Serum concentration of IL-2, IL-4, IL-10 and TNF-α in children with acute lymphoblastic leukemia – possible role of oxidative stress

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Abstract

Aim of the study was to measure interleukin 2 (IL-2), interleukin 4 (IL-4), interleukin-10 (IL-10) and tumor necrosis factor alpha (TNF-α) concentrations and their correlation with oxidative status and efficacy of antioxidative defense in peripheral blood of children with acute lymphoblastic leukemia (ALL). Study group consisted of 23 children, median age 5,9 years. Sampling was obtained twice: the first before therapy was started and the second after induction of the treatment. Cytokines quantitative assays were performed in serum, using an ELISA method. Superoxide dismutase (SOD), glutathione peroxidase (GPX) and total antioxidant status (TAS) levels were estimated using kits Randox Laboratories Ltd. Vitamin E and malonyldialdehyde (MDA) concentrations in plasma were measured fluorymetically.

IL-10 concentration was higher in serum of ALL children when compare to controls. Median TNF-α was significantly increased in newly diagnosed patients when compared to the same patients in remission and controls. MDA levels and GPX activity were higher in leukemia group then in controls. In our study, interleukin 10 was correlated with level of MDA. We concluded that serum IL-10 and TNF-α in children with ALL is increased compare to control group. Our data suggest that oxidative stress contribute to those changes.

Key words: *interleukin-10, tumor necrosis factor alpha, oxidative stress, acute lymphoblastic leukemia.*

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Introduction

Balance between T-helper type 1 (Th1), T-helper type 2 (Th2) and T-regulatory cytokine types is believed to be involved in pathogenesis of many diseases, one of them is leukemia. Interleukin 2 (IL-2) and tumor necrosis factor alpha (TNF-α) represent Th1 associated cytokines, whereas interleukin 4 (IL-4) related to Th2 and interleukin 10 (IL-10) is a member of regulatory cytokines. Multi-agent chemotherapy offers a chance to cure for most children, who suffer for acute lymphoblastic leukemia, however influence of the therapy on immunological system of these patients is not fully understood. Oxidative stress might be involved in acquired immunity by activation of nuclear factor κβ, which controls genes for various cytokines e.g. IL-2, IL-10 and TNF-α [1]. Oxidation can be measured by assessing aldehyde (malonyldialdehyde), which is a product of lipid peroxidation and this reaction usually reflects a level of oxidative stress [2]. Antioxidant defense consists of enzymes such as superoxide dismutase (SOD) or glutathione peroxidase (GPX). Vitamins (e.g. vitamin E) and physiological molecules (albumins, bilirubin) have also antioxidant properties [3], which capacity to neutralize free radicals could be measured by assessing total antioxidant status (TAS).

The aim of this study was to measure serum IL-2, IL-4, IL-10 and TNF-α concentrations and their correlation with oxidative status (MDA) and antioxidative defense efficacy (Zn-Cu SOD, GPX, Vitamin E and TAS) in peripheral blood of children with acute lymphoblastic leukemia.

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Material and Methods

Patients and blood samples

Study group consisted of 23 children admitted to our department between February 2004 and December 2005 and diagnosed as having acute lymphoblastic leukemia. There were 13 boys and 10 girls, the median age of this group was 5.9 years (range 0.5-17 years). The diagnosis of ALL was based on clinical features and bone marrow examination, which included cyto-morphological study, immuno-phenotyping and cytogenetic analysis.

The samples were carried out twice in each patient: the first before therapy was started and the second after induction of the treatment, when complete remission in bone marrow was confirmed. Results were compared with control group, consisting of 21 children (11 males and 10 females) aged 2-16 (median age 7.8). Patients in the control group were originally referred to otolaryngology ward for planned surgical procedures. Infection and cancer was excluded in the control group based on clinical examination.

Peripheral blood samples were collected as follows: 5 ml to heparinized vacutainers and 1.2 ml to EDTA vacutainers. All blood samples were obtained at the time of routine diagnostic tests.

Methods

Interleukin-2 (IL-2) and interleukin-4 (IL-4) quantitative assays were performed in serum, using an ELISA method according to manual provided by reagents manufacturer (Bender MedSystems GmbH, Vienna, Austria). Sensivity of the tests, described by manufacturer, were respectively for IL-2 9.9 pg/ml and for IL-4 1.32 pg/ml. Interleukin-10 (IL-10) and tumor necrosis factor α (TNF-α) quantitative assays were performed in serum, using *The Quantikine IL-10 and TNF HS Immunoassay kit* according to manual provided by reagents manufacturer (R & D Systems, Inc., Minneapolis, USA). Sensivity of the tests, described by producer, was for IL-10 0.5 pg/ml and for TNF-α 0.12 pg/ml, respectively.

Malondialdehyde (MDA) in plasma was estimated by test based on reaction with thiobarbituric acid at 95°C. Total thiobarbituric acid reactive substances were measured with spectrofluorimeter *Perkin-Elmer* at 553 nm. Results were calculated in nmol/ml. Total antioxidant status (TAS) in plasma measurements were performed using kit manufactured by Randox Laboratories Ltd. Results were calculated in mmol/l of plasma [mmol/l]. Superoxide dismutase (SOD) in erythrocytes was estimated using *Ransod* kit (Randox Laboratories Ltd). After sample preparation according to manufacturer instruction results were obtained using *Cobas Mira S* spectrophotometer at 37°C at 505 nm, and then converted to SOD units/g hemoglobin [U/g Hb]. Glutathione peroxidase (GPX) in whole blood was measured using *Ransel* kit (Randox Laboratories Ltd). Results were converted to GPX Units/g Hb [U/g]. Vitamin E was assessed in plasma using fluorimetric method, on spectrofluorimeter Perkin-Elmer at 310 nm. Results were calculated in μg/ml of plasma.

Analysis of the data and graphical presentation of the results were performed with *Statistica 6, 1 for Windows* (Stat Soft, Inc.) software. Statistical analyses were undertaken using Mann-Whitney *U*-test and Spearman's rank correlation test, respectively. A P value <0,05 was accepted as a limit of statistical significance.

Results

IL-10 concentration was significantly higher in serum of children with acute lymphoblastic leukemia at the time of diagnosis (28.16 pg/ml) when compared to controls (11.32 pg/ml) as well the ALL patients during remission phase (16.67 pg/ml). This results are presented in Figure 1. Median TNF-α serum level was significantly increased in newly diagnosed pediatric patients with ALL (9.52 pg/ml) when compared to the same patients after remission (2.69 pg/ml) and controls (3.68 pg/ml). Details are presented in Figure 2. Median serum concentration of IL-2 was 29.11 pg/ml in ALL children at the time of diagnosis and 21.75 pg/ml after remission. This results were not statistically different when compared to control group 22.84 pg/ml. Similarly, IL-4 median serum concentrations were not different in ALL patients at diagnosis, during treatment and in controls (48.31, 65.19 and 57.01 pg/ml, respectively). Results of serum cytokine concentrations are presented in Figures 1-2.

MDA levels were higher in leukemia group then in controls, the statistically significant difference was found not only between newly diagnosed children and controls (P=0.00003) but also between patients after achieving remission during treatment and healthy children (P=0.03). GPX activity was significantly higher in children with ALL

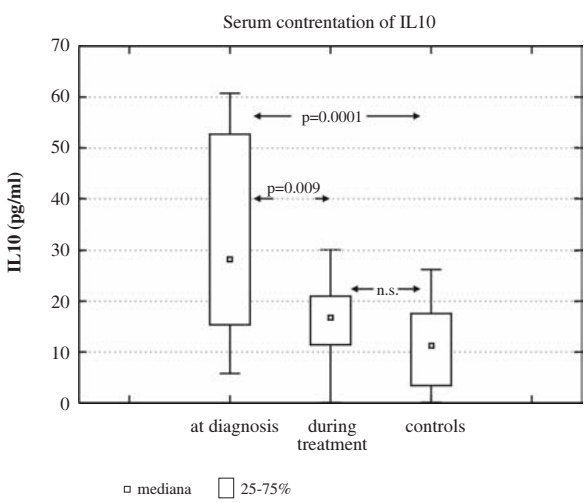

Fig. 1. Concentration of interleukin 10 (IL-10) in serum of children with ALL and controls

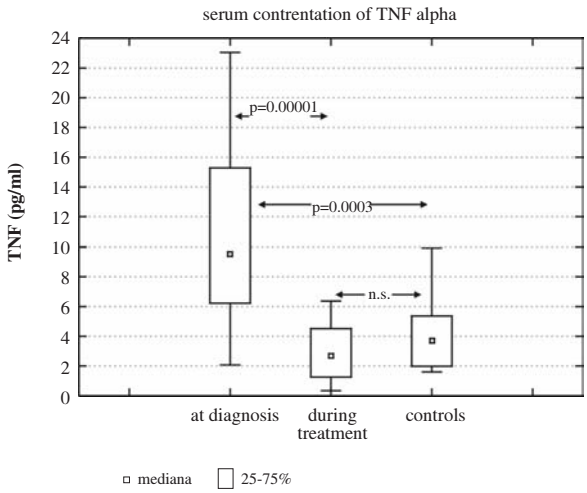

Fig. 2. Concentration of tumor necrosis alpha (TNF-α) in serum of children with ALL and controls

before treatment when compare to controls (P=0.04) and during treatment the difference between patients and healthy children was also very statistically significant (P=0.001). Values of estimated parameters of oxidative-antioxidative balance are presented in Table 1.

In our study, interleukin 10 was positively correlated with level of MDA (r=0.6) and TAS (r=0.46) in children with ALL at diagnosis. During therapy this correlation decreased, but remained significant between IL-10 and MDA (r=0.49). In healthy controls, correlation between IL-10 and MDA was also significant (r=0.51) but conversely the correlation between IL-10 and TAS was negative (r=–0.64). In control group, GPX activity negatively correlated with IL-10 serum concentration (r=–0.54) but we could not demonstrate this relationship in children with ALL neither before nor during therapy.

Discussion

Recent studies suggest, that abnormal cytokine production may play a role in pathogenesis of leukemia either by autocrine production by abnormal cells or by reaction of normal environment for leukemia cells invasion. Shultz at al. demonstrated, using RT-PCR method, that leukemic blasts express the mRNA for TNF-α and IL-10 [4]. Increased level of plasma IL-10 have been reported in pediatric patients with ALL at the time of diagnosis however the authors determined capacity to produce cytokines *ex vivo* and found it normal, so concluded that those changes are due to lower number of normal cytokine secreting cells (like monocytes) in leukemia patients at diagnosis [5]. Study with adult, T-cell leukemia patients showed, that increased IL-10 level was significant unfavorable predicting factor in those patients group, and correlated with lactate dehydrogenase concentration, and thus may reflect tumor burden [6]. Effect of IL-10 on B-cell chronic lymphocytic leukemia (B-CLL) have been reported as inhibition of leukemic cell function and this cytokine was proposed to have a role in therapy of B-CLL [7]. There were published reports regarding pediatric patients with ALL connected genotype of IL-10 or levels of TNF

Table 1. Parameters of oxidative-antioxidative balance in children with ALL and controls

Parametres	MDA [nmol/ml]	SOD [U/g Hb]	GPX [U/g Hb]	Vit E [µg/ml]	TAS [mmol/l]
ALL patients at diagnosis					
median value	2.02*	1157.12	71.51*	6.00	0.81
mean value	2.18*	1147.86	69.50*	5.98	0.95
range	(0.5-3.98)	(600-2194.78)	(23.57-145.98)	(2.02-12.40)	(0.48-1.61)
ALL patients during treatment					
median value	1.67*	1113.63	75.81*	7.25	1.01
mean value	1.82*	1090.41	82.02*	7.87	1.12
range	(1.01-3.47)	(573.77-1463.41)	(36.59-154.94)	(3.18-17.80)	(0.62-1.77)
control group					
median value	1.47	992.48	47.48	5.70	1.12
mean value	1.42	1011.04	51.68	6.43	1.12
range	(0.9-1.92)	(601.5-1428.57)	(29.59-78.51)	(3.3-12.11)	(0.45-2.07)

*SOD –superoxide dismutase, GPX – glutathione peroxidase, MDA – malonylidiahdehyde, Vit E – vitamin E, TAS – total antioxidant status, ALL – acute lymphoblastic leukemia; *p<0.05 compare to controls.*

receptors with sensivity for steroid therapy and proposed to include this features into prognostic factors, especially in children with disease relapse [8-10]. Secretion of IL-10 by leukemic cells have been confirmed in bone marrow specimens of children with relapsed ALL [11]. The increased level of TNF-α as compared to controls was reported by Mazur et al. in children after 12 months after cessation of treatment and authors speculated, that the cause of that may be hypogammaglobulinemia after chemotherapy [12]. Our results confirmed high TNF-α level in patients after diagnosis before chemotherapy. During treatment median serum concentration of this pro-inflammatory cytokine was comparable to controls.

Singh at al. reported increase of MDA concentration in active phase of the disease when compared to control group however the clinical significance of oxidative stress remains unclear [13]. Our study confirmed higher level of lipid peroxidation in newly diagnosed children with ALL as well as in the same patients after induction phase of treatment in comparison with healthy subjects. Increased activity of GPX in our patients corresponds with work done by Stammler et al. who found correlation between GPX and other antioxidant enzymes [14].Wide range of the GPX activity in ALL patients compared to control group may support hypothesis, that glutathione dependent enzymes play a crucial role in determining apoptosis in leukemia cells [15]. Based on the published reports we know that IL-10 besides its anti-inflammatory properties may act as a antioxidant inhibiting release of reactive oxygen species during oxidative stress [16]. In our opinion the positive statistically significant correlation between MDA and IL-10 suggest, that increase production and secretion of IL-10 by leukemic cells may be response for oxidative stress. Relationship between MDA and IL-10 was reported in other diseases like sickle cell disease or HIV. Authors speculated, that regulatory function of IL-10 may protect tissues from damage caused by pro-inflammatory cytokines [17, 18]. Liese et al. reported augmented TNF-α and IL-10 production by human monocytes in response to autologus damaged by oxidation erythrocytes [19]. More studies are necessary to identify the role of oxidative stress and antioxidant defense in pathogenesis of childhood ALL and its influence on cytokine network.

We concluded that serum IL-10 and TNF-α concentrations in children with ALL is different compared to control group. Our data suggest that oxidative stress may contribute to those changes.

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