CHAPTER IV
RESULTS

1. Characteristics of the healthy Thais

The study population was consisted of 189 unrelated healthy Thais, who were native-born in the Northeast region of Thailand. These subjects included 136 men and 53 women (male to female ratio was 2.57) with age ranged from 30 to 89 years. Smoking status was classified into three groups as current smoke, ex-smoke (quit smoking before assessment), and never smoked (Table 5). According to their smoking status, 41% of population was never smoked, and 59% was ex-smoke and current smoke. In ever smoked subgroup, the duration of smoking was between 3 to 57 years, and the pack-year of smoking ranged from to 0.9 to 90 (a pack-year of cigarette smoking was defined as the continuous smoking of 20 cigarettes per day for 1 calendar year).

2. Distribution of *NQO1* genotype and allelic frequencies in Thai population

The PCR products of exon 6, containing a C-to-T substitution at base pair 609, in the *NQO1* gene were digested by *Hin*fI, and we were able to identify the three *NOQ1* genotypes as shown in Figure 13. Genotype distribution and the allelic frequencies for NQO1 polymorphism in Thais are shown in Table 6. The frequency of *NQO1*1/*1* (or 609 C/C) wild type genotype was 32%, whereas the frequency observed for *NQO1*2* carrying genotype (609 C/T and 609 T/T) was 68%. This observed genotype pattern did not significantly deviate from the expected Hardy-Weinberg equilibrium ($\chi^2 = 0.89, p>0.05$).

Present data showed that *NQO1*1* (C or wild type) allele is commonly found in Thais, with the frequency as 59%, where that *NQO1*2* (T or mutant) allele was 41%. When compared the frequency of *NQO1*2* allele observed in Thais with other populations reported previously (Table 7), the prevalence found in this study was significantly higher than that in Caucasian ($p<0.0001$) and African-American ($p<0.0001$). However the allele frequencies are not significantly from other Asian populations, such as Korean, Chinese, and Japanese.

Table 5 Demographic characteristics of healthy controls.

Characteristics	n (%)
Sex	
Male	136 (72)
Female	53 (28)
Age	
Mean (years) ± S.D.	48.04 ± 11.36
Smoking status	
Never smoked	78 (41)
Ex-smoke	54 (29)
Current smoke	57 (30)
If ever smoked	
Mean duration of smoking (years) ± S.D.	24.86 ± 9.96
Mean number of pack-years ± S.D.	13.56 ± 10.01

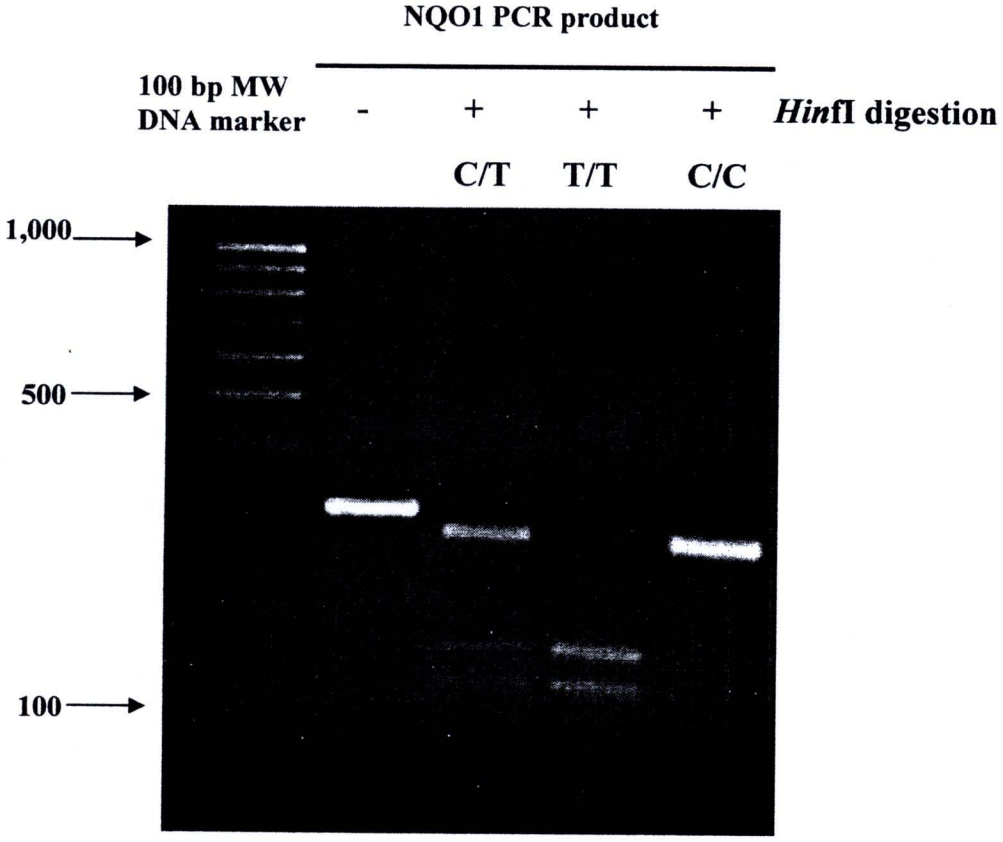

Figure 13 Agarose gel electrophoresis patterns of the NQO1 polymorphism identified by PCR-RFLP analysis using restriction enzyme *Hin*fI.

Table 6 Genotype and allele frequencies of NQO1 polymorphism in Thai population.

	Observed number (%)	95% CI[a]	Expected Frequency[b] (%)
Genotype			
*NQO1*1/*1 (609 C/C)*	61 (32)	25.61-38.94	35
*NQO1*1/*2 (609 C/T)*	100 (53)	45.79-60.02	48
*NQO1*2/*2 (609 T/T)*	28 (15)	9.75-19.88	17
Total	**189**		
Allele			
*NQO1*1 (C609)*	222 (59)	53.72-64.02	
*NQO1*2 (609T)*	156 (41)	36.43-46.32	
Total	**378**		

[a] 95% CI = 95% confidence interval

[b] The expected frequencies were calculated based on Hardy-Weinberg equilibrium

Table 7 Comparison of *NQO1 609T* allele frequency in different ethnic group.

Population	**N[a]**	**609T allele (%)**	***p*-value**	**Reference**
Thai	***378***	***41***		***Current study***
Korean	340	54	$p>0.05$	(Choi et al., 2003)
Chinese	330	42.4	$p>0.05$	(Zhang et al., 2003)
Japanese	408	40	$p>0.05$	(Hori et al., 2003)
Caucasian	1,366	16	$p<0.0001$	(Saldivar et al., 2005)
African-American	214	19	$p<0.0001$	(Saldivar et al., 2005)

N[a] = Number of alleles analyzed

3. Demographic characteristics of the CCA patients

The CCA patients groups comprised 210 unrelated Thais, who were native-born in the Northeast region of Thailand. They had been confirmed the diagnosis of CCA by tissue histopathology. The male to female ratio for the CCA group was slightly lower than that of the control group (1.84 and 2.57, respectively). CCA patients were between 24 to 78 years of age, with higher mean than that observed in the healthy controls (48 and 55 years, respectively). Information regarding smoking status of CCA patients showed 44% was never smoked and 56% was ex-smoke and current smoke (Table 8). In ever smoked subgroup, the pack-year of smoking of the CCA patients ranged from 0.4 to70, whereas duration of smoking was between 3 to 57 years. In our study population, there were no differences in pattern of smoking status between CCA patients and controls ($\chi^2 = 2.81$ $p>0.05$).

4. Distribution of *NQO1* genotype and allelic frequencies in CCA patients

Genotype distribution and the allelic frequencies for NQO1 polymorphism in CCA patients are presented in Table 9. Of the 210 CCA patients genotyped for NQO1 polymorphism, 42% was *NQO1*1/*1* genotype and 58% was *NQO1*2* variant allele carriers. The genotype distribution for *NQO1* gene in CCA patients was in Hardy–Weinberg equilibrium.

No significant difference was found in the *NQO1*2* variant allele frequency of the *NQO1* gene between both the CCA and control groups ($p = 0.20$) (Table 9). There was also no difference of the pattern of *NQO1* genotype between both study groups ($\chi^2 = 4.16$, $p>0.05$). Interestingly, the *NQO1*2* polymorphism showed a protective trend for a CCA development. In addition, we found that *NQO1*1/*2* genotype was less frequent in the CCA patients, as shown in Table 10 (OR = 0.63, $p = 0.04$). Data presented here suggested there was an association between *NQO1*1/*1* genotype and CCA risk.

Table 8 Demographic characteristics of CCA patients.

Characteristics	n (%)
Sex	
Male	136 (65)
Female	74 (35)
Age	
Mean (years) ± S.D.	55.27 ± 9.94
Smoking status	
Never smoked	81 (44)
Ex-smoke	39 (21)
Current smoke	64 (35)
If ever smoked	
Mean duration of smoking (years) ± S.D.	30.87 ± 12.66
Mean number of pack-year ± S.D.	12.02 ± 7.59
Pathology	
Papillary adenocarcinoma	43 (27)
Non-papillary	
Tubular	73 (45)
Well differentiation	25 (15)
Moderate differentiation	4 (2.5)
Poorly differentiation	9 (5)
Mucinous	5 (4)
Squamouse cell carcinoma	3 (1.5)

Table 9 Genotype and allele frequencies of NQO1 polymorphism in CCA.

	Observed number (%)	95% CI[a]
Genotype		
*NQO1*1/*1 (609 C/C)*	87 (42)	34.76-48.09
*NQO1*1/*2 (609 C/T)*	91 (43)	36.63-50.03
*NQO1*2/*2 (609 T/T)*	32 (15)	10.38-20.09
Total	**210**	
Allele		
*NQO1*1 (C609)*	265(63)	18.64-28.89
*NQO1*2 (609T)*	155(37)	17.16-30.58
Total	**420**	

[a] 95% CI = 95% confidence interval

Table 10 Association of *NQO1* genotype and allele with risk of CCA risk.

	No. CCA	No. Controls	OR (95% CI[a])	*p*-value
Genotype				
*NQO1*1/*1 (609 C/C)*	87	61	1.00	
*NQO1*1/*2 (609 C/T)*	91	100	0.63 (0.41-0.98)	0.04*
*NQO1*2/*2 (609 T/T)*	32	28	0.80 (0.44-1.46)	0.47
Total	**210**	**189**		
Allele				
*NQO1*1 (C609)*	265	222	1.00	
*NQO1*2 (609T)*	155	156	0.83 (0.63-1.10)	0.20
Total	**420**	**378**		

[a] 95% CI = 95% confidence interval

* *p*-value<0.05

5. Risk for NQO1 polymorphism in CCA patients

Risk estimates for NQO1 polymorphism in CCA patients and healthy controls are shown in Table 11. The *NQO1*1/*1* genotype in Thais had a higher risk of CCA, after adjusting for sex and smoking status (adjusted OR = 1.57; 95% CI = 1.03-2.40). Additionally, sub-analysis showed *NQO1*1/*1* genotype with aged more than or equal 48 years had a greater risk of CCA (adjusted OR = 2.04; 95% CI = 1.13-3.69), but this risk was not found in a young group (adjusted OR for *NQO1*1/*1* genotype with age <48 years = 1.33; 95% CI = 0.61-2.91). The analysis for the joint effect of *NOQ1* genotypes and sex on the CCA risk was performed (Table 11). However, among either female or male, a non-significant higher CCA risk was found in those with the *NQO1*1/*1* genotypes (1.63 and 1.45, respectively). In stratification analysis using smoking status, no subgroup classified by neither pack-year of cigarette smoking nor duration of smoking showed significance.

Table 12 presents the association between *NQO1*2* polymorphism and risk of various cancers previously reported. The current study showed a protective effects of *NQO1*2* polymorphism in CCA development (adjusted OR = 0.59; 95% CI = 0.38-0.93).

Table 11 CCA cancer risk estimates for NQO1 polymorphism.

Characteristic	No. CCA	No. Controls	Odds ratio (95% CI)[b]		*p*-value
			Crude	Adjusted	
NQO1 C609T					
C/T and T/T[a]	123	128	1.00		
C/C	87	61	1.48 (98.55 2.24)	1.57 (1.03-2.40)[c]	0.03*
***Age < 48 years* (by median value in control group)**					
C/T and T/T[a]	25	68	1.00		
C/C	16	37	1.18 (0.56-2.46)	1.33 (0.61-2.91)[c]	0.47
Age ≥ 48 years					
C/T and T/T[a]	98	60	1.00		
C/C	71	24	1.81 (1.03-3.17)	2.04 (1.13-3.69)[c]	0.02*
Male					
C/T and T/T[a]	78	90	1.00		
C/C	58	46	1.45 (0.89-2.37)	1.58 (0.92-2.70)[d]	0.09
Female					
C/T and T/T[a]	45	38	1.00		
C/C	29	15	1.63 (0.76-3.45)	2.22 (0.97-5.08)[d]	0.06

* *p*-value<0.05

[a] Reference group

[b] Odds ratios are for C/C versus C/T and T/T

[c] Adjusted for sex and smoking status (never smoked, ex-smoke and current smoke)

[d] Adjusted for age and smoking status (never smoked, ex-smoke and current smoke)

Table 11 CCA cancer risk estimates for NQO1 polymorphism (Cont.).

Characteristic	No. CCA	No. Controls	Odds ratio (95% CI)[b]		*p*-value
			Crude	Adjusted	
***Pack-years < 14* (by median value in control group)**					
C/T and T/T[a]	43	43	1.00		
C/C	25	20	1.25 (0.61-2.56)	1.38 (0.64-2.99)[c]	0.40
Pack-years ≥ 14					
C/T and T/T[a]	17	33	1.00		
C/C	18	15	2.33 (0.95-5.69)	2.33 (0.94-5.81)[c]	0.06
***Years of smoking <25* (by median value in control group)**					
C/T and T/T[a]	19	43	1.00		
C/C	14	21	1.51 (0.64-3.56)	1.51 (0.62-3.69)[c]	0.36
Years of smoking ≥ 25					
C/T and T/T[a]	41	33	1.00		
C/C	29	14	1.67 (0.77-3.63)	1.23 (0.51-2.97)[c]	0.64

* *p*-value<0.05

[a] Reference group

[b] Odds ratios are for C/C versus C/T and T/T

[c] Adjusted for age and sex

Table 12 Case-control studies reporting the association between NQO1 polymorphism and cancer risk.

Disease	*1/*2+*2/*2 Cases	Total Cases	*1/*2+*2/*2 Controls	Total Controls	OR	95% CI
CCA (current study)	***123***	***210***	***128***	***189***	***0.59***	***0.38-0.93***
Lung cancer						
(Alexandrie et al., 2004)	107	312	79	273	1.16	0.72-1.88
(Lewis et al., 2001)	26	82	34	145	1.31	0.66-2.58
(Chen et al., 1999)	109	353	117	360	0.80	0.40-1.50
Bladder cancer						
(Terry et al., 2005)	79	239	64	215	1.10	0.70-1.70
(Choi et al., 2003)	118	218	170	199	0.63	0.37-1.00
(Park et al., 2003)	90	232	76	239	1.51	1.01-2.25
Corolectal cancer						
(van der Logt et al., 2006)	144	369	123	415	1.60	1.03-2.40
(Mitrou et al., 2007)	300	889	290	936	1.15	0.94-1.40

6. Association of survival time and *NQO1* genotypes

To verify the association between *NOQ1* genotype and survival time of CCA patients, a total of 20 CCA patients was excluded from the analysis, because of their very short survival times after surgery (less than 30 days) possibly related to complications from surgery procedure. The overall median survival time of CCA patients in this study was 12.85 months. We found no impact of the NOQ1 polymorphism on overall survival of CCA, as presented in Table 13. Data showed that *NQO1*1/*1* wild type genotype had a trend of better 3-year and 5-year survival than *NQO1*2* carriers (heterozygous and homo-mutant genotype), but no significant difference was noted ($\chi^2 = 5.09, p = 0.08$).

Table 13 Summary of CCA survival data by *NQO1* genotype.

Genotype	N[a]	Median Survival (mo.)	Percentage of Survival (n)			*p*-value
			1 year	3 years	5 years	
CCA patients						
*NQO1*1/*2+*2/*2*	111	11.29	74 (82)	6.3 (7)	2.7 (3)	0.08
*NQO1*1/*1*	79	18.13	69.6 (55)	12.7 (10)	8.8 (7)	

[a] Number of patients with survival time >30 days

6.1 Analysis of independent factors on survival time

Survival analysis of the NQO1 polymorphism suggested that patients with *NQO1*1/*1* genotype had a trend of longer survival than *NQO1*2* carrying patients (Figure 14 & 15), however this observation did not reach statistically significant level. We did not find any association between smoking status and survival time also (Figure 16 & 17). Interestingly, univariate analysis for the effect of histopathology type on survival suggested patients with papillary type had a longer survival compared with those patients with non-papillary type (median time = 480 days versus 190 days, respectively; $p<0.001$) (Figure 18). In addition, data from multivariate analysis showed that the papillary type was only a unique independent prognostic relevance in CCA in this study ($p = 0.000$) (Table 10). In additional analysis, however, there was no combination effect of *NQO1* genotype and papillary type on the survival time (data not shown). As a result of Cox regression analysis (Table 14), neither *NQO1 C609T* nor smoking status could be the prognostic factors for survival for the CCA patients ($p>0.05$).

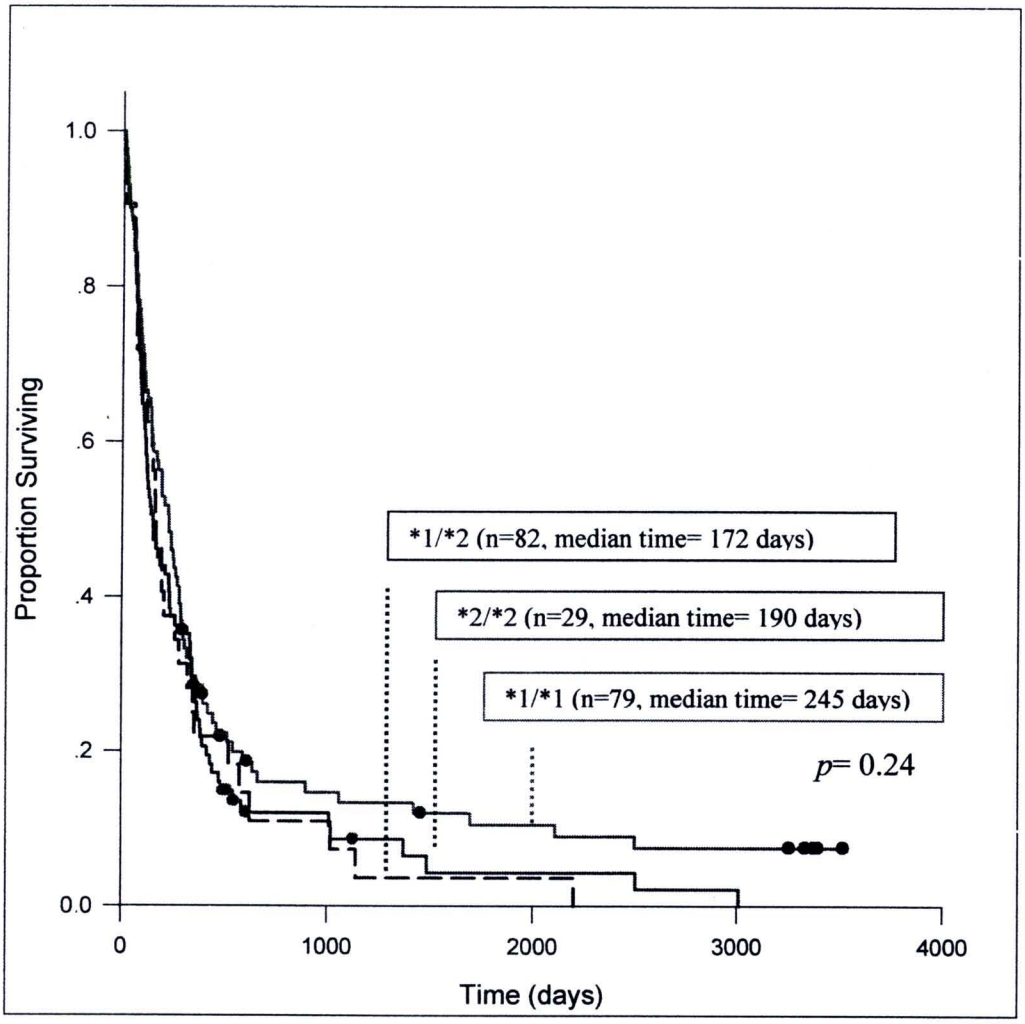

Figure 14 Survival after surgery, stratified by three *NQO1* genotypes (as *NQO1*1/*1*, *NQO1*1/*2*, and *NQO1*2/*2*), *p*-value= 0.24.

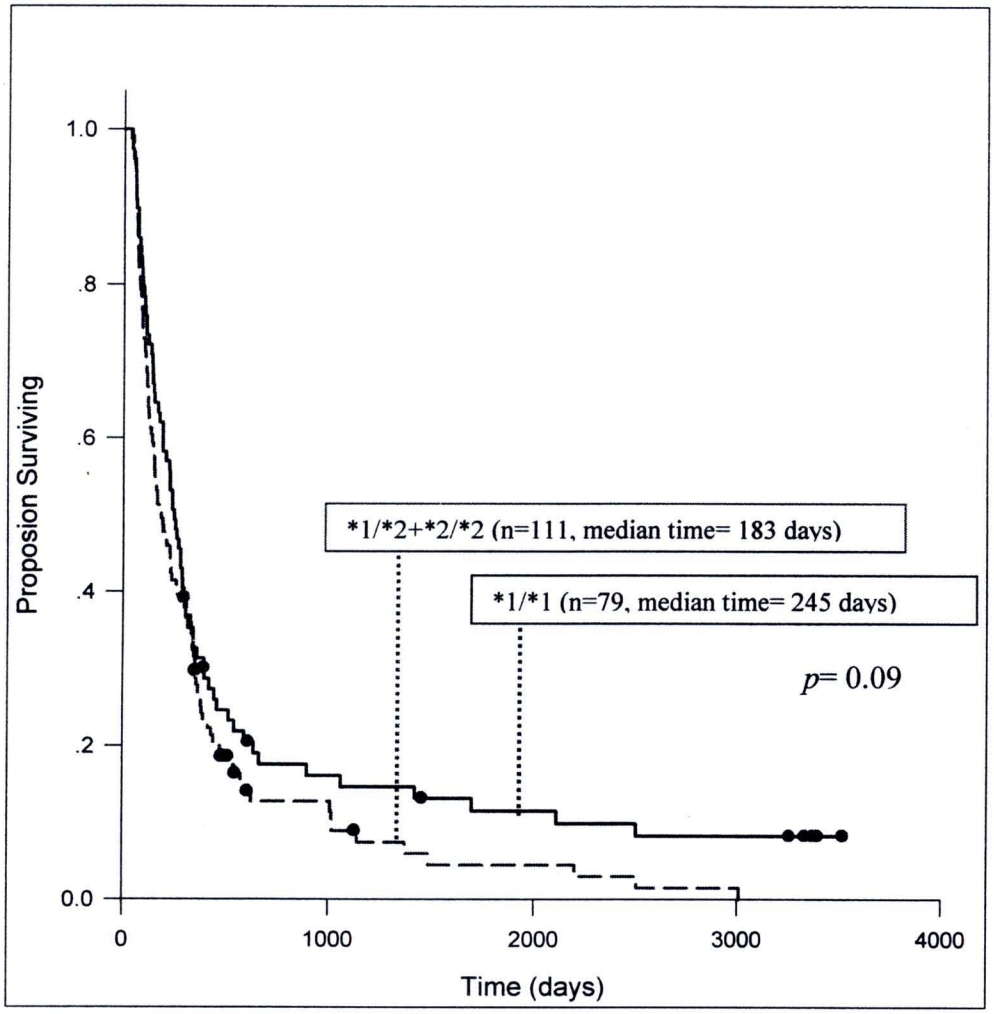

Figure 15 Survival after surgery, stratified by two groups of *NQO1* genotype (*NQO1*1/*1* versus *NQO1*1/*2+*2/*2*), *p*-value= 0.09.

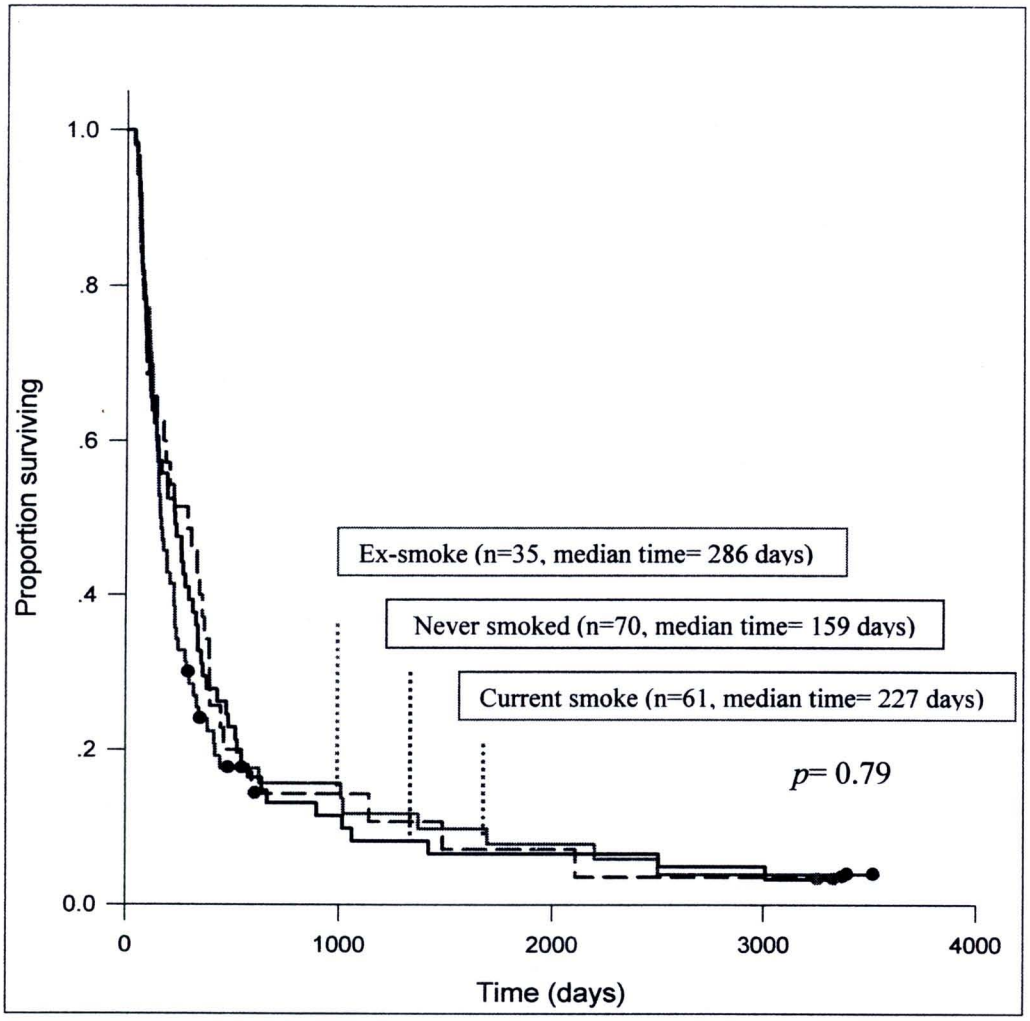

Figure 16 Survival after surgery, stratified by smoking status (as never smoked, ex-smoke, and current smoke), *p*-value= 0.79.

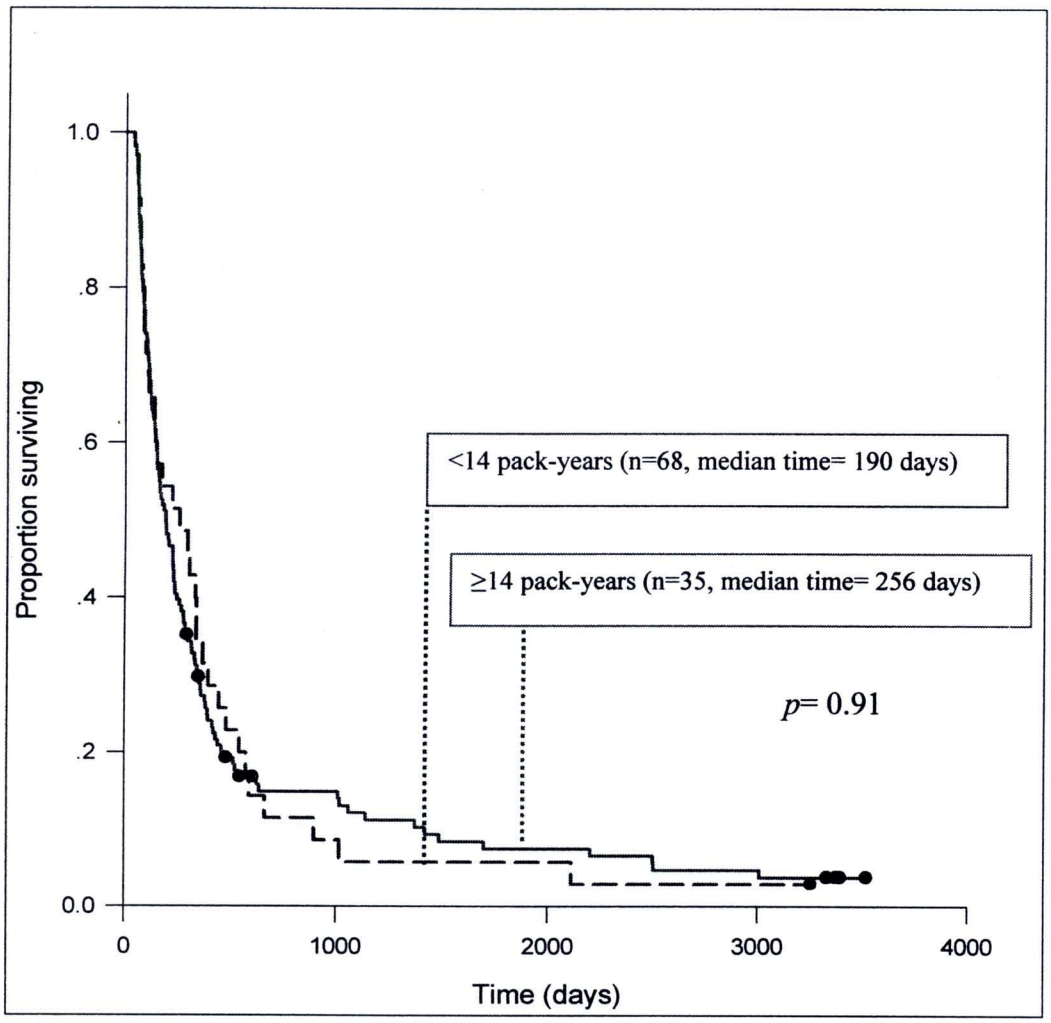

Figure 17 Survival after surgery, stratified by smoking status (as < 14 pack-years (by median value in control group) and ≥ 14 pack-years), *p*-value= 0.91.

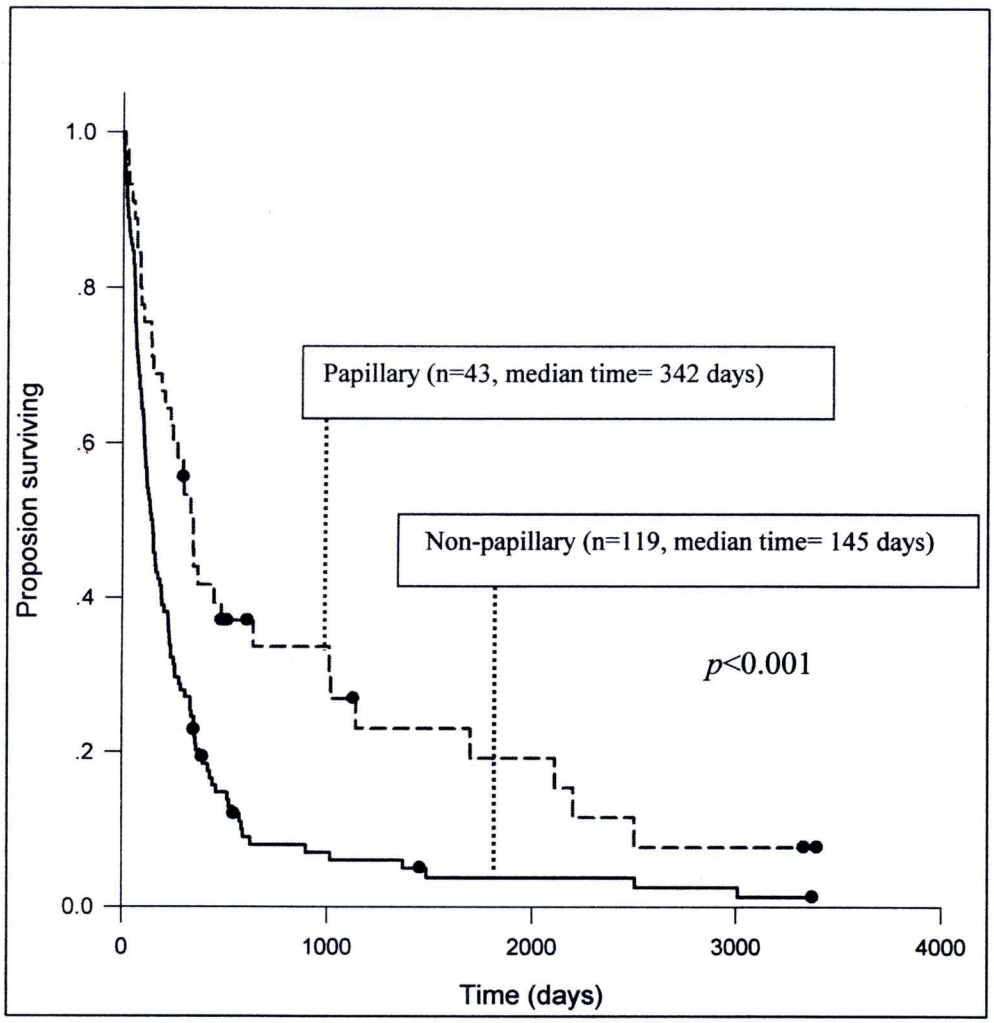

Figure 18 Survival after surgery, stratified by histopathology (papillary versus non-papillary), p-value < 0.001.

Table 14 Results of Cox regression analysis for the CCA patients.

Characteristic	Univariate analysis	Multivariate analysis	95% CI	*p*-value
	HR	HR		
NQO1 C609T				
*NQO1*1/*2+*2/*2*	1.00			
*NQO1*1/*1*	0.77	0.80[a]	0.57-1.10	0.18
***Age* (by median value in control group)**				
< 48 years	1.00			
≥ 48 years	1.16	1.14[b]	0.76-1.69	0.53
Sex				
Female	1.00			
Male	0.93	1.04[c]	0.72-1.52	0.81
Histopathology				
Non-papillary	1.00			
Papillary	0.51	0.53[a]	0.34-0.82	0.005*

* *p*-value<0.05

[a] Adjusted for age, sex and smoking status (number of pack-years)

[b] Adjusted for sex and smoking status (number of pack-years)

[c] Adjusted for age and smoking status (number of pack-years)

Table 14 Results of Cox regression analysis for the CCA patients (Cont.).

Characteristic	Univariate analysis	Multivariate analysis	95% CI	*p*-value
	HR	HR		
Smoking status				
Ever/current	1.00			
Never	0.84	0.69[d]	0.41-1.16	0.17
***Pack-years* (by median value in control group)**				
< 14	1.00			
≥ 14	0.88	0.84[d]	0.58-1.25	0.40

[*] *p*-value<0.05

[d] Adjusted for age and sex