic ulcers (n = 40), gastric ulcers (n = 35) and duodenal ulcers (n = 5). Each group was compared with non-ulcer dyspepsia (n = 40). There was no association between the combined genotypes of *vacA* s1, *vacA* m and *iceA* and clinical outcome.

Table 14. Multivariate analysis of interaction of *vac A* s1, *vacA* m and *iceA* subtypes in *H. pylori* and clinical outcome

Virulence Gene		*P* value		
		PUD vs NUD		
		GU vs NUD	DU vs NUD	Total PUD vs NUD
vacA s1	*vacA* m1	0.28	0.25	0.39
	vacA m1a	0.92	1.00	0.87
	vacA m1b	0.28	0.25	0.39
	vacA m2	0.22	0.42	0.31
	iceA1	0.51	1.00	0.46
	iceA2	0.27	1.00	0.18
	mixed *iceA*	0.24	0.21	0.16

PUD: Peptic Ulcer Disease (n =40)

NUD: Non-Ulcer Dyspepsia (n =40)

DU: Duodenal Ulcer (n =5), GU: Gastric Ulcer (n =35)

4.2.5 The interaction of genes *vacA* s2, *vacA* m and *iceA* subtype in *H. pylori* and clinical outcome

Analysis of *vacA* s-region type (s2), *vacA* m-region and subtype (m1, m2 m1a, m1b) and the *iceA* type (*iceA1, iceA2* and mixed *iceA*) with a single combined genotype was shown in Table 15. The patients were classified to three groups as having peptic ulcers (n = 40), gastric ulcers (n = 35) and duodenal ulcers (n = 5). Each group was compared with non-ulcer dyspepsia (n = 40). There was no association between the combined genotype of *vacA* s2, *vacA* m and *iceA* and clinical outcome.

Table 15. Multivariate analysis of interaction of *vacA* s 2, *vacA* m and *iceA* subtypes in *H. pylori* and clinical outcome

Virulence Gene		*P* value		
		PUD vs NUD		
		GU vs NUD	DU vs NUD	Total PUD vs NUD
vacA s2	*vacA* m1	0.22	0.43	0.31
	vacA m1a	0.92	1.00	0.87
	vacA m1b	0.22	0.43	0.31
	vacA m2	0.28	0.25	0.39
	iceA1	0.42	1.00	0.34
	iceA2	0.25	1.00	0.16
	mixed *iceA*	0.35	0.11	0.16

PUD: Peptic Ulcer Disease (n =40)

NUD: Non-Ulcer Dyspepsia (n =40)

DU: Duodenal Ulcer (n =5), GU: Gastric Ulcer (n =35)

4.2.6 The interaction of genes *vacA* s1 a, *vacA* m and *iceA* subtype in *H. pylori* and clinical outcome

The *vacA* subtype a , *vacA* m-region and subtype (m1, m2, m1a and m1b) and *iceA* type (*iceA1*, *iceA2* and mixed *iceA*) were combined and analyzed in relation to the clinical outcome (Table 16). The result demonstrated the association between *vacA* s1a, mixed *iceA* genotype and peptic ulcer disease ($P = 0.04$, OR = 2.51, 95 %, CI 1.05 – 6.04)

Table 16. Multivariate analysis of interaction of *vacA* s1a, *vacA* m and *iceA* subtypes in *H. pylori* and clinical outcome

Virulence Gene		*P* value		
		PUD vs NUD		
		GU vs NUD	DU vs NUD	Total PUD vs NUD
vacA s1a	*vacA* m1	0.61	0.16	0.78
	vacA m1a	0.20	1.00	0.16
	vacA m1b	0.61	0.16	0.78
	vacA m2	0.09	0.72	0.13
	iceA1	0.78	0.66	0.79
	iceA2	0.66	1.00	0.47
	mixed *iceA*	0.07	0.19	0.04[a]

[a] ($P = 0.04$, OR = 2.51, 95 %, CI 1.05 – 6.04)

PUD: Peptic Ulcer Disease (n =40)

NUD: Non-Ulcer Dyspepsia (n =40)

DU: Duodenal Ulcer (n =5)

GU: Gastric Ulcer (n =35)

4.2.7 The interaction of genes *vacA* s1c , *vacA* m and *iceA* subtype in *H. pylori* and clinical outcome

The *vacA* subtype c , *vacA* m-region and subtype (m1, m2, m1a and m1b) and *iceA* type (*iceA1*, *iceA2* and mixed *iceA*) were combined and analyzed in relation to the clinical outcome (Table 17). Although these difference between the groups were not statistically significant ($P > 0.05$). There was no association between the combined genotype of *vacA* s1c, *vacA* m and *iceA* and clinical outcome.

Table 17. Multivariate analysis of interaction of *vacA* s1c, *vacA* m and *iceA* subtypes in *H. pylori* and clinical outcome

Virulence Gene		*P* value		
		PUD vs NUD		
		GU vs NUD	DU vs NUD	Total PUD vs NUD
vacA s1c	*vacA* m1	0.10	0.51	0.14
	vacA m1a	1.00	1.00	1.00
	vacA m1b	0.10	0.51	0.14
	vacA m2	0.56	0.16	0.73
	iceA1	0.09	1.00	0.07
	iceA2	0.10	1.00	0.06
	mixed *iceA*	0.35	0.33	0.25

PUD: Peptic Ulcer Disease (n =40)

NUD: Non-Ulcer Dyspepsia (n =40)

DU: Duodenal Ulcer (n =5), GU: Gastric Ulcer (n =35)

From the statistic analysis for the association between *cagA, vacA* and *iceA* genes and clinical outcome, there was no difference in the prevalence of *cagA* between patients with peptic ulcer disease and those with non-ulcer dyspepsia. The *vacA* s region (s1, s2) and m region were not associated with peptic ulcer disease ($P > 0.05$). The genotype *iceA* which includes *iceA1*, *iceA2* and mixed *iceA* was not associated with clinical outcome. On the other hand, combination of *vacA* s1a, mixed *iceA* genotype was associated with peptic ulcer disease ($P = 0.04$). Statistical analysis showed the association of *vacA* s1a, mixed *iceA* genotype was the only predictive factor for peptic ulcer disease.