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# CONVENTIONAL POLYCHEMOTHERAPY OF ACUTE LYMPHOBLASTIC LEUKEMIA PATIENTS ASSOCIATED WITH OXIDATIVE STRESS AND ANTIOXIDANTS DEPLETION

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## ABSTRACT

**The aim of this study was to investigate whether conventional polychemotherapy of acute lymphoblastic leukemia patients contributed to the development of oxidative stress and antioxidants depletion. Plasma levels of thiobarbituric acid-reactive substances assessed by malonedialdehyde (MDA) content were measured as products of lipid peroxidation. Pretreatment MDA values and MDA values during therapy course were estimated to be above the normal range, indicating the occurrence of oxidative stress. Serum iron levels were monitored as a potential source of non-transferrin bound iron with a role in initiation of oxidative burst. Increased serum iron levels were measured during the whole course of chemotherapy. To analyze the effects of cytostatic therapy on the pro-oxidant/antioxidant parameters in plasma we measured the total antioxidant status (TAS) and a single plasma antioxidant - uric acid (UA). A significant reduction of TAS levels was found at the end of the therapy course, strongly correlating with UA content ($r=0,9$; $p<0,05$). Our data suggest that uric acid as a routine laboratory indicator could also serve as a marker of blood antioxidant capacity.**

**Key words**: acute lymphoblastic leukemia, total antioxidant status, uric acid, lipid peroxidation, total serum iron

## INTRODUCTION

Cancer is associated with increased formation of reactive oxygen species (ROS) and depletion of plasma and cellular antioxidants (1,3,7). The degree of lipid peroxidation in patients of acute lymphoblastic leukemia (ALL) was studied as a marker of disease activity (8). Chemotherapy and radiation therapy are also associated with increased plasma lipid hydroperoxides and thiobarbituric acid-reactive substances (TBARS) (4,9,12,13,15). What is more, there is evidence that a variety of anticancer drugs exert their cytotoxic activity by a free-radical-mediated mechanism (5,10). These drugs include anthracyclines, such as farmorubicin and cyclophosphamide (4,6,13). Treatment of acute leukemias with high doses of cytotoxic drugs could overwhelm the cell antioxidative resistance mechanisms and lead to a condition known as oxidative stress (5). It still remains unclear whether ALL patients who underwent conventional polychemotherapy would show signs of oxidative stress and antioxidant depletion in the course of their treatment. The assessment of their serum antioxidant status and levels of lipid peroxidation could be crucial for the evolution of future therapeutic strategies.

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## MATERIAL AND METHODS

The study was performed between October 2001 and March 2003 at the Clinics of Hematology of St. Marina University Hospital of Varna and the Department of Preclinical, Clinical Pharmacology and Biochemistry of Prof. Paraskev Stoyanov Medical University of Varna. Oxidative stress and antioxidant capacity were investigated in a selected group of 8 patients (3 males, mean age 31,7 years, and 5 females, mean age 32,4 years), hospitalized for treatment of ALL, who gave their informed consent to participation in the study. The morphological variant of leukemia was defined according to the FAB classification and patients' WHO performance status was estimated prior to the treatment (Table 1). Eligible for the study were only novel untreated lymphoblastic leukemia patients. Patients with high number of blast cells (over $30,10^9/l$) were excluded from the study due to expected chemotherapy-induced hyperuricemia as a result of tumor-lysis syndrome. All patients were treated with cytostatics according the protocols HCVAD applied in the Clinics of Hematology (Table 2). None of them had laboratory or clinical evidence of renal failure. Patients with clinical or biochemical features of

acute infection or inflammation were not enrolled in the study.

*Table 1. Laboratory indices and morphological variant of leukemia*

| Laboratory indices | ALL in acute phase of disease |
|---|---|
| Hemoglobin [g/l]* | 80.5 |
| White blood cells x 109/l* | 11.2 |
| Platelets x $10^9$/l* | 26.0 |
| Total bilirubin [ mol/l]* | 14.0 |
| WHO | $WHO_1$ - 1, $WHO_2$ - 2, $WHO_3$ - 4, $WHO_4$ - 1 |
| FAB classification | L2 - 8 |

*Data are presented as median

*Table 2. Cytostatic protocol HCVAD for treatment of ALL patents*

| Cyclophosphamide | 2 x 300mg/$m^2$ iv | 1-3 days |
|---|---|---|
| Vinblastin | 10mg iv | $4^{th}$, $11^{th}$ days |
| Farmorubicin | 50mg/$m^2$ iv | $4^{th}$ day |
| Dexamethazone | 40mg iv | 1 - 4 and 11 - 14 days |

Severe liver disease was excluded by means of clinical, biochemical and ultrasound examinations. No patient was affected by wasting syndrome (body mass index was between 20,9 and 28,0).

The applied restrictions reduced the number of the patients included in the study to 8. Routine laboratory tests were carried out before the treatment: differential blood count, blood hemoglobin, serum glucose, creatinine, and total protein levels, serum AsAT, AlAT, GGT, alkaline phosphatase, and LDH activities.

A group of 14 healthy volunteers (mean age 41,4 ±12,7 years: 6 males and 8 females) was used as a control. Exclusion criteria for control subjects were presence of neurological or endocrinological diseases, diabetus mellitus, arterial hypertension, prior myocardial infarction or stroke, infections, other inflammatory or malignant diseases.

Neither patients, nor controls were given vitamin-mineral supplements.

*Blood sample collection*

Blood samples from patients were collected one day before administration of cytotoxic drugs and on the $1^{st}$, $4^{th}$, $7^{th}$ day of chemotherapy, and on the day when leukocyte count was lower than 1 x $10^9$/l (nadir) under standardized conditions. To obtain serum the blood was incubated for 20min at 37°C and after that centrifuged at 3000xg for 10min. Plasma was obtained by centrifugation of blood (2000xg for 20min). Serum and plasma were immediately transferred into coded plastic tubes, snap frozen and stored at -22°C until analysis. The assays were carried out after thawing the frozen aliquots for 5min in a water-bath at 37°C and mixing them gently.

Differential blood count, total antioxidant status, and uric acid, malondialdehyde, serum iron, and total and direct bilirubin content were determined for all patients before and in the course of the chemotherapy cycle.

*Serum antioxidant status and lipid peroxidation assay*

Serum oxidative stress was measured as described by Porter *at al.* (17) by the content of TBARS used as a marker for lipid peroxidation in serum. Malondialdehyde (MDA), an end product of lipid peroxidation, was determined spectrophotometrically at 532nm on Specol 11 and was presented as nmol/ml serum. 1,1,3,3-tetramethoxypropan from Aldrich-Chemie (Steinheim, Germany) was used as a standard.

Serum iron levels were monitored as a potential source of NTBI with a role in initiation of oxidative burst. Serum iron content was determined by the standard colorimetric test with ferrozine (SPINREACT, S. A.). The measurements were performed on automated multichannal analyzer COBAS MIRA.

Total antioxidant status was estimated using the Total antioxidant status kit (RANDOX Laboratories Ltd., Ardmore, UK) based on the method of Miller *et al.* (14). This method measures the overall capacity of human serum antioxidants to quench ABTS radical cation ($ABTS^+$), expressed as mmol TROLOX equivalents per liter. The measurements were performed using an automated multichannal analyzer ABBOTT SPECTRUM EPX.

The levels of uric acid (UA) in serum were determined as compounds of the antioxidant defense (2). UA content was evaluated using enzymatic colorimetric test (URICASE-PAP) based on Trinder-Reaction. The measurements were performed on automated multichannal analyzer COBAS MIRA.

*Statistical analysis*

Paired *t* test was used to compare the baseline values (0 day) with the values on days 1, 4, 7, and on nadir. p -val. of <0,05 were regarded as significant. The independent *t* test was used to compare groups of patients. Correlation was quantified using Spearman rank correlation coefficient test. All statistical analysis was performed using SPSS 11.1.0 statistical software.

## RESULTS AND DISCUSSION

TAS levels of healthy volunteers were measured to be in the reference range of the method: 1,30-1,77mmol/l serum (mean value for the control group - 1,49 ±0,11mmol/l serum). Before chemotherapy serum TAS values of patients were below the lower range values of the controls - 1,27

±0,17 mmol/l (Table 3) followed by an increase of serum TAS values at the 1st and 4th days of chemotherapy: 11% increase at the 1st day and 9% at day 4 during chemotherapy. Slightly lower TAS values were measured at the 7th day of chemotherapy. On the nadir the decrease was of 15% and was statistically significant (p<0,05 vs. day 0).
UA values were 219 ±85 mol/l serum prior treatment and were within the normal range (mean value of the control group - 242 ±10 mol/l serum for women; 299 ±25 mol/l for men; reference range of the method: women - 148-357 mol/l; men - 200-416 mol/l) (Table 3).

Serum levels of bilirubin considered as a potential antioxidant in blood (11) did not show variations before and during chemotherapy of all ALL patients (data not shown).
The concentration of plasma MDA was estimated to be within the range of 2,31 to 5,70nmol/ml for the control group (mean value - 3,98 ±1,19nmol/ml). Pretreatment MDA levels were determined to be 7,72 ±2,72nmol/ml and significantly higher (p<0,05 vs. controls). A slight decrease in MDA values was measured in the whole course of chemotherapy, however, not statistically significant (p>0,05). On the nadir this parameter was determined to be lowest

*Table 3. Changes in blood antioxidant/procxidant parameters in the course of conventional chemotherapy of ALL patients in the acute phase of the disease (n=8)*

| Parameter | Day 0 (mean SD) | Day 1 (mean SD) | Day 4 (mean SD) | Day 7 (mean SD) | Nadir (mean SD) | Control group (mean SD) |
|---|---|---|---|---|---|---|
| TAS [mmol/l] | 1.27 ±0.17 | 1.41 ±0.22 | 1.38 ±0.16 | 1.20 ±0.20 | 1.07 ±0.10* | 1.49 ±0.11 |
| UA [ mol/l] | 219 ±85 | 249 ±90 | 295 ±123 | 198 ±90 | 167 ±76* | ‡242 ±10<br>†299 ±25 |
| MDA [nmol/ml] | 7.72 ±2.72 | 6.71 ±1.61 | 7.01 ±3.81 | 7.53 ±5.09 | 6.30 ±2.54 | 3.98 ±1.19 |
| Fe [ mol/l] | 26.0 ±12.6 | 33.4 ±11.4* | 36.8 ±13.2 | 35.6 ±14.1 | 32.8 ±11.0 | ‡13.8 ± 3.1<br>†19.8 ±4.3 |

* - p<0,05 vs. day 0; ‡ - women; † - men

UA concentration increased soon after administration of cytostatics (by 14% on the 1st day vs. day 0) and kept increasing up to the 4th day (by 35% on the 4th day vs. day 0).

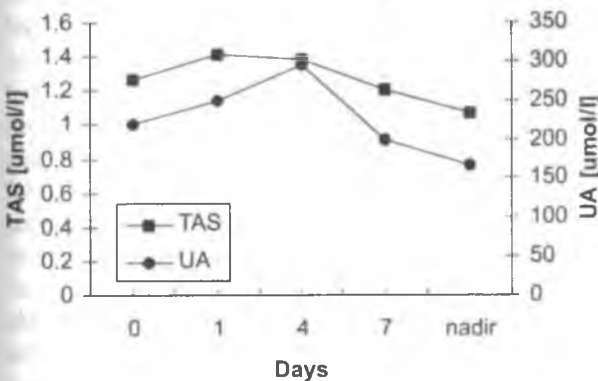


*Fig. 1. Comparison between the changes in TAS and UA average values in ALL patients during chemotherapy course (Spearman rank correlation coefficient was 0,9 (p<0,05)*

A reduction of serum UA concentrations in the course of chemotherapy was later measured, and was statistically significant (p <0,05) on the nadir (24% decrease). Data analysis revealed a strong correlation between UA as an antioxidant and serum TAS measured: r=0,9; p<0,05 (Fig. 1).

(6,3nmol/ml) as compared to the MDA pretreatment values but it was still exceeding the control values. Evidence of increased lipid peroxidation in serum as measured by MDA content did not correlate with decreased antioxidant capacity, estimated as TAS values (r=0,1; p<0,05).
Total serum iron significantly increased on the first day of the administration of cytostatics (p<0,05 vs. day 0) and kept increasing, exceeding the norm (reference values for women: 6,6-26,0 mol/l, mean value 13,8 ±3,1 mol/l; for men: 10,7-28,6 mol/l, mean value 19,8 ±4,3 mol/l) during the whole course of treatment. High serum iron still did not negatively correlate with TAS values (r=0,3) and with UA values (r=0,05; p=0,4).
The pathophysiology of cancer is associated with the increased generation of reactive oxygen species and lipid peroxidation processes (1,3,7). MDA in untreated newly diagnosed patients with acute ALL was estimated to be above the normal range, indicating the occurrence of oxidative stress. Our findings are in agreement with previously reported data demonstrating lipid peroxidation in acute leukemias (8) and free radical generation in patients with other types of cancer (13). MDA levels in treated patients did not remarkably change and were exceeding the control values over the time period studied, being thus a marker for prevalence of processes of lipid peroxidation above antioxidant defense. An increase in lipid peroxidation after high

dose chemotherapy has been previously reported (4), indicating a free radical generation occurrence. An initiator of free radical generation through Fenton reaction is NTBI. Elevated total serum iron could be a potential source of NTBI. We measured increased serum iron levels during the whole course of conventional chemotherapy of ALL patients.

Blood total antioxidant status is the result of the interaction of many different compounds such as uric acid and antioxidants of dietary origin and systemic metabolic interactions, and also by thiol groups of proteins. Despite the evidences for increased pretreatment lipid peroxidation, TAS and UA content measured were within the normal range before the chemotherapy. Chemotherapy-induced free radical generation and oxidative stress probably lead to the consumption of important antioxidants, including UA followed by a decrease in serum TAS. However, an elevated content and not a consumption of total serum antioxidants and UA were detected already on days 1 and 4 of chemotherapy course. This could rather be a result of the release of intracellular antioxidants during the cytostatics-induced lysis of blast cells than a result of an up-regulation of antioxidants induced by disease-related oxidative stress, as suggested earlier (12,16). Monitoring of serum bilirubin, considered in recent times as a potential antioxidant in blood (11), did not reveal changes in its levels during the course of treatment and thus could not contribute to the variations of TAS.

## CONCLUSION

Our data suggest that UA content as a routine laboratory index could also serve as a marker of blood antioxidant capacity. UA content monitoring in the course of chemotherapy could indicate the moment when antioxidant supplementation therapy would compensate certain side effects of many anticancer drugs. Supplementation of antioxidants might affect clinical outcome and improve quality of patients' life.

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