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Research Article

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Evaluation of the Efficacy of Asparaginase Added to Chemotherapeutic Drug Regimen in Acute Lymphoblastic Leukemia by Minimal Residual Disease Measurement

Akut Lenfoblastik Lösemide Kemoterapötik İlaç Rejimine Eklenen Asparajinaz Etkinliğinin Minimal Rezidüel Hastalık Ölçümü ile Değerlendirilmesi

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ABSTRACT

B-ALL is the most common subtype of childhood Acute Lymphoblastic Leukemia (ALL), affecting approximately 85% of children with ALL presents worldwide. Minimal Residual Disease (MRD) monitoring during treatment of ALL is important for the prognosis of the disease. MRD monitoring, which enables early detection of relapse, determination of risk percentage and understanding the effectiveness of treatment, can be performed with multiple methods such as flow cytometry, qRT-PCR and NGS. Asparaginase is an enzyme that has been used in the treatment of ALL since the 1960s, converting asparagine to ammonia and aspartic acid, lowering serum asparagine levels and causing the death of malignant cells. In this study, we investigated the effect of asparaginase added to the ALL IC BFM 2009 treatment protocol in 62 B-ALL patients aged 0-18 years with MRD monitoring by flow cytometry. In our study, Escherichia coli (E.coli.) asparaginase was used primarily and PEG-asparaginase was used after allergy development. The effect of asparaginase on treatment was evaluated by evaluating asparagine concentration and asparaginase activity measured at TP1 (4th week after induction treatment) together with MRD levels. As a result of the study, it was observed that the added asparaginase positively affected the treatment and increased the negativity in MRD levels.

Key Words

Acute lymphoblastic leukemia (ALL), asparagine, minimal residual disease (MRD), flow cytometry.

ÖZ

Çocukluk çağı Akut Lenfoblastik Lösemi (ALL) hastalığının en yaygın alt tipi olan B-ALL, dünya çapında yaklaşık %85 ALL hastası çocuğu etkilemektedir. ALL hastalığının tedavisi devam ederken yapılan Minimal Rezidüel Hastalık (MRD) takibi, hastalığın seyri için önem taşımaktadır. Relapsın erken tespitinin, risk yüzdesi saptanmasının ve tedavinin efektifliğinin anlaşılmasını sağlayan MRD takibi Akım sitometri, qRT-PCR, NGS gibi birden fazla yöntem ile yapılabilmektedir. Asparajinaz ALL tedavisinde 1960'lı yıllardan itibaren kullanılan, asparajini amonyağa ve aspartik asite dönüştürerek serum asparajin seviyesini düşeren ve malign hücrelerin ölümüne yol açan bir enzimdir. Bu çalışmada, akım sitometri ile MRD takibi yapılan, 0-18 yaş aralığındaki 62 kişilik B-ALL hastasının, ALL IC BFM 2009 tedavi protokolüne eklenen asparajinazın hastalığa etkisi incelenmiştir. Çalışmamızda, öncelikli olarak Escherichia coli (E.coli.) asprajinaz, alerji gelişimi sonrası ise PEG-asparajinaz kullanılmıştır. TP1 (indüksiyon tedavi sonrası 4. hafta) noktasında ölçülen asparajin konsantrasyonu ve asparajinaz aktivitesinin, MRD düzeyleri ile birlikte değerlendirilmesi sonucu asparajinaz tedaviye olan etkisi değerlendirilmiştir. Çalışma sonucunda eklenen asparajinazın tedaviyi olumlu yönde etkilediği ve MRD düzeylerinde negatifliği arttırdığı gözlenmiştir.

Anahtar Kelimeler

Akut lenfoblastik lösemi (ALL), asparajinaz, minimal rezidüel hastalık (MRD), akım sitometri.

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INTRODUCTION

Leukemia is a type of cancer caused by invasion of bone marrow cells. This disease, which is divided into two main categories as lymphoblastic and myeloblastic, accounts for 30% of childhood cancers [1,2]. Lymphoblastic leukemia is divided into two subgroups as T-cell and B-cell. The incidence of B-cell ALL in childhood Acute Lymphoblastic Leukemia (ALL) is 85% [1,3]. According to Cancer.net data, the 5-year survival rate of ALL patients under the age of 20 is 90% when adverse effects such as poor prognosis and biological factors are excluded [4]. Multiple treatment strategies are being developed to improve survival in ALL. One of these strategies is the use of asparaginase in addition to current therapy. The use of asparaginase, which has been used as a chemotherapeutic agent in the treatment of ALL since the 1960s, is one of the treatment strategies that has been proven to be effective by studies conducted since then [5-8]. L-Asparaginase, an enzyme, hydrolyzes asparagine to ammonia and aspartic acid, lowering the level of circulating serum asparagine and reducing the production of proteins required for cells [9]. Unlike healthy cells, malignant cells lack asparagine synthase, so asparagine synthesis cannot be performed and the cell undergoes apoptosis. Malignant cells can therefore be specifically killed by asparaginase supplementation [10].

In patients with ALL who appear to be in remission after or during treatment, residual blast cells that cannot be detected by clinical, radiological and morphological analyses can be detected by Minimal Residual Disease (MRD) analysis [11,12]. MRD analysis is an important prognostic marker for risk assessment and treatment planning in combination with well-known clinical, cytogenetic and molecular information [13]. MRD analysis is an immunophenotyping method that is performed by collecting bone marrow samples on selected days of treatment from diagnosis. The patient's response to treatment can be observed by calculating the risk percentage according to the immune barcode.

The group included in the study consisted of patients aged 0-18 years and diagnosed with B-ALL. Due to the apoptosis effect of asparaginase in malignant cells, PEG-asparaginase and L-asparaginase were used in addition to the BFM ALL IC 2009 treatment regimen. Blood asparaginase concentration and asparagine activity were measured and evaluated together with MRD levels in B-ALL patients who received *Escherichia coli (E.coli.)* L-asparaginase and PEG-asparaginase after allergy development in the TP1 (4th week after induction treatment) period based on MRD risk levels.

MATERIALS and METHODS

Patients and Treatment

The study included a group of patients aged 0-18 years who were followed up in the Pediatric Hematology Department of Lösante Children and Adult Hospital with a diagnosis of B-ALL between the years 2020 and 2022 and who received the ALL IC BFM 2009 chemotherapy protocol. In addition to the ALL IC BFM 2009 chemotherapy protocol, patients received L-asparaginase at a dose of 5000 IU/m^2 8 times until the 33rd day of induction treatment. When L-asparaginase allergy developed in patients during the treatment process, the treatment was continued by adding PEG-asparaginase at a dose of 2500 IU/m^2. Serum asparaginase activity and asparagine concentration were performed according to the research article of Nath et al. The asparaginase concentrations and asparagine activities of the patients were measured by HPLC method by using serum collected at the TP1 point from patients who were followed up for MRD [14].

MRD Analysis and Assessment

MRD analysis including the TP1 (day 33 after induction therapy) point was performed by constructing an individual panel according to the initial diagnostic immunophenotypes. The protocol and techniques applied were in consistent with EuroFlow standard operating procedures (SOP) [15]. Stained cells were detected at low speed on a BD FACSCanto II flow cytometry device. BD FACSDivaTM data analysis software program was used for data analysis of the results. Risk levels of the patients were determined according to the MRD analysis performed at TP1 point. Patients with MRD score less than 10^{-3} at TP1 point were considered to have a negative risk level and those with MRD score greater than 10^{-3} were considered to have a positive risk level.

RESULTS and DISCUSSION

Between the years 2020 and 2022, the MRD risk scores of patients receiving the ALL IC BFM 2009 protocol were determined according to the TP1 point. Detailed data of the patients examined for asparagine concentration and asparaginase activity, which were examined simultaneously with the TP1 point MRD, are given in the tables.

Table 1. Figure-of-merit (FOM) calculation of the sensor at different glycerol concentrations (FWHM represents full width at half maximum of the curves).

Patient Characteristics		
	Asparaginase Activity	Asparagine Concentration
Number of Cases	62	58
Gender (G/B)	24/38	23/35
Median age/years (range)	5.4 (0-18)	5.7 (0-18)
L-Asparaginase	41	40
PEG-Asparaginase	21	18
Number of MRD Negative Patient	43	42
Number of MRD Positive Patient	19	16

The study included 62 patients for asparagine activity determination and 58 patients for asparagine concentration. Approximately 38% of these patients were female. The age of the patients in this study ranged from 0 to 18 years with an overall mean age of 5.5 years. Approximately 32% of patients who received L-Asparagine in addition to the ALL IC BFM 2009 protocol continued treatment with PEG-Asparagine after the development of allergy. Approximately 70.8% of patients with B-ALL became negative according to the MRD risk score after treatment (Table 1).

In Table 2, asparagine activity and asparagine concentration were evaluated according to MRD risk scores at TP1 point of treatment. Among the patients with asparagine activity of 400 IU/L and above, 69.4% were MRD negative. When the serum asparagine concentrations of the patients are examined, it is seen that 84.4% of the patients entered complete depletion and 71.4% of the patients who entered complete depletion were MRD negative.

Table 2. Asparagine concentration and asparagine activity distribution according to MRD risk scores at TP1 point of treatment.

2020-2022		
	MRD Risk Score*	
PARAMETERS	MRD Negative** (n=43)	MRD Positive** (n=19)
Asparaginase Activity (IU/L) (n=62)		
<100 (n=1)	-	1
≥100 - <400 (n=25)	18	7
≥400 (n=36)	25	11
PARAMETERS / Asparagine Concentration (µM)*** (n=58)	MRD Negative** (n=42)	MRD Positive** (n=16)
Complete Depletion (n=49)	35	14
Incomplete Depletion (n=8)	6	2
Not-Depleted (n=1)	1	0

*MRD Risk Score: After induction therapy (week 4 (TP1))

**MRD Negative: <10-3; MRD Positive: ≥10-3

***Compelete Depletion: ≤0.5µM, Incomplete Depletion: >0.5 - <40 µM; Not-Depleted: ≥40 µM

Table 3. Distribution of mean values of asparagine concentrations and asparagine activities according to MRD risk scores at TP1 point of treatment.

2020-2022				
		MRD Risk Score*		
PARAMETERS		MRD Negative** (n=43)	MRD Positive** (n=19)	Mean
Asparaginase Activity (IU/L) (n=62)				
	<100 (n=1)	-	15.5	15.5
	≥100-<400 (n=25)	246.1 (128.2-394.6)	224.5 (126.8-333.8)	240.1 (126.8-394.6)
	≥400 (n=36)	736 (401-1546.2)	822.1(422.7-1655.3)	762.3 (401-1655.3)
PARAMETERS		MRD Negative** (n=42)	MRD Positive** (n=16)	Mean
Asparagine Concentration(µM)*** (n=58)				
	Complete Depletion (n=49)	0.2 (0-0.2)	0.06 (0-0.2)	0.03 (0-0.2)
	Incomplete Depletion (n=8)	6.4 (1-12.1)	11.3 (1.8-20.9)	8.6 (1-20.9)
	Not-Depleted (n=1)	43.4	-	43.4

*MRD Risk Score: After induction therapy (week 4 (TP1))

**MRD Negative: <10-3; MRD Positive: ≥10-3

***Compelete Depletion: ≤0.5µM, Incomplete Depletion: >0.5 - <40 µM; Not-Depleted: ≥40 µM

Table 3, demonstrates the distribution of mean values of asparagine concentrations and asparagine activities according to MRD risk scores. According to these data, it is seen that the only value with asparagine activity less than 100 IU/L is 15.5 and has a positive MRD risk score. When analyzing the asparagine activities greater than and equal to 100 IU/L and less than 400 IU/L, the overall average is 240.1 IU/L. Considering the MRD risk score negative and asparagine activities greater than and equal to 400 IU/L, it is seen that the average is 736. When asparagine concentrations were analyzed, it was observed that the average asparagine concentration of patients with complete depletion was 0.03 µM. When incomplete depletion is analyzed, it is seen that the general average is 8.6 µM and is near the lower threshold.

Asparaginase has been shown to be an effective drug in childhood ALL [16,17]. The use of asparaginase in the treatment of ALL has been reported to significantly improve clinical outcomes. Research has shown that its use in combination with anti-cancer drugs increases the rate of complete remission and improves the 5-year event-free survival rate by 90% compared to the use of anti-cancer drugs alone [6,18].

In a study of 377 newly diagnosed patients with ALL who were considered standard risk (SR) high risk (HR), indicating that when asparaginase was added to the treatment protocols, the estimated 5-year event-free survival (EFS) was superior to the previous protocols [19].

With advances in technology, MRD monitoring has made it possible to detect leukemia cells at levels lower than 10-4 leukemia cells. Therefore, MRD monitoring has become a useful tool to try different chemotherapy drugs and additional alternative therapies [20].

The paediatric haematology and oncology centre at LÖSANTE Children and Adult Hospital is the first centre in Turkey to perform real-time validated asparaginase activity measurements. Since various paediatric ALL treatment protocols, such as BFM, recommend monitoring of asparaginase activity, the number of centres able to perform this measurement is increasing. Aspa-

raginase therapy and MRD monitoring are the most important components of paediatric ALL treatment. In our study, we tested the efficacy of asparaginase used as an addition to the ALL IC BFM 2009 protocol with MRD monitoring. Based on the data of this study, the use of asparaginase as an addition to the ALL IC BFM 2009 protocol in the treatment of childhood B-ALL appears to reduce the risk rate of the disease. Considering other studies, we can say that the addition of asparaginase to the treatment goes in parallel with the increase in MRD negativity.

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