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RESEARCH

CORRELATION PERCENTAGE OF S AND G2/M WITH PERCENTAGE OF LYMPHOBLASTS IN PEDIATRIC ACUTE LYMPHOBLASTIC LEUKEMIA

(Kenasaban Persentase S dan G2/M dengan Persentase Limfoblas di Pasien Leukemia Limfoblastik Akut Anak)

Erawati Armayani[1], Yetti Hernaningsih[1], Endang Retnowati[1], Suprapto Ma'at[1], I Dewa Gede Ugrasena[2]

ABSTRAK

Leukemia Limfoblastik Akut (ALL) merupakan keganasan klonal di sumsum tulang/Bone Marrow (BM). Angka bertahan hidup 5 tahun saat ini >85%, tetapi 15-20% relaps sehingga perjalanan penyakit jelek. Perjalan penyakit jelek jika setelah tahap induksi limfoblas menetap di Darah Tepi (DT) dan BM >5% serta tahap S BM >6%. Tahap G2/M merupakan petunjuk prognosis ALL anak, selain itu sebagai target pengobatan. Tujuan penelitian menganalisis kenasaban persentase tahap S dan G2/M dengan persentase limfoblas DT pasien ALL anak sebelum dan sesudah kemoterapi induksi. Jenis penelitian analitik observasional longitudinal (kohor) di ALL anak kasus baru diperiksa sebelum dan sesudah induksi. Persentase limfoblas secara mikroskopis. Persentase fase S dan G2/M flowcytometryBD Facs Callibur. Kenasaban bermakna hanya persentase tahap S dan limfoblas sebelum induksi (r=0,449; p=0,007). Kelainan gen ALL pada ekspresi cyclins dan CDK sehingga hilang kendali checkpoint siklus sel, merangsang transisi tahap G1 menjadi tahap S. Persentase tahap S tidak berbeda pada remisi dan meninggal (p=0,138). Persentase tahap G2/M berbeda antara remisi dan meninggal (p=0,006) dan bernasab dengan luaran kemoterapi induksi (koefisien Eta= 0,744), G2/M ≥1,26% meramalkan remisi. Terdapat kenasaban antara persentase siklus sel tahap S dengan persentase limfobas sebelum kemoterapi induksi. Persentase siklus sel tahap S memberikan gambaran siklus sel pada sel limfoblas. Terdapat kenasaban antara persentase siklus sel tahap G2/M dengan luaran kemoterapi induksi tahap G2/M menjadi faktor peramal luaran kemoterapi induksi ALL. Perlu penelitian lanjutan dengan sampel BM, subtipe dan pengamatan semua tahap kemoterapi.

Kata kunci: *Limfoblas, siklus sel, tahap S, tahap G2/M, ALL*

ABSTRACT

Acute Lymphoblastic Leukemia (ALL) is a Bone Marrow (BM) clonal malignancy. At the moment, the five year survival rate is >85%, 15-20% relapse showing a bad prognosis. Persistant Peripheral Blood (PB) lymphoblasts, BM >5%, S phase BM >6% after induction give a poor prognosis. G2/M phase is an indicator for the prognosis and treatment target in ALL. The research aimed to analyze the correlation percentage of S phase and G2/M with the percentage of PB lymphoblasts in pediatric ALL patients before and after chemotherapy induction. This was an analytical observational longitudinal (cohort) research in new pediatric ALL cases, examined before and after induction. Percentage of lymphoblasts was examined microscopically, percentage of S phase and G2/M by flowcytometry BD FacsCallibur. A significant correlation was only found in the percentage of S phase and lymphoblasts before induction(r=0.449; p=0.007). ALL gene abnormalities were in the expression of cyclins and CDKs causing loss of control checkpoint, stimulating G1 phase transition into S phase. Percentage of S phase did not differ between remission and who died (p=0.138). Percentage of G2/M phase differed between remission and who died (p=0.006) and correlated with outcomes (coefficient Eta=0.744). G2/M ≥1.26% predicting an increased remission. There was a correlation between the percentage of cell cycle S phase and percentage of lymphoblasts before chemotherapy induction. The percentage of S phase gave an overview of lymphoblasts cell cycle. There was a correlation between G2/M phase percentages with chemotherapy induction outcomes. G2/M was a predictive factor for ALL chemotherapy induction outcomes. A further research is needed with BM samples, subtypes and observation of all phases of chemotherapy.

Key words: Lymphoblasts, cell cycle, phase S, phase G2/M, ALL

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INTRUDUCTION

Acute Lymphoblastic Leukemia (ALL) is a malignant (clonal) disease of the bone marrow in which early lymphoid precursors proliferate and replace the normal hematopoietic cells of the marrow.[1] Acute lymphoblastic leukemia is a common malignancy in the United States, the most common age is 1–4 years and occurance in males (45–55%) is more common than females.[2] A previous research in the Dr. Soetomo Hospital reported that there were 82 pediatric ALL new cases with outcomes after induction phase as remission 48.5%, no remission 14.7% and died 36.8%.[3] The five year survival rate for the last decade was 90.4% but about 15-20% relapsed.[4]

Prognosis in ALL is influenced by several parameters such as age, number of leucocytes, cell morphology immunophenotype and genotype. Blast clearence speed in the peripheral blood during therapy is a prognostic factor in the outcome of pediatric ALL.[5] A research by Posadzy et al[6] found out that lymphoblast Peripheral Blood (PB) which settled after one week and bone marrow >5% after induction chemotherapy gave a poor prognosis.[6] Early clearance of peripheral blood blasts after induction chemotherapy predicts early marrow blast clearance, complete remission, Relapse-Free Survival (RFS) and Overall Survival (OS).[7] S phase of the cell cycle can be a prognostic value in acute leukemia. Kumar[8] stated that it was low, S phase <2.6% before chemotherapy and > 6% after chemotherapy induction phase gave a poor response to induction chemotherapy and required intensive chemotherapy.[8] Cell cycle G2/M phase was related to the outcome of chemotherapy induction. A high fraction was corelated with poor outcomes in ALL patients. G2/M phase was the target of chemotherapy drugs so it could stimulate an apoptotic signal and induce apoptosis of cancer cells.[9]

Currently, there is no research on the correlation of the S phase percentage of the cell cycle and G2/M with the percentage of lymphoblast cells in peripheral blood in pediatric ALL in the Dr Soetomo Hospital.

The cell cycle is the sequence of events of cell growth and division into two cells. Cell cycle is divided into four stages, G1-S-G2-M. Cell cycle begins with the activation of cyclin D-CDK4/6 is and controlled by CDK inhibitor to activate the checkpoint signals so that cells stop proliferating. The DNA damage also activates signals checkpoint so the cell has time to repair DNA damage. Cell cycle abnormalities in ALL is on p 53 expression causing malfunction of CDK inhibitors and the cell can not stop proliferation if there is a stimulus checkpoint and results in uncontrolled proliferation.[10] Lekemic cells have a high proliferation compared to normal cells.[11]

The purpose of this study was to analyze the correlation between the percentage of cell cycle phases S and G2/M with the percentage of lymphoblasts in the peripheral blood before and after induction chemotherapy and the clinical benefit was to use the percentage of S and G2/M for stratification and predicting induction chemotherapy outcomes in pediatric ALL patients.

METHODS

This research was an observational analytical design with longitudinal (cohort), from March to June 2016. Sampling in the Outpatient Clinic and patient wards of the Department of Pediatrics Hemato-Oncology and sampling examination was performed in the Clinical Pathology Laboratory, Dr. Soetomo Hospital.

Inclusion criteria samples were ALL patients aged 1 month–16 years old who underwent regular chemotherapy, received the approval of the parents/guardians. Exclusion criteria were ALL patients with congenital abnormalities complex, multi-organ abnormalities, withdrawal from study participation.

Examination of the percentage of cell cycle was by using flowcytometry BD FACS Callibur with Propidium Iodine (PI) dyes. Microscopic examination of lymphoblast percentage with Wright staining. Statistical analysis for the difference in the percentage of cell cycle phases S and G2/ M with a percentage of lymphoblasts was done by t-test 2 samples and Wilcoxon Signed Rank, correlation of the percentage of cell cycle phases S and G2/M with a percentage of lymphoblasts with Pearson and Spearman correlation test, the difference between phase S and G2/M in induction chemotherapy outcomes used the Mann-Whitney U Test and correlation of cell cycle phase with induction chemotherapy outcomes with correlation Eta test.

RESULTS AND DISCUSSION

Total samples were 35 new pediatric ALL patients before chemotherapy then followed until completion induction phase of chemotherapy. The final subjects were 20 patients. Characteristics of the samples consisted of gender and age (can be seen in Table 1).

Table 1. Characteristics of research subjects

Characteristics of research subjects	f (%)
Gender	
Male	18 (51.4%)
Female	17(48%)
Age	
Mean ± SD	5.75 ± 3.73
< 2 years	4 (11.43%)
2-5 years	16 (45.71%)
6 – 9 years	8 (22.86%)
≥10 years	7 (20 %)

Research subjects were mostly males 18 (51.4%). This result was the same with Sandeep et al[12], 55 % males and 45% females. The high male incidence was correlated with the Single Nucleotide Polymorphism (SNP) that activated enhancers or activated promoter regions and had regulatory effects on gene expression levels, this gene may counteract the suppressor effect of estrogen-regulated in males.[12]

Most of these research subjects were 2-5 years (45.71%). These results were the same with Ugrasena et al[3], who also found that the age was 2-5 years in pediatric ALL patients. The age had a twice lower risk of death compared with below 2 years. This age was more common in type B ALL.[2,12]

Examination results of hemoglobin, leukocytes, platelets, the percentage of cell cycle and the percentage of lymphoblasts, can be seen in Table 2.

The mean Hb before induction chemotherapy was 8.73 g/dL. The cause of anemia can be caused due to the effects of a chronic disease, specific nutritional deficits, chronic bleeding, neoplastic infiltration of the BM, intercurrent, infections and autoimmune hemolytic processes. A new research found that reduced erythropoiesis was the other cause of anemia because of molecular changes in the regulation of cell growth in the bone marrow micro-environment.[13,14]

This research obtained 8 (22.86%) with leukocytes > 100 x$10^3/\mu$L and 4 (11.43%) < 3x$10^3/\mu$L. Acute lymphoblastic leukemia represented a group of B/T-precursor-stage lymphoid cell malignancies arising from genetic alterations that block lymphoid differentiation and drive aberrant cell proliferation and survival. When this happens, white blood cell production becomes abnormal and increases the number of white blood cell.[15]

The mean platelet count before induction chemotherapy was 84.26 x$10^3/\mu$L. Low platelets are an indication for lymphoblast cell infiltration in the bone marrow progenitor and causing suppression of megakaryocytes.[5]

Average lymphoblast PB was 18.37% in pre-induction chemotherapy, different from the research of Rashmi et al[5], with the average percentage of 87.3%. The difference was due to lymphoblast percentage variation in this study.[5] Percentage of lymphoblast after induction chemotherapy was 0%. This was similar to Rashmi[5] reporting, the percentage of lymphoblast after induction chemotherapy as 0.7%. Total lymphoblast PB would disappear in <10 days and would give a better prognosis. Pheripheral blood blast was negative in 6 days and predicted a reduction in BM blast occuring on day 14, which predicted complete remission.[7]

The mean and median percentage of S phase before induction chemotherapy were 6.26% and 4.45, this result was different from Kumar[8], who found that in type B ALL, the mean and median were 2.6% and 2.3%. The difference in the results of this study with a previous research was due to differences in sampling time, age and type of ALL which in this study did not distinguish the type. Patients with B type ALL had a more lower S phase cell cycle.[8]

Induction of chemotherapy outcomes in this study were mostly in remission 21 (60%) Table 3.

Table 2. Results of examination on hemoglobin, total leukocytes, platelets, S phase, G1 phase, phase G2/M and S phase ALL children patients before and after induction therapy

Parameter	Pre induction (n=35)		Post induction (n=20)		p
	$\bar{x}$ ± SD	Min-Max	$\bar{x}$ ± SD	Min-Max	
Hb (g/dL)	8.73±2.77	4.00–14.40	10.37±1.68	7.00–13.20	0.358
Leukocytes (10^3 /μL)	89.40±166.76	1.85–67.00	6.64±1.97	3.60–10.00	0.244
Platelets (10^3 /μL)	84.26±71.81	11.00–312.00	131.91± 65.28	70.00–254.00	0.002
Lymphoblasts (%)	18.37±21.87	0.00–80.00	0.00±0.00	0.00–0.00	0.000
G1(%)	92.60±5.47	70.56–98.55	94.34±6.21	72.78–99.33	0.063
G2/M (%)	1.14±1.82	0.00–7.19	0.86±1.64	0.00–7.36	0.113
S (%)	6.26±5.71	1.42–29.02	4.80±5.54	0.45–25.88	0.092

Table 3. Induction chemotherapy outcomes results

Outcomes	Frequency	%
Dead	14	40.0
Remission	21	60.0

This research result was different with Ugrasena et al in 2010, who reported that the remission was 40.9% and 48.5%. Remission rates improved more as time goes because protocols had been developed and the latest regimen was used in ALL chemotherapy.[3]

There was a significant correlation between the percentage of S phase and the percentage of lymphoblast before induction chemotherapy (r= 0.449; p=0.007) Figure 1.

Detection of a damage that were activated in normal hemotopoesis cells, the Ataxia-Telangiectasia Mutated (ATM) played a central role in the activation of the G1/S cell cycle checkpoint, preventing cells with damaged DNA from starting the S phase.[16] There was an abnormality in activity of ALL checkpoint. The gene abnormality that caused expression of cyclins and Cyclin-Dependent Kinases (CDKs) disrupted so it could make a loss of cell cycle checkpoint control. The gene abnormalities stimulated the G1 phase cell cycle transition into phase S. The proportion of cell cycle phases in leukemic cells was mostly in the S phase. The S phase could represent leukemic cell proliferation.[17]

There was no significant correlation between the percentage of G2/M phase and the percentage of lymphoblasts (r=-0.306; p=0.074) before chemotherapy (figure 2). The percentage of phase S and G2/M with percentage of lymphoblasts after induction chemotherapy could not be analysed because the percentage of lymfoblasts after chemotherapy was 0%.

Statistical analysis found no difference in outcomes according to the S phase after induction chemotherapy (p=0.138), Table 4. This result showed that the S phase could not be used to predict induction chemotherapy outcomes. This result differed with Kumar 2015, where S phase could be used to predict relapse. Percentages of S phase > 4% patient tended to relapse, relapse of patients was usually because of an increasing activity of proliferation in bone marrow that made the cell turnover and cell leukemic production increase. Increasing proliferation can be caused in effective chemotherapy and drug resistance.[8] The difference was because this research was performed only until induction phase, the first phase of sequences

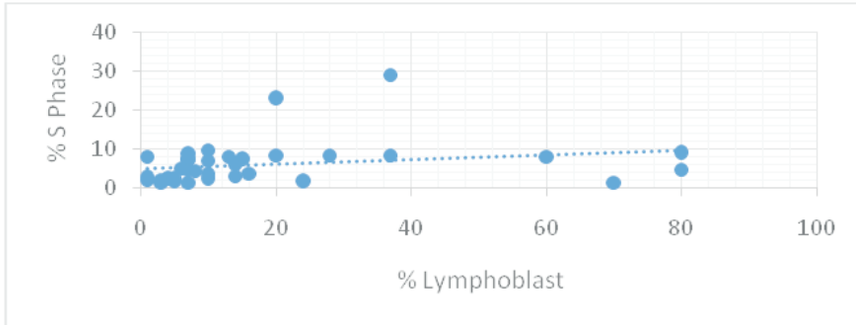

Figure 1. Graphic correlation percentages of G2/M phase and percentage lymphoblasts in peripheral blood before chemotherapy induction of pediatric acute lymphoblastic leukemia

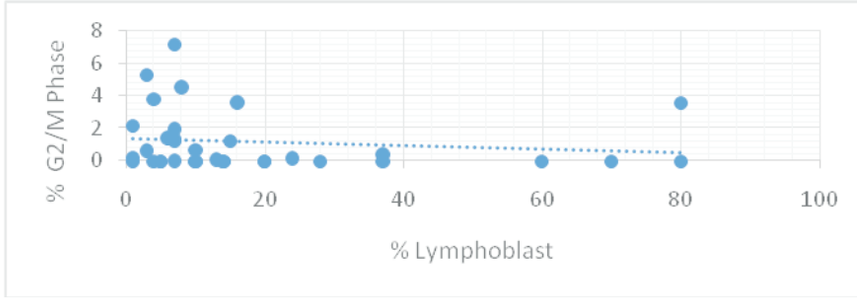

Figure 2. Graphic correlation percentages of G2/M phase and percentages of lymphoblasts before induction chemotherapy

Table 4. Statistical analysis result in differences percentages of S phase cell cycle between induction chemotherapy outcomes in all pediatric patients

Outcomes	n	Persentages S phase				p
		$\bar{x}$	SD	Minimum	Maximum	
Remission	21	5.50	5.98	1.42	29.02	0.138
Dead	14	7.39	5.29	1.86	23.22	

Table 5. Statistical analysis in percentages of G2/M phase between induction chemotherapy outcomes in all pediatric patients

Outcomes	n	Persentages G2/M phase				p
		$\bar{x}$	SD	Minimum	Maximum	
Remission	21	1.74	2.13	0.00	7.19	0.006
Dead	14	0.23	0.47	0.00	1.26	

of chemotherapy in ALL and the difference in total leukemic cells in S phase.

There was a difference in G2/M phase between outcomes of induction chemotherapy (p=0.006). G2/M phase was correlated to remission outcomes, with coefisient Eta result was p=0.744.

The G2 /M phase can be used for prognosis in pediatric ALL. This research showed that G2/M was >1.26%, the changes for remission were more higher. Leukemic cell in G2/M phase after treated with chemotherapy agent underwent death following mitotic arrest.[18] This research also showed that the G2/M phase < 1.26% predicted poor outcomes. The reason was because the chemotherapy agent for specific cell cycle Mitotic phase could not be effective, only cells that enter mitosis are killed or rendered senescent. Quiescent cells (cells that are in a temporary state of not dividing) or cycling cells that do not reach mitosis during drug exposure are spared.[19]

CONCLUSION AND SUGGESTION

In this research there was a correlation between percentages of S phase and percentages lymphoblasts, so S phase can be used as a picture of proliferation in lymphoblasts. There was a correlation between G2/M with the remission patients. This phase can be use as predictor for induction chemotherapy outcomes.

Futhers research must be done with bone marrow samples, determining the cluster differentiation, examinating the lymphoblast in several days and examination in all phase chemotherapy.

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