

Changes in Fibrinogen and Total Protein/Albumin Levels During Induction Chemotherapy in Children with Acute Lymphoblastic Leukemia

Akut Lenfoblastik Lösemili Çocuklarda İndüksiyon Kemoterapisi Süresince Fibrinojen ve Total Protein/ Albumin Düzeyinde Meydana Gelen Değişiklikler

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Abstract: L-Asparaginase is an important drug for treatment of childhood acute lymphoblastic leukemia (ALL). The impairment of protein synthesis due to depletion of asparagine by asparaginase leads to decreases in albumin and deterioration in hemostasis tests. The aims of the present study were to conduct a retrospective evaluation of levels of fibrinogen, total protein/albumin during induction chemotherapy in children with ALL treating according to the BFM 2009 protocol. For this purpose, the laboratory data of 45 pediatric ALL patients in the induction chemotherapy between August 2011 and March 2016 were retrospectively evaluated. This study was approved institutional review board of Eskişehir Osmangazi University Faculty of Medicine (Ethics approval No: 80558721/105). The median fibrinogen level on day 12 was already reduced by 62% compared to the before treatment values, but this difference was not statistically significant ($p>0.05$). After 15 days of therapy, the level of fibrinogen decreased to 27%, total protein decreased to 89%, albumin decreased to 92% of the initial value ($p<0.001, p<0.01, p<0.05$, respectively). The lowest fibrinogen and total protein/albumin levels were observed on the 21st and 27th days of chemotherapy. Thrombotic complication developed in three children (9.3%). The results showed that the levels of fibrinogen and total protein/albumin were significantly reduced during the induction chemotherapy.

Keywords: induction chemotherapy, L-asparaginase, children, fibrinogen, protein, albumin.

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Öz: L-Asparaginaz, çocukluk çağı akut lenfoblastik lösemi tedavisinde kullanılan önemli bir ilaçtır. Asparaginaz'ın asparagin tükenmesine bağlı olarak yol açtığı protein sentezindeki bozulma albüminde azalmaya ve hemostaz testlerinde bozulmaya neden olur. Bu çalışmanın amacı, BFM 2009 protokolüne göre tedavi alan akut lenfoblastik lösemili çocuklarda indüksiyon kemoterapisi sırasında fibrinojen, total protein/albumin düzeylerinin retrospektif olarak değerlendirilmesidir. Bu amaçla, Ağustos 2011 ile Mart 2016 tarihleri arasında indüksiyon kemoterapisi alan 45 hastasının laboratuvar parametreleri geriye dönük olarak incelendi. Çalışma için Eskişehir Osmangazi Üniversitesi Klinik Araştırmalar Etik Kurulu'ndan onam alındı (Etik kurul onam numarası: 80558721/105). Onikinci günde medyan fibrinojen düzeyi tedavi başlangıcına göre %62 oranında azalmıştı. Ancak bu farklılık istatistiksel açıdan anlamlı değildi ($p>0.05$). Tedavi sonrası 15. günde medyan fibrinojen düzeyi tedavi öncesi değerinin %27'sine, total protein % 89'una, albumin %92'sine düşmüştü ($p<0,001, p<0,01, p<0,05$, sırasıyla). Fibrinojen ve total protein/albumin düzeyleri 21. ve 27. günlerde en düşük düzeyde idi. Üç hastada tromboembolik komplikasyon gelişti (%9,3). Çalışmanın sonuçları, indüksiyon kemoterapisi süresinde fibrinojen, kan total protein/albumin düzeylerinin önemli ölçüde düştüğünü gösterdi.

Anahtar kelimeler: indüksiyon kemoterapisi, L-Asparaginaz, çocuk, fibrinojen, protein, albümin

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1. Introduction

L-Asparaginase is an enzyme with a well documented therapeutic effect in pediatric patients with acute lymphoblastic leukemia (ALL) (1,2). The drug induces a relative asparagine deficiency that leads to the death of human lymphoblasts. However, L-Asparaginase has also been shown to be toxic to the liver, pancreas, the central nervous system, and the kidneys (3). The impairment of protein synthesis leads to decreases in many proteins such as albumin, insulin and thyroxine-binding globulin (4). Furthermore, it has been reported that treatment with L-Asparaginase causes widespread impairment of hemostasis by inhibiting the biosynthesis of hepatic L-asparagine dependent proteins (3). Plasma antithrombin and fibrinogen levels decrease more significantly than any other hemostatic proteins (3). This effect may be associated with an increased tendency to bleeding or thrombosis. These hemostatic modifications induced by L-Asparaginase are relatively rare and milder in children (5). The aim of this retrospective study was to investigate the change in the levels of fibrinogen and total protein/albumin during induction chemotherapy in children with ALL.

2. Material and Methods

Forty-five children (1-17 years of age) newly diagnosed with ALL between

August 2011 and March 2016 were included in this study. All children were treated according to BFM 2009 protocol. The induction chemotherapy regimen consisted of 60 mg/m² prednisolone (oral) (days 1-33), vincristine (1.5 mg/m² on days 8, 15, 22 and 29), daunorubicin (30 mg/m² on days 8, 15; standard risk, on day 8, 15, 22, 29; medium and high risk), *Escherichia Coli* L-asparaginase (5000 U/m² on days 12, 15, 18, 21, 24, 27, 30, and 33).

At the time of diagnosis patients' age, sex, blast percentages in the peripheral blood and bone marrow, leukemia immunophenotype, risk group, sedimentation rate hemogram, fibrinogen and total protein/albumin levels were recorded. Blood samples for fibrinogen

and total protein/albumin studies were obtained before the L-Asparaginase infusions. The fibrinogen levels were determined using a commercially available kit (Thromborel-S) an autoanalyser. Total protein and albumin levels were determined with the colorimetric method on a Roche Hitachi Cobas C702 analyser. The patient files were retrospectively assessed and recorded by two physicians and both sets of data were compared.

Exclusion criteria

Children diagnosed with lymphoma, acute myeloid leukemia, relapsed ALL and not accessible to laboratory data of five children with newly diagnosed leukemia was excluded from the study.

Statistical analysis

All analyses were performed using the SPSS 21.0 statistics software package (SPSS for Windows 21.0; SPSS, Chicago, IL, USA). The normality of distributions was evaluated with the Kolmogorov Smirnov test. Continuous variables were expressed as mean values \pm standard deviation (SD) and median (interquartiles). The fibrinogen and total protein/albumin levels were compared with nonparametric Friedman's test. $P < 0.05$ was considered statistically significant.

3. Results

The ages of the forty-five pediatric patients (23 females and 22 males) with ALL ranged between 1 and 17 years and their median age was 4.5(3-10.2) years. Thirty-seven patients had pre-B ALL (82%), 4 had pro-B ALL (9%) and 4 had T-ALL (9%). Among the patients, 18 (40%) were in the SRG, 21 (47%) were in the IRG, and 6 patients (13%) were in the HRG. At time of diagnosis, the median hemoglobin value was 7.35 g/dl (5.07-9.52), the median leukocyte number was $10.95 \times 10^9/L$ (4.85-41.78), the median platelet number was $81.0 \times 10^9/L$ (23.75-131.75) and the median sedimentation rate was 87.5(62-105.25) mm/h. The mean blast ratio in the peripheral smear and bone marrow was 52.06 ± 29.51 and 86.30 ± 15.01 , respectively (Table 1).

Table 1.
Clinical characteristics and laboratory parameters of children

Değişkenler		n (%)
Age (years)		4.5(3-10.2)
Gender (Female/Male)	F/M	23/22 (51% / 49%)
Median hemoglobin (g/l)		7.35(5.07-9.52)
Median leucocyte number (10 ⁹ /L)		10.95 (4.85-41.78)
Median platelet number (10 ⁹ /L)		81.0(23.75-131.75)
Median sedimentation rate (mm/hr)		87.5(62-105.25)
Risk group	SRG	18 (40%)
	IRG	21 (47%)
	HRG	6 (13%)
Immuno-phenotyping	Pre-B	37 (82%)
	ALL	4 (9%)
	Pro-B	4(9%)
	ALL	
	T ALL	
Mean blast ratio in peripheral blood smear (%)		52.06±29.5
Mean blast ratio in bone marrow smear (%)		86.30±15.01

SRG: Standart risk group, IRG: Intermediate risk group, HRG: High risk group

The median fibrinogen and total protein/albumin levels on the 15th, 18th, 21st, 24th, 27th, 30th and 33rd days were significantly lower than the values of before treatment (Table 2).

P values used for the comparison of fibrinogen, protein and albumin levels were presented in Table 3.

The lowest fibrinogen and total protein/albumin levels were observed on the 21st and 27th days of chemotherapy (Figure 1 and 2). None of our patients developed a serious bleeding complication. Thrombotic complication developed in three children (9.3%). Two patients had deep vein thrombosis, one patient had central nervous system thrombosis.

Table 2.
Changes in fibrinogen and total protein/albumin levels during the induction chemotherapy.

	Fibrinogen (mg/dl)	Total protein (mg/dl)	Albumin (mg/dl)
Before treatment	386.70 (255-521)	6.4 (6.04-6.80)	4.34 (4.20-4.60)
12 th day	147.0 (108.0-184.30)	6.3 (6,10-6,60)	4.28 (3.80-4.30)
15 th day	106.0 (84.5-128.0)	5.70 (5.40-6.30)	4.0 (3.70-4.30)
18 th day	88.0 (63.60-109.80)	5,59 (5.20-5.70)	3.85 (3.50-4.20)
21 th day	72.0 (51.60-103.20)	5,50 (4.90-5.90)	3,70 (3.45-4.10)
24 th day	86.0 (51.0-124.80)	5,50 (4.90-6.0)	3.70 (3.30-4.0)
27 th day	82.60 (52.0-101.0)	5.30 (5.07-5.70)	3.58 (3.25-3.83)
30 th day	84.0 (56.0-138.0)	5.60 (5.20-5.80)	3.70 (3.330-3.90)
33 rd day	98.0 (72.0-158.20)	5,68 (5.40-5.90)	3.70 (3.41-4.16)

Table 3.
P values used for the comparison of fibrinogen, protein and albumin levels

	12 th day	15 th day	18 th day	21 th day	24 th day	27 th day	30 th day	33 rd day
Before treatment	>0.05	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	>0.05	0.006	<0.001	<0.001	<0.001	<0.001	<0.001	0.001
	>0.05	0.018	0.006	0.001	<0.001	0.004	<0.001	<0.001
12 th day		0.01	0.005	<0.001	0.002	<0.001	>0.05	>0.05
		0.008	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
		0.036	0.007	0.001	<0.001	<0.001	<0.001	<0.001
15 th day			>0.05	>0.05	>0.05	>0.05	>0.05	>0.05
			>0.05	>0.05	>0.05	>0.05	>0.05	>0.05
			0.032	0.028	0.018	0.001	0.009	0.030
18 th day				>0.05	>0.05	>0.05	>0.05	>0.05
				>0.05	>0.05	>0.05	>0.05	>0.05
				>0.05	>0.05	>0.05	>0.05	>0.05
21 th day					>0.05	>0.05	>0.05	>0.05
					>0.05	>0.05	>0.05	>0.05
					>0.05	>0.05	>0.05	>0.05
24 th day						>0.05	>0.05	>0.05
						>0.05	>0.05	>0.05
						>0.05	>0.05	>0.05
27 th day							>0.05	>0.05
							>0.05	>0.05
							>0.05	>0.05
30 th day								>0.05
								>0.05
								>0.05

1stline; fibrinogen, 2st line;total protein, 3rd line; albumin

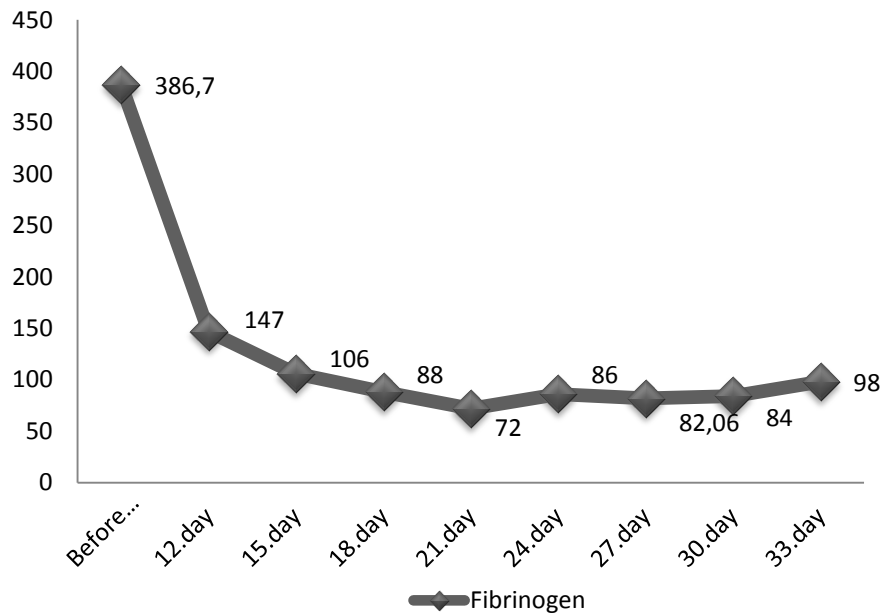


Figure 1. Median levels of fibrinogen during the induction chemotherapy

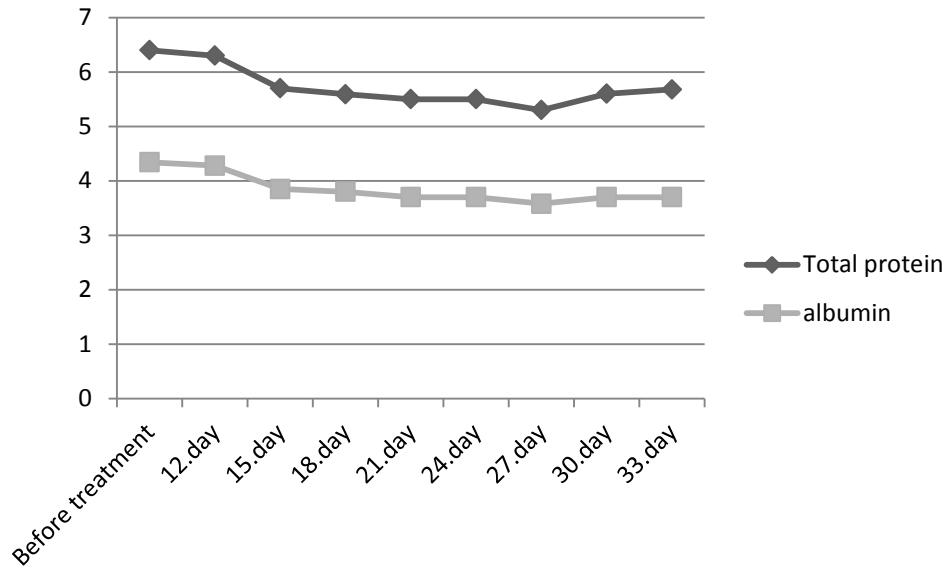


Figure 2. Median levels of total protein/albumin during the induction chemotherapy

4. Discussion

L-Asparaginase is an important chemotherapeutic agent used in pediatric leukemia. Previous study has been reported that treatment with L-Asparaginase causes widespread impairment of hemostasis by inhibiting biosynthesis of hepatic L-asparagine-dependent proteins (3). L-Asparaginase leads to marked reduction of plasma antithrombin and fibrinogen levels and this effect may be associated with an increased tendency to bleeding or thrombosis (3,11).

Giardona et al.(12) reported that antithrombin and fibrinogen levels dropped when L-asparaginase and steroid treatment were taken and that antithrombin and fibrinogen levels returned to normal after L-Asparaginase and steroid therapy were discontinued. They suggesting that the steroid and L-Asparaginase had negative effects on antithrombin and fibrinogen.

In the study by Sutor et al.(9) conducted on 14 patients receiving the ALL/NHL BFM 90 protocol, the fibrinogen level was observed to fall to 148 mg/dl while receiving glucocorticoids alone during the induction phase, while the reduction in the fibrinogen levels continued more rapidly after starting L-Asparaginase therapy after the 12th day,

reaching the lowest mean of 46 mg/dl on the 24th day. According to the results of our study, median fibrinogen values are significantly reduced while under steroid treatment in the first 12 days of treatment (Figure 1). Since the hemostatic parameters are tested before the each infusion of L-Asparaginase, we may state that the reduced fibrinogen values on the 12th day are not related to L-Asparaginase. Based on these results, we can say that the level of fibrinogen has already reduced before the start of L-Asparaginase treatment, and it continues to slightly decrease until the 21st day (Figure 1).

The fact that the median fibrinogen values were higher in our patients compared to the BFM 90 protocol may be associated with the 50% reduction in the L-Asparaginase dose in the BFM 2009 protocol. In the study by Attarbaschi et al.(13) conducted on 16 patients treated according to the BFM 2000 protocol, fibrinogen and antithrombin values were observed to be less significantly reduced when L-Asparaginase was administered on a dose of 5000 u/m²x 8 times with three-day intervals compared to a dose of 10.000 u/m². The results of our study also support the results of this study.

In this study, the total protein and albumin levels were found to be significantly reduced in the first 15 days of induction chemotherapy. We are of the opinion that this decrease occurs may be due to the catabolic processes caused by the malignancy, as well as the accompanying nourishment problems

and the suppression of the protein synthesis by the chemotherapeutic agents. We have not evaluated the malnutrition status of patients in this study. For this reason, we cannot say how much the effect of nutritional status on the reduction in protein and albumin levels.

Asparaginase and corticosteroid treatment in patients with ALL is associated with an increased risk of thrombotic complications (6). The frequency of thromboembolic complications (9.3%) in our study was similar to those reported in literature. The reported incidence of venous thromboembolism in childhood ALL varies from 1.1% to 36.7% with an overall average of 3.2% (7,8). A large retrospective multicentre survey of 1100 children treated on the Berlin Frankfurt

Munster (BFM)-90 trial found 19 (1.7%) with thromboses, whilst a smaller prospective study of similarly treated children showed a higher incidence of 14.3% (9,10).

5. Conclusion

Our results showed a significant decrease in fibrinogen and protein albumin levels during induction chemotherapy. Although the dose of L-Asparaginase has been reduced, thrombotic complications remain a significant problem.

Still, prospective studies including larger numbers of patients are needed in order to demonstrate the extent of the changes in the antithrombotic factor levels and determine the risk of these patients in terms of thrombotic complications.

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