Correlation of cell apoptosis index and hematology profile in children with acute myeloblastic leukemia with chemotherapy induction

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ABSTRACT

Background: Acute myeloblastic leukemia (AML) is one of the most prevalent pediatric neoplasms, with a poor prognosis and high risk of relapse. Response to induction of chemotherapy and complication occurrence are crucial in the AML patient's outcome. Chemotherapy can induce apoptosis in a cancer cell and the blood cell as a side effect that can cause severe complications. This study aims to evaluate the cell apoptosis index and its correlation with hematologic profile (hemoglobin, leucocyte, thrombocyte, absolute neutrophile count/ANC) between induction chemotherapy administration.
Methods: Observational study was performed on children with AML who will undergo induction chemotherapy. The cell apoptosis index and the hematologic profile were evaluated before and after induction chemotherapy. The data was then analyzed to compare the cell apoptosis index and its correlation with the hematologic profile.
Result: The mean age of the subjects was 13.6 years old, similar to previous epidemiologic studies, which showed the main incidence at 15 years old. There was a significant increase in cell apoptosis index (p=0.014) and a significant decrease in hemoglobin level (p=0.031) and ANC (p=0.05). The cell apoptosis index had a significantly strong negative correlation with all hematologic profiles.
Conclusion: Cell apoptosis index increased after induction chemotherapy in pediatric AML and correlated with the hematologic profile.

INTRODUCTION

Acute leukemia is the most frequent neoplasm in children, and the myeloblastic type (AML) accompanies 15% of them. The survivability of AML children has increased significantly in the last few decades, with 65-70% survival rates. However, this rate is still less than acute lymphoblastic leukemia (ALL).[1] Several factors contribute to AML incidences, such as genetics, radiation, chemical compound, and immunologic status. However, none of them are directly associated with the AML occurrence,[2,3]; hence the therapy still has a significant role in AML.

One of the main challenges of AML therapy is the high incidence of chemoresistance, which reduces the positive outcome.[4] Frequent relapses and risk of complications such as bleeding and infection also contribute to worsening the AML prognosis.[5] Response to the chemotherapy is crucial to the outcome of and relapse incident. Previous studies have shown several laboratory results, such as leucocytosis, thrombocytopenia, and anemia with low absolute neutrophil count (ANC) associated with less expected outcome.[6–8] Chemotherapy, in general, has a role in inducing apoptosis of the cancer cell, reduce the progressivity of the disease.[9]

The apoptosis process can be evaluated by the cell apoptosis index, which was studied previously to be associated with relapse incidence and chemotherapy resistance.[10,11] However, the measurement of the cell apoptosis index in the previous study was done before the chemotherapy regimen was started and not evaluated through the chemotherapy period. We aimed to evaluate the cell apoptosis index throughout the chemotherapy period and its correlation with the hematologic profile.

MATERIAL & METHODS

This is an observational study on children with newly-diagnosed acute myeloblastic leukemia (AML) who will undergo the induction chemotherapy by dr. Saiful Anwar General Hospital. The AML diagnosis is confirmed through peripheral blood morphology and bone marrow aspiration by identifying myeloblast and AML-positive immunophenotypes. The induction chemotherapy was performed on the patients following the national protocol of AML, revised in 2015, using a daunorubicin and cytarabine regimen.

The inclusion criteria were patients aged 0-18 months who had been diagnosed with AML before the induction phase of chemotherapy, and their parents gave consent to participate in the study.

The exclusion criteria were patients who have received chemotherapy and have an autoimmune disease. All of the procedures and data collection performed in this study were already approved by the Health Research Ethics Commission of Dr. Saiful Anwar Hospital, Malang (No. 400/126/K.3/302/2020).

A blood sample for each subject was taken twice, before and after two weeks of induction chemotherapy. A complete blood count examination was performed to evaluate the hematologic profile (hemoglobin, leucocyte, thrombocyte, and differential count). Cell apoptosis index was expressed in percent by calculating the number of cells undergoing apoptosis divided by the number of cells examined and multiplied by 100. The examination was carried out using flow cytometry examination using annexin V markers. All of the data collected in this study were then analyzed statistically using SPSS for Windows 25 (SPSS Inc.), and the correlation was considered significant if $p > 0.05$.

RESULT

Subject Characteristics
Five patients with AML are eligible for this study and complete the induction chemotherapy regimen. The mean age of the subjects was 13.6 years old, which ranged from 11 to 16 years old, with 4 (80%) of them being a woman. We also classified the subjects based on cell phenotype and various AML types obtained. Two issues were classified into M2-type AML and one for M1, M4, and M5-type AML.

Comparison of the parameter before and after the induction chemotherapy
We evaluate and compare the hematologic profile and cell apoptosis index before and after induction chemotherapy, as seen in Table 1. There was a significant increase in cell apoptosis index after induction chemotherapy. The cell apoptosis index increases occurred in all subjects, as seen in Figure 1. All of the parameters measured in the hematologic profile were decreased but not statistically significant on leucocyte count.

Table 1. Comparison of Each Variable Mean, Before & After Induction Chemotherapy

Variables	Before Chemotherapy	After Chemotherapy	P-value
Cell Apoptosis Index (%)	2.2 ± 1.22	8.13 ± 2.81	0.014
Hemoglobin (g/dL)	12.32 ± 1.74	8.3 ± 1.18	0.031
Leucocyte ($10^3/\mu$L)	5.75 ± 3.51	3.46 ± 2.83	0.12
Thrombocyte ($10^3/\mu$L)	239.2 ± 114.06	106.2 ± 25.26	0.09
ANC (cells/μL)	3754 ± 1296.81	1680 ± 832.7	0.05
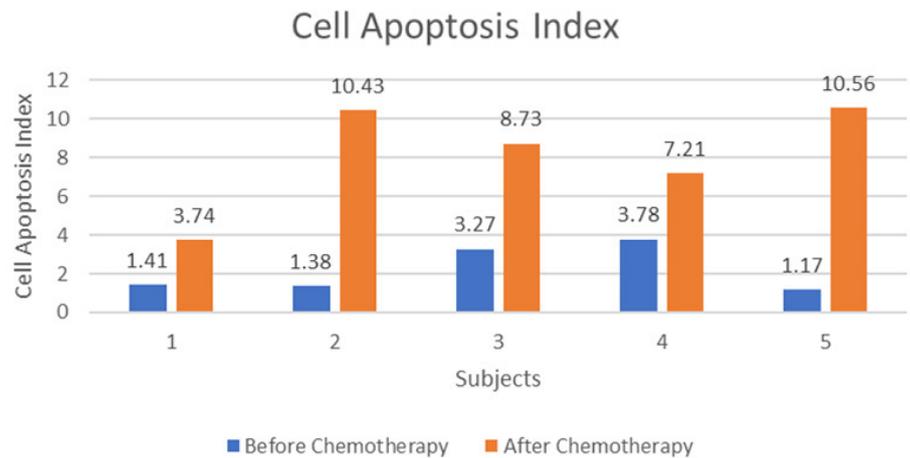
Figure 1. Cell Apoptosis Index of Each subject, Before and After Induction Chemotherapy

Table 2. Correlation of Differences After Induction Chemotherapy between Cell Apoptosis Index and Each Hematologic Profile

Correlation with Cell Apoptosis Index	R-value	P-value
Hemoglobin	- 0.89	0.04
Leucocyte	- 0.93	0.018
Thrombocyte	- 0.98	0.002
ANC	- 0.88	0.04

Correlation of the cell apoptosis index with hematologic profile
We analyzed the correlation between the differences in cell apoptosis index and the hematologic profile after the induction chemotherapy, as seen in Table 2. A significant and strong negative correlation occurred between the hematologic profiles and the cell apoptosis index.

DISCUSSION

AML was one of the important neoplasms in the adolescent, with its complexity to be treated. Previous epidemiologic studies showed the mean age of the AML patient was around 15, with an incidence of 7 cases per a million children,[12] similar to our subjects' characteristics. From a gender perspective, AML has a little higher incidence in males, with an incidence rate ratio of 1.88.[13] We performed consecutive sampling in this study due to the scarcity of the patient, which explains the differences in the gender pattern.

The significant increases in apoptosis after induction chemotherapy were the expected results, considering the regimen of daunorubicine and cytarabine have a cytotoxic effect. Daunorubicine inhibits the activity of topoisomerase II and polymerase, which is essential in the induction of the intrinsic pathway of apoptosis. In the other way, cytarabine

inhibits the progression of the G1 phase to the S phase in the cell cycle, which was suggested through the interaction with DNA polymerase.[14] The increases in the apoptosis index also suggest the induction regimen is still effective in inducing the apoptosis process and reducing cancer progression. However, apoptosis also affects other blood cells, which is described by the decreases in the blood profile. This indicates that the regimen is still working non-selectively to induce apoptosis.

A significant strong negative correlation occurred between the apoptosis index and hematologic profile, which showed the risk of myelotoxicity by induction chemotherapy. This feature could affect the complete administration of the chemotherapy regimen, which correlated with the outcome. Cytopenia susceptibility, hematopoietic recovery capacity, and the abnormal findings of bone marrow analysis determine the occurrence of the therapy complication.[15] The severity of myelotoxicity depends on the progress of the disease, host factor, and the therapy regimen. Late progression of the disease, comorbidities of the patient, and the period of chemotherapy-associated with the myelotoxicity of the therapy.[16]

This study had some limitations. First, the apoptosis process would be evaluated more comprehensively by measuring the apoptosis marker protein (e.g., Bax, Bcl-10, Bad) other than the apoptosis index. Second, some advanced examinations to establish the AML diagnosis, such as cytogenetic examination or immunophenotyping, were not performed due to limited resources and facilities. The genetic and phenotype variation certainly have a role in the therapy outcome.

CONCLUSION

There is a significant increase in apoptosis index after induction chemotherapy in pediatric AML. The hematologic profile also decreases after induction chemotherapy, and there is a significant negative correlation between the apoptosis index and the hematologic profile.

CONFLICT OF INTEREST

None of the authors has a conflict of interest to declare.

ETHICS CONSIDERATION

Ethics approval was obtained from the Health Research Ethics Commission, Dr. Saiful Anwar Hospital, Malang, Indonesia, with the number 400/126/K.3/302/2020 before the study.

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AUTHOR CONTRIBUTION

All authors equally contribute to the study from the conceptual framework, data acquisition, and data analysis until interpreting the study results through publication.

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