ORIGINAL ARTICLE

Caspase 3 Expressions in Children with Acute Lymphoblastic Leukemia During Induction Phase Chemotherapy

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ABSTRACT

Introduction: Caspase-3 is a crucial mediator of the extrinsic apoptosis pathway. The role of caspase-3 for extrinsic apoptosis signalling is still a challenge and should be exploited in childhood ALL. This study aimed to compare the caspase-3 expression in the patient's bone marrow before and after the induction phase of chemotherapy in childhood ALL. It will also to correlate the mean difference in caspase-3 expression between ALL standard-risk and ALL high-risk patients. **Methods:** Seventeen newly diagnosed ALL subjects were enrolled in this study. Caspase-3 expression in bone marrow was assessed using flow cytometry and monoclonal antibodies. A T-test and a paired T-test were used to compare between groups. The correlation coefficient between ALL groups was evaluated using Spearman's test and linear regression with a significant p-value of 0.05. **Results:** The caspase-3 expression is higher after induction therapy. However, it showed an insignificant difference (16.56+12.91% vs 27.71+12.33%; p = 0.08, p > 0.05). The mean difference of caspase-3 in ALL high-risk groups was significantly higher than in ALL standard-risk groups with a positive correlation (p = 0.007, r = 0.756). **Conclusion:** The caspase-3 expression after induction phase chemotherapy was increased in all standard-risk and high-risk patients; other lymphoblast apoptosis markers need to be confirmed alongside caspase-3.

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INTRODUCTION

Acute lymphoblastic leukemia (ALL) is the most common type in children, accounting for approximately 80% of leukemia cases with an 80–90% survival rate (1,2). Impressive clinical remission has been documented in patients who undergo chemotherapy, with more than 95% of children with ALL achieving remission after one month of induction treatment. The chemotherapeutic drugs mainly work through the activation of caspases to induce cancer cell apoptosis. It correlates with apoptosis, programmed cell death, a tightly regulated fundamental process for cell development, and eliminates damaged cells to maintain cellular homeostasis. Apoptosis is also an important cellular defense mechanism against viral invasion and is associated with abnormal regulation of apoptosis in many diseases and cancer cell development. Avoiding cellular apoptosis is essential for tumor cells to maintain genomic integrity (3,4,5). One

of the characteristics of human cancers is the ability to evade apoptosis. Theoretically, increasing anti-apoptotic molecules and decreasing or impairing the function of pro-apoptotic proteins could inhibit the signal that leads to cell death (6,7).

Caspase-3 is a cysteine protease that has a significant role in activating the apoptotic pathway and chemotherapeutic agent-induced cell death. Caspase-3 is a cysteine protease involved in the "execution" phase of cellular apoptosis that can be activated through the extrinsic (caspase-8) and intrinsic (caspase-9) pathways (8). Caspase 3 as an effector caspase will activate DNAase resulting in cell death (9). It is suggested that disruption in the pathway and expression of caspase-3 by leukemia cells in patients with ALL leads to apoptosis-insensitive stimuli and cytotoxic drug resistance. Low expression of caspase-3 as an effector caspase is associated with poor prognosis and low survival rates in patients with AML and ALL (10,11,12). Caspase-3 also played a pivotal role in activating the apoptotic pathway and was related to leukemia cells' sensitivity toward cytostatic drugs such as doxorubicin, etoposide, and cisplatin (8). The role of caspase-3 as a pro-apoptotic protein is still unclear

and has not been well studied in ALL (13,14). This study aimed to compare caspase-3 expression before and after receiving induction chemotherapy in pediatric ALL.

MATERIALS AND METHODS

This study was carried out in children with newly diagnosed childhood ALL who were admitted to the Pediatric Hematology and Oncology Department and were scheduled to receive the 2018 Indonesian ALL Chemotherapy Protocol. The diagnosis of ALL was based on morphological examination of bone marrow aspiration based on the French-American-British (FAB) classification and immunophenotyping (B-ALL and T-ALL). Caspase-3 expression was evaluated using flow cytometry and immunophenotyping at first diagnosis.
The bone marrow specimens that met the inclusion and exclusion criteria were further divided into groups. The inclusion criteria include 1) children aged 1-18 years with newly diagnosed ALL; 2) the legal guardian's approved and signed informed consent; and 3) patients who have completed the chemotherapy induction session. The exclusion criteria are 1) ALL L3 and 2) parents/guardians withdrawing from participating in the study. The drop-out criteria consist of 1) patients who have not completed the chemotherapy induction session and 2) incomplete research data.

The patients were divided into the following group based on stratified risk. The standard-risk group consists of ages 1 to 10 years with an initial leucocyte count of less than 50x109/l; immunophenotype of B-ALL; absence of mediastinal mass, and no blasts identified in the CSF (15). The patients excluded from the standard-risk group belong to high-risk ALL.

This study is a prospective design conducted from March to June 2021. It has been approved by the ethical committee, reference number 0116/LOE/301.4.2/IX/2020. Patient data were collected through anamnesis, physical examination, routine chemical investigations, complete blood count, morphological bone marrow examination, and immunophenotyping. A bone marrow aspiration was performed during the assessment to establish the ALL diagnosis and measure the caspase-3 expression before induction chemotherapy. These patients were followed up during induction therapy until remission or death for six months in 2021. A bone marrow aspiration was performed after the completion of induction chemotherapy. Caspase-3 expression was measured in 11 patients before and after chemotherapy induction using flow cytometry.

Bone marrow mononuclear cells (BMNCs) were isolated by Ficoll-Hypaque density gradient centrifuge. Intracellular staining of protein was performed according to the manufacturer's instructions. Phycoerythrin (PE) labeled anti-caspase-3 monoclonal antibodies were provided by BD Pharmingen (catalog #: C92-605-

RUO). Flow cytometry analyzed all labeled cells using the FACS Calibur instrument (Becton Dickinson) and the Cell Quest software package (Becton Dickinson). The percentage of caspase-3 was determined using a flow cytometry gating strategy.

Statistical analysis was carried out using the IBM SPSS statistic software version 25.0. The normality of data was tested using the Shapiro-Wilk test. T-test and paired T-test were used to compare the groups. Spearman correlation test was used to determine the relationship between variables. Qualitative data were presented as frequency and percentage. The mean and standard deviation were used to present quantitative data. The result of the statistical examination is significant if $p < 0.05$.

RESULTS

There were 37 children with suspicion of acute leukemia. A total of 19 children were excluded from this study; three children showed a hypoplastic marrow, seven children with myeloid lineage, and one child did not express a blast; eight children had no complete caspase-3 examination because the bone marrow aspiration sample volume was insufficient for the complete analysis. One child did not have parental consent (dropped out). Seventeen children had initial data for caspase-3 expression, and six children died during the induction phase of chemotherapy. Eleven children were thoroughly examined for the initial and final caspase-3 until the induction phase of chemotherapy was completed.

The characteristics of the primary data in 17 subjects found more boys than girls in a ratio of 15:2. The average age of the subjects was 7.56 ± 3.12 years which are 82.4% of children aged 1 to 10 years old. Statistical analysis showed no significant differences between sex, age, hemoglobin level, and leucocyte count in all groups. The baseline characteristics of the patients in this study are shown in Table I.

Seventeen children received induction phase chemotherapy, and only 11 children were able to complete the induction phase chemotherapy, while six children died while undergoing induction phase chemotherapy. Three children died of sepsis, and three children died of pneumonia, tumor lysis syndrome, and bleeding. Eleven subjects who completed the induction phase of chemotherapy showed complete remission and had less than 5% lymphoblast count in their bone marrow aspirates. The mean expression of caspase-3 before chemotherapy on survival was lower than the death outcome. However, the caspase-3 expression between the two groups of survival and death showed no significant differences (Table II).

Table III shows that the caspase-3 expression was higher

Table I: Patient characteristics at initial diagnosis based on chemotherapy induction phase outcome

Characteristics (n=17)	n (%)		p
	Live n=11 (64.7)	Died n=6 (35.3)	
Sex			
Male	9 (52.9)	6 (35.3)	0.515[a]
Female	2 (11.8)	0	
Age, years			
1-10	9 (52.9)	5 (29.4)	1.000[a]
> 10	2 (11.8)	1 (5.9)	
Sign and symptoms			
Fever	10 (58.8)	6 (35.3)	1.000[a]
Anemia	8 (47.1)	6 (35.3)	0.515[a]
Bone pain	10 (58.8)	4 (23.5)	0.515[a]
Hepatomegaly	8 (47.1)	5 (29.4)	1.000[a]
Splenomegaly	2 (11.8)	4 (23.5)	0.109[a]
Lymphadenopathy	1 (5.9)	3 (17.6)	0.099[a]
FAB classification			
ALL L1	10 (58.8)	6 (35.3)	1.000[a]
ALL L2	1 (5.9)	0	
Immunophenotyping result			
B-ALL	8 (47.1)	5 (29.4)	1.000[a]
T-ALL	3 (17.6)	1 (5.9)	
Risk stratification			
Standard-risk	5 (29.4)	5 (29.4)	0.304[a]
High-risk	6 (35.3)	1 (5.9)	

[a]Fisher exact test

Table II: The caspase-3 expression difference before induction phase chemotherapy

	Caspase 3		
	n (%)	Mean (SD)	p-value
Alive	11 (64.7)	16.56 (12.91)	0.491
Died	6 (35.3)	22.57 (22.58)	

The statistical test used was the paired T-test.

Table III: Caspase-3 expression before and after induction phase chemotherapy

	Caspase-3			
	n (%)	Mean (SD)	Range	p-value
Before chemotherapy	11 (100)	16.56 (12.91)	2.24-39.53	0.08
After chemotherapy	11 (100)	27.71 (12.33)	14.43-50.71	

The statistical test used was the paired T-test.

after induction therapy, but there was no significant difference. Spearman's correlation analysis showed a significant (p=0.007) with a strong positive correlation (r=0.756), which means that there was a linear relationship between induction phase chemotherapy and the expression of caspase-3 shown in Table IV. It could be interpreted that induction phase chemotherapy would increase caspase-3 expression in ALL patients. Increased expression of caspase-3 was higher in the high-risk than in the standard-risk ALL group.

DISCUSSION

The characteristics data of 17 subjects found more males than females, with a ratio of 15:2. The percentage of the age group 1 to 10 years is higher than over 10 years old. ALL accounts for 25% of cancer diagnoses in children and the peak incidence occurs at the age of 2-5 years (16,17). Aisyi et al. (18) reported that 94 ALL children at Dharmais Hospital from January 2016 to December 2018 was male sex, with percentage of 60.6%, with a median age of 5 (1-8) years. Kakaje et al. (19) reported that the highest incidence of ALL occurred at the age of 5-9 years. Mairuhu et al. (20) also reported that there are more males than females with a ratio of 1.5:1. The molecular mechanisms underlying the sex prevalence of cancer are still unclear. Several genome-wide association studies (GWAS) attempted to identify male sex-specific genetic markers on the risk of childhood ALL (21). Healy et al. (22) reported that there was a statistically significant correlation between the specific effects of 2 SNPs on the ARID5B gene (AT-rich interactive domain 5b) and childhood acute lymphoblastic leukemia (rs10994982 P=0.01) and rs10740055 (P=0.03)). The risk of ALL in boys on SNPs was rs10994982 with OR=3.79 and rs10740055 with an OR of 4.35 compared to girls. Gender differences in cancer prevalence arise from a complex combination of environmental, genetic, and epigenetic factors, as well as differences in gene regulation and expression (23).

In our study, most patients presented with fever, pale skin, and hepatomegaly. ALL patients' common signs and symptoms are fever (65.1-75.5%), anemia (42.8-79.2%), and bone pain (39.6%) (24). On physical examination, lymphadenopathy (62.6-69.2% omit), splenomegaly (59.4-60.8%), and hepatomegaly (50.1-59.5%) occur in most cases (25).

Approximately 35.3% of children with ALL die during the induction phase of chemotherapy. It consist of three deaths due to sepsis and the others due to pneumonia, tumor lysis syndrome, and hemorrhage. Kiem Hao et al. (26) found that the mortality rate of pediatric ALL patients was 31.1%. Deaths were most frequent in the chemotherapy phase at 35.1% in maintenance phase, the induction phase at 24.3%, and 12.2% in the intensified phase. The most frequent cause of death is infection by 43.2%, bleeding 8.1%, and hyperleukocytosis by 1.35%. Another study in Islamabad stated that 20.8% of child ALL deaths occurred in the induction phase. The cause of death was predominantly bleeding 50%, 40% due to infection or sepsis, and 1% due to tumor lysis

Table IV: The caspase-3 expression difference between the stratified risk groups before and after initial chemotherapy

	Caspase-3				p	r
	n (%)	Before Mean (SD)	After Mean (SD)	Delta Mean (SD)		
Standard-risk ALL	5 (45.5)	22.14 (6.99)	24.29 (18.99)	4.09 (15.26)	0.007	0.756
High-risk ALL	6 (54.5)	10.66 (7.63)	32.35 (14.43)	23.86 (11.55)		

The statistical test used was the paired T-test and Spearman correlation test.

syndrome. Appropriate supportive therapy, availability of blood components, and a fine selection of antibiotics lower the risk of child ALL death in Islamabad (27).

Expression of caspase-3 before chemotherapy on survival was lower than in death subjects (16.56±12.91 vs. 22.57±22.58), p>0.05). Huang et al. (28) reported that low caspase-3 expression was associated with better disease-specific survival in patients in the early pathological phase compared to high expression. Caspase-3 could be used as a biomarker in tumorigenesis and a prognostic biomarker for specific stages in cancer patients.

The caspase-3 expression after induction phase chemotherapy was higher than before chemotherapy (16.56±12.91 vs. 27.7±12.33) yet statistically non-significant (p=0.08, p>0.05). Gebyarani et al. (2013) reported different results that the caspase-3 expression after chemotherapy was significantly elevated (p=0.03) in 12 children with ALL (29).

The expression of caspase-3 could cause the difference in the result in our study as the main effector of caspase of apoptosis in normal mammalian cells. The expression of caspase-3 was also found to increase the proliferation and differentiation of keratinocytes, B cells, myoblasts, osteoblasts, erythroblasts, and platelets. Another factor affecting the result of caspase-3 expression in our study is the difference in the time of examination of each sample after induction phase chemotherapy. Failure to detect increased expression may also be due to the half-life of caspase-3 in the circulation of blood (30).

Several studies reported the increasing activity level of caspase-3 after chemotherapeutic agent administration in ALL. Fortney et al. (31) reported that levels of caspase-3 increased up to 12 times in 8 hours after administration of cytarabine and etoposide in leukemia B cells (JM1). A specific caspase-3 inhibitor (DEVDFMK) was administered to confirm the role of the caspase cascade. After 48 hours of DVEDDFMK administration, there was significant leukemic B cell viability compared with no caspase inhibitor administration. The decrease in the effectiveness of chemotherapy by cytarabine and etoposide after administration of caspase inhibitors suggests that the caspase cascade plays a vital role in the apoptosis in chemotherapy. Kuttikrishnan et al. (32) found that caspase-3 expression increased after 24 hours of administration of chemotherapeutic agents. The administration of pan-caspase inhibitor (z-VADfmk) showed a decrease in cells undergoing DNA degradation using the Comet assay examination, which indicates that the caspase pathway cascade primarily mediates apoptosis due to the administration of cytostatic agents. Xia et al. (33) reported an increase in caspase-3 expression in an in vitro study using the anti-tumor ginsenoside Rh2(GRh2) in cultured B cells and T ALL cells. Mendivil-Perez et al. (34), in an ex

vivo study, reported that pro-oxidant agents significantly induce apoptosis in CD34+/CD19+-expressing ALL B cells. Pro-oxidants significantly increase pro-apoptotic proteins, one of which is caspase-3 as an active indicator of the apoptotic pathway.

Another in vitro study reported that administration of anticancer agents to ALL T cells (Jurkat and Molt4) showed an increase in cell apoptosis up to 79-83% expressed by apoptotic markers (Annexin V-FITC/7-AAD). The increase in apoptosis is linear to the elevated level of caspase-3 activity, and ALL T cells stop growing in the cell cycle's S and G0/G1 phases (35). Fu (36) reported that after administration of Lithospermum erythrorhizon extract as a cytotoxic agent on Human leukemic lymphoblast (CCRF-CEM cell line) cells resulted in inhibition of cell viability and downregulation of CDK2, CDK4, CDK6, cyclin D1, and cyclin E1 but also increased caspase-3 expression and apoptosis.

There is an elevated CD95 (FAS) level, caspases-3, 8, and 9 after 48 hours of anti-tumor administration in leukemic cell cultures (37). A similar result was reported by Lonetti et al. (38) in an in vitro study that showed the cytotoxic effect of nelarabine on chemotherapy-sensitive ALL T cells (JURKAT and MOLT4) was correlated with the induction of apoptosis indicated by the cleavage of caspase-3 as an effector caspase. The administration of nelarabine showed apoptosis activation through the extrinsic pathway characterized by caspase-8 cleavage and intrinsic, which was characterized by caspase-9 cleavage.

Analysis of the mean difference of caspase-3 expression between the ALL stratified risk groups showed a higher trend in the standard-risk group and the high-risk group (4.1±15.26 vs. 23.86±11.55) with a statistically significant difference and a positive correlation (p=0.007, p<0.05; r=0.756). However, the clear evidence for caspase-3 expression between stratified risk groups was limited and not fully understood. Salama et al. (40) reported that caspase-3 levels in B-ALL cases showed a higher difference before and after induction chemotherapy compared to T-ALL patients (p=0.025). Gebyarani et al. (41) reported different results that the mean expression of caspase-3 after chemotherapy was higher than before chemotherapy with a statistically significant difference (p=0.03). Faderl et al. (11) suggested possibility to complete remission was significantly higher in the cut-off of caspase-3 > 0.37. Another study also stated that patients with a greater caspase-3 level after induction therapy have more remarkable survival (37). T-lymphocytes, which belong to the high-risk group, are revealed to express a lower level of caspase (39).

Fortney et al. (31) reported an in vitro study that 8 hours after administration of cytarabine and etoposide in leukemia B cells (JM1,) there was an increase in caspase-3 expression up to 12 times, and it disappeared

after 24. A specific caspase-3 inhibitor (DEVDFMK) was administered to confirm the role of the caspase cascade. After 48 hours of DVEDDFMK administration, there was significant leukemic B cell viability compared with no caspase inhibitor administration. The decrease in the effectiveness of chemotherapy by cytarabine and etoposide after administration of caspase inhibitors suggests that the caspase cascade plays a vital role in the apoptosis in chemotherapy. Another study reported that the intrinsic apoptotic pathway proapoptotic protein also increased fourfold after induction phase chemotherapy, especially in acute lymphoblastic leukemia patients who were in remission (41).

CONCLUSION

In this study, we found that caspase-3 expression before and after induction phase chemotherapy is insignificant. Meanwhile, the mean difference of caspase-3 between the stratified risk groups was significantly increased with a positive correlation, especially in high-risk groups. Due to the limited sample, short duration, and single-center study, the significance of caspase-3 and its correlation with cancer cell apoptosis and overall survival should be validated in further studies. We recommend that future research use a larger sample size, a more extended follow-up period, and collaboration with multiple centers to achieve a more remarkable result. Investigating caspase-3 during the maintenance phase of chemotherapy and other lymphoblast apoptosis markers is also essential to determine the role of caspase-3 in pediatric ALL.

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REFERENCES

1. Jin M, Xu S, An Q, Wang P. A review of risk factors for childhood leukemia. Eur Rev Med Pharmacol Sci 2016; 20(18): 3760-3764.PMID: 27735044
2. Kaplan JA. Leukemia in Children. Pediatrics in Review 2019; 40(7): 319-331. doi:10.1542/pir.2018-0192
3. Fernald K, Kurokawa M. Evading apoptosis in cancer. Trends in Cell Biology. Trends Cell Biol 2013; 23(12): 620-33. doi:10.1016/j.tcb.2013.07.006
4. Banerjee S, Uppal T, Strahan R, Dabral P, Verma SC. The modulation of apoptotic pathways by gammaherpesviruses. Front Microbiol 2016; 7: 585. doi:10.3389/fmicb.2016.00585
5. Huang FL, Yu SJ, Li CL. Role of Autophagy and Apoptosis in Acute Lymphoblastic Leukemia. Cancer control: Journal of the Moffitt Cancer Center 2021; 28: 10732748211019138. doi:10.1177/10732748211019138
6. Fulda S. Tumor resistance to apoptosis. International Journal of Cancer. Int J Cancer 2009; 124(3): 511-5. doi:10.1002/ijc.24064
7. Malczewska M, Kośmider K, Bednarz K, Ostapińska K, Lejman M, Zawitkowska J. Recent Advances in Treatment Options for Childhood Acute Lymphoblastic Leukemia. Cancers 2022; 14(8): 2021. doi:10.3390/cancers14082021
8. Chen GQ, Lu Y. Effector caspases and leukemia. International Journal of Cell Biology 2011; 2011. doi:10.1155/2011/738301
9. Chinnasamy S, Zameer F, Muthuchelian K. Molecular and Biological Mechanisms of Apoptosis and its Detection Techniques. Journal of Oncological Sciences 2020; 6: 49-64. doi:10.37047/jos.2020-73477
10. Estrov Z, Thall P, Talpaz M, Estey EH, Kantarjian H, et al. Caspase 2 and caspase 3 protein levels as predictors of survival in acute myelogenous leukemia. Blood 1998; 92(9): 3090-7. doi:10.1182/blood.V92.9.3090
11. Faderl S, Thall P, Kantarjian H, Talpaz M, Harris D, et al. Caspase 2 and caspase 3 as predictors of complete remission and survival in adults with acute lymphoblastic leukemia. Clin Cancer Res. 1999; 5(12): 4041-7. PMID: 10632337
12. Yadav P, Yadav R, Jain S, Vaidya A. Caspase-3: A primary target for natural and synthetic compounds for cancer therapy. Chem Biol Drug Des 2021; 98: 144– 165. doi:10.1111/cbdd.13860
13. Gamen S, Anel A, Lasierra P, Alava MA, Martinez-Lorenzo MJ, et al. Doxorubicin-induced apoptosis in human T-cell leukemia is mediated by caspase-3 activation in a Fas-independent way. FEBS Lett 1997; 417(3): 360-4. doi:10.1016/s0014-5793(97)01282-9
14. Bennett JM, Catovsky D, Daniel M -T, Flandrin G, Galton DAG, et al. Proposals for the classification of the acute leukemias. French-American-British (FAB) co-operative group. Br J Haematol 1976; 33(4): 451-8. doi:10.1111/j.1365-2141.1976.tb03563.x
15. Schrappe M. Prognostic Factors in Childhood Acute Lymphoblastic Leukemia. Symposium on Advances in Oncology. Indian J Pediatric 2003; 70(10): 817-24. doi:10.1007/BF02723806
16. Inaba H, Pui CH. Advances in the Diagnosis and Treatment of Pediatric Acute Lymphoblastic Leukemia. J Clin Med 2021; 10(9): 1926. doi:10.3390/jcm10091926
17. Tebbi CK. Etiology of Acute Leukemia: A Review. Cancers 2021. 13(9): 2256. doi:10.3390/cancers13092256
18. Aisyi M, Dwijayanti F, Wigati R, Mahdi H, Yunus L, et al. Sepsis in children with acute lymphoblastic

leukemia. Indonesian Journal of Cancer 2020; 14(4): 121–6. doi:10.33371/ijoc.v14i4.738

19. Kakaje A, Alhalabi MM, Ghareeb A, Karam B, Mansour B, Zahra B, Hamdan O. Rates and trends of childhood acute lymphoblastic leukaemia: an epidemiology study. Sci Rep 2020; 10: 6756. doi:10.1038/s41598-020-63528-0

20. Mairuhu AM, Andarsini MR, Setyoningrum RA, Cahyadi A, Larasati MCS, Ugrasena IDG, Permono B, Budiman S. Hospital acquired pneumonia risk factors in children with Acute Lymphoblastic Leukemia on chemotherapy. Heliyon 2021; 7(6): e07209. doi: 10.1016/j.heliyon.2021.e07209

21. Singh SK, Lupo PJ, Scheurer ME, Saxena A, Kennedy AE, et al. A childhood acute lymphoblastic leukemia genome-wide association study identifies novel sex-specific risk variants. Medicine Baltimore 2016; 95(46): e5300. doi:10.1097/MD.0000000000005300

22. Healy J, Richer C, Bourgey M, Kritikou EA, Sinnett D. Replication analysis confirms the association of ARID5B with childhood B-cell acute lymphoblastic leukemia. Haematologica 2010; 95(9): 1608–1611. doi:10.3324/haematol.2010.022459

23. Lopes-Ramos CM, Quackenbush J, DeMeo DL. Genome-Wide Sex and Gender Differences in Cancer. Front Oncol 2020; 10: 597788. doi: 10.3389/fonc.2020.597788

24. Dai Q, Zhang G, Yang H, Wang Y, Ye L, et al. Clinical features and outcome of pediatric acute lymphoblastic leukemia with low peripheral blood blast cell count at diagnosis. Medicine Baltimore 2021; 100(4): e24518. doi: 10.1097/MD.0000000000024518

25. Al-Mulla NA, Chandra P, Khattab M, Madanat F, Vossough P, et al. Childhood acute lymphoblastic leukemia in the Middle East and neighboring countries: a prospective multi-institutional international collaborative study (CALLME1) by the Middle East Childhood Cancer Alliance (MECCA). Pediatric Blood Cancer 2014; 61(8): 1403-10. doi:10.1002/pbc.25031

26. Kiem-Hao T, Nhu Hiep P, Kim Hoa NT, Van Ha C. Causes of Death in Childhood Acute Lymphoblastic Leukemia at Hue Central Hospital for 10 Years (2008-2018). Glob Pediatr Health 2020; 7: 2333794X20901930. doi: 10.1177/2333794X20901930

27. Khan MI, Naseem L, Manzoor R, Yasmeen N. Mortality Analysis in Children during Induction Therapy for Acute Lymphoblastic Leukemia. J Islamabad Med Dent Coll 2017; 6(2): 69-72.

28. Huang JS, Yang CM, Wang JS, Liou HH, Hsieh IC, Li GC, Huang SJ, Shu CW, Fu TY, Lin YC, Ger LP, Liu PF. Caspase-3 expression in tumorigenesis and prognosis of buccal mucosa squamous cell carcinoma. Oncotarget 2017; 8(48): 84237–84247. doi:10.18632/oncotarget.20494

29. Gebyarani I, Nugroho S, Widjajanto E. The

Expression of Caspase-3 and Survivin Related with Induction Chemotherapy in Childhood Acute Lymphoblastic Leukemia. IOSR Journal of Dental and Medical Sciences 2013; 8(4): 23-29. doi: 10.9790/0853-0842329

30. Darzynkiewicz Z, Huang X, Okafuji M, King MA. Cytometric methods to detect apoptosis. Methods Cell Biol 2004; 75: 307-41. doi: 10.1016/S0091-679X(04)75012-8

31. Fortney JE, Zhao W, Wenger SL, Gibson LF. Bone marrow stromal cells regulate caspase 3 activity in leukemic cells during chemotherapy. Leuk Res 2001; 25(10): 901-7. doi: 10.1016/s0145-2126(01)00051-0

32. Kuttikrishnan S, Siveen KS, Prabhu KS, Khan AQ, Ahmed EI, Akhtar S, et al. Curcumin Induces Apoptotic Cell Death via Inhibition of PI3-Kinase/AKT Pathway in B-Precursor Acute Lymphoblastic Leukemia. Front Oncol 2019. doi: 10.3389/fonc.2019.00484

33. Xia T, Zhang J, Zhou C, Li Y, Duan W, Zhang B, Wang M, Fang J. 20(S)-Ginsenoside Rh2 displays efficacy against T-cell acute lymphoblastic leukemia through the PI3K/Akt/mTOR signal pathway. J Ginseng Res 2020; 44(5): 725–737. doi: 10.1016/j.jgr.2019.07.003

34. Mendivil-Perez M, Velez-Pardo C, Quiroz-Duque LM, Restrepo-Rincon A, Valencia-Zuluaga NA, et al (2022). TPEN Selectively Eliminates Lymphoblastic B cells from Bone Marrow Pediatric Acute Lymphoblastic Leukemia Patients. Biometals 2022. doi:10.20944/preprints202103.0611.v2

35. Gao H, Lou S, Hong H, Ge Q, Zhao H (2020). Autophagy Inhibition Potentiates the Anticancer Effects of a Bendamustine Derivative NL-101 in Acute T Lymphocytic Leukemia. BioMed Research International 2020; 2020. doi: 10.1155/2020/1520651

36. Fu Z, Wang L, Zhang W, Wang J, Zhu S, et al. Cytotoxic activity of lithospermum erythrorhizon root extract against childhood acute leukemia cells via regulation of PI3K/AKT/mTOR pathway. Trop J Pharm Res 2019; 18(11): 2379-2394. doi: 10.4314/tjpr.v18i11.21

37. Jeon SR, Lee JW, Jang PS, Chung NG, Cho B, et al (2015). Anti-leukemic properties of deferasirox via apoptosis in murine leukemia cell lines. Blood Res 2015; 50(1): 33–39. doi:10.5045/br.2015.50.1.33

38. Lonetti A, Cappellini A, Bertaina A, Locatelli F, Pession A, et al. Improving nelarabine efficacy in T cell acute lymphoblastic leukemia by targeting aberrant PI3K/AKT/mTOR signaling pathway. J Hematol Oncol 2016; 9(1): 114. doi: 10.1186/s13045-016-0344-4

39. Salama MA, Hassab HM, Gendy W, Zeid MY. Study of Caspase-3 Level in Relation to Prognostic Risk Factors in Children with Acute Lymphoblastic Leukemia. Alex J Pediatr 2005. 9(1): 40-50. ID: emr-69479

40. Cutrona G, Tasso P, Dono M, Roncella S, Ulivi M, et al. CD10 is a marker for cycling cells' propensity to apoptosis in childhood ALL. Br J Cancer 2002; 86(11): 1776-85. doi: 10.1038/sj.bjc.6600329

41. Cahyadi A, Ugrasena I, Andarsini MR, Larasati M, Aryati A, Arumsari DK. Relationship between Bax and Bcl-2 Protein Expression and Outcome of Induction Phase Chemotherapy in Pediatric Acute Lymphoblastic Leukemia. Asian Pacific journal of cancer prevention 2022; 23(5): 1679–1685. doi:10.31557/APJCP.2022.23.5.1679