Genetic Polymorphisms Associated with Cyclophosphamide Outcome and Risk of Toxicity in Patients with Lupus Nephritis

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ABSTRACT

A 6-month cyclophosphamide induction therapy followed by maintenance therapy every three months is the first-line treatment for Class III, IV, and V lupus nephritis. Among the 139 single nucleotide polymorphisms (SNPs) associated with cyclophosphamide, four SNPs, namely rs4244285, rs4802101, rs7254579 and rs3957356, are related to the response and risk of toxicity in patients with lupus nephritis. Although pharmacogenetic studies in patients with lupus nephritis (LN) have not been conducted previously in Indonesia, data on rs4244285 are available for several ethnic groups, including Papuans, Bataks, Balinese, Dayaks, Javanese, Bugis, Chinese, Timorese and Malays, even though direct evidence in LN patients is less detectable. However, this can be followed up prior to cyclophosphamide therapy based on the identification of genetic markers. Therefore, clinical studies in patients with lupus nephritis are deemed necessary to evaluate the potential of these markers.

Keywords**:** *Cyclophosphamide, SNP, response, risk of toxicity.*

INTRODUCTION

The major goal of treatment for lupus nephritis (LN) is to prevent loss of nephron function and the development of chronic kidney disease, especially in advanced stages.[1] In this regard, the International Society of Nephrology/Renal Pathology (ISN/RPS)[2] stated that immunosuppressants play an crucial role in the management of proliferative Class III, IV, and V lupus nephritis with an increased risk of kidney damage. Meanwhile, the short-term goal of treatment for this disease is complete or partial response to its clinical signs.[3] In Indonesia, 402 patients with systemic lupus erythematosus (SLE) received treatment at Hasan Sadikin Hospital Bandung, 125 of them (31.1%) took cyclophosphamide and almost all of them (97.4%) also took methylprednisolone.[4]

In a multicenter randomized clinical trial, the efficacy of cyclophosphamide therapy in

LN patients was observed during maintenance therapy, with the primary endpoint being complete remission after six months of treatment.[5] Meanwhile, renal response includes parameters of urine protein/creatinine ratio, serum creatinine, and glomerular filtration rate within six months.[6] Once clinical remission is achieved, patients with proliferative LN should undergo maintenance therapy.[7] In some patients, the prescription of intravenous cyclophosphamide can help improve medication adherence.[8] Studies on this topic need to provide the main reasons for the clinical efficacy of cyclophosphamide in relation to genetic factors and pharmacokinetic parameters. Evaluation of LN patients in Romania demonstrated an increasing trend of complete response.[9] Meanwhile, another study that compared disease profiles or treatment outcomes between early-onset and late-onset lupus nephritis found no significant differences between the two.[10] Understanding the factors contributing to cyclophosphamide treatment failure in patients with LN is particularly important to minimize mortality and morbidity.

PREVALENCE OF SLE AND LUPUS NEPHRITIS

The overall incidence rates of SLE vary from 3.7 to 49.0 per 100,000 persons per annum in North America, 1.5 to 7.4 per 100,000 persons per year in Europe, 1.4 to 6.3 per 100,000 persons per annum in South America, and 2.5 to 8.6 per 100,000 persons per year in Asia. Several relevant studies done in North America, Europe, and Asia have indicated an increase in the prevalence of SLE over time.[11]

Lupus nephritis (LN) is the most common and most serious manifestation of SLE, occurring in 60% of SLE patients and contributing to their morbidity and mortality.[12] The development of kidney disease in SLE patients is caused by the deposition and formation of immune complexes in the mesangial, subendothelial, or subepithelial space and subsequent complement activation.[13] One of the most important factors that influence the incidence of LN is ethnicity, with the prevalence in populations varying by ethnicity.[14] Cohort studies conducted in various countries have shown the prevalence of LN in SLE patients based on their ethnic groups, i.e., 39.9% American, 49.3% Hispanic, 36.8% Asian, and 20.3% Caucasian.[15] Notably, patients with LN had a higher standardized mortality rate (SMR) than patients without LN in Asia (9.0 vs. 4.8), and those with proliferative LN had a higher SMR than those with membranous LN.[16]

Based on cohort data register at Hasan Sadikin Hospital Bandung in 2017, there were 813 SLE patients in Indonesia (95.6% of them are women), with an average age of 27.7$\pm$9.4 years and an average illness duration of 76.5$\pm$51.1 months. According to the data, the ethnic variations in SLE patients include Sundanese (69.6%), Javanese (4.7%), Batak (0.7%) and others (24.9%). Furthermore, the number of SLE patients with impaired kidney function based on the ACR criteria was 341 patients (41.9%).[4]

PHARMACOGENETIC STUDY OF CYLOPHOSPHAMIDE

Genetic markers such as single nucleotide polymorphisms (SNPs) reflect the characteristics of each individual. The more data available on SNPs and other types of genetic mutations will contribute significantly to pharmacogenetic variation. Therefore, research is absolutely necessary if there is no data in the literature regarding the effects of genetic polymorphisms on the efficacy and safety of the drug being studied.[17]

Literature was searched on the PubMed and PharmGKB databases, with keywords ("Cyclophosphamide") AND ("Pharmacogenetic" OR "Pharmacogenomic") being used to search for articles regarding the effects of genetic polymorphisms on cyclophosphamide efficacy, risk of toxicity, and metabolism. Total of 392 associations of SNP on cyclophosphamide efficacy, metabolism, and risk of toxicity. The inclusion criteria used for the literature search with PubMed were original articles that discussed the significance of genetic variations or SNPs in association with the clinical response of cyclophosphamide on SLE and Lupus Nephritis. A total of 8 associations of SNP on cyclophosphamide efficacy, metabolism, and toxicity risk were significant in SLE and Lupus Nephritis. Eight associations of

cyclophosphamide involving patients with renal involvement who had their response evaluated using SLEDAI and clinical parameters of lupus nephritis (**Table 1**). CYP2B6 and CYP2C19 enzymes play a vital role in the bioactivation of cyclophosphamide, and these inducers enhance its activation and therapeutic efficacy, whereas GSTA1 plays a role in the detoxification of cyclophosphamide metabolites.

The primary end-point of LN was response to urine protein, serum creatinine (Scr), and estimated glomerular filtration rate (eGFR).[7,22,23] During six months of induction therapy with cyclophosphamide, patients in the extensive metabolism categories (*CYP2C19*1*1* and *CYP2B6*-750TT polymorphisms) had a significantly faster response time to achieve remission (complete and partial) than those

Table 1. Four SNPs associated with efficacy, metabolism and toxicity of cyclophosphamide

SNP Variant	Literature	Genes	Association	Sample Size	Biogeographical Groups	Phenotype Categories
rs4244285 (G>A)	[18]	*CYP2C19*	*CYP2C19*2* is associated with decreased response to cyclophosphamide in people with Lupus Nephritis as compared to genotype GG.	136	Central/South Asian	Efficacy
rs3957356 (T>C)	[19]	*GSTA1*	Genotype CT is associated with decreased response to cyclophosphamide in people with Lupus Nephritis as compared to genotype CC.	77	East Asian	Efficacy
rs4802101 (T>C)	[20]	*CYP2B6*	The C allele is associated with decreased cyclophosphamide metabolism in people with SLE, compared with the T allele.	189	East Asian	Metabolism/ PK
rs7254579 (T>C)	[20]	*CYP2B6*	The C allele is associated with decreased cyclophosphamide metabolism in people with SLE, compared with the T allele.	189	East Asian	Metabolism/ PK
rs4244285 (G>A)	[20]	CYP2C19	The A allele is associated with decreased cyclophosphamide metabolism in people with SLE, compared with the G allele.	189	East Asian	Metabolism/ PK
rs4244285 (G>A)	[21]	*CYP2C19*	The GG genotype is associated with an increased risk of toxicity in women with Systemic Lupus Erythematosus (SLE) when treated with cyclophosphamide, compared with the AA + AG genotypes.	71	East Asian	Toxicity
rs4244285 (G>A)	[20]	*CYP2C19*	The A allele is associated with a reduced risk of gastrointestinal toxicity and leukopenia in people with SLE when exposed to cyclophosphamide, compared with the G allele.	189	East Asian	Toxicity
rs4802101 (T>C)	[20]	*CYP2B6*	The C allele is associated with a reduced risk of gastrointestinal toxicity and leukopenia in people with SLE when exposed to cyclophosphamide, compared with the T allele.	189	East Asian	Toxicity

in the intermediate and slow metabolism categories (*CYP2C19*2* and *CYP2B6*-750CC).[20] Theoretically increase systemic exposure to cylophosphamide improve therapeutic effects. The response of the homozygous allele (CC) of rs3957356 was better than heterozygote (CT).[19] Decreased detoxification rates of 4-hydroxycyclophosphamide can result in prolonged exposure to activated cyclophosphamide and hyperresponsiveness.[24]

Prolonged exposure may increase the occurrence of adverse drug reactions. Some of the most common side effects of cyclophosphamide are nausea, vomiting, thinning hair, and reversible alopecia. Meanwhile, severe and rare side effects of this medication affects gonads (amenorrhea) and bladder (hemorrhagic cystitis), as well as includes its myelotoxicity (risk of thrombocytopenia and granulocytopenia leading to bacterial and opportunistic infections) and malignancy (myelodysplastic syndrome, acute leukemia, and non-Hodgkin's lymphoma, especially for prescription of > 2-3 years and cumulative dose of >100 grams).[25] Prevention of hemorrhagic cystitis and bladder cancer can be done by administering mesna to reduce deposits of acrolein (a cyclophosphamide metabolite) in the bladder. However, as this is controversial, mesna can be substituted with appropriate hydration with 6 L of water per day plus a diuretic, or hydration with a volume of 3 L/m^2 per day.[8]

IMPACT OF GENETIC POLYMORPHISM TO METABOLIC PATHWAYS

The metabolic pathways of cyclophosphamide include activation, deactivation, and toxicity. Activation of cyclophosphamide to 4-hydroxycyclophosphamide is catalyzed by the hepatic cytochrome P450 (CYP) isoenzymes, namely CYPs 2B6, 2C9, and 3A4 (with 2A6, 2C8, and 2C19 also making minor contribution)[26]. As an alkylating agent, cyclophosphamide has no effect in vitro but acts in vivo after being oxidized by cytochrome P450 in the liver to produce 4-hydroxycyclophosphamide, which is further oxidized and metabolized to form the nontoxic 4-ketocyclophosphamide or formed into aldehyde compounds such as aldophosphamide through tautomerization.[27] The part of aldehyde compounds which cannot carry out metabolism undergoes non-enzymatic reaction to form into phosphoramide mustard (PM) and acrolein. PM is cytotoxic by alkylating DNA and affecting cell replication through the formation of crosslinks, thus stopping the cell cycle and preventing proliferation, whereas acrolein is a metabolic byproduct that increases toxic effects.[28] Immunosuppression induced by cyclophosphamide metabolites affects both cell-mediated and humoral immunity. Phosphoramide mustard is responsible for anticancer activity, and acrolein exhibits immunosuppressive and cardiotoxic effects.[29,30]

Substitutions in the nucleotide bases of the SNP produce variations that affect transcription and gene regulation so that effectiveness, risk of toxicity and metabolism are found in the use of cyclophosphamide for LN patients. The normal variants of *CYP2C19* produce a better response even though the risk of toxicity increases, while the polymorphism variants of *GSTA1* produce a better response. Polymorphism variants of *CYP2C19* and *CYP2B6* produce a decrease in efficacy, metabolism, and risk of toxicity (**Figure 1**).

Cytochrome P450 enzymes that metabolize to activate 4-hydroxycyclophosphamide are associated with variations in the concentration of protein expression or metabolic activities.[20,31] The *CYP2C19*1*1* and *CYP2B6*-750TT alleles tend to have high concentrations of 4-hydroxycyclophosphamide, a better response, and a high risk of leukocytopenia and gastrointestinal toxicity, whereas the *CYP2C19*2* and *CYP2B6*-750T>C alleles have great potential for an increased dose.[20] Glutathione (GSH) conjugation by Glutathion S-transferase (GST) detoxifies.[19] GST plays a cytoprotective role by catalyzing reduced glutathione (GSH) conjugation and reactive electrophiles generated by cytochrome P450 metabolism to form GSH conjugates.[32]

MAPPING OF ASSOCIATED POLYMORPHISM IN INDONESIA

The literature search of national journals and/or Indonesian journals was done using Google

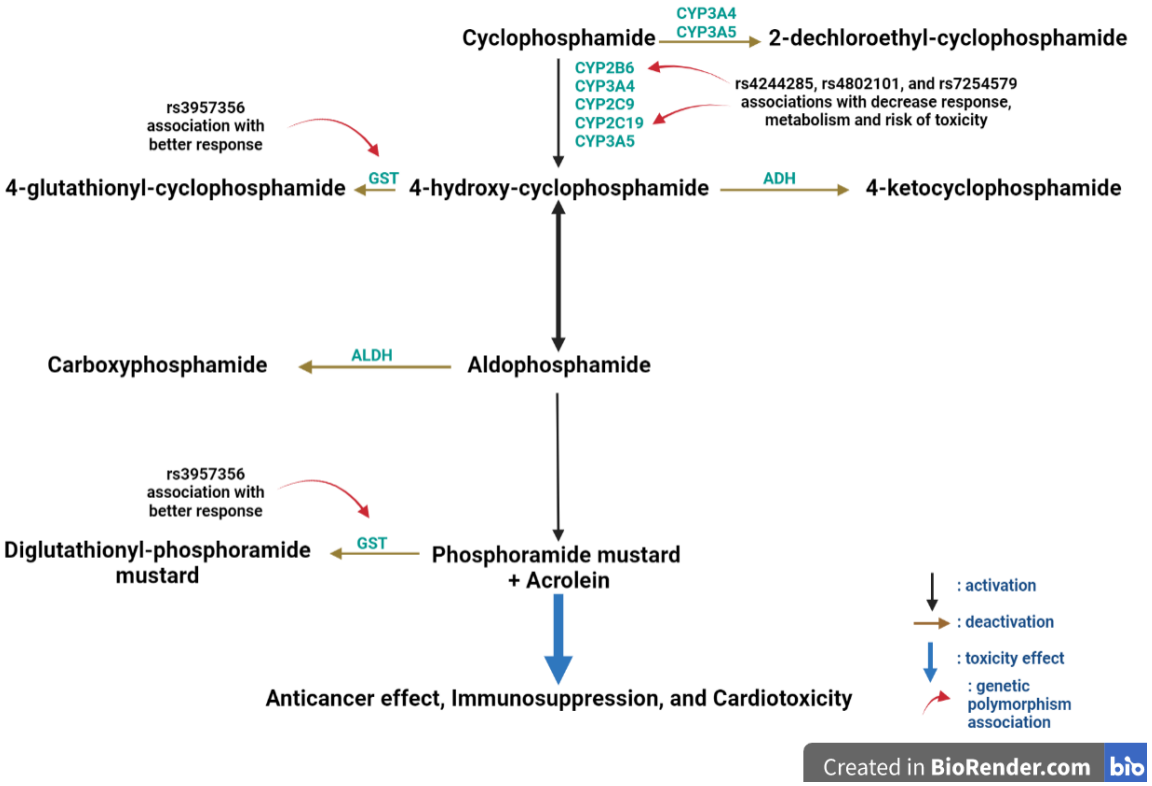

Figure 1. Association of four SNPs to metabolic pathways of cyclophosphamide. Phase I metabolism of cyclophosphamide requires metabolic enzymes such as CYP2C19 and CYP2B6. In phase II metabolism, the GST enzyme inactivates phosphoramide mustard before it is excreted. A SNP within a gene encoding an enzyme affects the amount and activity of the enzyme according to the position of the SNP within the gene. Created with BioRender.com.

Scholar with the keywords "rs4244285" OR "*CYP2C19*2*", obtaining 3 results[33–35]. The inclusion criteria used for from the literature search with Google Scholar were articles which involved Indonesian patients by mentioning their race/ethnicity. A similar study was also carried out in Indonesia using the PCR-RFLP method to detect the same SNP gene, namely rs4244285 in *CYP2C19*, while SNPs rs4802101 and rs7254579 in *CYP2B6* and also rs3957356 in *GSTA1* were not found. All ethnic groups have minor alleles frequency (MAF) greater than 5% based on genotypic samples (**Table 2**).

Table 2. Frequency of Variant rs4244285 (G>A) in Indonesia

Literature	Ethnic Groups	Sample Genotypes	% Allel G (Reference)	% Allel A (Altered)
35	Javanese	25	84	16
33	Javanese	28	71	29
34	Malay	30	72	28
33	Batak	27	70	30
33	Balinese	25	68	32
33	Timorese	8	69	31
33	Bugis	37	66	34
33	Dayak	10	65	35
33	Chinese	17	62	38
33	Papuan	14	54	46

For the *CYP2C19*2* polymorphism, the homozygous wild-type allele was found in 77 (46.4%) of the 166 patients with *Helicobacter pylori*.[33] Another study on 25 patients with coronary heart disease who received antiplatelet therapy found homozygous (AA) alleles in 18 patients, heterozygous (GA) alleles in 6 patients, and wild type (GG) alleles in 1 patient.[35] Furthermore, in a study of 30 patients who took omeprazole showed that the prevalence of the wild type (GG) alleles, heterozygous (GA) alleles, and homozygous AA alleles of the *CYP2C19*2* genotypes was 56.7%, 30%, and 13.3%, respectively.[34]

The *CYP2C19* rs4244285 polymorphisms produce clinical phenotypic effects, with the A allele being associated with a decreased response to therapy.[18] The most common type of mutation is nucleotide substitution which results in single nucleotide polymorphisms (SNPs) that are used in genomic maps. Nucleotide differences in humans vary from 1 in 1000 to 1 in 2000 nucleotide sequences or about 2.5 million SNPs in the whole genome. The mutation rate is much lower in larger genomes. In humans, the mutation occurs $5.0x10^{-8}$ per 1000 base pairs.[36] However, recommendations for adjusting the dose of cyclophosphamide based on genetic polymorphisms as biomarkers have not been established in the CPIC guidelines based on the type of metabolism[37]. Therefore, proper management of cyclophosphamide, especially in patients with lupus nephritis, is highly necessary.

CONCLUSION

Improved detection of the significance of SNPs associated with cyclophosphamide response and risk of toxicity can increase the accuracy of drug administration in lupus nephritis patients. Data on the prevalence of cyclophosphamide used in the treatment of lupus nephritis may provide benefits for implementing SNP detection. However, the data should also include several SNPs of other genes associated with the pathogenesis of lupus nephritis that are related to treatment efficacy and targets.

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