CHAPTER V
CONCLUSION AND DISCUSSION

Previous studies has been explained the role of genetic susceptibility in the etiology of childhood ALL. It is thought that genetic factors and environmental exposures predispose children to leukemogenesis. In fact, the etiology of sporadic cancers cannot be explained by allelic variability at a single locus. This study presents the first simultaneous analysis of loci encoding both phase I and II xenobiotic-metabolizing enzymes and pesticides exposure in childhood ALL in Thailand.

This is the first report to determine the frequencies of *CYP1A*, *NQO1*, *GSTM1* and *GSTT1* genetic polymorphisms in childhood ALL population of Thailand by matched case-control study, these xenobiotic-metabolizing enzyme genes are well known for the presence of association with the risk of cancers (Krajinovic *et al.*, 2002; Belta *et al.*, 2003; Francesco *et al.*, 2004). The overall frequencies of the genotypes in control subjects and ALL subjects are in with Hardy-Weinberg Equilibrium (HWE), reassuring that the result is unbiased, and are representative for each population.

The variants of *CYP1A1* allele that have increased activity and product more harmful metabolites (Vineis *et al.*, 1999). The *CYP1A1*2A* allele is associated with elevated enzymatic activity, supporting the hypothesis linking the risk of ALL with the reducing of the xenobiotics-metabolizing enzyme (Guengerich *et al.*, 1991). Consequently, carriers of variant alleles are expected to be at a greater risk when exposed to carcinogens such as PAH (Kadlubar *et al.*, 1987). In this study, the *CYP1A1**2A variant (21.6%), is similar to a previous report for Thai population (Pakakasama *et al.*, 2005), but lower than in French-Canadians (42%) and other Asian populations (30%) (Krajinovic et al., 1999;). For the *CYP1A1*2B* were present in 6% of ALL cases, lower than Canadian (10.3%) and similar to Asians (8.3%) (Krajinovic *et al.*, 2002). Similar to previous study (Pakakasama *et al.*, 2005), there was no statistical difference of genotype or allele frequency of *CYP1A1**2A variant between cases

(20.3%) and controls. However, we found that combination of genetic polymorphisms of the *CYP1A1*2A* and *GSTM1* are associated increased risk of childhood acute lymphoblastic leukemia.

Our finding that *GSTM1* null were 53%, is lower than a previous study in Thai children ALL, but is similar to Caucasians (53.1%) and others Asians (52.9%), but higher than Africans (26.7%) (Bolt *et al.*, 2006), but lower than Thai children (71%) (Pakakasama *et al.*, 2005). In our study *GSTT1* null were 36%, lower than Chinese (49%) and higher than in Turkish (20%) (Bolt *et al.*, 2006). The other study analyzing the *GST* genotypes in the combined gene with ALL risk reported that the double-null genotype for *GSTM1* and *GSTT1* was significantly more frequent among blacks with childhood ALL (Kadlubar *et al.*, 1987). Children carrying the *GSTM1* null were also at greater risk of developing ALL (Krajinovic *et al.*, 2002; Belta *et al.*, 2003; Francesco *et al.*, 2004). *GSTs* are involved in the metabolism of a wide range of xenobiotics, including environmental carcinogens, chemotherapeutic agents, and reactive oxygen species. Several previous studies have associated the null genotype of *GSTT1* with a higher risk of lung cancer (Liang G, *et al.*, 2004), this is probably related to an inability to metabolize tobacco-related carcinogens, such as benzo(a)pyrene.

A polymorphism of *NQO1* 609C→T has been associated with reduced enzymatic activity or no enzyme function. The frequency of the *NQO1* were 25% in ALL cases that seems similar to Asians (20.3%) and higher than Mexican-American (15.5%) and African-American (5.2%) (Daniel *et al.*, 2002). Our study of *NQO1* variant in childhood ALL in Thai population is the first report in Thailand, we would not demonstrate significant difference between cases and controls. Similarly *NQO1* were not found associated with increased risk of childhood ALL in Turkey, Japan and Egypt (Sirma S *et al.*, 2004; Eguchi-Ishimae M *et al.*, 2005; Eyada TK *et al.*, 2007). The others studies, the combination between *GSTT1* null and *NQO1*2* increased the risk of ALL in Spain (Bolufer P *et al.*, 2007), Children carrying the *NQO1*2* were also at greater risk of developing ALL in Canada and USA (Krajinovic *et al.*, 2002; Smith MT *et al.*, 2005).

In this study, *GSTM1* null genotype of ALL subjects was more common than but not significantly different from control group. However, more subjects may confirm these findings. The molecular epidemiology study in childhood diseases makes it difficult to predict how these genotypes at risk will modify the host response to different exposures. The association of parental exposures to various chemicals suggests that variability at loci encoding xenobiotic- metabolizing enzymes could influence susceptibility to childhood ALL. Infants and children may be at greater risk than adults from a variety of environmental toxicants, including, pesticides, and tobacco smoke (Krajinovic *et al.*, 2002). In this regard, we found that exposure to pesticides are associated with increased risk of ALL (OR=2.29, 95% CI 1.54-3.47). The se results suggest that the risk of ALL may indeed be associated with pesticides exposure, particularly with exposure before pregnancy (OR=13.63, 95%CI 6.7-27.4), as well as during pregnancy (OR=8.27, 95%CI 4.2-16.3) and after pregnancy (OR=3.14, 95%CI 1.8-5.5). The characteristics of pesticides exposure in this study, the most common use household pesticides were mosquito/insect spray (85%) due to specific combination of active ingredients including Propoxur, Cyfluthrin and Dichlovos that the Organophosphate/ Carbamate/ Pyrethroid compound and mosquito coil/stick (10.78%) due to specific gradient from Transflutin that the Pyrethroid compound (Kaufman *et al.*, 1997; www.baygon.com).

The timing of pesticide exposure, led to the suggestion that in utero and postnatal exposures to various biological, physical, and chemical factors may be important determinants of childhood ALL. The previous studies, the parental occupational exposure with pesticide associated increased risk of childhood ALL (Reynolds et al., 2005). In this study, pesticide exposure in these analyses estimated for exposure in the period (1) 3 months before pregnancy, (2) first trimesters of pregnancy, (3) second trimesters of pregnancy, (4) third trimester of pregnancy, (5) breath feeding, (6) 0-1 year, (7) 1-2 years, (8) 2-3 years. We suggest that the pesticides exposure in these periods, post pregnancy, during pregnancy, and after pregnancy year were associated increased risk of childhood ALL in Thai population.

To the best of our knowledge, the present match case-control study is the first report of association of the *GSTT1, GSTM1, CYP1A1* and *NQOI* polymorphisms with ALL risk in the population characterized, and determined prevalence of pesticides exposure in childhood ALL in Thai population, the matched case-control study seems better than the case-control study, because of sex and age matched. One of the previous studies is detailed information of household pesticide usage in aplastic anemia patients in Thai population, and they not found the association (Kaufman *et al.*, 1997). Previous report has shown that household pesticide is associated with childhood ALL (Buffler *et al.*, 2002). The xenobiotic mechanism, pesticides manifested the involvement in free radical generation. The xenobiotic-metbolizing production is principally correlated to the enzymes release and consequently executing the programmed cell death and leukemogenesis, pointing out a role of pesticide in etiology of ALL. In addition, to minimize the risk of population stratification bias, we restricted our analyses to subjects with birth place in Thailand and using age and sex matched. However, there are others chemicals that were not investigated may involve to be the risk of leukemogenesis. In this study, we found that there was tendency for the higher frequency of *GSTM1* in patients with ALL. However, it did not support associations with the variant genotypes. In addition, it has been demonstrated that some genes may related to the detoxification are associated with ALL susceptibility.

In summary, a disease model of ALL pathogenesis is may be undoubtedly involved in interaction between genes. There is may be involved in gene and environmental exposure interaction. Our study provides support to the hypothesis that susceptible the combination of *GSTM1* null and *CYP1A1*2A* associated increased risk of childhood acute lymphoblastic leukemia in Thai population. For the maternal household pesticide exposure factor, in this study the mosquito/insect spray were associated to increase ALL risk in Thai children.