P-Glycoprotein Expression on Patients with Acute Lymphoblastic Leukemia

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Abstract
Objective: P-glycoprotein (P-gp) overexpression on neoplastic cells can deteriorate the therapeutic outcome on cancer patients. P-gp plays important role on drug efficacy and toxicity. This research aimed to measure P-gp expression on children with Acute Lymphoblastic Leukemia (ALL) on Sanglah Hospital, Denpasar. Method: Flowcytometry method was used to measure P-gp expression level on Bone Marrow samples from pediatric patients (0-12 years old) who were newly diagnosed with ALL in Sanglah Hospital. P-gp overexpression were based on the percentage of cell stained. Ten percent of P-gp expression were considered as the cut-off value of P-gp overexpression. Result: On this study, 11 samples were obtained with the range value of 56-97% on P-gp expression. Conclusion: All 11 patients had P-gp overexpression.

Keywords : **ALL children, BMA, P-gp**

I. INTRODUCTION

P-glycoprotein (P-gp) overexpression can decrease intracellular drug concentration, such as vinblastine, vincristine, and daunomycin. P-gp may overexpressed on neoplastic cells. It can deteriorate the therapeutic response and prognosis on cancer therapy, such as on Acute Myeoblastic Leukemia (AML). P-gp plays important role in drug efficacy and toxicity [1], [2].

On leukemia, *myeloma, lymphoma*, and solid tumor (such as breast and ovarium cancer), the detection of drug resistances marker can help to choose drugs therapy. Low expression of P-gp can be a prediction marker on therapeutic result, such as in ovarium cancer [3], [4].

This study was aimed to evaluate P-gp expression on children with ALL in Sanglah Hospital, Bali.

II. METHODS AND PROCEDURES

This laboratory exploration study was approved by Ethical committee of Sanglah Hospital – Faculty of Medicine Udayana University.

K. Samples

Bone marrow aspirates (BMA) (1 ml) were collected in ethylenediamine tetraacetic acid (EDTA) tubes from subjects (newly diagnosed children (0-12 years old) with ALL, who did not receive chemotherapy yet and signed inform consent) on the period of June 2015 – January 2016. The samples were sent to Clinical Pathology Laboratory, Dr Sutomo Hospital-Faculty of Medicine Airlangga University, Surabaya. P-gp Expression were measured in the period of 24 hours after the samples were drawn.

L. P-gp expression

BD reagent anti P-gp PE dan PE isotype control kappa IgG1A was used to study P-gp expression. The procedure was based on the manufacture instruction. Samples were run using FACS Calibur. P-gp positivity was determined based on the percentage (%) of cells stained. Ten percent (10%) of P-gp expression was considered as P-gp overexpression [5]-[7].

III. RESULTS AND DISCUSSION

Eleven samples were able to be studied. Only limited samples were collected because of limited patients, cell viability, and availability of reagents. Based on Mudita (2007), the total ALL cases on children at Sanglah Hospital were 51 cases on the period of 2000-2005 [8].

On this study, all the subjects had P-gp overexpression with the range of 56 – 97% (Table I and Figure 1).

TABLE I
P-GP EXPRESSION ON PATIENTS WITH ALL

Subject	Number of Cell Stained	Cell Total	% P-gp positivity (A/B)x 100%	P-gp overexpression [a] + / -
	A	*B*	*C*	
1	5298	7440	71%	+
2	7683	9327	82%	+
3	5147	9147	56%	+
4	3303	4032	82%	+
5	3304	3899	56%	+
6	7210	7383	97%	+
7	8368	8589	97%	+
8	4233	6168	68%	+
9	3550	6228	57%	+
10	4372	5989	73%	+
11	5105	6006	85%	+

[a]P-gp expression on patients with ALL; (+) = P-gp overexpression; (-) = normal P-gp expression.

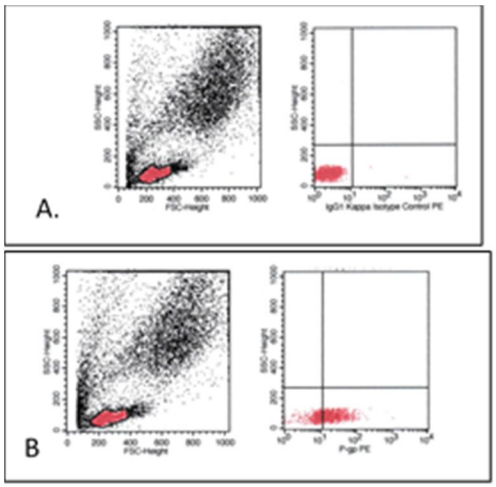

Fig. 1. Example of Flow Cytometry Results on Patients number 1
(*A)* Cell stained with Isotype Control,
(B) P-gp positive Cells (Upper Right and Lower Right Quadrant)

On all 11 patients (Table 1 and Figure 1) had P-gp overexpression. P-gp is the member of ATP-binding cassette (ABC) transporter family. Hydrophobic medicines (such as vincristine, vinblastine, doxorubicin, and daunorubicin) entry across plasma membrane by diffusion. The activation of energy dependent transport systems can keep the medicines out of cells. It may reduce the drug accumulation on cancer cells [3], [9].

On this study, the P-gp expression on all 11 samples were on the range of 56-97% (Table 1). Genetic variation on Multi Drug Resistance-1 (MDR-1) gene, which encoded P-gp, was considered as one of the factors that alter P-gp function and expression [10]-[14]. Many studies in pharmacogenetics and pharmacogenomics showed that variation on *Single Nucleotide Polymorphisms* (SNP) of MDR-1 gene may affect the function and expression of P-gp in different ethnic-populations [2], [15]-[17]. There were 28 exon in MDR-1 gene. Based on the data from *National Center for Biotechnology Information (NCBI)*, more than 50 SNPs from MDR-1 gene were found in human. High frequency of variant 3435, 2677, and 1236 MDR-1 Gene were found in Chinese, Malays, Iran, and India [3], [9], [18]-[21]. Haplotype may affect the secondary structure of mRNA and its activity. It will influence the efflux transporter activity and the drug therapeutic response [11], [22]. The difference on haplotype and phenotype can make alteration on *mRNA level*, protein expression, *protein folding*, and a *substrate specificity* [3], [11], [23].

Variation on MDR-1 gene may influence on diseases progression and therapeutic outcome [3], [24]. In AML, addition of P-gp inhibitor on chemotherapy regimen can increase the probability of remission and survival rate [9], [25]. Mutation on MDR-1 gene may influence the P-gp inhibitor activity. The mutation on haplotype MDR-1 gene may alter the *substrate binding site* [3]. Therefore, further research need to be conducted to identify MDR-1 Gene variation and its association with the P-gp expression level and function in children with ALL in Bali.

IV. CONCLUSION

P-gp overexpression was detected on all 11 patients in this study. Further research need to be conducted to identify polymorphisms on MDR – 1 Gen MDR-1 and its impact on P-gp expression level and function

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