UNIVERSITI TEKNOLOGI MARA

INTERPATIENT VARIATION OF CLINICAL RESPONSES AMONG ACUTE LYMPHOBLASTIC LEUKAEMIA (ALL) PATIENTS TREATED WITH 6-MERCAPTOPURINE (6-MP): A PHARMACOGENOMICS - METABOLOMICS PERSPECTIVE

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Thesis submitted in fulfilment of the requirements for the degree of
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AUTHOR'S DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Teknologi MARA. It is original and is the result of my own work, unless otherwise indicated of knowledge as reference work. This thesis has not been submitted to any other academic institution or non-academic institution for any other degree or qualification.

I, hereby, acknowledge that I have been supplied with the Academic Rules and Regulations for Post Graduate, Universiti Teknologi MARA, regulating the conduct of my study and research.

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ABSTRACT

Leukaemia chemotherapy had advanced considerably, enabling higher rates of survival and cure. This success is largely attributable to optimization of the use of chemotherapeutic agents such as 6-mercaptopurine (6-MP). However challenges still remain in striking a delicate balance between therapeutic outcome and risk of toxicity due to the narrow therapeutic window of anticancer drugs. This is compounded by the variability in patient response even when given the standard dose. Identification of patients at risk of adverse events would be beneficial, thus pharmacogenomics could play a role in reducing the related adverse drug reaction and hospitalization by the development of diagnostic tools to tailor appropriate therapies for patients. The aim of this study is to investigate the impact of pharmacogenomics in the clinical outcome of acute lymphoblastic leukaemia (ALL) patients that were on 6-MP. Therefore, 313 healthy Malaysian volunteers from each ethnic group (Malay, Chinese and Indians) and 63 ALL patients were recruited for the study and their blood sampled. The samples were then processed to obtain DNA, RNA and also metabolites. Investigations proceeded with the genotyping of *TPMT* common variants and *ITPA 94C>A* and the only *TPMT* common variant detected was heterozygous *TPMT*3C*. Whereas for *ITPA 94C>A* was detected at a high frequency, in accordance with other studies on Asian populations. There is higher likelihood of developing fever, liver toxicity and risk for relapse for ALL patients bearing the variant *ITPA 94A*. Variability in patient response was still observed therefore the patient samples were also subjected to denaturing high performance liquid chromatography (DHPLC) analysis to navigate the entire exon regions of *TPMT* for any variants other than the common variants assayed in PCR. There were a total of 15 variant sites detected that include both reported and unreported variations. Even though there is low frequency of *TPMT* common variants, the genotyping of other detected polymorphisms and haplotypes may prove more useful. Measurement of *TPMT* gene expression by absolute quantification of mRNA in patients' blood utilizing quantitative PCR (qPCR) was done for patient samples and found that the expression level was not uniform and this suggest that although there were no common variants of *TPMT* detected in patients, there may be other variants in the gene that affect the mRNA expression. Analyses have shown that there exist relationship between the DHPLC-detected variants and the variable level of TPMT expression. Global metabolite profiling of patient samples reveal that there exist differential expression among patients and healthy subjects as well as among patients with different genotype groups. Some metabolites detected such as glycerophosphocholine, hypoxanthine, linoleic acid metabolites and prostaglandin 2F alpha were previously associated with clinical outcome of cancer patients. At the end of study there were a total of nine metabolites that were identified to have potential to be used as biomarker either for disease progression, onset of adverse reaction and relapse. However the use of these metabolites in the clinical setting has to be validated further. An initial understanding of the biochemical processes that affect clinical outcome could aid in tailoring therapies that suit individual patient needs and be a positive step toward personalized medicine. At the end of this study establishment of the correlation between genotype, metabolite levels and TPMT expression with treatment outcome is hoped to lead to more effective therapy for patients in the future.

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