Akut lenfoblastik lösemili çocuk hastaların beyin omurilik sıvısı oksidan/antioksidan seviyeleri

Cerebrospinal fluid oxidant/antioksidant status in children with acute lymphoblastic leukemia

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Abstract

Objective: To assess the effect of leukemia therapy on the cerebrospinal fluid (CSF) antioxidant and oxidant status in children with acute lymphoblastic leukemia (ALL).

Material and Method: Twenty-four consecutive children (15 boys and 9 girls) newly diagnosed with ALL were enrolled in the study. CSF concentrations of ceruloplasmin ferroxidase activity, total thiol group, lipid hydroperoxide (LOOH) and total oxidant status (TOS) were evaluated at diagnosis, remission induction, consolidation, continuation, and the end of the continuation. Nineteen children (10 boys and 9 girls) were enrolled as a reference group.

Results: The study group at diagnosis had significant higher mean ferroxidase activity (50.2 ± 10.4 U/L and 30.3 ± 7.9 U/L), total thiol (0.34 ± 0.02 μmol/L and 0.18 ± 0.01 μmol/L), LOOH (6.5 ± 1.9 μmol/L and 5.3 ± 0.8 μmol/L), and TOS (9.3 ± 4.3 μmol H_2O_2 equiv./L and 6 ± 1.5 H_2O_2 equiv./L) levels compared with the references, respectively ($P < 0.05$).. Ferroxidase activity remained elevated during the entire treatment course. Total thiol levels stayed elevated from diagnosis to week 80 of ALL treatment and significantly decreased thereafter. LOOH levels were progressively increased to week 80 of the treatment course. TOS levels were progressively decreased two times at continuation week 7 and end of the treatment.

Conclusions: Our data indicate that CSF oxidative stress and antioxidants are higher at diagnosis, significant changes to be during therapy, and is already high at the end of therapy in children with ALL.

Key Words: Acute lymphoblastic leukemia, antioxidants, cerebrospinal fluid, oxidants, oxidative stress

Özet

Amaç: Akut lenfoblastik lösemili çocuk hastalarda tedavinin beyin omurilik sıvısında (BOS) antioxidant ve oksidan seviyelerini araştırmak.

Materyal ve Metod: 24 (15 erkek 9 kız) yeni tanı almış hastaların tanı anında, remisyon indüksiyon, güçlendirme, idame ve tedavi sonunda BOS seruloplazmin ferroksidaz aktivitesi, total tiyol, lipid hidroperoksit (LOOH), ve total oksidant seviyeleri (TOS) çalışıldı. Çalışmaya 19 çocuk (10 erkek 9 kız)

(kontrol) olarak alındı.

Bulgular: İlk tanıda ALL grubunda kontrol grubuna göre sırasıyla, ortalama ferrokisdaz aktivitesi (50.2 ± 10.4 U/L ve 30.3 ± 7.9 U/L), total tiyol (0.34 ± 0.02 µmol/L ve 0.18 ± 0.01 µmol/L), LOOH (6.5 ± 1.9 µmol/L ve 5.3 ± 0.8 µmol/L), ve TOS (9.3 ± 4.3 µmol H_2O_2 equiv./L ve 6 ± 1.5 H_2O_2 equiv./L) bulundu ($P < 0.05$). Ferroksidaz aktivitesi tüm tedavi süresince yüksek seyrettiği, total tiyol seviyesinin ise tedavinin ilk 80 haftasında yüksek kaldığı daha sonra azlamaya başladığı, total oxidant seviyenin ise idamenin 7 haftası ile 120 haftasında düşüşler gösterdiği saptandı.

Sonuç: ALL'li çocuk hastaların BOS oksidan stresi ve antioksidanları yüksek seviyededir ve tedavi süresince bu yükseklik devam etmektedir.

Anahtar kelimeler: Akut lenfoblastik lösemi, antioksidanlar, beyin omurilik sıvısı, oksidanlar, oksidan stress

Introduction

The prognosis for children with acute lymphoblastic leukemia (ALL) has improved dramatically, with 5-year event-free survival rates as high as 75% to 82% (1-3) Improved supportive care, more precise risk stratification, and personalized chemotherapy based on the characteristics of leukemic cells and hosts (e.g., pharmacokinetics and pharmacogenetics) have pushed the cure rate of childhood ALL to near 90% (4). Higher survival rates have resulted in heightened focus on improving the quality of life of survivors by reducing treatment-related late effects.

Reactive oxygen species (ROS) are defined as a family of molecules derived from the partial reduction of molecular oxygen, and their generation is a consequence of aerobic life and is unavoidable. ROS are known to play a dual role in biological systems, since they may be either harmful or beneficial to living systems (5). Because of their free-radical nature, they are chemically more reactive with organic molecules than oxygen, and are cytotoxic, mutagenic, and strongly implicated in the etiology of cancer (6,7). Beneficial effects of ROS occur at low/moderate concentrations and involve physiological roles in cellular defense against infectious agents and in the function of a number of cellular signaling systems (7). Moreover, they play a role in the anticancer effect of treatment and may be an important factor in improving outcome of ALL therapy (8-10).

The effect of ALL on serum malondialdehyde (MDA) (8,11,12) and total antioxidant capacity (TAC) (8); vitamin A, 8-oxo-dG, and total antioxidant capacity (ORAC) (13); plasma thiol level (14); activities of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) (10,11,14); cerebrospinal fluid 8-isoprostane and antioxidative capacity;[9] and oxidized phosphatidylcholine (15,16) have been investigated previously in children with ALL. The purpose of the present study was to investigate the effect of leukemia and its treatment on the levels of cerebrospinal fluid (CSF) ceruloplasmin ferroksidaz aktivitesi, total thiol (total –SH), and total oxidant status (TOS) in children with ALL.

Materials and Methods

Subjects

The sample consisted of 24 children (15 boys and 9 girls) newly diagnosed with acute lymphoblastic leukemia who were 2–16 years of age and treated according to the modified St. Jude Total XIIIA protocol (a locally adapted ALL therapy protocol based on the St. Jude Children's Research Hospital in Memphis, TN, Total XIIIA protocols) (2,3,17,18). The subjects were admitted to Harran University's

Faculty of Medicine Pediatric Hematology Clinic in Sanliurfa. Parents completed the informed consent process, and assent was obtained from patients when appropriate. Patients were not eligible for the study if they had received another form of therapy or traumatic lumbar puncture or had central nervous system involvement at diagnosis. The sample consisted of 24 children (15 boys and 9 girls) with newly diagnosed ALL. No more patients could be enrolled because the St. Jude Total XIIIA ALL therapy protocol was changed to the St. Jude Total XV protocol. One patient who moved away from Sanliurfa dropped out of the study, and 3 were lost during follow-up. Nine children were classified as standard risk, nine were intermediate risk, and one was high risk. CSF samples from children with ALL were collected during lumbar punctures performed at diagnosis, during the consolidation phase (on day 23), after induction treatment (on day 43), during the consolidation phase (on day 51), during maintenance therapy (in week 7, 15, 31, 53, 80), and at the end of treatment (in week 120); approximately 2 ml of cerebrospinal fluid was collected with the prior consent of the parents of all patients. Cerebrospinal fluid was not collected in the control groups because our ethics committee does not permit cerebrospinal fluid collection in healthy control subjects. Nineteen children (10 boys and 9 girls) were enrolled as a reference group that had meningeal signs and required analysis of cerebrospinal fluid but no cytologic or biochemical evidence of meningitis in their cerebrospinal fluid and serum. A lumbar puncture was performed and collected for diagnostic purposes after informed consent was obtained. There was no apparent site of infection or a noninfectious diagnosis upon further investigation.

CNS prophylaxis consisted of triple intrathecal therapy (methotrexate, cytarabine, and prednisolone), irrespective of the CNS status and the risk group; the total doses administered were based on the CNS status and the risk classification and ranged from 13 to 25. The total duration of treatment for all risk groups was 120 weeks for both males and females.

In all subjects a detailed history was taken concerning passive smoking and antioxidant medication and fruit juice consumption. Children with severe malnutrition, those that had taken any antioxidant medications (vitamin C, vitamin E, etc.) prior to or during the study, and any child with central nervous system (CNS) disease at the time of diagnosis or throughout the duration of the study were excluded. The local scientific ethics committee approved this study.

Methods of analyzing the oxidants and antioxidants

Cerebrospinal fluid was withdrawn into tubes and was stored at -80 °C. Ceruloplasmin ferroxidase activity and total oxidant status levels were measured by Erel's methods (19,20). The results were expressed in U/L and H_2O_2 equiv./L, respectively. The total thiol (-SH) level was estimated in samples by the method of Hu et al. and was expressed as μmol/L (21). Lipid hydroperoxide concentrations of serum were measured using the automated ferrous oxidation-xylenol orange (FOX-2) assay (22). All measurements were performed using the same autoanalyzer (Aeroset, Abbott, Illinois, USA).

Statistical Analyses

The data were analyzed using SPSS for Windows Release 11.5 (SPSS Inc.), and were expressed as mean SD or 95% confidence interval for mean (CI). Variables' homogeneity of variance was tested by Levene's test. Changes in ceruloplasmin, total –SH group, LOOH, and TOS over time were investigated by repeated measures of ANOVA and

paired *t* test. Differences between the study group and references were compared by Student's *t*-test. A *P* value <0.05 denoted statistical significance.

Results

The mean age at diagnosis was 5.3 ± 3.1 years (range: 2–16 years) in the study patients (15 boys and 9 girls) and was 4.6 ± 4.1 years (range 1.8–15 years) in the references (9 boys and 10 girls). There were no significant differences between mean age and ratio of sexes ($P > 0.05$). Nineteen patients had WBC < 50.000/mm^3, 5 patients had WBC >50.000/mm^3 (range 1.500–92.700/mm^3), and mean WBC count was 28.200 ± 36.200 / mm^3.

We found no significant differences between the groups with regard to male/female distribution, mean age, or height or weight values. Cerebrospinal fluid ferroxidase activity, total thiol, LOOH, and TOS levels at diagnosis and week 120 in children with ALL and the references are shown in Table 1.

Because there are no normal ranges of cerebrospinal fluid ferroxidase activity, total thiol, LOOH, or TOS levels for children, we compared our results to the data taken from the study group at diagnosis and the control group.

Ferroxidase activity were higher in the study group than in the references and remained elevated during the entire treatment with chemotherapy (Figure 1A). Mean values were 50.2 ± 13.1 U/L and 30.3 ± 7.9 U/L at diagnosis and in the references, respectively ($P < 0.001$).

Total thiol levels stayed elevated from diagnosis to week 80 of ALL treatment and significantly decreased thereafter (Figure 1B) (paired *t* test $P = 0.038$; repeated measures of ANOVA $P < 0.001$). The mean total thiol levels were 0.34 ± 0.02 μmol/L and 0.18 ± 0.01 μmol/L at diagnosis and in the reference group, respectively ($P < 0.001$).

Progressively significant increases in LOOH levels were observed from diagnosis to week 80 of ALL treatment, thereafter slightly decreasing (Figure 1C) (repeated measures of ANOVA, $P < 0.001$). The mean LOOH levels were 6.5 ± 1.9 μmol /L and 5.3 ± 0.8 μmol/L at diagnosis and in the reference group, respectively ($P < 0.001$).

The changes in TOS levels were observed throughout the treatment course (repeated measures of ANOVA, $P < 0.001$); these changes were significantly different between diagnosis and consolidation, and continuation weeks 80 and 120 (Figure 1D). Of greatest interest was the approximately 50% decrease over the value at diagnosis in the mean TOS during the consolidation day 43 of treatment ($P < 0.001$). The mean TOS level at diagnosis was 9.3 ± 4.3 μmol H_2O_2 equiv. /L, while it was 6 ± 1.5 μmol H_2O_2 equiv. /L in the reference group ($P < 0.001$).

Discussion

Despite such experimental and clinical evidence for ROS as important mediators of leukemia-associated pathophysiological changes, there has been little research on changes in the oxidant status during continuation therapy for ALL (23).. Recent studies have suggested that ROS play an important role in several pathologic processes in bacterial meningitis, cerebrospinal fluid pleostosis, including vascular damage, and leukemia (8,24,55). The high reactivity of the oxidants coupled with their very short life span is a stumbling block in the direct measurement of these species in human subjects and clinical studies have focused on the measurement of stable markers of oxidant activities in blood samples from patients with leukemia. Previous published reports have found significant decreases in blood antioxidant levels status (8,13,26), but one study (27) reported that oxidative stress did not change in children with lymphoid leukemias. Another previous study reported that cerebrospinal fluid oxidative stress is associated with chemotherapy

used to treat ALL and that MTX may have a central role in this process (9). In our study, we examined the direct automated measurement of CSF oxidative stress in children with ALL before and after treatment. In the present study, the major findings were as follows: CSF oxidative and antioxidative parameters were significantly increased at diagnosis and continued to be high during therapy, antioxidant and oxidants were lower after 80 weeks, and all parameters were higher compared with those of the references. To the best of our knowledge, these findings have not been reported previously.

Chemotherapy for ALL in children is an intensive, comprehensive, risk-directed, long-term treatment, and typically includes an induction of remission phase, consolidation phase, and continuation therapy (28). Combined chemotherapy has potent systemic anticancer effects but penetration to the CSF is limited. CNS-directed therapy consists of triple intrathecal therapy and high-dose systemic methotrexate infusion (29). Triple intrathecal therapy was given on days 2, 22, and 43 of remission induction, and then every 8 weeks during the first year of continuation therapy (3). It is stated that this therapy, however, may also cause acute or chronic neurotoxicity, which, including seizures, encephalopathy, ataxia, or hemiparesis, has been linked to antifolate therapy, particularly in patients receiving systemic and intrathecal MTX therapy with little or no systemic leucovorin rescue (9,30). We did not encounter any case of neurotoxicity during the study period.

Protas et al. reported a significant difference in CSF levels of 8-isoprotane (as a oxidative marker) observed during CNS-directed chemotherapy, including high-dose systemic and intrathecal methotrexate (9). High levels of 8-isoprostane and lower CSF total antioxidative capacity during protocol M points to a positive relationship between methotrexate and oxidative stress. Increased oxidative stress during protocol M may reflect a depletion of the nonenzymatic antioxidant system due to the higher production of ROS in the CNS. Furthermore, it has been reported that oxidative stress plays an important role in the anticancer effect of the treatment, and the use of exogenous ROS-generating agents such as arsenic trioxide may significantly enhance the antileukemia activity (31). In our study, lipid hydroperoxide, ceruloplasmin, and total thiol levels persisted at high levels until week 80, but TOS level was significantly lower in spite of intrathecal and high dose intravenous methotrexate therapy. Our results also showed that serum lipid hydroperoxide concentrations were higher in the ALL patients than in the references (Table 1).

In our study, the level of TOS showed a bimodal pattern: it was significantly reduced for the first 7 weeks and afterwards it was slightly increased until week 80, after which it decreased. Consequently, we have been able to show strong oxidative stress throughout all treatment phases. We also found that serum lipid hydroperoxide was higher after treatment than before, implying that ALL causes a substantial increase in serum oxidant formation during treatment, and after healing these oxidants decrease. This may be a significant factor in improving outcome of ALL therapy. All patients went into complete remission and none relapsed during the study period.

Ceruloplasmin ferroxidase activity is an acute phase protein with a response of intermediate magnitude compared with other acute phase proteins; the plasma concentration is increased two- to threefold during inflammation and after traumatic injury, including surgery (19). It is reported that ceruloplasmin is increased in

infections and other inflammatory diseases (32,33).

Osterlund et al. (27) reported that neurochemical markers such as neuron-specific enolase, which can be interpreted as early signs of brain damage, and ascorbyl radicals as a marker of oxidative stress do not show any convincing signs of oxidative stress in CSF during induction treatment for ALL in children. Our results support this finding: the levels of CSF LOOH, which could serve as a marker of oxidative stress, did not change significantly during the induction period. However, they gradually increased and reached their highest level at week 80 of continuation. Al-Tonbary et al. (8) argued that oxidative stress may participate in leukemia pathogenesis and chemotherapeutic agents may further increase oxidative stress and apoptosis in ALL. The increase in oxidative stress seems not to be a result of the treatment with chemotherapeutic agents but may be involved in the pathogenesis of leukemia, since in the diagnostic results the levels were more increased than in those during treatment. Moreover, lipid peroxidation may be an irreversible lesion as this parameter was high in the continuation therapy for ALL (14). It has been suggested that oxidative damage accumulates in biological molecules during chemotherapy and that oxidative stress is relevant to the disease and anticancer treatment.

It is reported that ASA in CSF and serum had a good correlation for patients undergoing chemotherapy but not for patients after the therapy, and ascorbate may play an important role during chemotherapy.[31]

This study had several limitations that should be addressed in future investigations. The overall sample size (n=24) was small for specific marker analyses due to inadequate quantities of some CSF samples, thereby restricting the power to detect effects. This study should be replicated in a larger sample and with risk-based classification with more complete data to see if the same and/or different relationships hold. Another limitation involves the use of children with meningismus to evaluate controls. Ideally, healthy children's CSF would be assessed. Generally, it is not possible to collect CSF from these subjects due to ethical considerations. Whether these CNS effects are transient, reversible, or progressive is not yet clear and studies conducted during and after cessation of treatment are warranted. Moreover, there is an ethical impediment to CSF sampling after stopping chemotherapy. It is difficult to state which of the parenteral and intrathecal drugs used during treatment is implicated in the CNS findings. In the future, the contribution of individual drugs to the effect on the CNS and underlying mechanisms might be clarified in new studies.

In conclusion, our data indicate that CSF oxidative stress and antioxidants were higher at diagnosis, continues to be high during therapy, and is already high at the end of therapy. Further studies of ALL should be pursued to better delineate the effect on CSF oxidative stress.

Conflict of interest statement

The authors of this paper have no conflicts of interest, including specific financial interests, relationships, and/or affiliations relevant to the subject matter or materials included.

Table 1. Cerebrospinal fluid antioxidative/oxidative parameters in children with ALL and reference. Data are given mean ± SD.

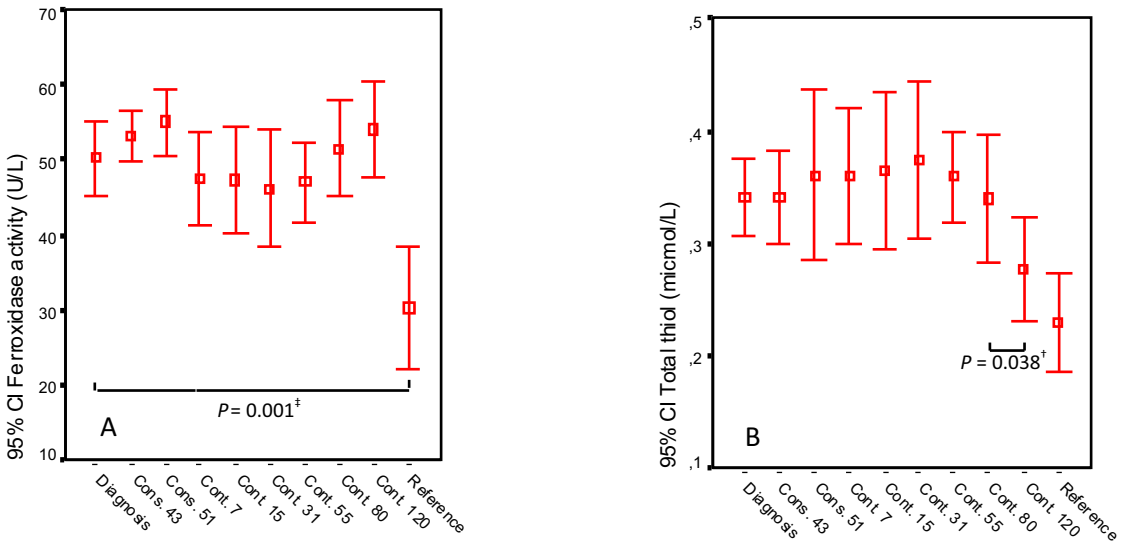
	Acute lymphoblastic leukemia			
	Diagnosis (*n*= 24)	Week 120 (*n*= 20)	$P^{\dagger\ddagger}$	Reference (*n*= 19)
Ceruloplasmin (U/L)	50.2 ± 10.4	54.2 ± 12.5	0.471	30.3 ± 7.9
Total thiol (-SH) µmol/L	0.34 ± 0.02	0.28 ± 0.02	0.015	0.18 ± 0.01
LOOH (µmol H_2O_2/L)	6.5 ± 1.9	6.4 ± 1.8	0.296	5.3 ± 0.8
TOS (µmol H_2O_2/L)	9.3 ± 4.3	7.1 ± 1.4	0.045	6 ± 1.5

Abbreviations: LOOH, lipid hydroperoxide; TOS, total oxidant status.

† Paired *t* test, diagnosis and continuation week 120, in the ALL group.

‡ The data for four subjects' diagnosis were omitted.

Fig. 1



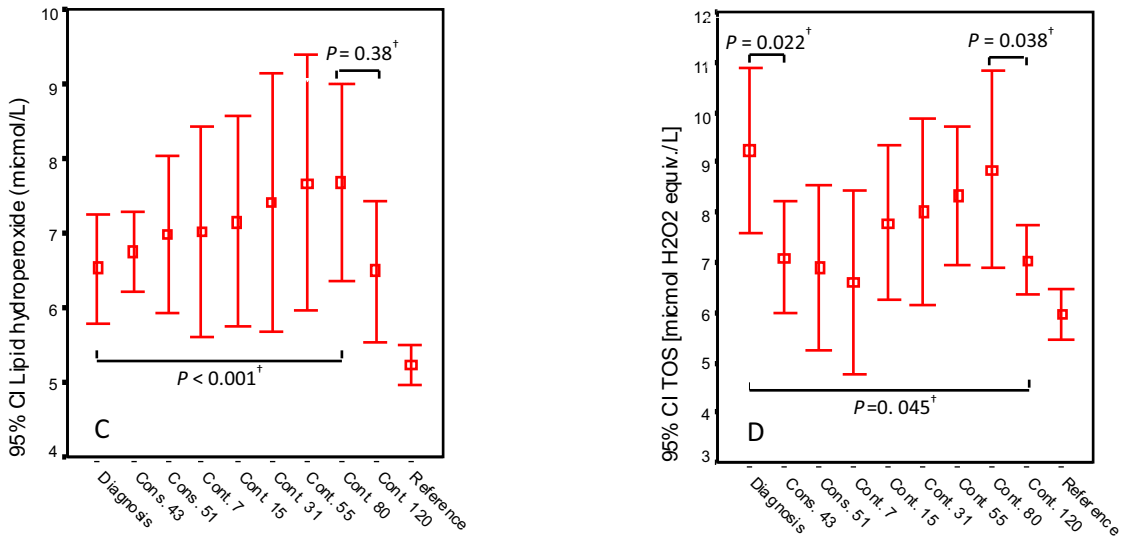


Fig 1. The level of cerebrospinal fluid ferroxidase activity (A), total thiol (B) , lipid hydroperoxide (C) and total oxidant status (D) in the study group and the reference group.

Abbreviations: Cons, consolidation day; cont, continuation week.

‡Student *t* test

†Paired *t* test

Yazarlarla ilgili bildirilmesi gereken konular (Conflict of interest statement) : Yok (None)

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