ORIGINAL REPORT

Influence of Chemotherapy on the Lipid Peroxidation and Antioxidant Status in Patients with Acute Myeloid Leukemia

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Abstract- Chemotherapeutic agents used in patients with cancer cause to generate the enormous amounts of free radicals associated with cell injury. In this study we assess the effects of chemotherapy regimen on oxidant/antioxidant status in patients with acute myeloid leukemia (AML). 38 newly diagnosed patients with acute myeloid leukemia were recruited in this study. All patients received cytarabine and daunorubicin as chemotherapy regimen. Plasma levels of malondialdehyde (MDA), total antioxidant status (TAS), and the levels of erythrocyte activity of superoxide dismutase (SOD) and glutathione peroxidase (GPx) were determined before chemotherapy and 14 days after chemotherapy with cytarabine and daunorubicin. Plasma MDA concentrations increased significantly (from 2.68±0.89 nmol/L to 3.14±1.29 nmol/L) during the 14days post-chemotherapy period (P=0.04). Plasma TAS concentrations changed with chemotherapy from 1.09±0.15 mmol/L to 1.02±0.14 mmol/L with P=0.005. Erythrocyte SOD and GPX activity decreased overtime from 1157.24±543.61 U/g Hb to 984.01±419.09 U/g Hb (P=0.04) and 46.96±13.70 U/g Hb to 41.40±6.44 U/g Hb (P=0.02) respectively. We report here that there is an increase in malondialdehyde levels and a decrease in the levels of antioxidant enzymes and total antioxidant status. This suggests that chemotherapy causes these changes as a result of enormous production of reactive oxygen species in the patients with AML. Antioxidant supplementation must be approached with caution because of the probability of reduction the therapeutic efficacy of these cytotoxic drugs.

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Keywords: Acute myeloid leukemia; Chemotherapy; Oxidative stress; Antioxidant

Introduction

Acute myeloid leukemia (AML) is a neoplasm of the myeloid line of white blood cells that is characterized by the accumulation of abnormal blast cells principally in the bone marrow that interfere with the production of normal blood cells (1). AML occurs more commonly in adults and its incidence increases with age (2). The incidence rate for adults under 65 years old is 1.800/100,000; whereas it is 17.9/100,000 for those who are over 65 (3). About 30% of those who are diagnosed with AML and age between 18 and 60 can be cured. However, chemotherapy can be destroying, especially in older patients and the disease-free survival prognosis is poor (4). The possible risk factors found to be related to AML are: benzene, radiation, cigarette smoking, prior chemotherapy treatment of cancer and pesticide exposure (1). Moreover, the association between oxidative stress and AML has also been reported (1,5).

Many chemotherapeutic agents used in cancer treatment form free radicals as a result of their metabolic activities and their efficacy may be due to an increase in reactive oxygen species (ROS) generation (6). A combination of anthracycline and cytarabine has been the standard induction therapy for AML since 1973 (7). It has been shown that both of them cause the generation of free radicals and induction of oxidative stress, associated with cellular injury (8-11). The removal of free radicals is done by numerous enzymatic and non-enzymatic mechanisms and they protect cells from reactive oxygen species (ROS) (6). Among antioxidant enzymes, superoxide dismutase (SOD) and glutathione peroxidase (GPx) play a major role in protecting cells from the oxidative damage (12).

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Table 1. Clinical characteristics of the patients.

Total number of subjects	
Gender	38
Male	21,55.3%
Female	17,44.7%
Age	
Range	18-71
Mean	34.05 ± 12.49
Diagnosis	
M0 (%)	2.6
M1 (%)	7.9
M2 (%)	44.7
M3 (%)	31.6
M4 (%)	13.2

Mo-M4: Subgroups of acute myeloid leukemia

The aim of this study was to assess the effects of chemotherapy on antioxidant status and lipid peroxidation in AML patients. Therefore we examined the alteration of oxidant/antioxidant parameters in the circulation of patients with AML by measuring a) malondialdehyde (as the indices of lipid peroxidation), b) total antioxidant status, c) superoxide dismutase, d) glutathione peroxidase

Materials and Methods

Subjects

The study subjects were 38 patients; women 17(44.7%); men 21, (55.3%); mean age: 34.05±12.49 y, range: 18-71 y. They included 2.6%, 7.9%, 44.7%, 31.6% and 13.2 %patients with M0, M1, M2, M3 and M4 respectively (Table 1). All patients were recruited from the Shahid Ghazi Tabatabaii Hospital of the Hematology and Oncology Department of Tabriz University of Medical Sciences, Tabriz, Iran.

Eligible study patients (≥18 y of age) were newly diagnosed with acute myeloid leukemia and they were selected on the basis of the following exclusion criteria: 1) taking any form of the antioxidant supplementations ≤ 3 months before the enrollment; 2) primary cancer diagnosis other than AML; and receiving any previous chemotherapy treatment. Blood samples were drawn from the antecubital vein before chemotherapy and on the day 14 after chemotherapy for the biochemical investigation. The study was approved by ethical committee of Tabriz University of Medical Sciences and informed consent was obtained from all participants of the study before the blood collection.

All patients received citarabine at 100 mg/m² daily by continuous infusion on days 1 through 7 and daunorubicin (an anthracycline antibiotic) at 45 mg/m² on days 1 through 3, the so-called "7 and 3 regimen" as their standard induction chemotherapy regimen.

Reagents

All chemicals used in this study were of analytical grade and obtained from Randox Laboratories (Randox Laboratories, Crumlin, Antrim, UK) and Merck (Darmstadt, Germany).

Determination of superoxide dismutase activity

Heparinized whole blood sample were used to measure the activity of SOD. The activity of the enzymes was evaluated spectrophotometrically using the commercial Randx-Ransod kit (Randox Laboratories, Crumlin, Antrim, UK). This method is described by the McCord and Fridovich (13). Results were expressed in units/g of hemoglobin (g Hb).

Determination of glutathione peroxidase activity

To assess the activity of GPx, we used heparinized whole blood which was diluted with double strength Drabkin's by the method that described by Paglia and Valentine (14) using the commercial Randox-Ransod kit (Randox Laboratories, Crumlin, Antrim, UK). The activity of the enzymes was evaluated spectrophotometrically and the results were expressed in units/g Hb.

Measurements of total antioxidant status (TAS)

Freshly drawn serum was used to determine the level of TAS. To do this, the commercial total antioxidant status kit (Randox Laboratories, Crumlin, Antrim, UK) was applied. In this method, level of TAS was measured spectrophotometrically too and the results were expressed as nmol/L.

Measurements of malondialdehyde (MDA)

Serum level of MDA was determined spectrophotometrically. In this procedure, thiobarbituric acid-reactive substances (TBARs) assay measures peroxidative damage to lipids that occurs by excessive amounts of ROS. Lipid peroxidation was estimated according to the method of lapenna *et al.* (15). The results were expressed as nmol TBARs/ml serum, using MDA as standard.

Statistical analysis

The results were expressed as mean ± SD. Differences in the level of MDA, TAS and the activities of antioxidant enzymes before and after chemotherapy were analyzed by paired t-test.

Table 2. Erythrocyte and plasma oxidant/antioxidant levels in 38 patients with acute.

Myeloid leukemia undergoing chemotherapy	**Pre-chemotherapy**	**Post-chemotherapy**	***P*-value**
Parameters			
Plasma			
TAS (nmol/L)	1.09±0.15	1.02±0.14	0.005
MDA (nmol/ml)	2.68±0.89	3.14±1.29	0.04
Erythrocytes			
SOD (U/g Hb)	1157.24±543.61	984.01±419.09	0.04
GPx (U/g Hb)	46.96±13.70	41.40±6.44	0.02

All values are expressed as mean ± S.D.,

P-values<0.05 were considered statistically significant. Analysis was performed using SPSS software (spss Inc; Chicago, Illinois, USA).

Results

Changes in the levels of MDA, TAS, SOD and GPx activity were assessed by comparing pre-chemotherapy blood values with values obtained 14 days after chemotherapy (Table 2). Plasma MDA concentration increased significantly (from 2.68±0.89 nmol/L to 3.14±1.29 nmol/L) during the 14 days post-chemotherapy period (*P*=0.04). Plasma TAS concentrations changed with chemotherapy and were 1.09±0.15 nmol/L before chemotherapy and 1.02±0.14 nmol/L after chemotherapy. So the level of TAS decreased significantly (*P*=0.005). Erythrocyte SOD and GPx activity decreased overtime from 1157.24±543.61 U/g Hb to 984.01±419.09 U/g Hb (*P*=0.04) and 46.96±13.70 U/g Hb to 41.40±6.44 U/g Hb (*P*=0.02) respectively (Table 2).

Notably, there were no significant differences of MDA, TAS, GPx and SOD levels before and after chemotherapy according to gender with *P*=0.80, *P*=0.13, *P*=0.74 and *P*=0.77 respectively. Our results also showed that the levels of these parameters did not change significantly according to the kind of AML as we assessed the differences between patients with AML M3 and non M3 (*P*=0.12, *P*=0.12, *P*=0.49 and P=0.66, respectively). The patients with AML M3 received all-trans-retinoic acid (ATRA) as a standard component of their induction therapy.

Discussion

In this study, we assessed the levels of erythrocyte GPx and SOD activity and the plasma levels of MDA and TAS by comparing pre-chemotherapy blood values with values obtained 14 days after the initiation of chemotherapy. It was demonstrated that the plasma MDA concentrations have markedly increased after chemotherapy with cytarabine and daunorubicin whereas the levels of TAS, GPx and SOD have reduced significantly.

Arise in MDA determined in our study reflects the increase of oxidative stress and lipid peroxides in our series of 38 patients. Hepatic microsomal monooxygenase system is a primary site where many chemotherapeutic drugs such as anthracyclines generate ROS, although other enzymatic (e.g., xantine oxidase) and non-enzymatic (Fenton and Haber-Weiss reactions) mechanisms may play a role. The electron transport chain of cardiac mitochondria is another site where ROS are generated by anthracyclines (16,17).

Danorubicin is an anthracycline antibiotic used to treat many human malignancies such as acute leukemias, lymphomas, stomach, breast and ovarian cancers, bone tumors and Kaposi's sarcoma (18). Several mechanisms have been suggested for the metabolic activity of anthracyclines as the anticancer drugs. The most attractive evidence is via interaction with double-stranded DNA and inhibition of topoisomerase II activity. The quinone moiety of anthracyclines can be reduced **"**a one-electron reduction**"** enzymatically, which can donate an electron to molecular oxygen producing a superoxide and subsequent generation of hydroxyl radicals from superoxide (17). Daunorubicin distributed the oxidative state because of its quinone structure. In other words, the NADPH-cytochrome P-450 converts the drug to a semi-quinone free radical that is subsequently re-oxidized and regenerated by oxygen resulting in generation of superoxide anions (19). These processes stimulated the enzymatic activity of SOD leading to increased production of hydroperoxides. Hence, GPx enzyme stimulated in response to high levels of peroxides leading to the formation of hydroxyl radicals using metal ions. Hydroxyl radicals may react with polyunsaturated fatty acids, resulting in formation of diffusible aldehydes (e.g. MDA) (19), as shown in our results.

The chemotherapeutic agent cytarabine (cytosine arabinoside) is used to treat leukemia (9). It increases the generation of ROS and promotes an oxidative DNA damage and P53-dependent apoptosis. The mechanism by which cytarabine causes ROS generation is not well understood. One speculation is that cytarabine increases the production of ATP and elevates the activity level of cytochrome c oxidase in leukemic cells, resulting in ROS production. Alternatively, cytarabine can effect on lipid metabolism. Cholinephosphotransferase use cytarabine to produce diradylglycerol and the choline derivative of cytarabine. diradylglycerol can be de-esterified by diglyceride lipase lead to formation of fatty acids such as arachidonic acid and the subsequent metabolism of these fatty acids resulting in ROS production. However, the precise mechanism by which cytarabine produces ROS and increases the oxidative stress is not clear (9).

Antioxidants that make up the antioxidant defense system have three parts: primary, secondary and tertiary defense (1). The primary antioxidants prevent the formation of ROS. These include SOD, GPx and metal binding proteins (e.g. ferritin or ceruloplasmin). Secondary antioxidants block chain reactions, so trap free radicals. They include vitamin E, vitamin C, beta-carotene, uric acid, albumin and bilirubin. Tertiary antioxidants are enzymes that repair biomolecules like DNA, damaged by free radicals. By measuring the total antioxidant system (TAS), the total antioxidant effects of these three groups in circulation are evaluated (1). So, determination of TAS is a tool to measure the antioxidant capacity (20), and the depletion of TAS as is seen in this study may represent the utilization of antioxidants to counteract ROS.

SOD, is the first line of enzymatic defense against oxygen free radicals, catalyzes dismutation of super oxide anion radical into hydrogen peroxide (H_2O_2), while can be transformed into water and oxygen by GPx or catalase (16). Besides H_2O_2, GPx in conjugation with reduced glutathione (GSH) reduces lipid or non-lipid hydroperoxides while oxidizing glutathione (12, 21). The depletion of the antioxidant enzymes may be due to attempt to counteract the elevated lipid peroxidation. Another speculation is that as GPx and SOD are themselves vulnerable to oxidation by ROS and lipid peroxides, they could be inactivated by their own substrates (22,23). MDA and superoxide anion could partially inhibit GPx activity (23,24).

Increased level of the plasma MDA with concomitant depletion in the antioxidant defense system in our patients after chemotherapy is in accordance with previous findings of elevated levels of lipid peroxidation products and decreased activities of antioxidant enzymes in patients with acute lymphoblastic leukemia (25) and Hodgekin's lymphoma patients (26). Similar results were observed in patients with prostate cancer (23), melanoma (27) and those with oral cancer treated with radiotherapy (6).

Our laboratory normal reference concentration ranges of TAS, SOD and GPx for healthy adult subjects are as follows, TAS: 1.30-1.77 nmol/L, SOD (level of activity): 1102-1601 U/g Hb and GPx (level of activity): 27.5-73.6 U/g Hb. So, it is clear that the mean level of TAS has been lower than the normal range even prior to therapy (1.09±0.15 nmol/L), and the mean level of SOD has been reached the below the normal range of this parameter after the chemotherapy (984.01±419.09 U/g Hb). There are many evidences indicating that ROS have a role in the initiation and promotion of cancer. However, whether an oxidative stress is involved in AML remains unclear and highly considerable.

On the basis of these results, we report here that after chemotherapy with daunorubicin and cytarabine in patients with AML, there is an increase in the mean serum level of MDA and a significant decrease in the levels of SOD, GPx and TAS as the important parts of the body's antioxidant defense system. It seems that chemotherapy causes these changes as a result of enormous production of ROS in the patients with AML and assessment the relationship between oxidant/antioxidant status and chemotherapy related toxicities are needed. Moreover, the study of the effect of antioxidant supplementation to improve antioxidant status in these patients is worth to be carried out in future. Although it must be done with caution as it cannot be said with certainty whether or not the oxidative stress is the major part of these chemotherapeutic agents mechanism of action.

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