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OXIDATIVE STRESS AND ANTIOXIDANT STATUS IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA

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ABSTRACT

Chronic myeloid leukemia is a myeloproliferative disorder with a unique rearrangement, the Philadelphia chromosome. Oxidative stress, a pervasive condition of an increased number of reactive oxygen species, is now recognized to be prominent feature of various diseases and their progression. Thus antioxidants, which control the oxidative stress state, represent a major line of defense regulating overall true state of health. The relationship between antioxidants status and levels of well-known markers of oxidative stress that are measured as lipid peroxides and oxidized proteins reflect better health indices and postures. The aim of this study was to evaluate the role of oxidative stress in pathophysiology of Chronic myeloid leukemia by measuring the circulating plasma lipid peroxide levels in terms of malonyldialdehyde, total lipid hydroperoxide and oxidized proteins as protein carbonyl whereas antioxidant status were estimated in terms of reduced glutathione and total thiol in plasma of Chronic myeloid leukemia patients. The present study included 47 Chronic myeloid leukemia patients and 20 age-and sex-matched healthy subjects. Out of 47 Chronic myeloid leukemia patients, 31 were in chronic phase (CML-CP) and 16 in accelerated phase (CML-AP). The median age of Chronic myeloid leukemia patients was 33 years and that of controls was 32 years. Oxidative stress and antioxidant status in plasma were evaluated by spectrophotometric procedures. There was a significant increase ($p<0.05$*) in plasma malonyldialdehyde, total lipid hydroperoxide and protein carbonyl levels in Chronic myeloid leukemia patients as compared to healthy subjects. Our results also showed that plasma malonyldialdehyde and protein carbonyl levels were markedly elevated (*$p<0.05$*) in both chronic phase (CML-CP) and accelerated phase (CML-AP) as compared to healthy volunteers. Antioxidant status was found to be significantly decreased (*$p<0.05$*) in Chronic myeloid leukemia patients and its phases as compared to healthy participants. It could be concluded that oxidative stress may be associated with the pathophysiology of Chronic myeloid leukemia.*

KEY WORDS

Chronic myeloid leukemia, Oxidative stress, Antioxidants, Malonyldialdehyde, Total lipid hydroperoxide, Protein carbonyl.

INTRODUCTION

Chronic myeloid leukemia (CML) is characterized by neoplastic proliferation of hematopoietic cells. It is the first human malignancy where a specific marker, the Philadelphia (Ph) chromosome, was associated with CML (1). At the gene level, breaks occur in the ABL and BCR genes on chromosome 9 and 22 respectively (2). The resulting BCR-ABL fusion on the Ph chromosome is transcribed to chimerical RNA and then translated into a fusion tyrosine kinase protein of varying size P190 kDa to P210 kDa(2). Classic CML has three phases, chronic (CML-CP), accelerated (CML-AP), and terminal blast crisis (CML-BC). CML has an incidence of 1 to 1.5 cases per 100,000 per year in western countries whereas the incidence of CML is not well known in India and occurs slightly more often in men. Although the median age at appearance is 40-60 years, 10% of patients are below the age of 20.

Reactive oxygen species (ROS) or free radicals are generated

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as byproducts of normal cell metabolism. They can be produced and act inside the cell, or they can be generated within the cell and released to extra cellular space (3). Reactive oxygen species may also arise from exogenous sources, like electromagnetic and particulate radiation, air pollutants and tobacco smoke, or through the metabolism of certain drugs, like anthracyclic antineoplastic agents (doxorubicin), pesticides, and solvents (4). Oxidative stress defines as a pervasive condition of increased and/or inadequate removal of ROS (4, 5). To counteract ROS induced oxidative stress, aerobic organism rely on defenses, including enzymes (superoxide dismutase, catalase), as well as on small molecular weight endogenous antioxidants which include reduced glutathione (GSH) and total thiols (T-SH) (4,5).

Direct measurement of ROS is not reliable due to short half-life of ROS and none of the methods are sensitive enough for quantification of ROS. The indirect methods for estimation of ROS or oxidative stress include estimation of oxidized products of lipid, protein and status of endogenous antioxidants (6). Lipid oxidation is evaluated in term of malonyldialdehyde (MDA) and total lipid hydroperoxides (LOOH) (6, 7). Protein carbonyl (PC) is a product of irreversible non-enzymatic oxidation of protein (6). Oxidative stress is now recognized to be a prominent feature of many acute and chronic diseases, and even cancer and leukemias (4-10). Moreover body's defense mechanism would play an important role in the form of antioxidants and try to minimize the damage, adapting itself to above stressful situation. Antioxidants are compounds that dispose, scavenge, and suppress the formation of ROS, or oppose their actions so that cellular antioxidants could play a major role in various diseases including cancer and their clinical manifestations (6, 11-15).

The present study was planned to evaluate the possible role of oxidative stress and antioxidants status in pathobiology of CML patients.

MATERIALS AND METHODS

The present study was conducted in the Departments of Biochemistry and Medicine, Chhatrapati Shahuji Maharaj Medical University, Lucknow, UP, India. Forty seven diagnosed CML patients were chosen for the study. Out of 47 CML patients, 31 were in chronic phase (CML-CP) and 16 in accelerated phase (CML-AP). Twenty age and sex matched healthy subjects were also included in the study. The median age of CML patients was 33 years (range 15-65 years) whereas the median age of healthy subjects was 32 years (range 21-45 years). Patients were given treatments and the dosage of drug was adjusted on each follow-up so as to maintain total leukocyte counts between 5000/mm^3 to 10000mm^3. There were no dietary or supportive antioxidant medication given to patients and no control subjects were allowed to take any dietary antioxidants as well which could affect the status of free radicals and antioxidants.

Informed consent was obtained from all the individuals included in the study. The present investigations were approved by the Institutional Ethical Committees for biomedical research. At the time of blood collection, there were no evidence of infections, tissues injuries or any inflammatory manifestation in the patients as well as in controls. Blood samples were taken from patients and healthy subjects following an overnight fasting period into K_2 EDTA vials. Thus, avoiding the possible influence of dietary factors on the ROS level.

The plasma layer was separated by centrifugation at 3000 rpm for 15 minutes. MDA in the plasma was evaluated by slightly modified spectrophotometric method based on the reaction between MDA and thiobarbituric acid (TBA) (16). Absorbance was measured spectrophotometrically at a wavelength 532 nm with molar extinction coefficient ε_{532} =1.56 x 10^5 M^{-1} cm^{-1}. All values were expressed as nmole MDA/ml plasma. LOOH level in plasma was evaluated using the ferrous oxidation in xylenol orange Fox-2 assay by spectrophotometerically (17). Values of LOOH were expressed as mM/ml plasma. Plasma PC content was measured according to the method described by Levine et al. based on the spectrophotometric detection of the reaction between 2,4-dinitrophenyl hydrazine (DNPH) with PC to form protein hydrazone (18). The results were expressed as nmoles of PC content per milligram of protein by using wavelength 370 nm with molar extinction coefficient ε_{370} = 22000 $M^{-1}cm^{-1}$. The protein content was determined by the Lowry method using bovine serum albumin as a standard (19). Plasma antioxidants status in the form of reduced GSH and T-SH was measured by using 5, 5'-dithiobis (2- nitrobenzoic acid) (DTNB) spectrophotometerically method (20, 21) Plasma GSH was expressed as µ mol/ml whereas plasma T-SH was expressed mM/ml.

The findings were expressed as the mean ± standard deviation. The differences were compared with the use of unpaired Student's *'t'*-test.

RESULTS

The present study was conducted to assess MDA, LOOH and

Table 1: Plasma levels of malondialdehyde, total lipid hydroperoxides and protein carbonyl in CML, CML-CP, CML-AP patients and healthy subjects (Mean ± SD)

		Healthy subjects (n=20)	CML (n=47)	CML-CP (n=31)	CML-AP (n=16)
Malondialdehyde	Mean±SD	2.63±0.62	4.22±2.11	3.91±1.16	4.84±2.79
(nmole/ml plasma)	Median	2.69	3.69	3.58	5.11
	Range	1.41 – 4.15	1.15 – 10.94	1.19 – 8.29	1.15 – 10.94
Total Lipid	Mean±SD	0.33±1.58	0.75±0.36	0.58±0.22	1.08±0.33
Hydroperoxides	Median	0.33	0.72	0.63	1.05
(mM/ml plasma)	Range	0.10 – 0.62	0.25 – 1.93	0.25 – 0.96	0.59 – 1.93
Protein carbonyl	Mean±SD	1.99±1.67	4.70±3.21	3.14±1.86	7.71±3.17
(nmole/mg protein)	Median	1.58	3.94	2.61	7.36
	Range	0.28 – 6.28	0.28 – 14.29	0.28 – 7.29	2.09 – 14.29

Statistically significant (p<0.05) in comparison to CML, CML-CP, CML-AP patients with healthy subjects and also between each patients group.

PC for oxidative stress in patients with CML whereas reduced GSH and T-SH to evaluate antioxidants status in CML patients. Mean plasma MDA, LOOH and PC levels were found to be significantly increased (p<0.05) in CML patients as compared to healthy subjects (Table 1). The mean plasma MDA, LOOH and PC levels in CML-CP and CML-AP patients were significantly different (p<0.05) as compared to healthy subjects (Table 1). We have also found significant difference (p<0.05) in these parameters when compared between CML-CP and CML-AP (Table 1).

Cellular antioxidant status was measured in form of GSH and T-SH and were found significantly decreased (p<0.05) in CML patients as compared to healthy subjects (Table 2). Mean plasma GSH and T-SH levels in CML-CP and CML-AP were significantly different (p<0.05) as compared to healthy subjects (Table 2). We found significant association between the levels of mean plasma GSH and T-SH among clinical phases of CML (Table 2).

DISCUSSION

The current interest in clinical significance of oxidative stress and antioxidants status in pathophsiology of CML and its progression is due to widespread effect on cell constituents and cell functions of the hematopoietic system. Chronic myeloid leukemia is the most common form of chronic leukemia in India. The etiology of CML is not well known. Of-late free radicals or ROS have been implicated in the pathogenesis of leukemia (10).

MDA and LOOH are well-characterized end products of lipid peroxidation. They can also modulate the expression of genes related to tumor promotion (22). The level of MDA and LOOH reflect the extent of lipid peroxidation. On the other hand PC is a product of irreversible non enzymatic oxidation or carbonylation of protein and indicators of free radical generation in cells (6). Carbonylation of protein often leads to a loss of protein function, which is considered a widespread marker of severe oxidative stress, damage and disease-derived protein dysfunction (6, 23).

A significant increase in the plasma levels of MDA and LOOH were observed in patients with CML and its different phases (CML-CP, CML-AP) as compared with healthy subjects. Similarly, significantly higher plasma levels of PC was found in CML, CML-CP and CML-AP patients as compared to healthy controls. Elevated levels of lipid peroxidation products support

Table 2: Plasma levels of reduced glutathione and total total thiol in CML patients and healthy subjects (Mean ± SD)

		Healthy subjects (n=20)	CML (n=47)	CML-CP (n=31)	CML-AP (n=16)
Reduced	Mean±SD	1.25±0.54	0.79±0.30	0.92±0.27	0.53±0.10
Glutathione	Median	1.00	0.72	0.87	0.55
(µ mol/ml plasma)	Range	0.55 – 2.31	0.38 – 1.81	0.54 – 1.81	0.38 – 0.79
Total thiol	Mean±SD	431.67±83.97	307.19±88.57	347.64±71.76	288.83±61.89
(mM/ml plasma)	Median	419.91	323.57	340.69	230.39
	Range	314.47 – 601.25	130.25 – 692.05	256.53 – 692.05	130.25 – 348.25

Statistically significant (p<0.05) in comparison to CML, CML-CP, CML-AP patients with healthy subjects and also between each patients group.

the hypothesis that the cancer or malignant cells produce large numbers of ROS and that there exists a relationship between ROS activity and malignancy (24, 25). Present observations are in agreement with other reports on various human cancers including hematological malignancies (7, 10, 26-29). A significantly increased level of plasma protein carbonyl was observed in patients with Hodgkin's lymphoma, bladder cancer, lung cancer and in children with various malignancies (30-33).

Furthermore, a significant depletion of GSH and total T- SH in patients with CML and its different phases (CML-CP, CML-AP) was noticed as compared with healthy volunteers. Low levels of plasma reduced GSH and T-SH in patients with CML could be due to increased rate of ROS generation in the hematopoietic cells as compared to their normal counterparts.

Cellular antioxidants known as ROS scavengers protect cells against toxic free radicals (34). Reduced GSH is a principal constituent of thiol pool and vital intracellular scavengers of ROS (34). The most important biological function of GSH is to work as a non-enzymatic reducing agent to support in keeping cysteine thiol side in a reduced state on the surface of proteins (4, 34). GSH is involved in the synthesis and repair of DNA. GSH serves as a reductant mean antioxidant in oxidation reaction resulting oxidized GSH, thereby decreased GSH levels may reflect depletion of non-enzymatic antioxidant reserve (34). On the other hand, total T-SH play a prominent role in antioxidant defense system, and also in reactions of catalysis, regulation, electron transport and those preserving the correct structure of proteins (4).

Cellular antioxidants have been shown to limit carcinogenesis and counteract cell immortalization and transformation (35). Actions of different antioxidants show different patterns during neoplastic transformation and cancer or leukemic cells exhibit atypical activities of antioxidant enzymes as well as concentrations of endogenous antioxidants, when compared to their appropriate normal cell counterparts (36-38). Decreased level of total T-SH was reported in various pathophysiologies including cancers and can be used to evaluate excess ROS generation in both physiological and pathological conditions (21, 26, 39-43).

The decreased plasma levels of reduced GSH and total T-SH in the present study may be due to over production of ROS in hematopoietic cells. These non enzymatic antioxidants may be taken up by hematopoietic cells to bring down the oxidative stress.

It is well accepted that ROS play a critical role in tumor metastasis. As a signaling messenger, ROS are able to oxidize the critical target molecules such as protein kinase C (PKC) and protein tyrosine phosphates (PTPs), which are involved in tumor cell invasion. Mitogen activated protein kinase (MAPK) and p21 activated protein kinase (PAK) have been proposed to be regulated by ROS as well (44). Philadelphia chromosome produces a fusion protein, BCR-ABL in CML patients and the resultant protein causes a perturbation of stem cell function through unclear mechanisms involving increased tyrosine kinase activity (2). It was demonstrated that BCR-ABL fusion protein is associated with increased levels of ROS in hematopoietic cell lines compared with their non-transformed parental cell lines (45). High ROS with the increase in the activity of tyrosine kinase protein could play an important physiological role in signal transduction and induced proliferative pathway in the cell (45). A recent report state that BCR-ABL kinase stimulates ROS, which causes oxidative DNA damage, resulting in mutations in the kinase domain. This suggested that ROS may play a major role in resistance to given therapy which could contribute to progression of CML (46).

The proposed mechanism for elevated levels of lipid and protein oxidation products through mature and immature myeloid cells could be the major source of ROS which lead to oxidative stress in the cells, if antioxidant system is not influential. There may be a parallelism between numbers of immature myeloid cells and BCR-ABL fusion protein as disease progresses to vicious blast crisis via accelerated phase. BCR-ABL fusion protein has also been associated with genomic instability, progression of the disease, increased ROS production and additional cytogenetic alterations. These situations create an intracellular environment more favorable for macromolecule damage, mutations and disease progression. An elaborate study with a larger sample size is needed to ascertain the precise role of oxidative stress in pathophysilogy of CML.

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