# CHAPTER IV
RESULTS

1. Patient characteristics

1.1 Charcteristics of leukemia subtype.

One hundred and thirty nine children who were diagnosed with acute lymphoblastic leukemia (ALL = 139) at King Chulalongkorn Memorial Hospital were recruited for the study. Of these patients, 69 were boys and 70 were girls (mean age 9.08 ± 4.13 years). The subtypes of ALL were 71% precursor B-cell, 6% precursor T-cell, 1% mature B-cells, 1% infantile and 21% unclassified.

Figure 11 Pie charge representing distribute of childhood acute lymphoblastic leukemia subsype of study patients.
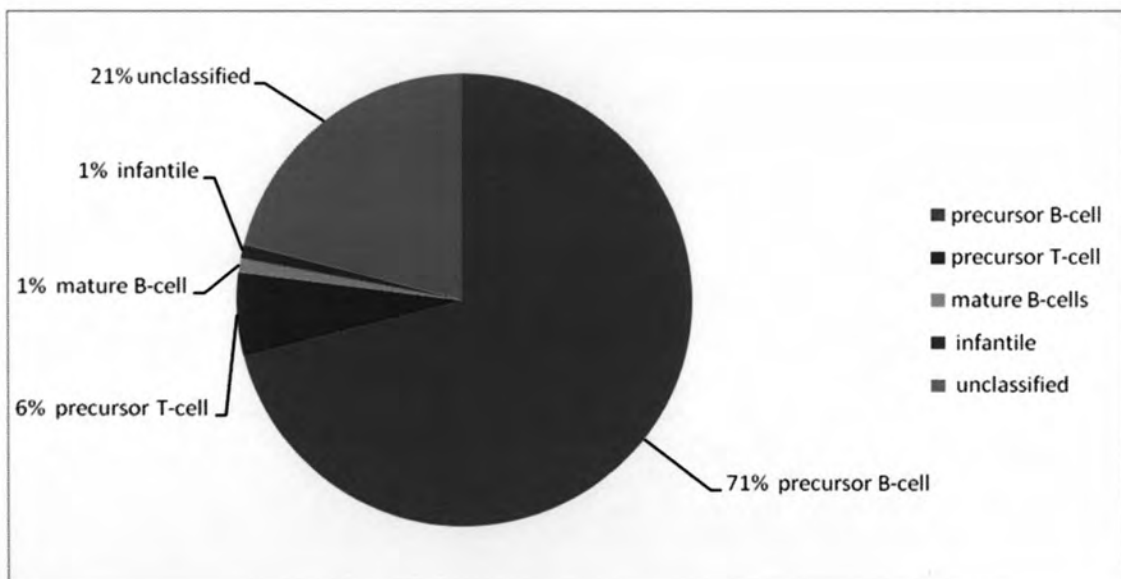
1.2 Identification of *XRCC1* gene polymorphisms.

Codon 194

Analysis of *XRCC1* codon 194 is shown on Figure 12. Using appropriate primer and PCR condition (Table 6, p. 33), a 138 bp amplicon is digested with *Pvu*II. Wild type (C/C) is not digestible, but polymorphic variant (T/C, T/T) created a *Pvu*II site and digested by *Pvu*II into 63+75 bp band.

Figure 12 RFLP analysis of *XRCC1* codon194 PCR product: Lane 1,2,3 shows Arg/Arg wild genotype, lane 4 homozygous and lane 5,6,7 heterozygous variant respectively. Lane M is the DNA molecular weight marker.
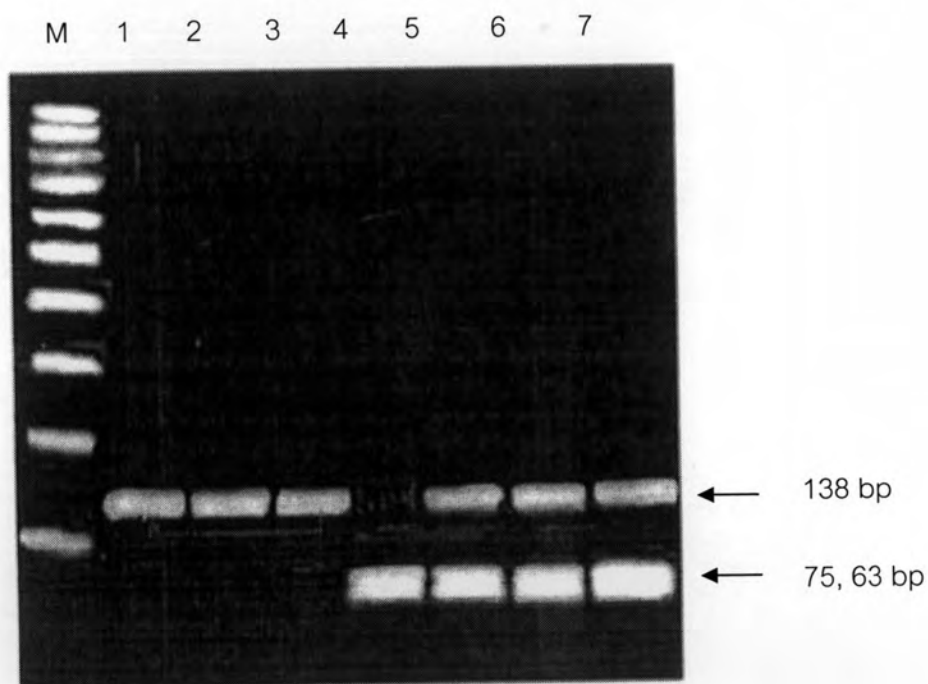
Codon 280

Analysis of *XRCC1* codon 280 is shown on Figure 13. Using appropriate primer and PCR condition (Table 6, p. 33), after *Rsa*I digestion, the 861 bp amplicon is digested into a 660 bp and 201 bp bands with wild type (G/G). A polymorphic variant (A/G, A/A) created the *Rsa*I site, and digestion with *Rsa*I produced 3 bands.

Figure 13 RFLP Analysis of *XRCC1* codon 280 genotype analysis: lanes 2,4 and 6—Arg/Arg wild genotype, lanes 3 and 5—Arg/His heterozygous genotype and lane 1—His/His homozygous variant. LaneM is the DNA molecular weight marker.
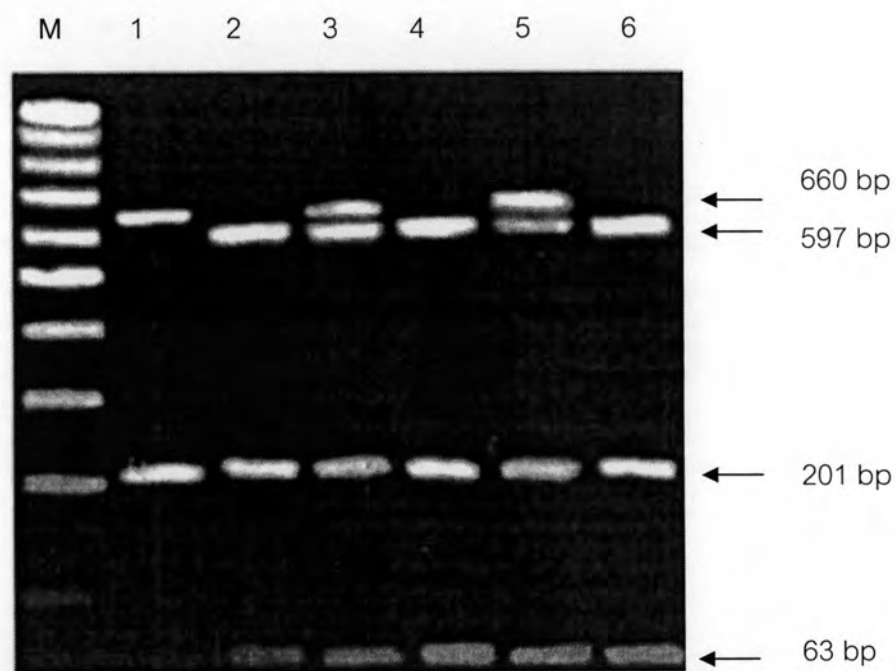
Codon 399

Analysis of *XRCC1* codon 399 is shown on Figure 14. Using appropriate primer and PCR condition (Table 6, p. 33), after *Msp*I digestion, the 1437 bp amplicon is digested into a 576 bp, 461 bp and 285 bp bands with wild type (G/G). A polymorphic variant (A/G, A/A) created the *Msp*I site, and digestion with *Msp*I produced 4 bands.

Figure 14 RFLP Analysis of *XRCC1* codon 399 PCR product: lanes 1 and 2 show a Arg/Gln heterozygous genotype and Gln/Gln homozygous polymorphic genotype, respectively. Arg/Arg wild genotype is seen in lane 3. Lane M—DNA molecular weight marker.
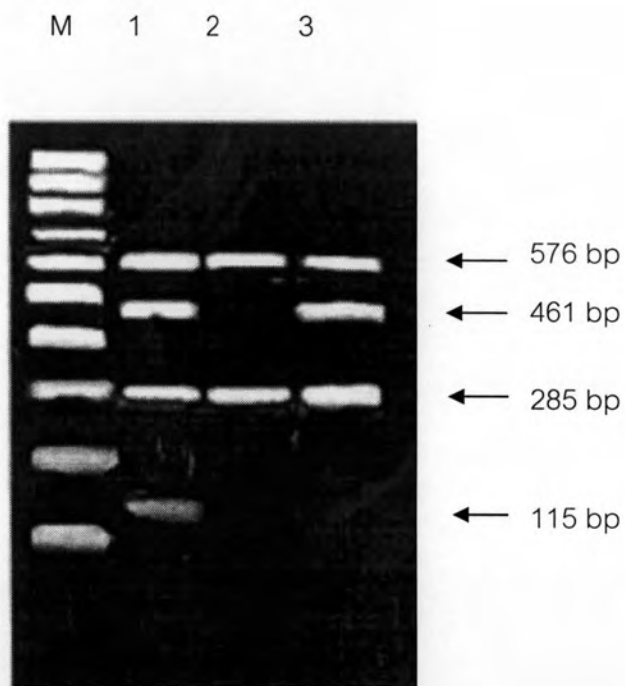
1.3 Characteristics of *XRCC1* gene polymorphisms.

Distribution of *XRCC1* genotypes in 139 ALL cases were compared to 139 apparently normal age and sex matched controls. For ALL, the distribution of the allele and genotype frequency of *XRCC1* codon 399 Arg to Gln is associated with increased risk of ALL. The heterozygous variant is at higher risk (Odds ratio, OR=3.17, 95% CI=1.42-7.06) than homozygous variant (OR=2.94, 95% CI=1.73-5.00). For codon 194 Arg to Trp, the heterozygous variant had an increased risk of ALL lower than codon 399 (OR=2.00, 95% CI=1.14-3.53)(Table 8 and 9). In contrast, the distribution of the allele and genotype frequency of *XRCC1* codon 280 is not associated with increased risk of ALL among those with heterozygous and homozygous variant. In ALL the three variant forms, the Arg/Arg wild type allele was taken as the reference category. The distributions of each allele were compatible with the Hardy–Weinberg equilibrium.

Table 8 Distribution of *XRCC1* allele frequencies in childhood ALL cases and controls.

Polymorphisms	Cases (allele=278)	Control (allele=278)	*P* - value	OR (95% CI)
Codon 194				
C	216 (77.7)	242 (87.0)		1.00 (Reference)
T	62 (22.3)	36 (13.0)	0.01	1.93 (1.23-3.01)
Codon 280				
G	224 (80.6)	235 (84.5)		1.00 (Reference)
A	54 (19.4)	43 (15.5)	0.22	1.32 (0.85-2.04)
Codon 399				
G	174 (62.6)	221 (79.5)		1.00 (Reference)
A	104 (37.4)	57 (20.5)	0.00	2.32 (1.59-3.39)

Table 9 Distribution of *XRCC1* gene frequencies in childhood ALL cases and controls.

Polymorphisms	Cases (N=139)	Control (N=139)	*P* - value	OR (95% CI)
Codon 194				
C/C	10(7.2)	5(3.6)		1.00 (Reference)
T/C	42(29.5)	26(18.7)	0.01	2.00 (1.14-3.53)
T/T	87(63.3)	108(77.7)	0.09	2.48 (0.81-7.53)
Codon 280				
G/G	4(2.9)	2(1.4)		1.00 (Reference)
A/G	46(33.1)	39(28.1)	0.35	2.20 (0.39-12.32)
A/A	89(64.0)	98(70.5)	0.31	1.29 (0.77-2.17)
Codon 399				
G/G	21(15.1)	11(7.9)		1.00(Reference)
A/G	62(44.6)	35(25.9)	0.01	3.17 (1.42-7.06)
A/A	56(40.3)	93(66.2)	0.00	2.94 (1.73-5.00)

1.4 Haplotype of *XRCC1* polymorphisms.

The estimated haplotype frequencies of *XRCC1* polymorphisms of ALL patients and controls are shown in Table 10. The *XRCC1* haplotype consisted of polymorphisms of codon 194, 280, and 399, respectively. The possible eight haplotypes were demonstrated in Thai population; however, one of them had the frequencies of less than 0.1% in controls. The haplotype A, containing all three wild-type alleles (C-G-G), was the most frequent one in both cases and controls. The haplotype D, E, F, H (C-G-A, T-G-A, C-A-G, T-G-G) were associated with an increased risk of childhood ALL (OR=4.38 95% CI=2.64-7.33, OR=4.19 95% CI=1.55-2.44, OR=3.03 95% CI=1.69-5.48 and OR=2.55, 95% CI=1.42-4.60)(Table 10).

Table 10 Haplotype Frequencies of *XRCC1* polymorphisms in childhood ALL cases and controls.

Haplotype (194-280-399)	Cases (allele=278)	Controls (allele=278)	*P* - value	OR (95% CI)
A (C-G-G)	90 (32.4)	165 (59.4)		1.00 (Reference)
B (C-A-A)	6 (2.2)	18 (6.5)	0.31	0.61 (0.19-1.68)
C (T-A-A)	1 (0.4)	0	-	-
D (C-G-A)	79 (28.4)	33 (11.9)	0.00	4.38 (2.64-7.33)
E (T-G-A)	16 (5.8)	7 (2.5)	0.01	4.19 (1.55-2.44)
F (C-A-G)	43 (15.5)	26 (9.4)	0.01	3.03 (1.69-5.48)
G (T-A-G)	4 (1.4)	1 (0.4)	0.08	3.63 (0.70-7.33)
H (T-G-G)	39 (13.9)	28 (9.9)	0.01	2.55 (1.42-4.60)

1.5 The cigarette smoke exposure in childhood ALL cases and control.

The cigarette smoke exposure was separated 2 types, active and passive exposure. Active exposure means mother smoke by myself and passive exposure mean other people smoke. For ALL cases and controls, no significant differences of active and passive exposure were observed between ALL cases and controls (Table 11).

Table 11 The cigarette smoke exposure in childhood ALL cases and controls.

Cigarette smoke exposure	Cases (N=139)	Control (N= 139)	P - value	OR (95% CI)
Active exposure				
Not exposed	136 (97.8)	139 (100.0)		1.00 (Reference)
Exposed	3 (2.2)	0(0.0)	-	-
Passive exposure				
Not exposed	2 (51.8)	6 (40.3)		1.00 (Reference)
Exposed	7 (48.2)	3 (58.7)	0.05	0.62 (0.37 - 1.03)