CHAPTER III

RESULTS

3.1 GENOMIC DNA PREPARATION

The 130 genomic DNA samples were extracted from peripheral blood of both, 89 cases normal control and 41 cases gastric cancer patients. The yield of every sample were approximately range 150-850 μg/ml and OD260/OD280 ratio were between 1.5-2.0.

3.2 POLYMERASE CHAIN REACTION

3.2.1 Optimization of PCR

The PCR process is widely employed in a tremendous variety of experimental applications to produce high yields of specific DNA target sequences. Since no single set of conditions can be applied to all PCR amplifications, individual reaction component concentrations (and time and temperature parameters) must be adjusted within suggested ranges for efficient amplification of specific targets. While there are a number of possible concentration parameters, logical titrations of interrelated reaction components can be readily defined. In addition, the time and temperature optima can often be determined within a few experiments.

In this study, for the optimization of PCR reaction components, the primer were varied from 100, 150 and 250 pmol. Too much primer, possibly because of primer-dimers (Figure 17). In this experiment, the annealing temperatures were varied from 56°C to 67°C (56 °C, 59 °C, 61 °C, 64 °C and 67°C) as shown in Figure 16. We found that at 64°C represented obviously annealing result without non-specific bands. Finally, the $MgCl_2$ were varied from 1.5-3.5 mM, the most efficient $MgCl_2$ concentration shown at 2.5 mM in Figure 18.

3.2.2 PCR-base diagnosis of IL-1B

After the genomic DNA was amplified by using appropriate conditions. The PCR products, which provided a clear single band at the expected size of 155 bp, as shown in the "a"-lanes of Figures 19-31. The 50 bp of nucleic acid molecular weight markers was shown in the lane "M" of each figures.

3.3 RESTRICTION ENZYME *Ava*I CUT

Samples generated the DNA fragments of 155 bp were interpreted as homozygousity of T base or T/T genotype that uncut by *Ava* I, whereas samples generated the DNA fragments of 88 bp and 67 bp interpreted as homozygousity of C base or C/C genotype that completely cut by *Ava*I. The samples shown partially cut that generated the DNA fragments of 155bp, 88 bp and 67 bp interpreted as heterzygosity or C/T genotype.

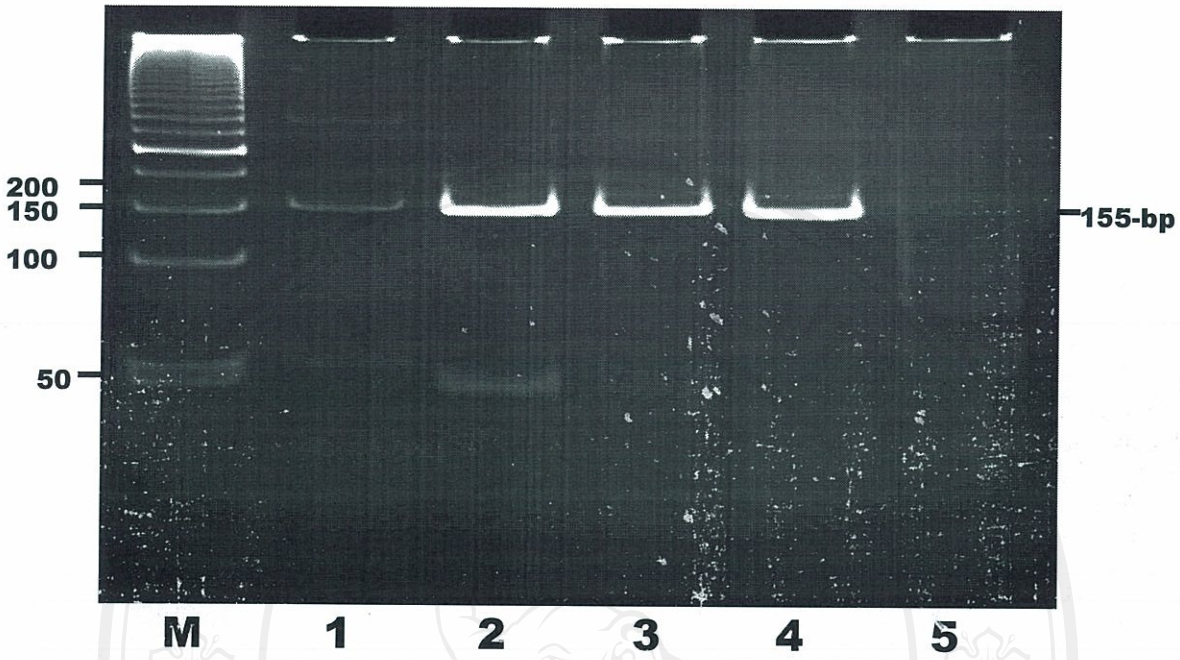

Figure 16. The optimization of the annealing temperatures for detection of -511 promoter mutation of IL-1β gene by PCR.

The anncaling temperatures were adjusted to provide the best result. The annealing temperatures were varied from 56°C to 67°C respectively. The PCR products were subjected to electrophoresis on 12% polycrylamide gel in 1x TBE buffer. Specifically sized PCR products were obtained for the 155-bp.

Lane M = standard DNA 50-bp ladder
Lane 1 = annealing temperature at 56°C
Lane 2 = annealing temperature at 59°C
Lane 3 = annealing temperature at 61°C
Lane 4 = annealing temperature at 64°C
Lane 5 = annealing temperature at 67°C

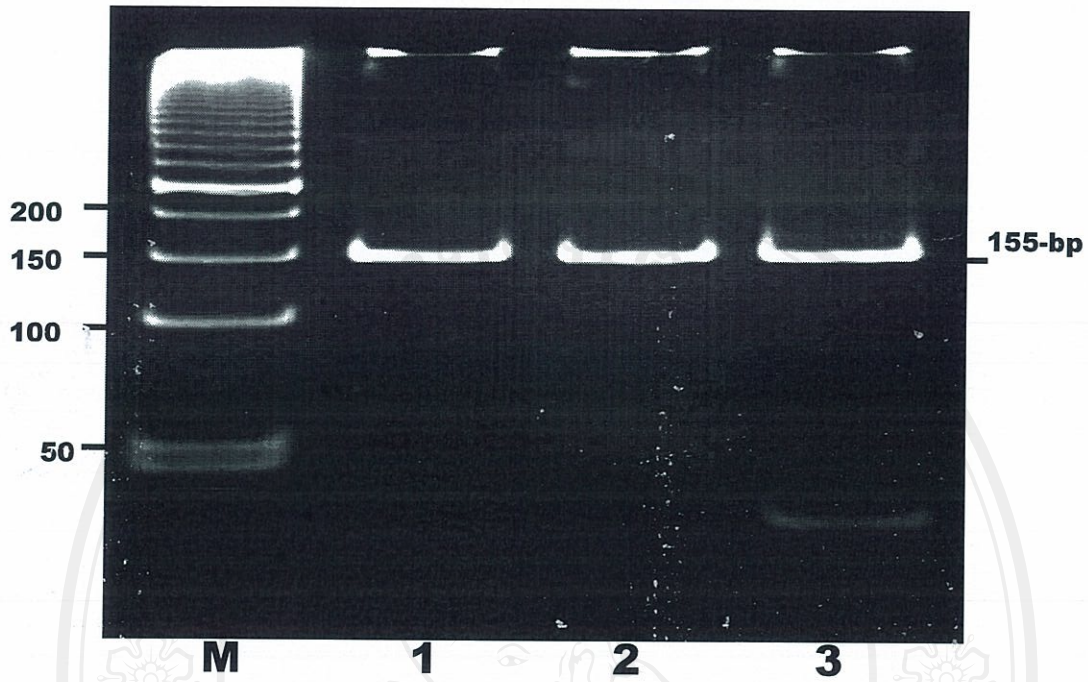

Figure 17. The optimization of the primer for detection of -511 promoter mutation of IL-1β gene by PCR.

The primer were adjusted to provide the best result. The primer were varied from 100 pmol to 250 pmol respectively. The PCR products were subjected to electrophoresis on 12% polycrylamide gel in 1x TBE buffer. Specifically sized PCR products were obtained for the 155-bp.

Lane M = standard DNA 50-bp ladder

Lane 1 = 100 pmol of primers

Lane 2 = 150 pmol of primers

Lane 3 = 250 pmol of primers

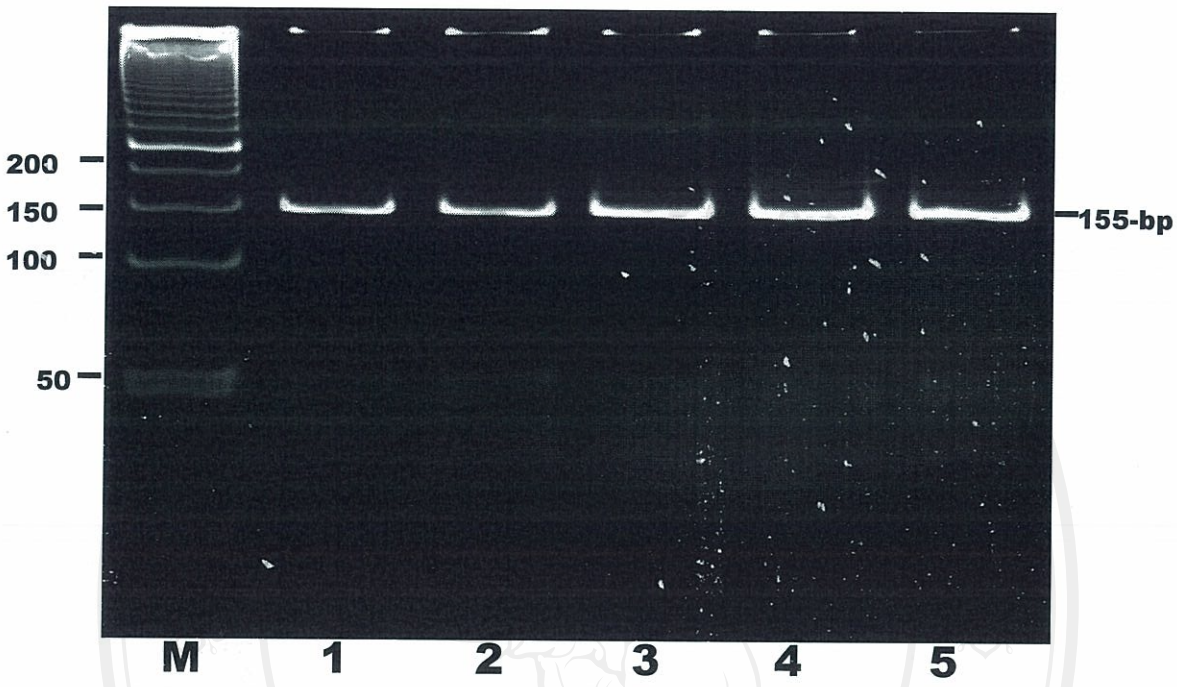

Figure 18. The optimization of the $MgCl_2$ concentration for detection of -511 promoter mutation of IL-1β gene by PCR.

The $MgCl_2$ were adjusted to provide the best result. The $MgCl_2$were varied from 1.5 mM to 3.5 mM respectively. The PCR products were subjected to electrophoresis on 12% polycrylamide gel in 1x buffer. Specifically sized PCR products were obtained for the 155-bp.

Lane M = standard DNA 50-bp ladder

Lane 1 = 1.5 mM of $MgCl_2$

Lane 2 = 2.0 mM of $MgCl_2$

Lane 3 = 2.5 mM of $MgCl_2$

Lane 4 = 3.0 mM of $MgCl_2$

Lane 5 = 3.5 mM of $MgCl_2$

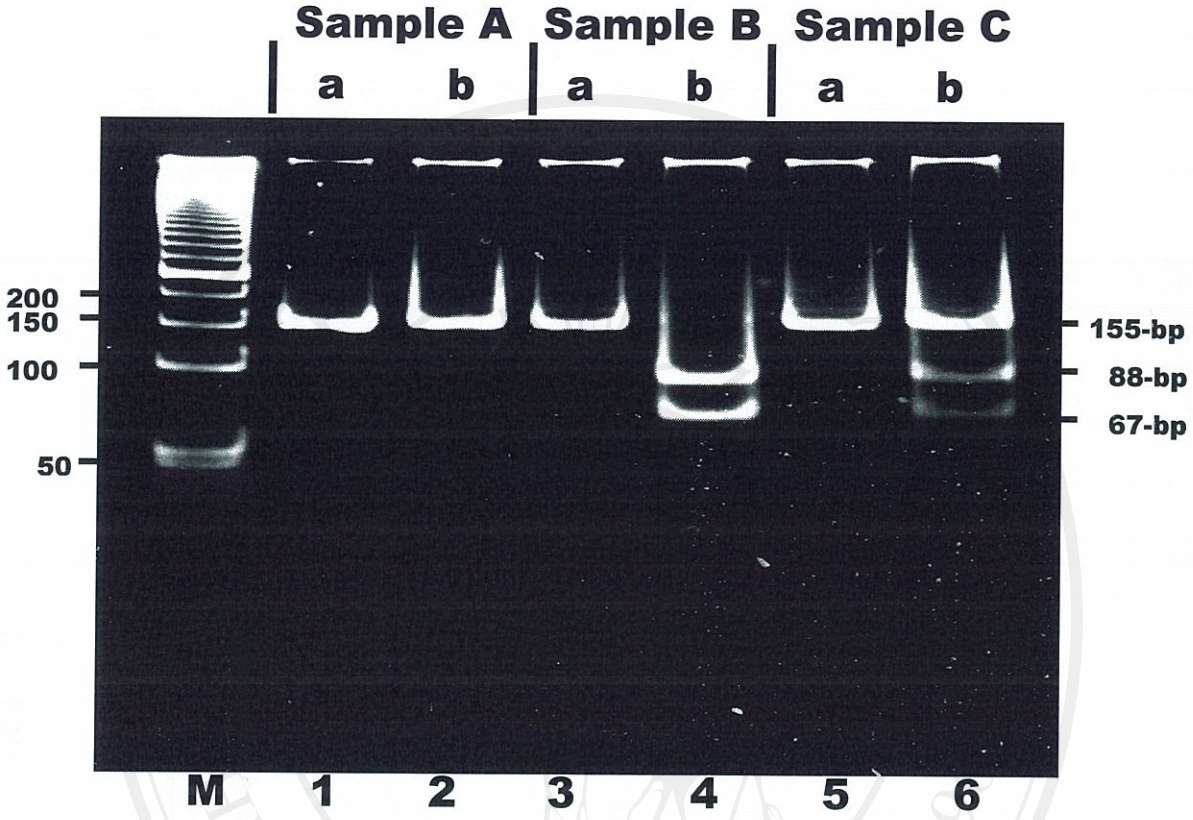

Figure 19. RFLP Pattern from Peripheral Blood Samples after Cut with *Ava*I Restriction Enzyme.

The sample A, B and C representing the different of C-T base transition of -511 from the transcription start site of IL-1B gene. The PCR products before cutting with *Ava*I are shown in lane "a" and after cutting are shown in lane "b". The standard DNA 50-bp ladder was shown in the lane "M". Sample A lane "b" were interpreted as homozygousity of T base or T/T genotype that uncut by *Ava*I, whereas sample B lane "b" interpreted as homozygousity of C base or C/C genotype that completely cut with *Ava*I. The sample C lane "b" that shown partially cut interpreted as heterzygosity or C/T genotype. They were run on 12 % polyacrylamide gel in 1x TBE buffer.

Lane M = standard DNA 50-bp ladder

Lane 2 = T/T genotype

Lane 4 = C/C genotype

Lane 6 = C/T genotype

Lane 1, 3, 5 = PCR products before cutting with *Ava*I size 155-bp

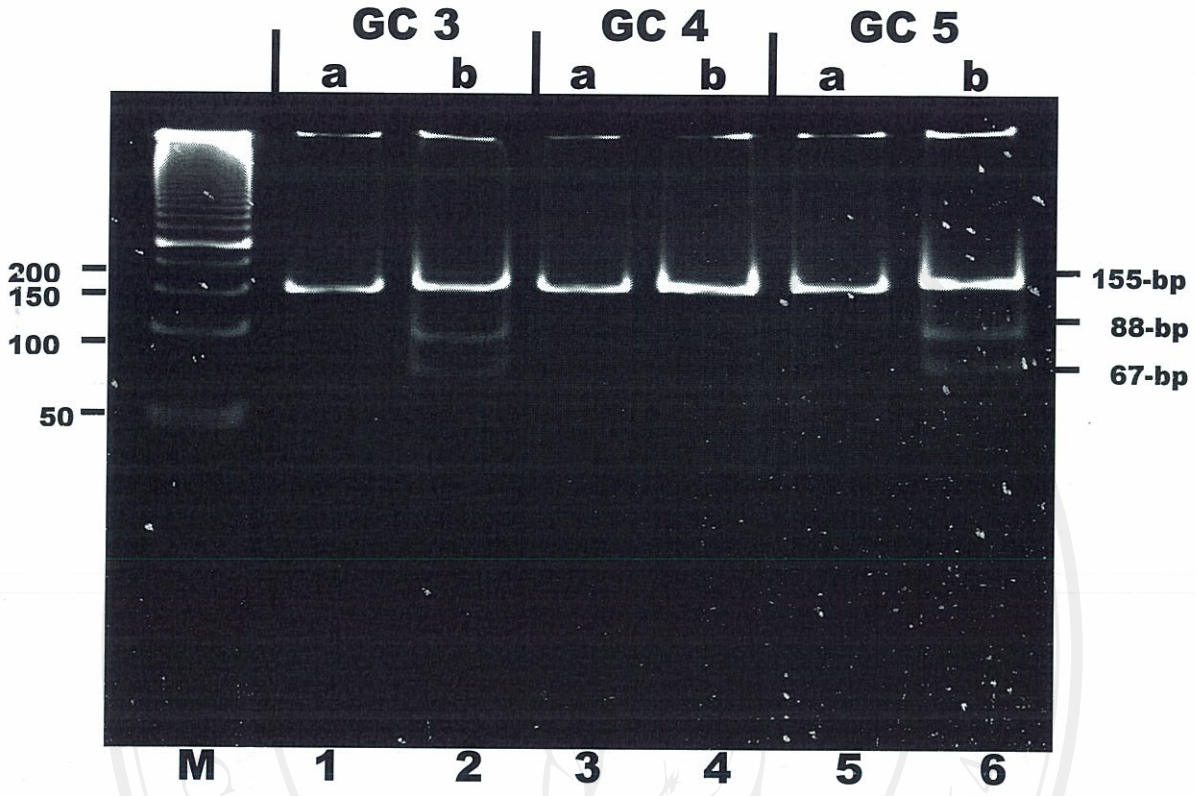

Figure 20. RFLP Pattern from Gastric Cancer's Peripheral Blood Samples after Cut with *Ava*I Restriction Enzyme.

The samples representing the different of C-T base transition of -511 from the transcription start site of IL-1B gene. The PCR products before cutting with *Ava*I are shown in lane "a" and after cutting are shown in lane "b". The standard DNA 50-bp ladder was shown in the lane "M". The number of each sample is shown at the above line, GC represents Gastric Cancer patients. They were run on 12 % polyacrylamide gel in 1x TBE buffer.

Lane M = standard DNA 50-bp ladder

Lane 2 = C/T genotype

Lane 4 = T/T genotype

Lane 6 = C/T genotype

Lane 1, 3, 5 = PCR products before cutting with *Ava*I size 155-bp

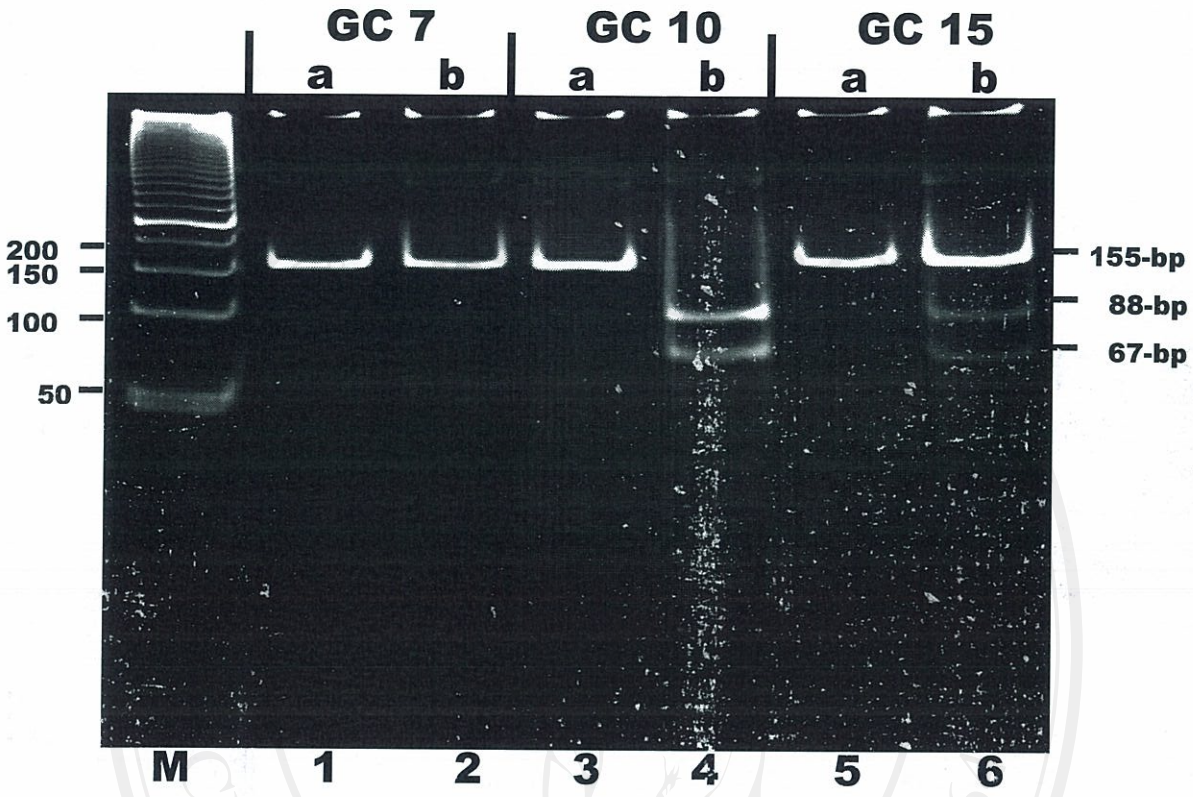

Figure 21. RFLP Pattern from Gastric Cancer's Peripheral Blood Samples after Cut with *Ava*I Restriction Enzyme.

The samples representing the different of C-T base transition of -511 from the transcription start site of IL-1B gene. The PCR products before cutting with *Ava*I are shown in lane "a" and after cutting are shown in lane "b". The standard DNA 50-bp ladder was shown in the lane "M". The number of each sample is shown at the above line, GC represents Gastric Cancer patients. They were run on 12 % polyacrylamide gel in 1x TBE buffer.

Lane M = standard DNA 50-bp ladder

Lane 2 = T/T genotype

Lane 4 = C/C genotype

Lane 6 = C/T genotype

Lane 1, 3, 5 = PCR products before cutting with *Ava*I size 155-bp

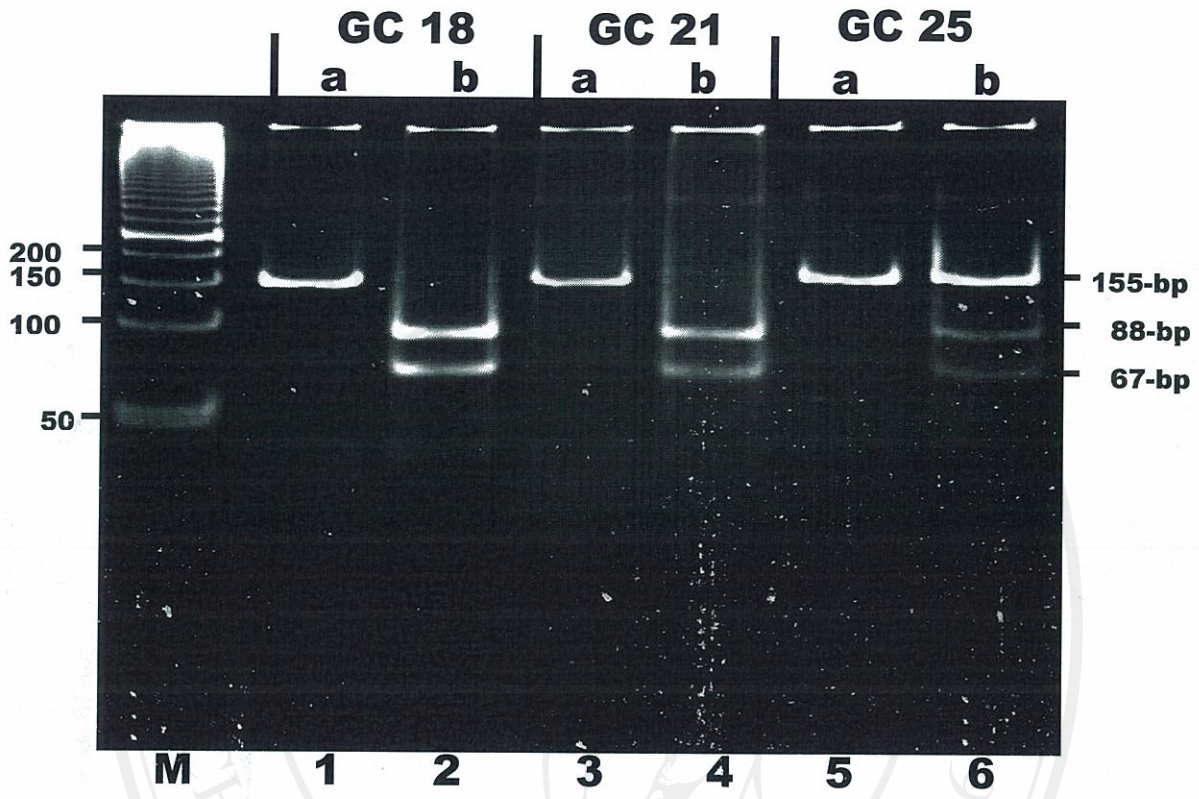

Figure 22. RFLP Pattern from Gastric Cancer's Peripheral Blood Samples after Cut with *Ava*I Restriction Enzyme.

The samples representing the different of C-T base transition of -511 from the transcription start site of IL-1B gene. The PCR products before cutting with *Ava*I are shown in lane "a" and after cutting are shown in lane "b". The standard DNA 50-bp ladder was shown in the lane "M". The number of each sample is shown at the above line, GC represents Gastric Cancer patients. They were run on 12 % polyacrylamide gel in 1x TBE buffer.

Lane M = standard DNA 50-bp ladder

Lane 2 = C/C genotype

Lane 4 = C/C genotype

Lane 6 = C/T genotype

Lane 1, 3, 5 = PCR products before cutting with *Ava*I size 155-bp

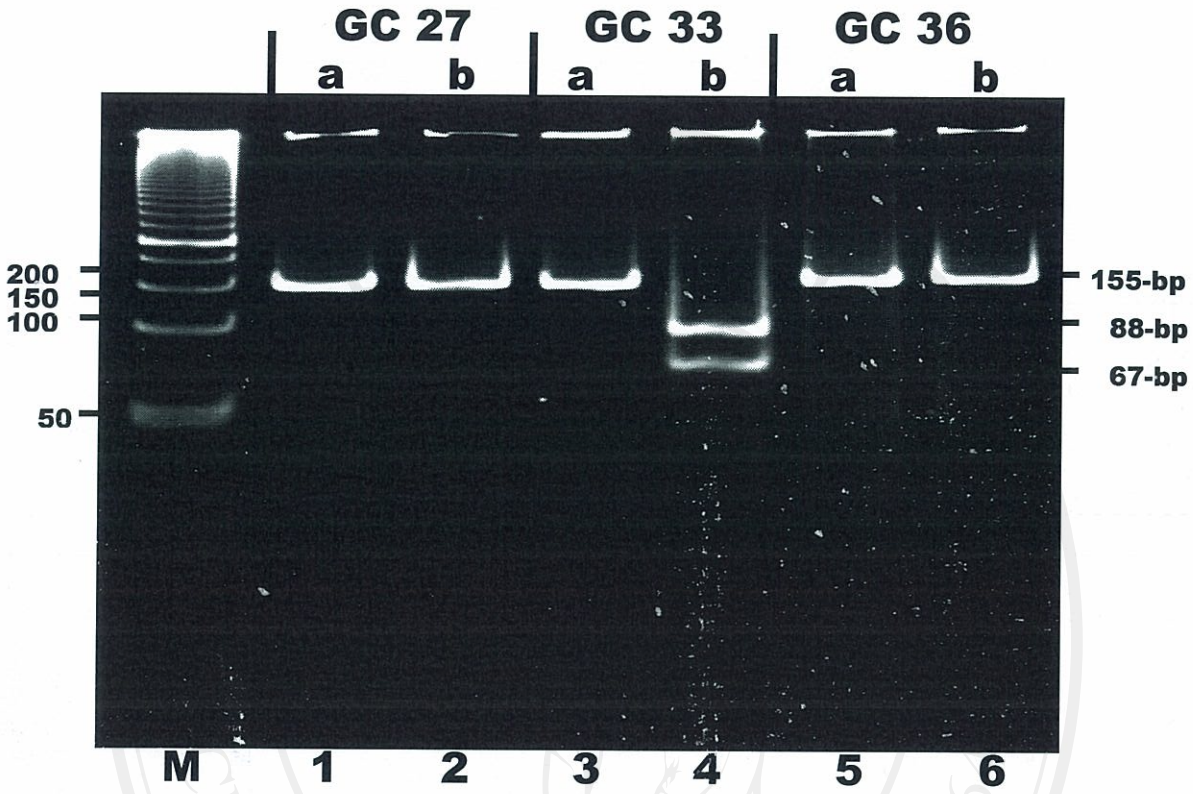

Figure 23. RFLP Pattern from Gastric Cancer's Peripheral Blood Samples after Cut with *Ava*I Restriction Enzyme.

The samples representing the different of C-T base transition of -511 from the transcription start site of IL-1B gene. The PCR products before cutting with *Ava*I are shown in lane "a" and after cutting are shown in lane "b". The standard DNA 50-bp ladder was shown in the lane "M". The number of each sample is shown at the above line, GC represents Gastric Cancer patients. They were run on 12 % polyacrylamide gel in 1x TBE buffer.

Lane M = standard DNA 50-bp ladder

Lane 2 = T/T genotype

Lane 4 = C/C genotype

Lane 6 = T/T genotype

Lane 1, 3, 5 = PCR products before cutting with *Ava*I size 155-bp

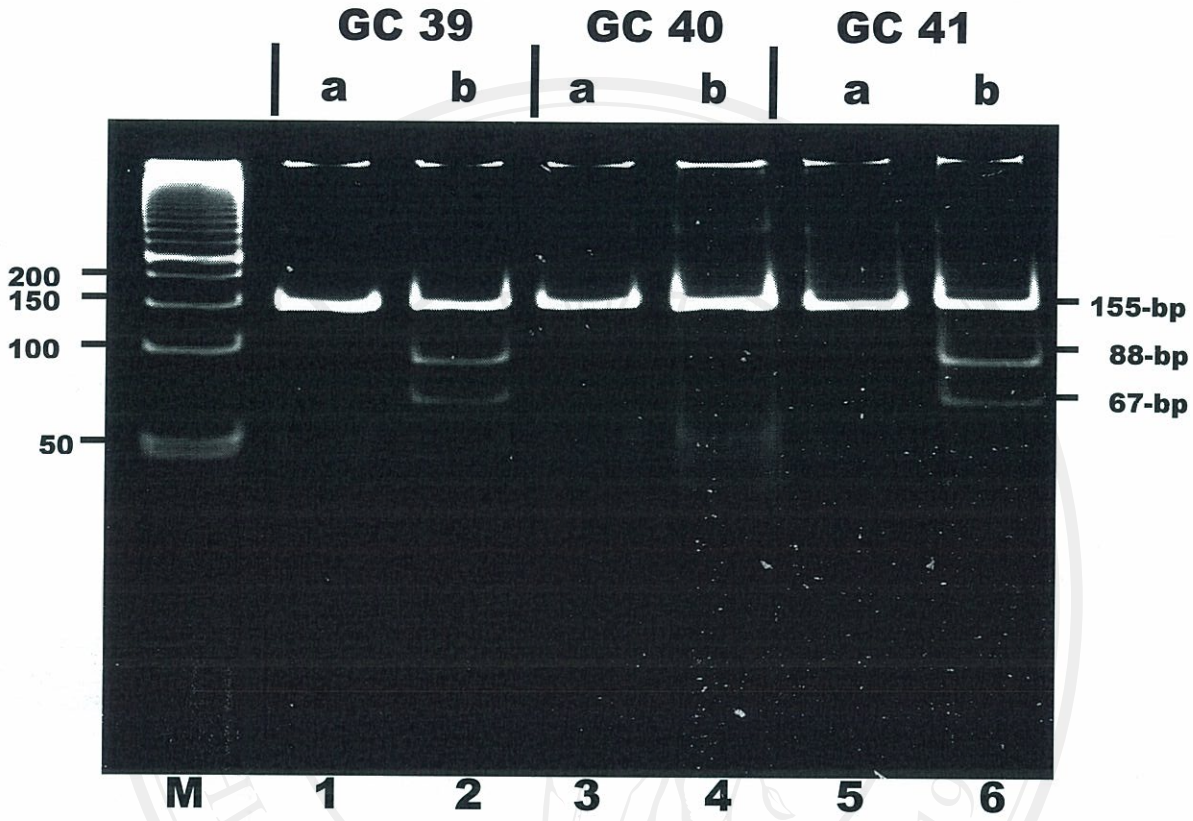

Figure 24. RFLP Pattern from Gastric Cancer's Peripheral Blood Samples after Cut with *Ava*I Restriction Enzyme.

The samples representing the different of C-T base transition of -511 from the transcription start site of IL-1B gene. The PCR products before cutting with *Ava*I are shown in lane "a" and after cutting are shown in lane "b". The standard DNA 50-bp ladder was shown in the lane "M". The number of each sample is shown at the above line, GC represents Gastric Cancer patients. They were run on 12 % polyacrylamide gel in 1x TBE buffer.

Lane M = standard DNA 50-bp ladder

Lane 2 = C/T genotype

Lane 4 = T/T genotype

Lane 6 = C/T genotype

Lane 1, 3, 5 = PCR products before cutting with *Ava*I size 155-bp

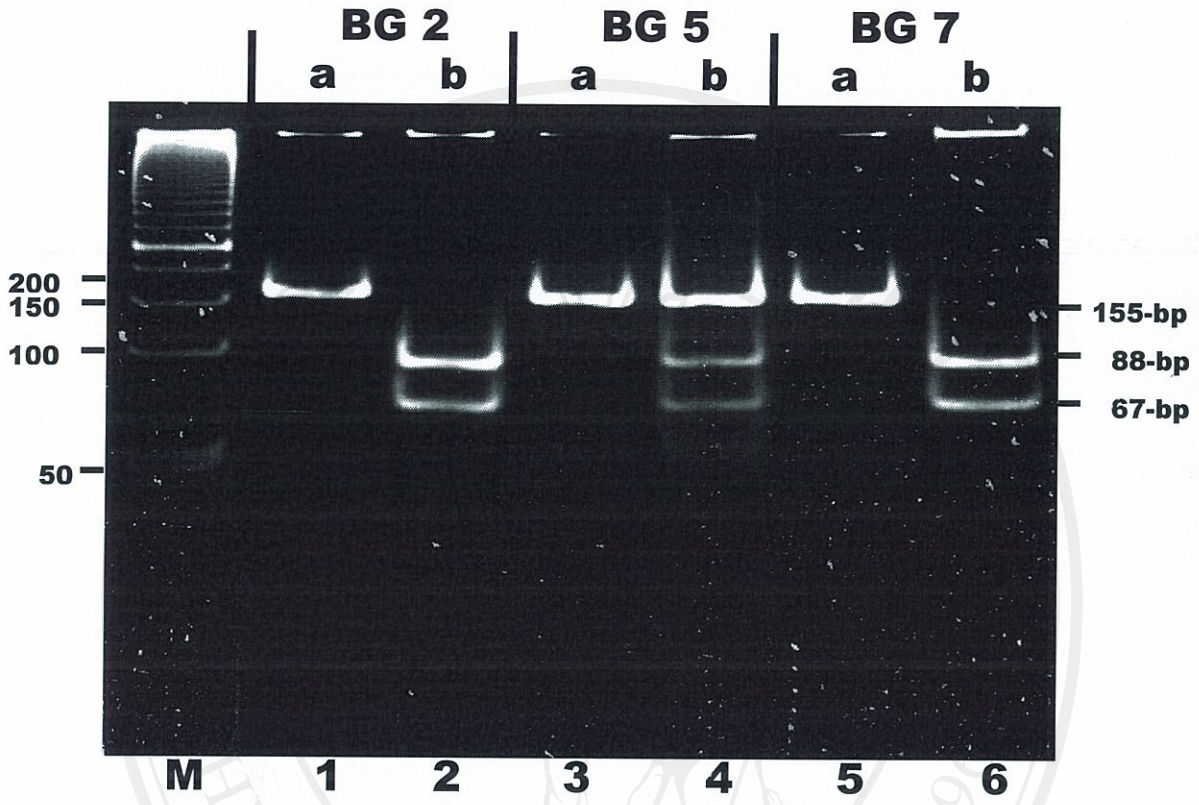

Figure 25. RFLP Pattern from Benign Gastritis Group's Peripheral Blood Samples after Cut with *Ava*I Restriction Enzyme.

The samples representing the different of C-T base transition of -511 from the transcription start site of IL-1B gene. The PCR products before cutting with *Ava*I are shown in lane "a" and after cutting are shown in lane "b". The standard DNA 50-bp ladder was shown in the lane "M". The number of each sample is shown at the above line, BG represents benign gastritis groups. They were run on 12 % polyacrylamide gel in 1x TBE buffer.

Lane M = standard DNA 50-bp ladder

Lane 2 = C/C genotype

Lane 4 = C/T genotype

Lane 6 = C/C genotype

Lane 1, 3, 5 = PCR products before cutting with *Ava*I size 155-bp

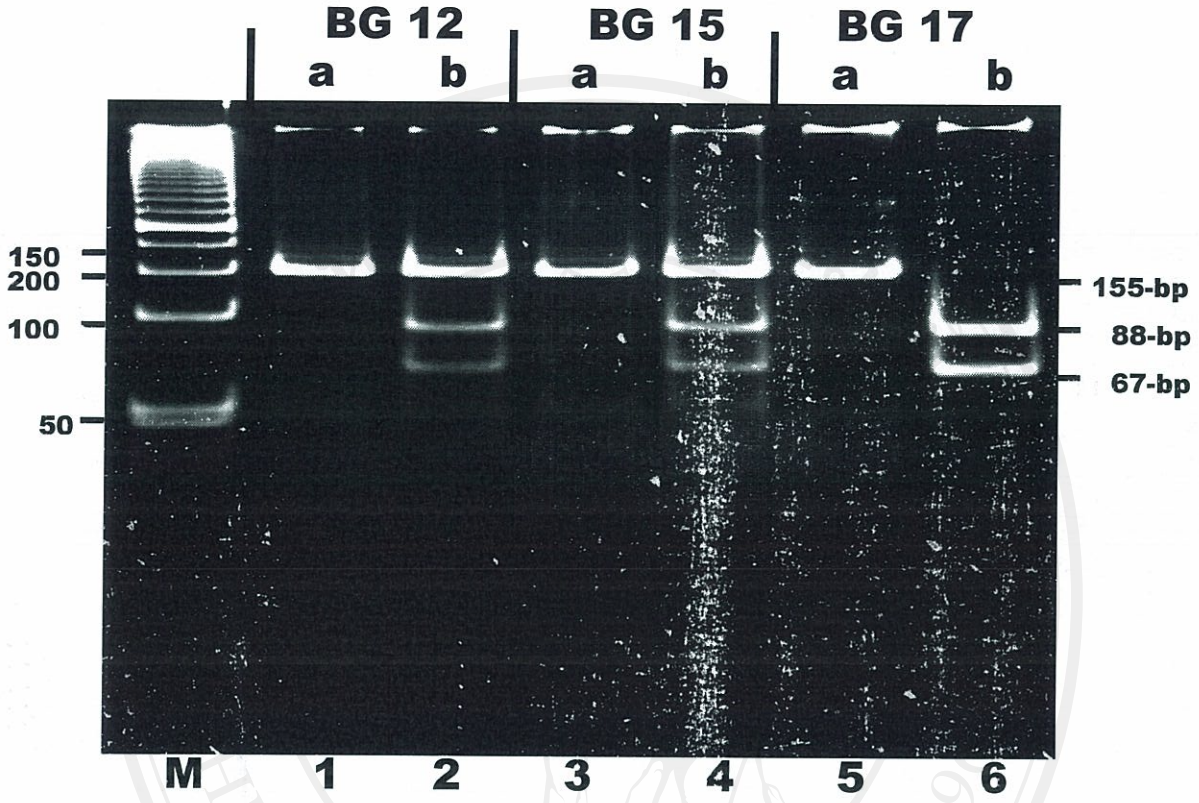

Figure 26. RFLP Pattern from Benign Gastritis Group's Peripheral Blood Samples after Cut with *Ava*I Restriction Enzyme.

The samples representing the different of C-T base transition of -511 from the transcription start site of IL-1B gene. The PCR products before cutting with *Ava*I are shown in lane "a" and after cutting are shown in lane "b". The standard DNA 50-bp ladder was shown in the lane "M". The number of each sample is shown at the above line, BG represents benign gastritis groups. They were run on 12 % polyacrylamide gel in 1x TBE buffer.

Lane M = standard DNA 50-bp ladder

Lane 2 = C/T genotype

Lane 4 = C/T genotype

Lane 6 = C/C genotype

Lane 1, 3, 5 = PCR products before cutting with *Ava*I size 155-bp

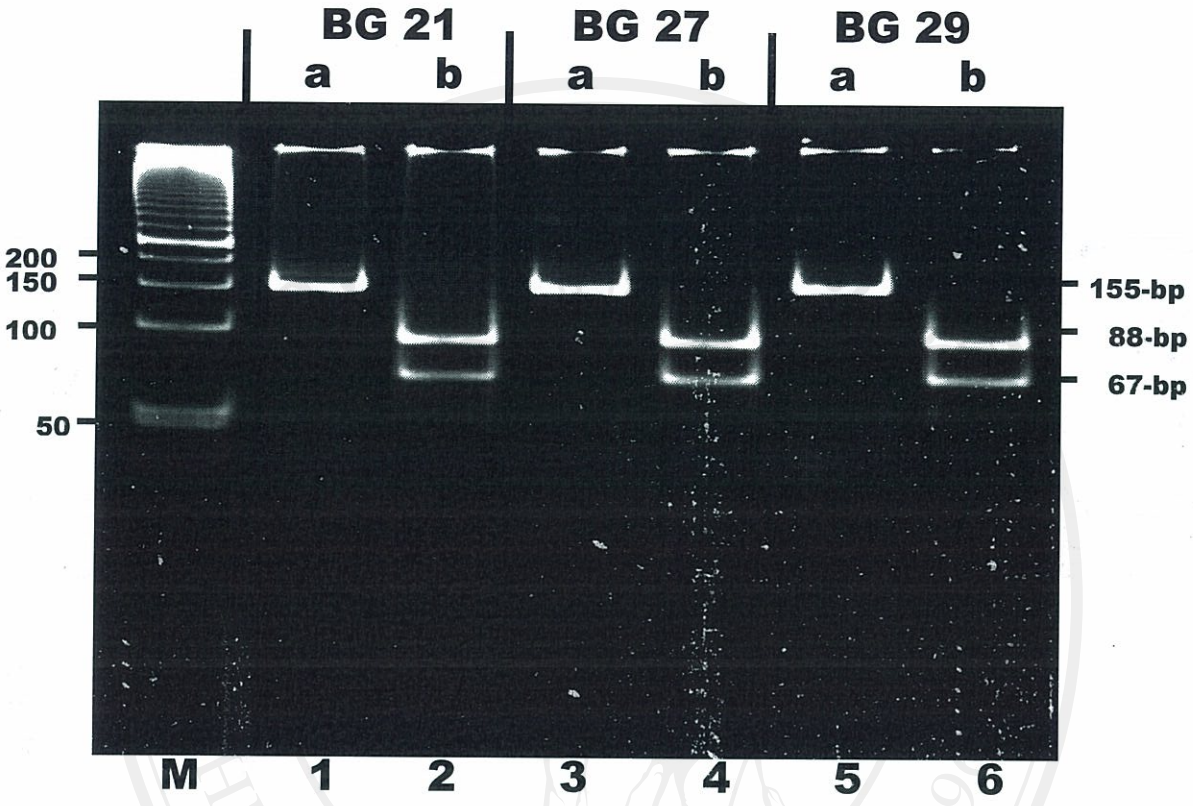

Figure 27. RFLP Pattern from Benign Gastritis Group's Peripheral Blood Samples after Cut with *Ava*I Restriction Enzyme.

The samples representing the different of C-T base transition of -511 from the transcription start site of IL-1B gene. The PCR products before cutting with *Ava*I are shown in lane "a" and after cutting are shown in lane "b". The standard DNA 50-bp ladder was shown in the lane "M". The number of each sample is shown at the above line, BG represents benign gastritis groups. They were run on 12 % polyacrylamide gel in 1x TBE buffer.

Lane M = standard DNA 50-bp ladder

Lane 2 = C/C genotype

Lane 4 = C/C genotype

Lane 6 = C/C genotype

Lane 1, 3, 5 = PCR products before cutting with *Ava*I size 155-bp

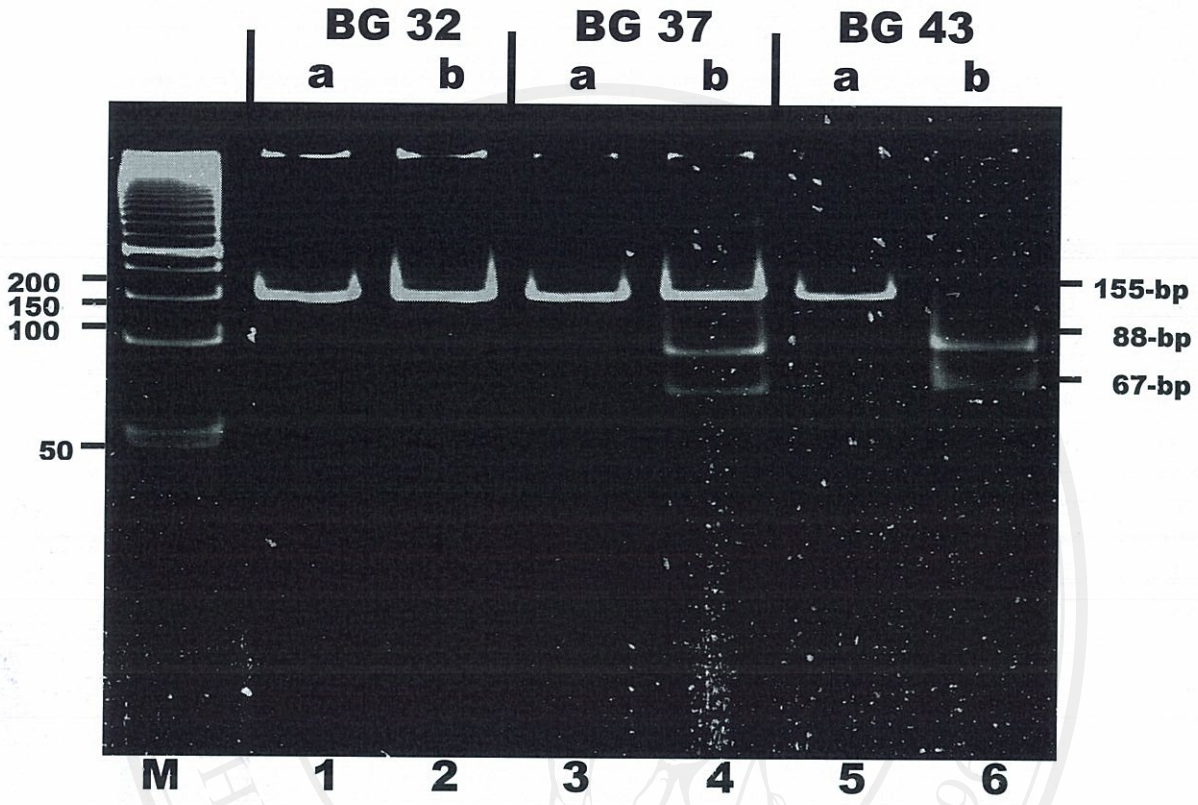

Figure 28. RFLP Pattern from Benign Gastritis Group's Peripheral Blood Samples after Cut with *Ava*I Restriction Enzyme.

The samples representing the different of C-T base transition of -511 from the transcription start site of IL-1B gene. The PCR products before cutting with *Ava*I are shown in lane "a" and after cutting are shown in lane "b". The standard DNA 50-bp ladder was shown in the lane "M". The number of each sample is shown at the above line, BG represents benign gastritis groups. They were run on 12 % polyacrylamide gel in 1x TBE buffer.

Lane M = standard DNA 50-bp ladder

Lane 2 = T/T genotype

Lane 4 = C/T genotype

Lane 6 = C/C genotype

Lane 1, 3, 5 = PCR products before cutting with *Ava*I size 155-bp

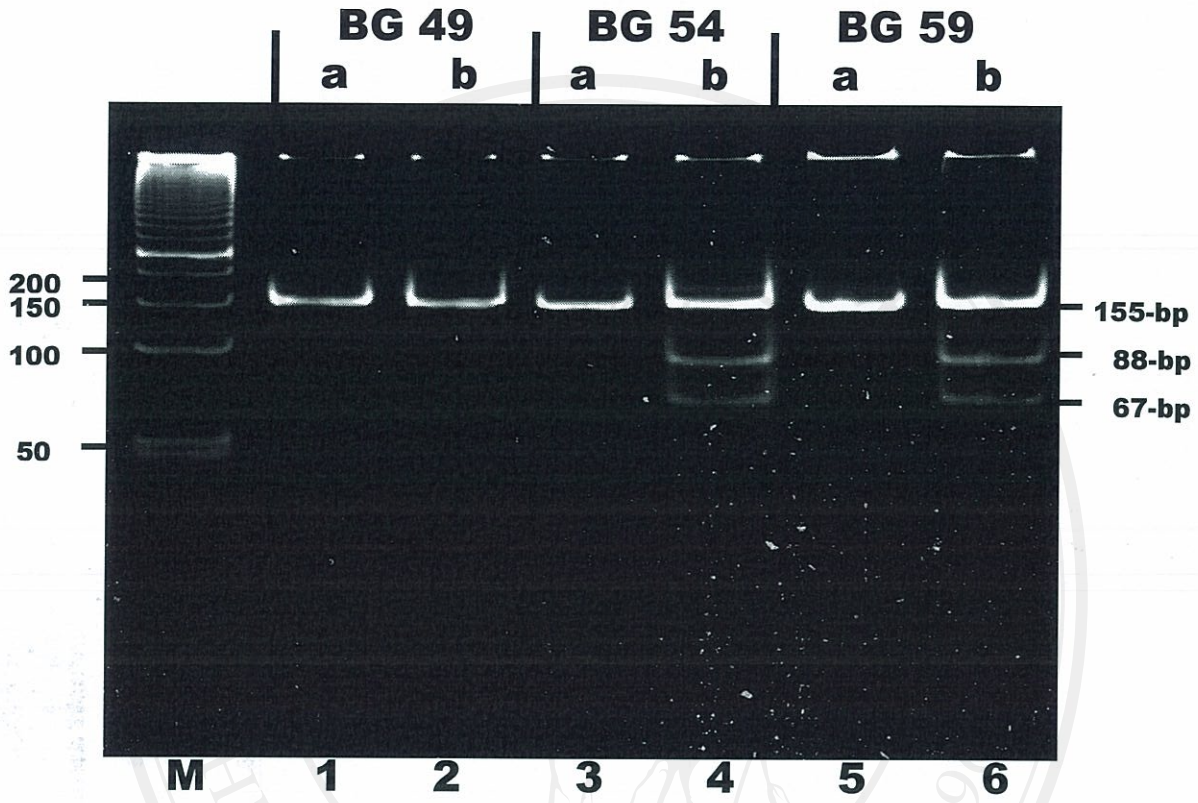

Figure 29. RFLP Pattern from Benign Gastritis Group's Peripheral Blood Samples after Cut with *Ava*I Restriction Enzyme.

The samples representing the different of C-T base transition of -511 from the transcription start site of IL-1B. PCR products before cutting with *Ava*I are shown in lane "a" and after cutting are shown in lane "b". The standard DNA 50-bp ladder was shown in the lane "M". The number of each sample is shown at the above line, BG represents benign gastritis groups. They were run on 12 % polyacrylamide gel in 1x TBE buffer.

Lane M = standard DNA 50-bp ladder

Lane 2 = T/T genotype

Lane 4 = C/T genotype

Lane 6 = C/T genotype

Lane 1, 3, 5 = PCR products before cutting with *Ava*I size 155-bp

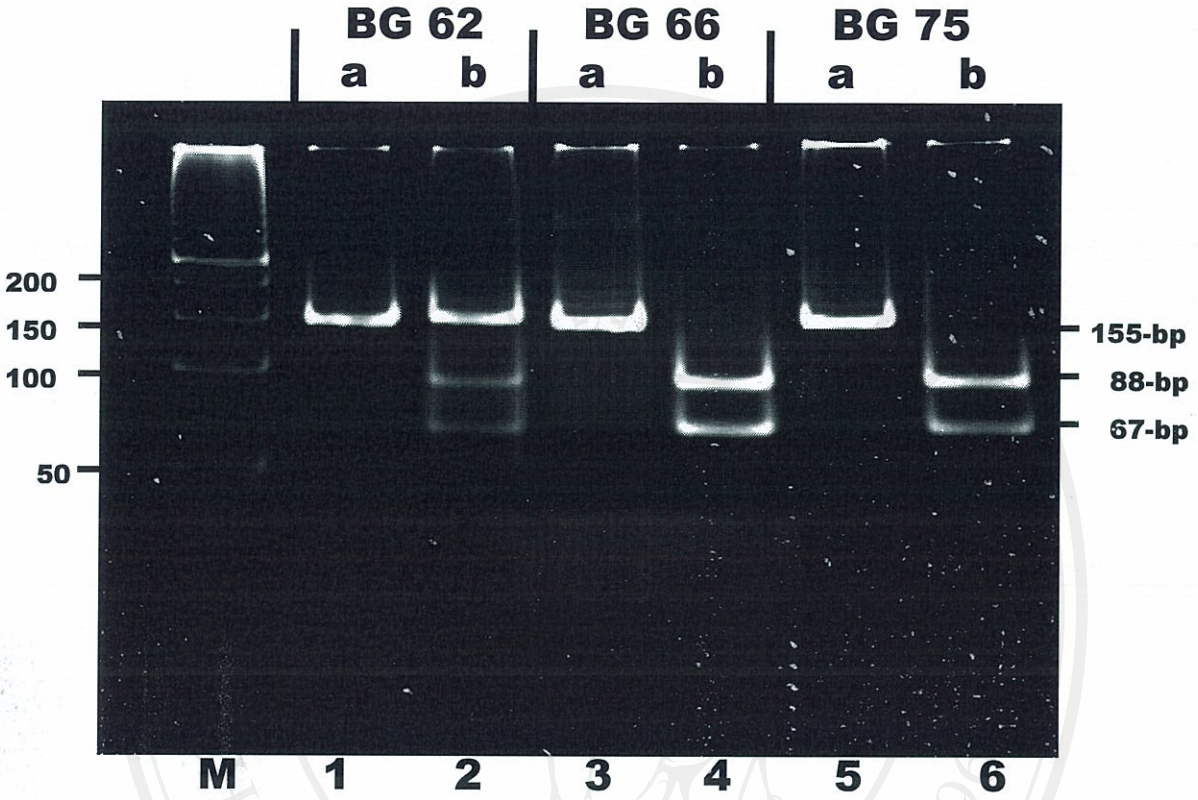

Figure 30. RFLP Pattern from Benign Gastritis Group's Peripheral Blood Samples after Cut with *Ava*I Restriction Enzyme.

The samples representing the different of C-T base transition of -511 from the transcription start site of IL-1B. PCR products before cutting with *Ava*I are shown in lane "a" and after cutting are shown in lane "b". The standard DNA 50-bp ladder was shown in the lane "M". The number of each sample is shown at the above line, BG represents benign gastritis groups. They were run on 12 % polyacrylamide gel in 1x TBE buffer.

Lane M = standard DNA 50-bp ladder

Lane 2 = C/T genotype

Lane 4 = C/C genotype

Lane 6 = C/C genotype

Lane 1, 3, 5 = PCR products before cutting with *Ava*I size 155-bp

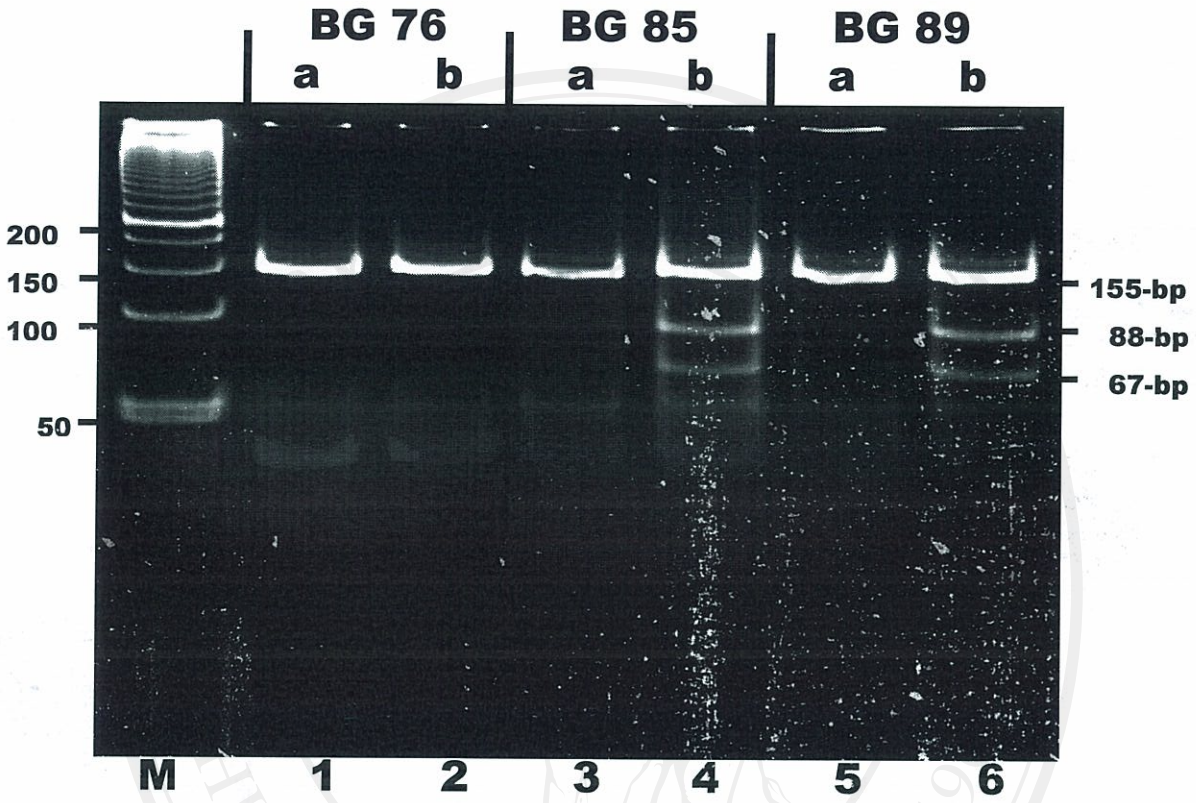

Figure 31. RFLP Pattern from Benign Gastritis Group's Peripheral Blood Samples after Cut with *Ava*I Restriction Enzyme.

The samples representing the different of C-T base transition of -511 from the transcription start site of IL-1B. PCR products before cutting with *Ava*I are shown in lane "a" and after cutting are shown in lane "b". The standard DNA 50-bp ladder was shown in the lane "M". The number of each sample is shown at the above line, BG represents benign gastritis groups. They were run on 12 % polyacrylamide gel in 1x TBE buffer.

Lane M = standard DNA 50-bp ladder

Lane 2 = T/T genotype

Lane 4 = C/T genotype

Lane 6 = C/T genotype

Lane 1, 3, 5 = PCR products before cutting with *Ava*I size 155-bp

3.4 The results of IL-1B -511 genotype, PG I, PG II and PG I/ PG II Levels.

Table 3. Show the result of of IL-1B -511 genotype, PG I, PG II and PG I/ PG II Levels from gastric cancer groups.

No	Genotype	PG I (ng/ml)	PG II (ng/ml)	PG I/PG II ratio
GC 1	T/T	42.1	24.8	1.7
GC 2	C/C	28	8.2	3.41
GC 3	C/T	2.8	1.6	1.75
GC 4	T/T	65.4	50.5	1.3
GC 5	C/T	21.8	5.5	3.96
GC 6	C/T	44.3	10.4	4.26
GC 7	T/T	28.3	13.8	2.05
GC 8	C/T	43.5	11.1	3.92
GC 9	T/T	91.4	13.5	6.77
GC 10	C/C	ND	ND	ND
GC 11	T/T	37.3	5.4	6.91
GC 12	C/T	111	27.4	4.05
GC 13	C/C	29.8	7.6	3.92
GC 14	C/C	38.2	8.8	4.34
GC 15	C/T	29.1	10.2	2.85
GC 16	C/T	100	29.4	3.4
GC 17	C/T	34.3	7.7	4.45
GC 18	C/C	242	36	6.72
GC 19	C/T	20.6	18.1	0.11
GC 20	C/C	74.8	22.1	3.38
GC 21	C/C	40.6	4.6	8.83
GC 22	T/T	49	8.3	5.9
GC 23	C/C	45.4	10.3	4.41

No	Genotype	PG I (ng/ml)	PG II (ng/ml)	PG I/PG II ratio
GC 24	C/C	14.3	2.4	5.96
GC 25	C/T	10.1	2.5	4.04
GC 26	C/T	24.1	7	3.44
GC 27	T/T	29.1	30.5	0.95
GC 28	C/T	19.7	5.9	3.34
GC 29	C/C	89.6	11.1	8.07
GC 30	C/T	44	20.3	2.17
GC 31	C/T	17.9	3.3	5.42
GC 32	C/T	59.7	16.9	3.53
GC 33	C/C	32.9	4.8	6.85
GC 34	C/T	4.9	ND	ND
GC 35	T/T	31	7.4	4.19
GC 36	T/T	5.2	2.9	1.79
GC 37	C/C	135	12.7	10.63
GC 38	C/C	72.3	13.7	5.28
GC 39	C/T	276.3	37.1	7.45
GC 40	T/T	12.9	4.2	3.07
GC 41	C/T	57.5	9.8	5.87

GC represents gastric cancer groups and BG represents benign gastritis groups.

PG I represents pepsinogen I levels;PG II represents pepsinogen II levels and PG I/ PG II represents pepsinogen I/pepsinogen II ratios.

C/C represents homozygous C base; C/T represents heterozygous and T/T represents homozygous T base.

ND represents not detection.

Table 4. Show the result of of IL-1B -511 genotype, PG I, PG II and PG I/ PG II Levels from benign gastritis groups.

No	Genotype	PG I (ng/ml)	PG II (ng/ml)	PG I/PG II ratio
BG 1	C/T	80.4	12.2	6.59
BG 2	C/C	126.5	36.5	3.47
BG 3	C/T	42.6	9.9	4.3
BG 4	C/C	68.7	21.1	3.26
BG 5	C/T	45	13.8	3.26
BG 6	T/T	77.5	16.6	4.67
BG 7	C/C	31.4	8.7	3.61
BG 8	C/C	52.2	17.1	3.05
BG 9	C/C	62.1	11.4	5.45
BG 10	C/C	28	7.3	3.84
BG 11	C/C	79.5	17.4	4.57
BG 12	C/T	60.2	13.7	4.39
BG 13	T/T	33.7	6.6	5.11
BG 14	T/T	37.4	8.7	4.3
BG 15	C/T	80.8	16.4	4.93
BG 16	C/C	76.9	20.7	3.71
BG 17	C/C	75.8	28.9	2.62
BG 18	C/T	38.7	8.8	4.4
BG 19	C/C	69.2	15.7	4.41
BG 20	C/T	82.4	21.3	3.87
BG 21	C/C	51.9	14.7	3.53
BG 22	C/T	65.5	28.2	2.32
BG 23	T/T	36.6	6.6	5.55
BG 24	T/T	42.9	9.8	4.38

No	Genotype	PG I (ng/ml)	PG II (ng/ml)	PG I/PG II ratio
BG 25	T/T	55.4	13.5	4.1
BG 26	C/T	70.1	22.7	3.09
BG 27	C/C	40	13.9	2.88
BG 28	C/T	59.4	13.4	4.43
BG 29	C/C	31	8.1	3.83
BG 30	C/T	73.5	40.9	1.8
BG 31	C/T	22.7	4.8	4.73
BG 32	T/T	57.5	12.3	4.67
BG 33	C/C	40.7	8.6	4.73
BG 34	C/T	73.2	15.2	4.82
BG 35	T/T	77.7	25.7	3.02
BG 36	T/T	67.5	27.8	2.43
BG 37	C/T	55.8	23.9	2.33
BG 38	C/T	53.1	9	5.9
BG 39	C/T	30	4.7	6.38
BG 40	T/T	27.8	6.3	4.41
BG 41	C/T	33	7.1	4.65
BG 42	C/T	282.9	46.2	6.12
BG 43	C/C	40.5	13.1	3.09
BG 44	C/T	61.5	40	1.54
BG 45	C/T	61.2	17.6	3.48
BG 46	C/T	55.3	8.3	6.66
BG 47	C/T	69	11.9	5.8
BG 48	C/T	55.2	7.8	7.08
BG 49	T/T	67.5	21.7	3.11
BG 50	C/T	40.2	8.6	4.67

No	Genotype	PG I (ng/ml)	PG II (ng/ml)	PG I/PG II ratio
BG 51	C/T	39	6.4	6.09
BG 52	T/T	42.7	19.5	2.19
BG 53	C/T	41.7	7.7	5.42
BG 54	C/T	33.3	4.7	7.09
BG 55	C/C	56.8	14.8	3.84
BG 56	C/T	23.5	5.5	4.27
BG 57	C/T	30.9	4.6	6.72
BG 58	T/T	37	6	6.17
BG 59	C/T	24	7.5	3.2
BG 60	T/T	52.9	13.4	3.95
BG 61	C/C	44.1	14.1	3.13
BG 62	C/T	20.8	4	5.2
BG 63	C/T	23	7.4	3.11
BG 64	T/T	33.4	9.8	3.41
BG 65	C/T	41.7	8.6	4.85
BG 66	C/C	119.3	30.1	3.96
BG 67	T/T	39.3	11.7	3.36
BG 68	C/T	57	7.7	7.4
BG 69	C/T	78.8	18.5	4.26
BG 70	C/T	54.1	15.6	3.47
BG 71	C/T	84	22.7	3.7
BG 72	C/T	31.2	5.8	5.38
BG 73	C/T	42.2	11.1	3.8
BG 74	C/T	26.4	4.7	5.62
BG 75	C/C	46.2	9.2	5.02
BG 76	T/T	53.7	12.8	4.2

No	Genotype	PG I (ng/ml)	PG II (ng/ml)	PG I/PG II ratio
BG 77	C/T	66	22.1	2.99
BG 78	C/T	34.2	10.8	3.17
BG 79	C/C	31.2	6.2	5.03
BG 80	C/T	27.4	6.6	4.15
BG 81	C/T	47.1	11.9	3.96
BG 82	C/T	ND	ND	ND
BG 83	C/T	29.5	4.5	6.56
BG 84	T/T	94.1	28.5	3.3
BG 85	C/T	40.4	6.2	6.52
BG 86	C/T	27.5	5.4	5.09
BG 87	T/T	40.6	10.3	3.94
BG 88	T/T	37.1	10.1	3.67
BG 89	C/T	40.5	17	2.38

GC represents gastric cancer groups and BG represents benign gastritis groups.

PG I represents pepsinogen I levels;PG II represents pepsinogen II levels and PG I/ PG II represents pepsinogen I/pepsinogen II ratios.

C/C represents homozygous C base; C/T represents heterozygous and T/T represents homozygous T base.

ND represents not detection.

3.5 Result of the different of C-T base transition of -511 from the transcription start site of IL-1B in Northern Thai population.

In this study, a total of 130 samples (41 gastric cancer and 89 benign gastritis) were studied. As shown in Table 5, all tested cancer cases and controls were reported as gene frequency. The frequency of C/C homozygote of IL-1B -511 was 0.25 (13 cases of gastric cancer and 20 cases benign gastritis), 0.52 (18 cases of gastric cancer and 49 cases benign gastritis) were frequency of C/T heterozygote and 0.23 (10 cases of gastric cancer and 20 benign gastritis) were T/T genotype.

Table 5. Analysis of genotype and gene frequency of IL-1B -511 from 130 samples.

Genotype	number (%)	Gene Frequency
C/C	33 (25.4)	0.25
C/T	67 (51.5)	0.52
T/T	30 (23.1)	0.23

3.6 Plasma Pepsinogen Levels in Benign Gastritis and Gastric Cancer.

The plasma pepsinogen (PG) level were determine by using radio immunoassay method. The median PG I and PG II value among the study subjects is shown in Table 6. The benign gastritis group had the median pepsinogen I level 45.6 (range, 34.8-67.1 ng/ml) and significant higher than gastric cancer group ($p<0.05$) Figure 32 (A). Unlike in finding the median PG II value and PG I/II ratio of both group are not significantly different ($p>0.05$) Figure 32 (B) and Figure 33.

Table 6. Median plasma pepsinogen I, pepsinogen II levels and pepsinogen I/II ratio in benign gastritis and gastric cancer.

		n	Median	Mean	SD	*p* value
PG I (ng/ml)	Benign gastritis	88	45.6	53.9	32.4	0.03
	Gastric cancer	40	37.7	53.2	56.4	
PG II (ng/ml)	Benign gastritis	88	11.8	13.9	8.9	0.25
	Gastric cancer	39	9.8	17.6	29.1	
PGI/II ratio	Benign gastritis	88	4.3	4.3	1.3	0.83
	Gastric cancer	39	3.9	4.4	2.3	

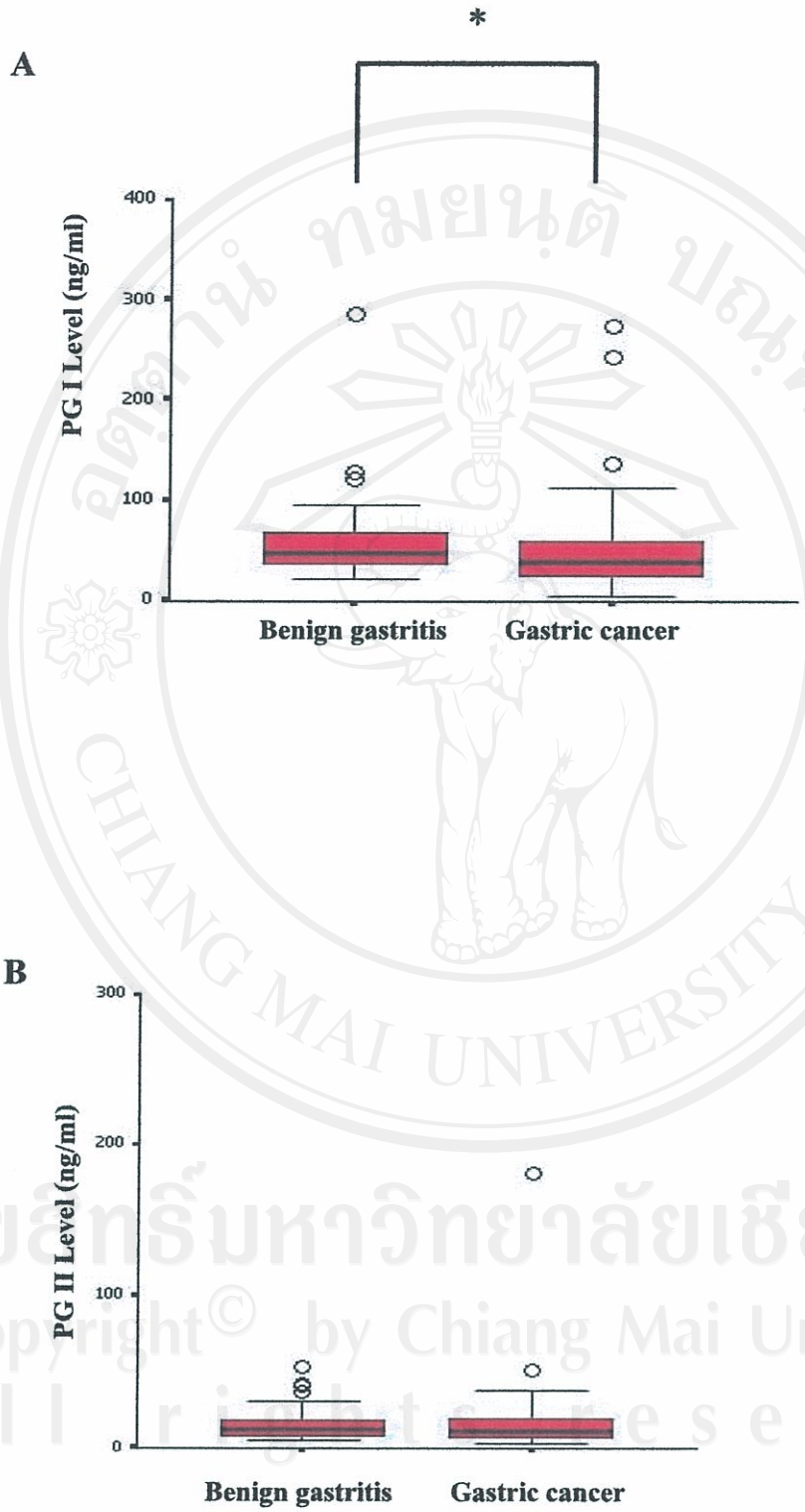

Figure 32. Median scores for (A) serum PG I levels, (B) serum PG II levels in comparison with benign gastritis and gastric cancer groups. Data are expressed as median with interquartile ranges (box: 25%-75%; line within the box: median; bars: 10%-90%; **O**: out of interquartile ranges; *: $p< 0.05$).

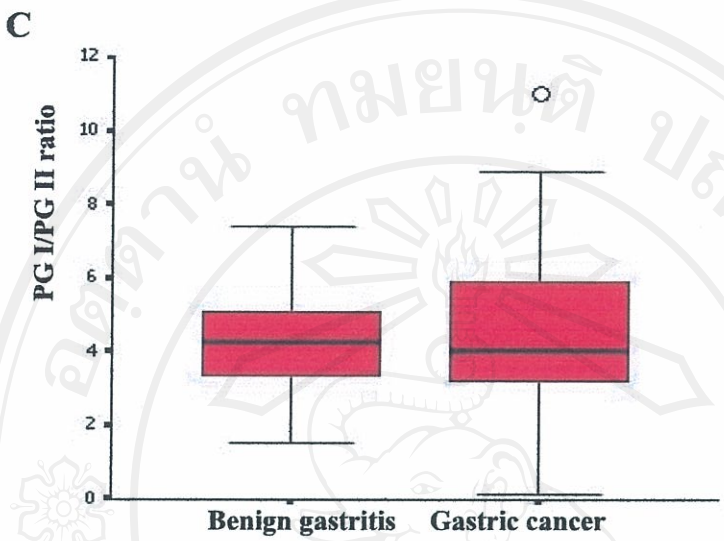

Figure 33. Median scores for serum PG I / PG II ratios in comparison with benign gastritis and gastric cancer groups. Data are expressed as median with interquartile ranges (box: 25%-75%; line within the box: median; bars: 10%-90%; **O**: out of interquartile ranges; *: $p < 0.05$).

3.7 Effect of different age on Pepsinogen I/II ratio Levels.

In the benign gastritis subjects, the median plasma pepsinogen I/II ratio was significantly lower in benign gastritis subjects with age≥40 years group (4.93; range, 3.18-4.84) compared with subjects with age<40 years group (4.52; range, 3.71-6.10; p=0.047) Figure 34 A, and this significance was higher in benign gastritis subjects with age≥50 years group (3.86; range, 3.22-4.67) compared with subjects with age<50 years group (4.40; range, 3.33-6.53; p=0.047) Figure 35 A, but was not significantly different from the median plasma pepsinogen I/II ratio in the gastric cancer group Table 7.

Table 7. Median pepsinogen I/II ratio of various age in benign gastritis and gastric cancer.

	n	Median	Mean	SD	*p* value
Benign gastritis					
age <40 years	28	4.52	4.79	1.46	0.047
age≥40 years	58	4.03	4.09	1.17	
Gastric cancer					
age <40 years	6	4.91	5.10	1.79	0.280
age≥40 years	32	3.94	4.20	2.37	
Benign gastritis					
age <50 years	57	4.40	4.55	1.35	0.041
age≥50 years	29	3.86	3.85	1.12	
Gastric cancer					
age <50 years	14	4.05	4.19	1.75	0.880
age≥50 years	24	3.94	4.44	2.59	

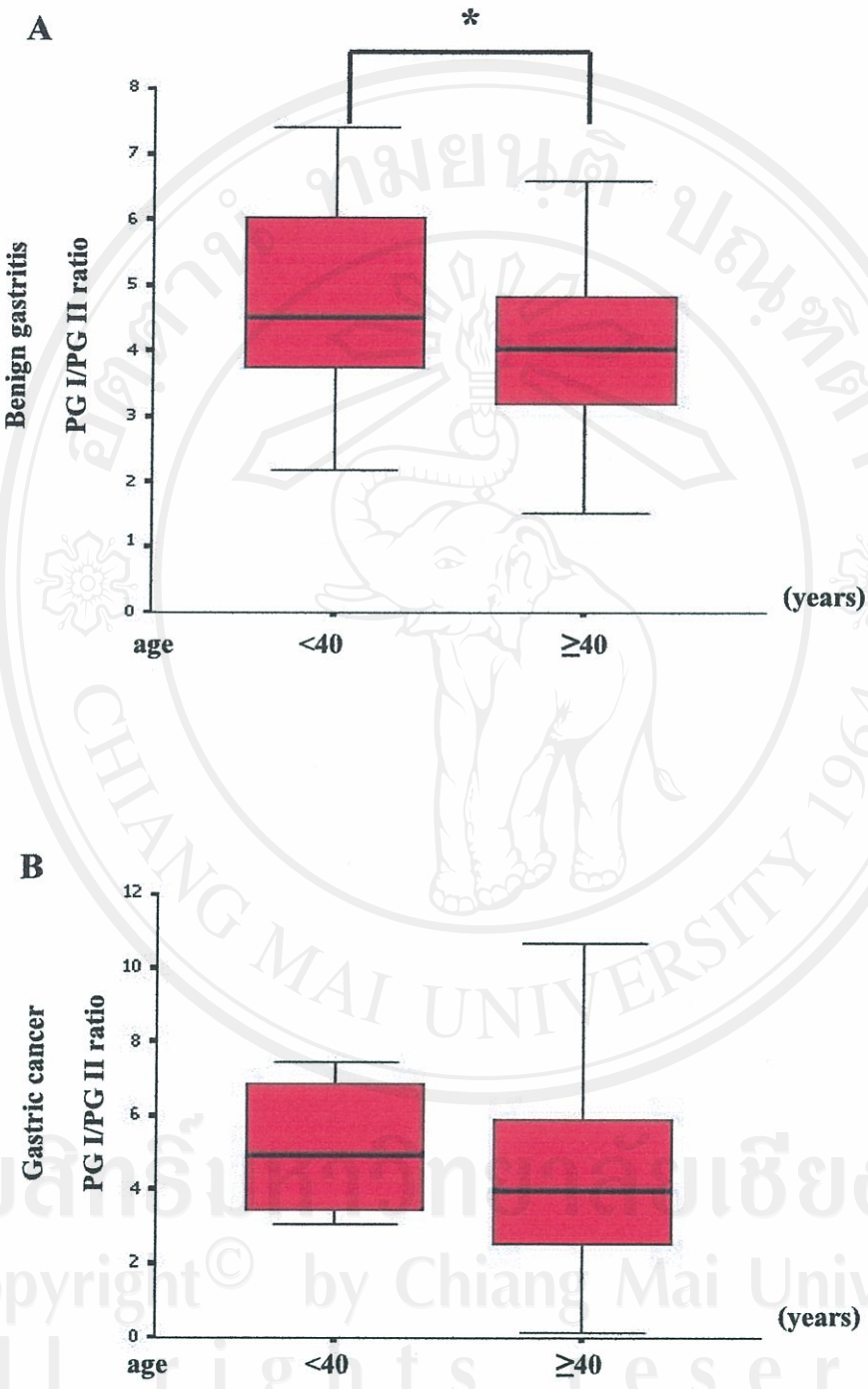

Figure 34. (A) Median scores for serum PG I/PG II ratios of benign gastritis in the two age groups (<40 and ≥40). (B) Median scores for serum PG I/PG II ratios of gastric cancer in the two age groups(<40 and ≥40). Data are expressed as median with interquartile ranges (box: 25%-75%; line within the box: median; bars: 10%-90%; *: $p< 0.05$).

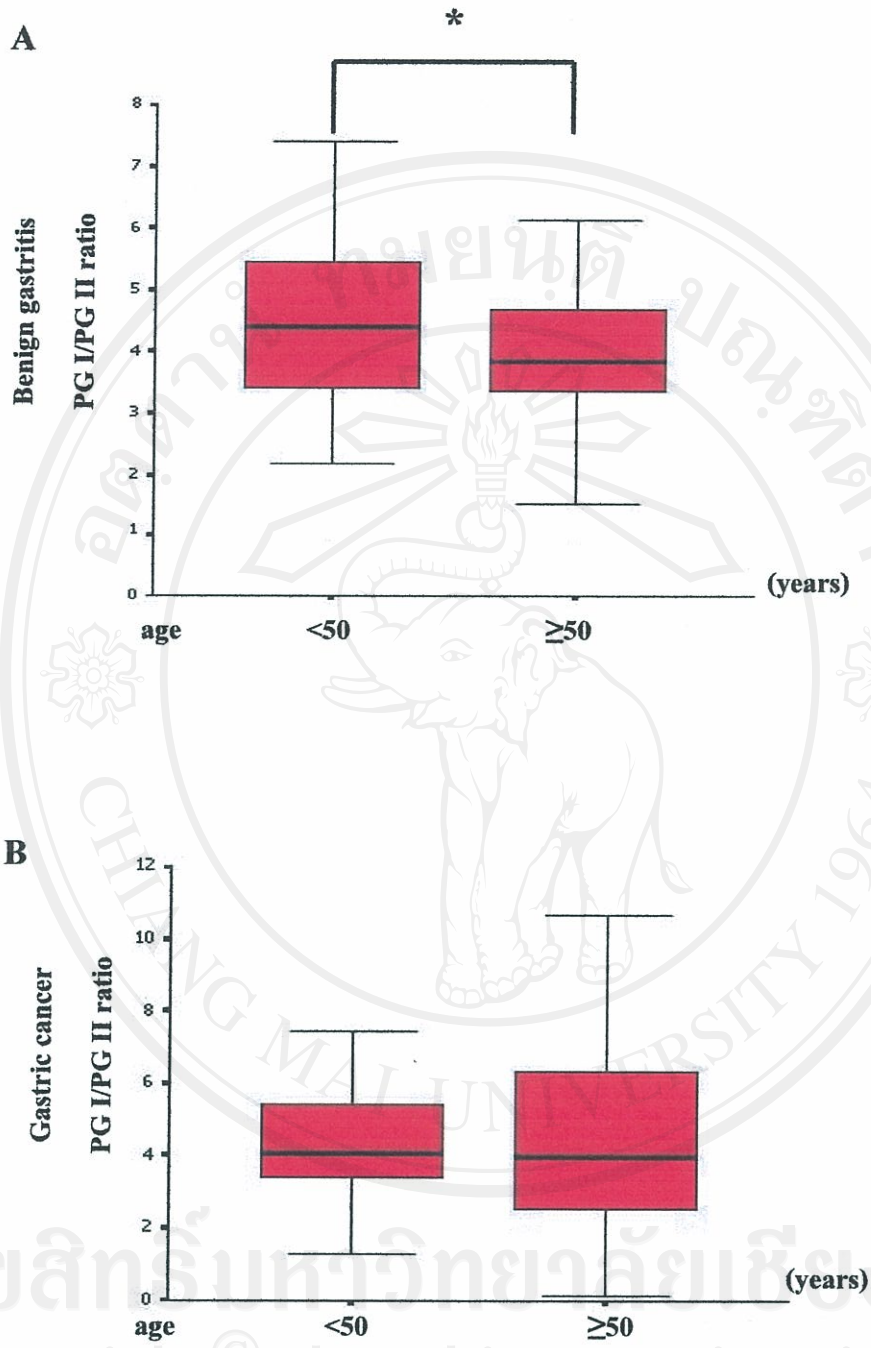

Figure 35. (A) Median scores for serum PG I/PG II ratios of benign gastritis in the two age groups (<50 and ≥50). (B) Median scores for serum PG I/PG II ratios of gastric cancer in the two age groups(<50 and ≥50). Data are expressed as median with interquartile ranges (box: 25%-75%; line within the box: median; bars: 10%-90%; *: $p< 0.05$).

3.8 Serum Pepsinogen Levels and Gastric Cancer Risk.

In the present study, the dose-response relationships of pepsinogen I, pepsinogen II, and the pepsinogen I/II ratio with the risk of gastric cancer, stated in the form of age-adjusted odds ratios, are shown in Table 8. The odd ratio for pepsinogen I was significantly elevated for levels below 50 ng/ml (OR 2.4, 95% CI 1.07-5.4), and the odd ratio for the pepsinogen I/II ratio was significantly elevated for levels below 2.4 (OR 4.2, 95% CI 1.3-14). None of the odd ratio for pepsinogen II was significant.

Table 8. The effect of the PGI, PG II and the PG I/II ratio on risk of gastric cancer was expressed as odds ratios (OR) with 95% confidence interval (CI).

	Benign Gastritis (N)	Gastric Cancer (N)	OR(95% CI)
PG I (ng/ml)			
≥50.0	42	11	1.0
<50.0	46	29	2.4 (1.07-5.4)
Total	88	40	
PG II (ng/ml)			
≥25.0	78	32	1.0
<25.0	10	7	1.7 (0.6-4.9)
Total	88	39	
PG I/II			
≥2.4	83	31	1.0
<2.4	5	8	4.2 (1.3-14.0)
Total	88	39	

3.9 IL-1B -511 Genotype and Gastric Cancer Risk.

Using the IL-1B C/C genotype as a reference group, neither C/T nor T/T genotype was associated with an increased risk of gastric cancer (Odd Ratio (OR) 0.56, 95%Confidence Interval (CI) 0.23-1.3; OR 0.76, 95% CI 0.27-2.1, respectively) (Table 9).

Table 9. Odd ratio (OR) of benign gastritis and gastric cancer according to Il-1β -511 genotype.

Genotype	Benign Gastritis		Gastric Cancer		OR (95%CI)
	N	(%)	*N*	(%)	
All subjects	89	(100)	41	(100)	
C/C	20	(22.5)	13	(31.7)	1.00
C/T	49	(55.0)	18	(43.9)	0.56 (0.23-1.3)
T/T	20	(22.5)	10	(24.4)	0.76 (0.27-2.1)
C/C	20	(22.5)	13	(31.7)	1.00
T carrier (C/T and T/T)	69	(77.5)	28	(68.3)	0.62 (0.27-1.4)

3.10 Effect of the IL-1B -511 Polymorphism on Plasma Pepsinogen Levels.

In the benign gastritis subjects, the median plasma pepsinogen I level with the IL-1B -511 C/C genotype was 52.05 ng/ml (range, 40.12-74.15 ng/ml), which was not significantly different from the median in the C/T group (43.8 ng/ml; range, 31.65-64.5 ng/ml) or that in the T/T group (42.8 ng/ml; range, 37.02-65.0 ng/ml) shown in Figure 36 A. The median plasma pepsinogen II level in benign gastritis subjects with the IL-1B -511 C/C genotype was 14.4 ng/ml (range, 8.82-19.87 ng/ml) and was not significantly different from the median in the C/T group (9.9 ng/ml; range, 6.45-16.85 ng/ml) or the T/T group (12.0 ng/ml; range, 8.97-18.77 ng/ml) (Figure 37 A). The median plasma pepsinogen I/II ratio was significantly lower in benign gastritis subjects with the IL-1B -511 C/C group (3.77; range, 3.15-4.53) compared with the C/T genotype (4.54; range, 3.47-5.87; p=0.033) but was not significantly different from the T/T group (3.9; range, 3.32-4.6; p=0.57) (Figure 38 A) or T carrier (C/T and T/T) group (4.34; range, 3.37-5.4; p=0.75).

The median (interquartile range) plasma pepsinogen I level in gastric cancer subjects with the IL-1B -511 C/C genotype was 42.45 ng/ml (range, 30.57-85.9 ng/ml), which was not significantly different from the median in the C/T group (31.7 ng/ml; range, 19.25-58.05 ng/ml) or that in the T/T group (34.15 ng/ml; range, 24.45-53.1 ng/ml) (Figure 36 B). The median plasma pepsinogen II level in gastric cancer subjects with the IL-1B -511 C/C genotype was 8.46 ng/ml (range, 5.5-12.3 ng/ml) and was not significantly different from the median in the C/T group (10.2 ng/ml; range, 5.73-23.85 ng/ml) or the T/T group (10.91 ng/ml; range, 5.1-26.22 ng/ml) (Figure 37 B). The median plasma pepsinogen I/II ratio was significantly higher in gastric cancer subjects with the IL-1B -511 C/C genotype (5.62; range, 4.03-7.77) compared with C/T group (3.92; range, 3.1-4.36; p=0.012), T/T group (2.56; range, 1.6-6.12; p=0.025) (Figure 38 B), and T carrier (C/T and T/T) group (3.53; range, 2.05-4.45; p=0.005).

There was not significantly different from the median in the C/T group compared with T/T group in neither Pepsinogen I and pepsinogen II levels nor pepsinogen I/II ratio.

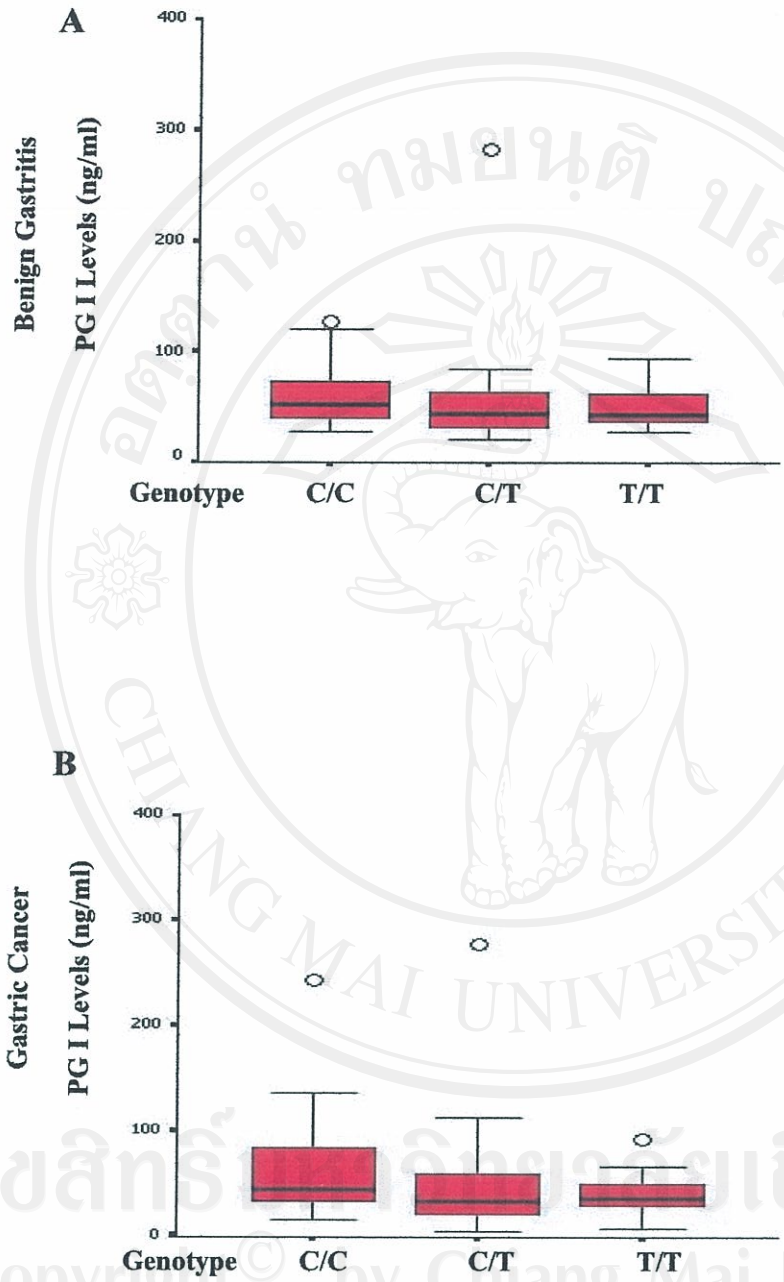

Figure 36. (A) Median scores for serum pepsinogen I levels of benign gastritis in the three IL-B -511 genotype groups (C/C, C/T and T/T). (B) Median scores for serum pepsinogen I levels of gastric cancer in the three IL-B -511 genotype groups. Data are expressed as median with interquartile ranges (box: 25%-75%; line within the box: median; bars: 10%-90%; **O**: out of interquartile ranges).

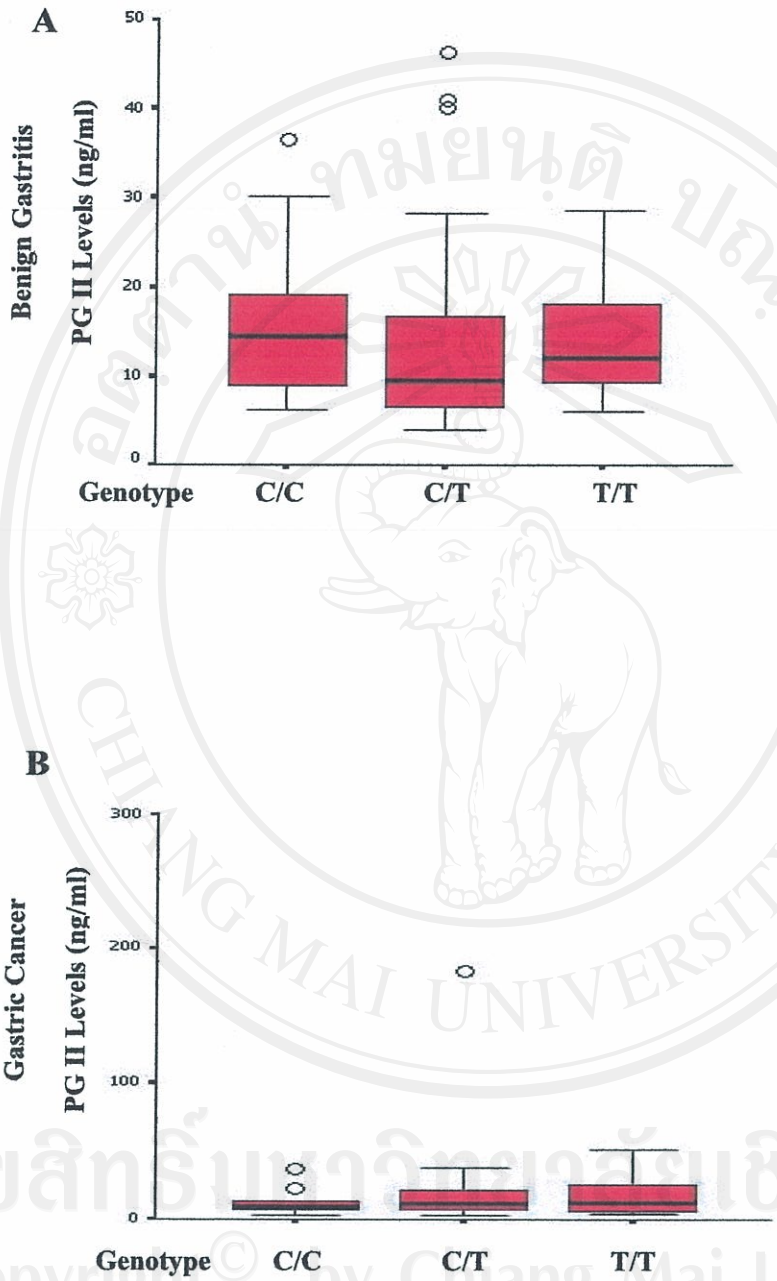

Figure 37. (A) Median scores for serum pepsinogen II levels of benign gastritis in the three IL-B -511 genotype groups (C/C, C/T and T/T). (B) Median scores for serum pepsinogen II levels of gastric cancer in the three IL-B -511 genotype groups. Data are expressed as median with interquartile ranges (box: 25%-75%; line within the box: median; bars: 10%-90%; **O**: out of interquartile ranges).

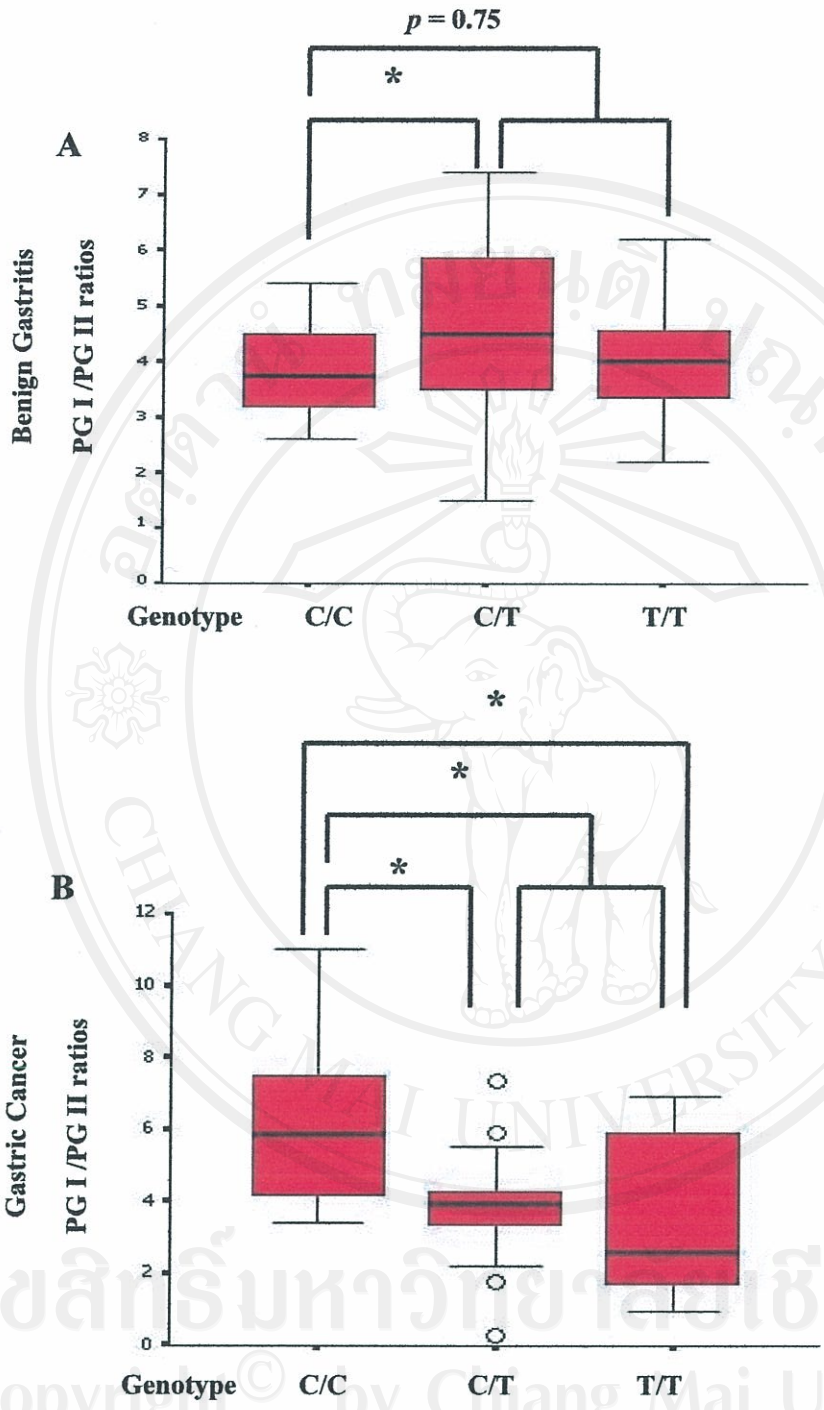

Figure 38. (A) Median scores for serum PG I/PG II ratios of benign gastritis in the three IL-B -511 genotype groups (C/C, C/T and T/T). (B) Median scores for serum PG I/PG II ratios of gastric cancer in the three IL-B -511 genotype groups. Data are expressed as median with interquartile ranges (box: 25%-75%; line within the box: median; bars: 10%-90%; **O**: out of interquartile ranges; *: $p < 0.05$).