CHAPTER IV

DISCUSSION

Gastric cancer is associated with many factors, such as sex, age, food, ethnicity, smoking, environmental risk, and more. In the last decade, much attention has been focused on the role of *H. pylori* infection in the development of the disease. Correa, in his multistep multifactorial model of gastric carcinogenesis, has proposed that *H. pylori* play a pivotal role in trigging chronic inflammation of the stomach, leading to the stepwise development of malignancy (1). El-Omar *et al.* first showed the correlation between the genetic factor of IL-1B polymorphism and gastric carcinogenesis in Scottish and Polish subjects (8). This finding was subsequently confirmed in other ethnic groups in the US (43), European (19), Japanese (44), Chinese (45) and Korean (46) populations.

Samloff and his co-workers purified pepsinogen I and pepsinogen II from gastric mucosa and developed a radioimmunoassay for serum pepsinogen II and an improved radioimmunoassay for serum pepsinogen I (42). After that, this method was widely used and applied to many techniques such as chemiluminescent enzyme immunoassay (CLEIA), chemiluminescent immunoassay (CLIA), enzyme immunoassay (EIA), enzyme-linked immunosorbentassay (ELISA), and immunoradiometric assay (IRMA). Thus, plasma pepsinogen measurement should be a useful method for gastric cancer screening and many gastro-intestinal abnormalities (16, 17).

Previous studies have shown that IL-1β is also a potent inhibitor of gastric acid secretion. On a molar basis, it is estimated to be 100-fold more potent than proton pump inhibitors and 6,000-fold more potent than histamine antagonists. There are conflicting data regarding the functional effects of these polymorphisms on IL-1β production (4, 5). Furuta *et al.* found that different genotypes of IL-1B influence gastric acid secretion (7).

In this study, we have optimized various parameters of PCR to detect the IL-1B -511 mutation. Especially important for successful PCR assays are the cycling temperature and time, the concentration of the PCR buffer, and the balance between the dNTP and $MgCl_2$ concentration. These parameters were titrated to provide the best results. A clear single band of amplicon and

RFLP products, each specially sized products, was observed without any complications neither nonspecific background band nor the formation of primer-dimer.

The annealing temperature was found to be one of the most important parameters. Variation of the annealing temperature from 56 °C to 67 °C had an alteration of the amplification efficiency. In this study, the best result of annealing temperature was found at 64 °C (Figure 16. lane 4). The temperature and length of time required for primer annealing depend upon the base composition, length, and concentration of the amplification primers.

The extension time depends upon the length and concentration of the target sequence and upon temperature. The primer extension temperature was chosen from the temperature that was optimal for extension primers on the genomic DNA template. This temperature was found to be 74 °C for 1 minute, which gave high amounts of product and no non-specific binding.

The dNTP concentration was kept constant (200 µmole), while the $MgCl_2$ concentration was increased stepwise from 1.5 mM to 3.5 mM. At a $MgCl_2$ concentration of 2.5 mM, the results showed an optimum of the PCR reaction with a satisfactory amount of product (Figure 18.lane 3).

The primer concentration in this study varied from 100 pmol to 250 pmol. A value of 150 pmol of primers gave the best result, without visible dimer-primer formation and satisfactory amounts of product (Figure 17. lane 2). We also used 12% non-denaturing polycrylamide gel to determine the products of restriction enzyme *Ava*I. Because the resolving power is so great that separate molecules of DNA whose lengths differ little can be separated.

The present study shows that in northern Thai populations, decreased plasma pepsinogen I is a risk factor for gastric cancer. We found that pepsinogen I levels were significantly lower in subjects who developed gastric cancer than in the benign gastritis group. In contrast, no difference was found in plasma pepsinogen II levels between the two groups. Hence, the trend in the pepsinogen I/II ratio was lower in gastric cancer patients than in the benign gastritis group. Cut-offs found in this study were pepsinogen I $\leq$ 50 ng/ml, and the pepsinogen I/II ratio was $\leq$ 2.4. These data show the usefulness of monitoring abnormal plasma pepsinogen levels for the identification of gastric cancer. Our results were quite similar to the Japanese data, as first reported by Miki *et al.*, where the serum pepsinogen levels of stomach cancer patients, especially pepsinogen I and the pepsinogen I/II ratio were significantly lower than those of normal controls and correlated well with the extent of chronic gastritis associated with the cancerous stomach with

cut-offs of pepsinogen I $\leq$ 50 ng/ml and pepsinogen I/II $\leq$ 3.0. Although Miki *et al.* reported later that pepsinogen I $\leq$ 70 ng/ml and pepsinogen I/II $\leq$ 3.0 were more suitable cut-offs for the detection of gastric cancer (16, 17).

The presence of the IL-1B -511 T/T genotype did not appear to increase further the risk of gastric cancer in the northern Thai population. This T/T genotype lack of synergistic effects to gastric cancer might be related to the equal frequency of the C/C genotype (0.25) and T/T genotype (0.23) in this population or the presence of another potent environmental factor, such as dietary habit, age, *H.pyroli* etc. In contrast with studies in western countries, the relationship between IL-1B polymorphism and gastric cancer has been less clear cut in studies from Asia. In Korea, Ryu *et al.* reported that Il-1B polymorphisms at loci -511 and -31 were not associated with *H.pyroli* infection and the risk of gastric cancer (47). Kato *et al.* failed to demonstrate a positive association between the IL-1B -511 C allele and gastric cancer in their Japanese cohort of 699 subjects; they suggested that Japanese individuals with the IL-1B-511 allele might be more likely to progress to mucosal atrophy with intestinal metaplasia (48).

We should consider the interaction between *H.pylori* infection and IL-1B genotype polymorphisms. The process of *H.pylori* induced malignant transformation in the gastric mucosa takes decades to develop. Zeng *et al.* attempted to study the interaction between *H.pylori* infection and IL-1B polymorphism in Chinese populations. They found that if an infected individual also had the proinflammatory genotype IL-1B-511 T/T, the risk of gastric carcinogenesis was dramatically elevated to over 17-fold (45).

We proceeded to show that a decrease in the pepsinogen I/II ratio was most evident in gastric cancer groups with the most proinflammatory genotype IL-1B-511 T/T. This observation suggests the development of gastric cancer in the T/T genotype, C/T genotype, and T carrier groups and might explain the correlation with IL-1B-511 polymorphism and the increase in gastric juice pH. As Furuta *et al.* reported a result of having more extensive gastritis and less acid production, it is possible that the T/T or C/T genotypes might be more likely to be exposed to exogenous and endogenous stimulants, including procarcinogens, compared with an increased risk of gastric cancer (7). Furuta *et al.* also reported later that the most proinflammatory IL-1B-511 genotype, namely T/T, was associated with the highest median fasting gastric juice pH, the highest gastritis score, and the highest atrophy scores in both antrum and corpus. They proceeded

to show that age-dependent decreases in pepsinogen I levels and pepsinogen I/II ratio were most evident in subjects with the most proinflammatory genotype IL-1B-511 T/T. This finding suggests that the progression of severe gastritis toward gastric atrophy in the T/T genotype group was accelerated in comparison with the C/T and C/C genotype groups, and this might explain the association with the age dependent increase in gastric juice pH. Another interpretation is that the proinflammatory genotype leads to profound functional inhibition of gastric acid secretion and thus greater damage to the parietal cell mass within the corpus, leading to progressive atrophy and the onset of permanent hypochlorhydria (44).

In benign gastritis groups, we found that the pepsinogen I/II ratio was decreased in the C/C genotype groups, but there was no correlation with C/C genotype and T carrier groups. It is possible that benign gastritis with C/C genotype group have more severe gastritis. This phenomenon should be clarified by investigating the degree of inflammation and atrophy scores for subjects undergoing gastroscopy. The gastritis region is also associated with pepsinogen levels because the main source of pepsinogen is from the corpus rather than antral part of stomach. Keith *et al.* found significantly raised levels of serum pepsinogen I in cases of antral-predominant gastritis, but lacked evidence of corpus or fundus atrophy. The few cases of severe atrophic gastritis of the corpus in his study had very low levels of serum pepsinogen I and a reduced serum pepsinogen I/II ratio (49). Kitahara et al. also shown the effects of sex, age, smoking and drinking were reduce the level of pepsinogen I/II ratio and also pepsinogen II level in non-gastric cancer group (50). According to his study, we divided the benign gastritis and gastric cancer into two groups by age, more than 40 years and 50 years. The lower ratio of pepsinogen I/II was seen in subjects $\geq$ 40 years of age ($p<0.05$) and also in subjects $\geq$ 50 years of age ($p<0.05$) when compared with < 40 and < 50 years of age in benign gastritis subjects (Figure 33 A and Figure 34 A). While in the gastritis cancer subjects, the age was independent of the pepsinogen I/ II ratio ($p>0.05$).

H.pylori should be another one factor that might be related with a decrease of the pepsinogen I/II ratio. However, the status of *H.pylori* infection in the subjects was not determined in the present study. The prevalence of *H.pylori* is known to be considerably higher in Japanese populations. Fukuda *et al.* reported in a case control study that the percentage of positive anti-*H.pylori* antibody in control subjects was 73.9% (17). Asaka *et al.* reported that 74.3% of control

subjects were *H.pylori*-positive in the Japanese population. They showed that *H.pylori* infection is associated with a raised serum pepsinogen II level and a decreased pepsinogen I/II ratio (51). Furthermore, the majority of *H.pylori* strain was expressed as cytotoxin-associated gene A (CagA), which is known to be associated with more active mucosal inflammation and thus higher levels of serum pepsinogen I and II than other strains (52).

Our study verified that both the study of IL-1 β mutation and the measurement of pepsinogen levels can be used as screening tests for high risk subjects with gastric cancer in the northern Thai population. That is, it is more sensitive, it is easy to carry out, and patients do not feel much discomfort compared to screening by gastrointestinal endoscopies and barium X-rays. Determination of the IL-1B-511 gene and measurement of the plasma pepsinogen levels are safe, there is no radiation exposure, and there are no side effects similar to those experienced with barium ingestion. These methods are inexpensive, fast, and many blood samples can be analyzed simultaneously.