JPP
Journal of
Pharmacy and Pharmacology

JPP 2007, 59: 409–417
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Received February 1, 2006
Accepted October 23, 2006
DOI 10.1211/jpp.59.3.0011
ISSN 0022-3573

Measurements of oxidative stress status and antioxidant activity in chronic leukaemia patients

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Abstract

There is an interactive relationship between leukaemia and oxidative stress. Leukaemic cells produce larger amounts of reactive oxygen species (ROS) than non-leukaemic cells as they are under a continual state of oxidative siege. So, this study was performed on 20 patients with chronic leukaemia from the Oncology Centre, Mansoura University. We measured leucocytic H_2O_2 concentrations and lipid peroxidation as serum malondialdehyde (MDA) concentration, serum total antioxidant activity, plasma ascorbic acid and dehydroascorbic acid concentrations, blood reduced glutathione concentration, haemolysate G6PD activity, blood catalase activity, serum superoxide dismutase (SOD) activity and serum anti-dsDNA concentration. We found that chronic leukaemia patients showed a significant increase ($P < 0.05$) in leucocytic H_2O_2, serum MDA concentration and total antioxidant activity either before or after treatment as compared with control group. Also, there was a significant increase in the other parameters (glutathione, catalase and SOD) either before or after treatment, but we found a significant decrease in ascorbic acid concentration and G6PD activity. There was a significant increase in anti-dsDNA concentration either before or after treatment. It can be concluded that leukaemic patients produce larger amounts of ROS than non-leukaemic patients. Also, the increase in antioxidant activity in leukaemic patients is not high enough to counteract the harmful effects of free radicals. This scenario becomes worse after administration of chemotherapy.

Introduction

Chronic leukaemia progresses slowly and permits the growth of a greater number of more developed cells. In general, these more mature cells can carry out some of their normal functions (Rozman & Montserrat 1995). It is classified into chronic lymphocytic leukaemia (CLL) and chronic myelogenous leukaemia (CML). CLL is characterized by accumulation of non-proliferative mature-appearing lymphocytes in blood, marrow, lymph nodes and spleen (O'Brien et al 1995). CML is a clonal stem-cell disorder characterized by proliferation of myelogenous elements at all stages of differentiation (Bennett 1994).

Reactive oxygen species (ROS) can initiate lipid peroxidation and DNA damage, leading to mutagenesis, carcinogenesis and cell death if the antioxidant system is impaired (Cross et al 1998). Carcinogenesis and mutagenesis by ROS could contribute to the initiation of cancer in addition to being important in the promotion and progression phase. There is evidence that hydroxyl radicals and singlet oxygens are formed in tumour cells and that they are the most powerful oxidizing radicals known to arise in biological systems (Gutowski et al 1998).

Superoxide anions (generated by polymorph nuclear leucocytes) are increased in leukaemic patients. The production of ROS in tumour cells by the action of superoxide anions may follow two pathways (Abou-Seif et al 2000). Firstly, the superoxide anion can react with Fe^{3+} to produce Fe^{2+}, which can catalyse a Fenton-type production of hydroxyl radicals from H_2O_2. This pathway can lead to vast metabolic consequences due to large changes in oxidation reduction potential of the cell. Reduction of H_2O_2 with Fe^{2+} in the presence of the superoxide anion can produce singlet oxygen. Secondly, increased load of superoxide anion can leak through leukaemic leucocytes and can either diffuse directly into the interior of red blood cells (RBCs) or generate an iron-mediated oxidation reduction flux, which can possibly

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Acknowledgement: Deep appreciation is for all the staff of the Oncology Centre, Mansoura University, especially Dr Maha Ebrahim Esmael, Lecturer of Medical Oncology.

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potentiate antioxidant defence in plasma and RBCs, thereby regulating lipid peroxidation based on the stage of the disease (Devi et al 2000).

Transmembrane changes in RBCs of leukaemic patients occur due to phospholipid assymetry. Membrane lipids are vital for the maintenance of cellular integrity and survival. Peroxidation of membrane lipids can result in changing the properties of RBC membrane cross-linking of membrane lipids and proteins, and cell death (Abou-Seif et al 2000).

Some recent studies (Mizutani et al 2005) reported oxidative DNA damage occurring due to H_2O_2 production by some anti-cancer drugs such as doxorubicin. Also, it is evident that attenuation in DNA repair capability would increase the risk of cancer. Indeed, some rare human diseases showing defects in genes for repair proteins are characterized by a high incidence of cancer. However, the extent of variation in DNA repair in the general population, resulting from genetic polymorphism, or from intrinsic or environmental modulation of gene expression or enzyme activity, is unknown, largely because of the lack of a simple DNA repair assay suitable for use in biomonitoring (Collins & Horvathova 2001).

Information on the activity of the antioxidant enzymes is conflicting and reports on antioxidant enzymes and lipid peroxidation are mainly limited to cell lines. Studies in leukaemia patients are scanty. Hence, our study was carried out to investigate the level of H_2O_2, as an indicator of the amount of free radical present in the body, and lipid peroxidation product (malondialdehyde), as a marker of the harmful effects of the free radicals that take place in different body tissues due to leukaemia. In addition, the antioxidant potential was measured in these patients to gain a complete view on the free radical/antioxidant system in leukaemic patients, and to monitor the possible changes that may occur in this system because of the disease. In addition, the effect of chemotherapy on the free radical/antioxidant system and the possibility of production of ROS through the action of anti-cancer drugs was investigated.

Materials and Methods

Patients

This study was carried out on 20 patients with chronic leukaemia. They were selected from the outpatient clinics of the Oncology Centre, Mansoura University (Mansoura, Egypt) in the period between November 2003 and February 2005.

Patients' consent was obtained according to the regulations of the Egyptian Ministry of Health. As a baseline study, all prospective patients were subjected to history taking (personal, menstrual, past and family history) and general and local examinations. Patients included in this study were classified into two groups. Group I consisted of 11 patients (3 female, 8 male) with CML, aged 15–56 years (mean ± s.e.: 43.36 ± 3.75 years). This group received daily doses of interferon-α 3–9 × 10^6 IU, hydroxyurea 40 mg kg^{-1} and busulfan 0.1 mg kg^{-1}. During treatment 2 patients (1 male, 1 female) died in this group. Group II consisted of 9 patients (3 female, 6 male) with CLL, aged 21–66 years (mean ± s.e.: 45.33 ± 5.72 years). This group received cyclophosphamide (500 mg m^{-2}, i.v., on the first day), vincristine (1.4 mg m^{-2}, i.v., on the first day) and prednisone (100 mg m^{-2}, i.v., on the first 5 days). This treatment was repeated every three weeks. During treatment 3 patients (2 female and 1 male) died in this group. The control group consisted of 10 (4 female, 6 male) apparently healthy subjects, aged 27–50 years (mean ± s.e.: 38.8 ± 2.78 years).

Sample collection

Fasting blood samples were collected from patients before administering any drug and after three months of treatment. The samples were divided into two portions, the first of which was collected into an EDTA-containing tube, and was used for estimation of glutathione concentration and catalase activity. Then blood was centrifuged at 3000 rev min^{-1} for 10 min. The clear plasma was investigated for ascorbic and dehydroascorbic acid concentrations. The second portion was collected into a clean dry tube, allowed to coagulate and then centrifuged at 3000 rev min^{-1} for 10 min. The clear non-haemolysed serum was investigated for malondialdehyde (MDA), total antioxidant activity, and superoxide dismutase (SOD) activity.

Biochemical analysis

Leucocytic hydrogen peroxide concentration was measured according to the method of Devi et al (2000). This method depends on the amount of hydrogen peroxide released from leucocytes and was estimated by the horseradish peroxidase (HRPO) method. The assay was based on the HRPO-mediated oxidation of phenol red by H_2O_2, which resulted in the formation of a compound that could be read at 610 nm.

Serum MDA was measured according to the method of Draper & Hadley (1990), in which serum proteins are precipitated by the addition of trichloroacetic acid. Then thiobarbituric acid reacts with MDA to form thiobarbituric acid-reactive substance (TBARS) that is measured at 532 nm. Serum total antioxidant activity was measured using the method of Koracevic et al (2001). A standardized solution of Fe–EDTA complex reacts with hydrogen peroxide by a Fenton type reaction, leading to the formation of hydroxyl radicals ($^{\bullet}$OH). These ROS degrade benzoate, resulting in the release of TBARS. Antioxidants from the added sample of human fluid cause suppression of the production of TBARS. This reaction can be measured spectrophotometrically and the inhibition of colour development is defined as the AOA.

Plasma ascorbic acid and dehydroascorbic acid concentrations were measured according to the method of Okamura (1980). For determination of ascorbic acid, the ferric ion is reduced in acidic solution by ascorbic acid to the ferrous ion, which is coupled with α,α'-dipyridyl to form a complex with a characterized absorption at 525 nm (A). The amount of both ascorbic acid and dehydroascorbic acid can be measured by reducing dehydroascorbic acid within 10 min to ascorbic acid by dithiothreitol (DTT) at room temperature and pH 6.5–8.0. After removing the excess DTT with *N*-ethylmaleimide (NEM), total ascorbic acids (reduced and original) are determined by the α,α'-dipyridyl method (B). Finally, the amount of dehydroascorbic acid can be calculated by subtracting A from B.

Blood reduced glutathione was determined by the method of Beutler et al (1963). It depends on the fact that virtually all of the non-protein sulfhydryl of red cells is in the form of reduced glutathione (GSH). 5,5′-Dithiobis (2-nitrobenzoic acid) (DTNB) is

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a disulfide compound that is readily reduced by sulfhydryl compounds, forming a highly coloured yellow anion. The optical density of this yellow substance is measured at 412 nm.

Haemolysate-glucose 6-phosphate dehydrogenase (G6PD) was measured according to the method of Roper et al (2002). It depends on the ability of G6PD to react with glucose 6-phosphate (G6P) to produce 6-phosphogluconate (6PG) and conversion of nicotine amide adenine dinucleotide phosphate (NADP) into $NADPH^+ + H^+$. The latter can be measured at 340 nm over the first 5 min of the reaction.

Blood catalase activity was determined by the method of Cohen et al (1970). In this method catalase reacts with a known excess of H_2O_2; the unreacted H_2O_2 reflected the catalase activity. It was determined with potassium permanganate using a rapid spectrophotometric determination. SOD activity in serum was determined using the method of DeChatelet et al (1974); the ability of the enzyme to inhibit the phenazine methosulfate-mediated reaction of nitroblue tetrazolium dye is measured as an indication of the activity of SOD.

Anti-dsDNA concentration in serum was determined by the method of Okamura et al (1993).

Statistical analysis

Statistical analysis was achieved by using the GraphPad InStat program version 3.05 (GraphPad Software Inc. (2000)). Analysis of variance test, Student's *t*-test and paired *t*-test were employed throughout this study.

Results

Oxidative stress

Chronic leukaemic patients (CML and CLL), either before or after treatment, showed a significant increase in leucocytic hydrogen peroxide and serum MDA concentration ($P < 0.05$) when compared with control group (Table 1). Treatment caused no significant difference in leukaemia patients regarding the hydrogen peroxide concentration of leucocytes, but a significant increase in serum MDA concentration as compared with the control subjects or their counterpart groups before treatment ($P < 0.05$).

Antioxidant activity

Serum total antioxidant activity and plasma ascorbic acid concentration (mean ± s.e.) of chronic leukaemic patients (before and after treatment) and control group are shown in Table 2. CML and CLL patients, either before or after treatment, showed a significant increase in serum total antioxidant activity ($P < 0.05$) when compared with the control group. Leukaemic patients after treatment showed no significant difference in serum total antioxidant activity when compared with their counterparts before treatment.

Table 1 Leucocyte hydrogen peroxide (H_2O_2) and serum malondialdehyde (MDA) concentrations in chronic leukaemic patients (before and after treatment) and control group

Group	Leucocyte H_2O_2 concn (μM)				Serum MDA concn (nmol mL^{-1} h^{-1})			
Control (n = 10)	51.5 ± 4.95				157.66 ± 13.79			
Leukaemia patients	Before treatment		After treatment		Before treatment		After treatment	
	n	mean ± s.e.	n	mean ± s.e.	n	mean ± s.e.	n	mean ± s.e.
	CML 11	75.09 ± 7.01[a]	9	86 ± 9.33[a]	11	401.26 ± 40.07[a]	9	541.38 ± 30.5[ab]
	CLL 9	79.78 ± 7.18[a]	6	96 ± 9.78[a]	9	368.91 ± 40.12[a]	6	498.09 ± 56.77[ac]

CML, chronic myelogenous leukaemia; CLL, chronic lymphocytic leukaemia.
[a]$P < 0.05$, compared with control group; [b]$P < 0.05$, compared with CML before treatment; [c]$P < 0.05$, compared with CLL before treatment

Table 2 Serum total antioxidant (TAO) activity and plasma ascorbic acid concentration of chronic leukaemic patients (before and after treatment) and control group

Group	Serum TAO activity (mM)				Plasma ascorbic acid concn (mg dL^{-1})			
Control (n = 10)	2.57 ± 0.25				9.54 ± 0.64			
Leukaemia patients	Before treatment		After treatment		Before treatment		After treatment	
	n	mean ± s.e.	n	mean ± s.e.	n	mean ± s.e.	n	mean ± s.e.
	CML 11	4.02 ± 0.41[a]	9	4.04 ± 0.30[a]	11	6.51 ± 0.65[a]	9	5.30 ± 0.46[ab]
	CLL 9	3.73 ± 0.45[a]	6	4.38 ± 0.43[a]	9	5.93 ± 0.45[a]	6	4.89 ± 0.36[ac]

CML, chronic myelogenous leukaemia; CLL, chronic lymphocytic leukaemia.
[a]$P < 0.05$, compared with control group; [b]$P < 0.05$, compared with CML before treatment; [c]$P < 0.05$, compared with CLL before treatment

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Table 3 Plasma dehydroascorbic acid concentration and ascorbic/dehydroascorbic acid ratio of chronic leukaemic patients (before and after treatment) and control group

Group	Plasma dehydroascorbic acid level (mg dL^{-1})				Plasma ascorbic/dehydroascorbic acid ratio			
Control (n = 10)	0.55 ± 0.66				18.46 ± 1.21			
Leukaemia patients	Before treatment		After treatment		Before treatment		After treatment	
	n	mean ± s.e.	n	mean ± s.e.	n	mean ± s.e.	n	mean ± s.e.
	CML 11	2.41 ± 0.23[a]	9	2.73 ± 0.27[a]	11	3.07 ± 0.52[a]	9	2.21 ± 0.39[ab]
	CLL 9	2.48 ± 0.23[a]	6	2.93 ± 0.11[a]	9	2.61 ± 0.41[a]	6	1.68 ± 0.12[ac]

CML, chronic myelogenous leukaemia; CLL, chronic lymphocytic leukaemia.
[a]$P < 0.05$, compared with control group; [b]$P < 0.05$, compared with CML before treatment; [c]$P < 0.05$, compared with CLL before treatment

Table 4 Blood reduced glutathione concentration (GSH) and haemolysate glucose-6-phosphate dehydrogenase (G6PD) activity of chronic leukaemic patients (before and after treatment) and control group

Group	Blood GSH concn (mM)				Haemolysate G6PD activity (mol mL^{-1})			
Control (n = 10)	5.39 ± 0.41				9.13 ± 0.58			
Leukaemia patients	Before treatment		After treatment		Before treatment		After treatment	
	n	mean ± s.e.	n	mean ± s.e.	n	mean ± s.e.	n	mean ± s.e.
	CML 11	9.06 ± 0.89[a]	9	8.85 ± 0.85[a]	11	5.65 ± 0.43[a]	9	7.01 ± 0.48[ab]
	CLL 9	8.22 ± 0.61[a]	6	8.61 ± 0.75[a]	9	6.51 ± 0.46[a]	6	8.03 ± 0.31[c]

CML, chronic myelogenous leukaemia; CLL, chronic lymphocytic leukaemia.
[a]$P < 0.05$, compared with control group; [b]$P < 0.05$, compared with CML before treatment; [c]$P < 0.05$, compared with CLL before treatment

Chronic leukaemic patients (CML and CLL), either before or after treatment, showed a significant decrease ($P<0.05$) in plasma ascorbic acid concentration as compared with the control group. Also, the leukaemic groups after treatment showed a significant decrease ($P<0.05$) in ascorbic acid concentration in plasma when compared the same groups before treatment.

We found a significant increase in plasma dehydroascorbic acid concentration and a significant decrease in the plasma ascorbic/dehydroascorbic acid ratio of CML and CLL patients, either before or after treatment, as compared with the control group ($P < 0.05$) (Table 3). Treatment caused no significant difference in the plasma dehydroascorbic acid concentration of leukaemic patients and a significant decrease ($P < 0.05$) in the ascorbic/dehydroascorbic acid ratio as compared with the same group of patients before treatment.

CML and CLL patients, either before or after treatment, showed a significant increase in blood reduced glutathione concentration when compared with the control group ($P < 0.05$). No significant difference in blood reduced glutathione level was observed in leukaemic patients after treatment (Table 4). Also, before treatment, chronic leukaemic patients showed a significant decrease ($P < 0.05$) in haemolysate G6PD activity when compared with the control group. After treatment, CML patients showed a significant decrease in haemolysate G6PD activity as compared with the control group ($P < 0.05$). Also, all groups of chronic leukaemia after treatment showed a significant increase ($P < 0.05$) in G6PD activity in haemolysate when compared with their counterpart groups before treatment (Table 4).

As illustrated in Table 5, there was a significant increase in blood catalase and serum SOD activity ($P<0.05$) in chronic leukaemic patients (CML and CLL), either before or after treatment, as compared with control group. After treatment, groups of chronic leukaemic patients showed a significant increase ($P<0.05$) in both parameters as compared with patients before treatment.

Measurement of serum anti-dsDNA concentration

The serum anti-dsDNA concentration (mean ± s.e.) of leukaemic patients (before and after treatment) and the control group is shown in Table 6. There was a significant increase ($P < 0.05$) in serum anti-dsDNA concentration of chronic leukaemic patients, either before or after treatment, as compared with the control group. After treatment, leukaemic patients showed no significant difference in serum anti-dsDNA concentration as compared with their counterpart groups before treatment.

Discussion

In recent years, evidence has developed that supports a key role for free radicals in modifying cellular reactions, and

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Table 5 Blood catalase and serum SOD activity of chronic leukaemic patients (before and after treatment) and control group

Group	Blood catalase activity (mg mL^{-1})				Serum SOD activity (%)			
Control (n = 10)	18.87 ± 1.68				25.12 ± 2.04			
Leukaemia patients	Before treatment		After treatment		Before treatment		After treatment	
	n	mean ± s.e.	n	mean ± s.e.	n	mean ± s.e.	n	mean ± s.e.
	CML 11	58.47 ± 5.61[a]	9	81.57 ± 4.81[ab]	11	51.24 ± 4.91[a]	9	61.86 ± 5.32[ab]
	CLL 9	62.56 ± 5.87[a]	6	88.12 ± 6.83[ac]	9	46.92 ± 4.71[a]	6	63.05 ± 6.04[ac]

CML, chronic myelogenous leukaemia; CLL, chronic lymphocytic leukaemia.
[a]$P < 0.05$, compared with control group; [b]$P < 0.05$, compared with CML before treatment; [c]$P < 0.05$, compared with CLL before treatment

suggests that oxidative stress might be important in the pathophysiology of common diseases including many types of cancer (Young & Woodside 2001).

Cancer cells are under intrinsic increased oxidative stress and are vulnerable to free radical-induced apoptosis. There is also evidence that cancer cells can produce larger amounts of oxygen-derived species than non-neoplastic cells and that the antioxidant potential of cancer cells is suppressed (Senturker et al 1997). The metabolism of ROS in cancer cells is a research area that has not been intensively pursued. It is important to identify whether cancer cells may produce larger amounts of ROS than non-neoplastic cells or whether the antioxidant system of cancer cells is suppressed. There is evidence favouring both mechanisms. Large amounts of hydrogen peroxide are reportedly produced in-vitro without exogenous stimulation in several human carcinoma cell lines (Toyokuni et al 1995). Hydrogen peroxide freely passes through membranes and can reach any cellular compartment.

In this work, the H_2O_2 content of leucocytes increased in all groups of leukaemic patients as compared with control group. These results agree with those of Toyokuni et al (1995) and Devi et al (2000).

The increase in leucocyte H_2O_2 content is an indicator of oxidative stress in leukaemic patients, as the origin of the disease is located in leucocytes. These higher concentrations of H_2O_2 are assumed to accumulate in leucocytes rather than in the serum (Young & Woodside 2001). In addition, leucocytes can generate $^{\bullet}OH$, an extremely potent radical (Aruoma et al 1991) that is non-ionized and capable of diffusion through hydrophobic membranes. Therefore, H_2O_2 formed in one location might translocate into another before decomposing to yield highly reactive hydroxyl radical (Halliwell & Gutteridge 1990). Finally, H_2O_2 is weak oxidizing agent and might directly damage proteins and enzymes by oxidizing their reactive thiol groups (Young & Woodside 2001). Leukaemic lymphocytes are unlikely to generate reactive oxygen intermediates (ROIs) at an unusually high rate, given that the oxidative stress observed is of predominantly mitochondrial origin and that, being quiescent, they do not have large numbers of respiring mitochondria. Furthermore, leukaemic cells are known to express high levels of glutathione peroxidase, the predominant H_2O_2-decomposing enzyme in mammalian cells (Jacobson 1996). The most likely explanation as to why the abundant intracellular GSH peroxidase of leukaemic cells cannot adequately protect them from endogenous oxidative stress is that the function of the enzyme is in some way impaired.

Recent studies indicate that ROS, such as H_2O_2, generated by anti-cancer drugs, can damage cells and then induce apoptotic cell death. Studies on leukaemic cells indicate that H_2O_2 causes cell death via the iron-dependent instability of the lysosome (Shim et al 2003). Some anti-cancer drugs, such as doxorubicin and methotrexate, also work by increasing H_2O_2 production and cause DNA damage (Miketova et al 2005; Mizutani et al 2005).

An increased serum level of TBARS, which represents a maker of lipid peroxidation, was detected in all leukaemic patients in this study. This was consistent with the findings of Kumerova et al (1998) (in CLL and CML patients), Oltra et al (2001) and Bakan et al (2003) (in CLL patients) and Ghosh et al (2005) (in all leukaemia patients).

Serum MDA concentration was increased in all groups of treated leukaemic patients compared with patients before treatment and the control group. On the other hand, the study of Kazanova et al (1997) discovered no significant difference in serum MDA concentration between acute lymphoblastic leukaemia (ALL) patients before or after treatment.

The increase in serum MDA level after treatment may be possibly due to the production of oxygen-derived radicals by most anti-cancer drugs during their action. The mechanism of generation of these reactive oxygen metabolites is thought to be through an interaction of the drug with oxyhaemoglobin (Azizova & Roitman 1997).

Table 6 Serum anti-dsDNA concentration in leukaemic patients (before and after treatment) and control group

Group	Serum anti-dsDNA concentration (IU mL^{-1})			
Control (n = 10)	13.9 ± 0.43			
Leukaemia patients	Before treatment		After treatment	
	n	mean ± s.e.	n	mean ± s.e.
	CML 11	70.27 ± 6.88[a]	9	67.11 ± 6.61[a]
	CLL 9	54.44 ± 3.96[a]	6	60 ± 5.73[a]

CML, chronic myelogenous leukaemia; CLL, chronic lymphocytic leukaemia.
[a]$P < 0.05$, compared with control group; [b]$P < 0.05$, compared with CML before treatment; [c]$P < 0.05$, compared with CLL before treatment.

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In this study, the serum total antioxidant activity in leukaemic patients, either before or after treatment, was higher than that of control subjects and there was no significant difference between patients before and after treatment. None of the previous studies has ever measured serum total antioxidant activity in leukaemic patients either before or after treatment; some studies had only measured individual antioxidant parameters in serum or in tissue culture.

The total antioxidant activity is not a single sum of the activity of the various antioxidant substances. It is a dynamic equilibrium that is influenced by the interactions between all serum antioxidative constituents. This cooperation of antioxidants in human serum provides a greater protection against attack by free radicals than any antioxidant alone.

In this study, there was a significant decrease in plasma ascorbic acid concentration of leukaemic patients as compared with the control group. In a study by Nakagawa (2000), there was a significant decrease in the serum ascorbate level of ALL patients while the CSF ascorbate level was twice that in serum but still lower than normal.

In addition, there was a significant increase in the plasma dehydroascorbic acid level as compared with control subjects. As a result of diminished ascorbic acid level and elevated dehydroascorbic acid concentration, a decreased ascorbic/dehydroascorbic acid ratio was observed in all patients with leukaemia in this study.

There was a significant decrease in plasma ascorbic acid level in patients after treatment as compared with either the control subjects or the same patients before treatment.

Also, a significant increase in plasma level of dehydroascorbic acid was observed in patients after treatment when compared with control group, while no significant difference was detected between patients either before or after treatment.

The proposed mechanisms of vitamin C activity in the prevention and treatment of cancer include prevention of cellular free radical damage (Dumitrescu et al 1993), enhancement of the immune system by increasing lymphocyte production (Carr & Frei 1999), stimulation of collagen formation necessary for walling off tumours (Head 1998), inhibition of hyaluronidase, keeping the ground substance around the tumour intact and preventing metastasis (Head 1998), chemoprotection against mutagenic compounds as nitrosamines (Hecht 1997), inhibition of oncogenic viruses (Head 1998), expedition of wound healing after cancer surgery (Head 1998), enhancement of the effect of certain chemotherapy drugs, such as tamoxifen, cisplatin, adriamycin and others (Kurbacher et al 1996), and neutralization of carcinogenic substances (Aidoo et al 1994).

In this study, there was a significant increase in blood reduced glutathione (GSH) level in leukaemic patients as compared with the control group. This result was also reported by Oltra et al (2001) (in CLL patients). On the other hand, a study by Arruda et al (1996) demonstrated no significant difference between leukaemic patients and control group. Also, Bakan et al (2003) reported a significant decrease in blood reduced glutathione concentration in CLL patients when compared with control group.

Ascorbic acid and GSH are among the most active reducing substances in living tissues (Winkler et al 1994). Both of these chemicals undergo redox cycling in-vivo and there seems to be a significant interrelationship in this cycling. For example, the toxic effects due to GSH deficiency can be prevented by administering ascorbate (Meister 1994). Conversely, GSH seems to be required for the regeneration of ascorbic acid from its oxidized form, dehydroascorbate (Wang & Ballatori 1998).

The cell is protected against oxygen free-radical-induced DNA damage by the continuous sequestration of metal ions (Fe^{2+}/Cu^{2+}). GSH has three antioxidant mechanisms of action: first, as a scavenger of free radicals such as $O_2^{\bullet -}$, $^{\bullet}OH$ and lipid hydroperoxides; second, as a substrate for glutathione peroxidase; and third, in the direct repair of oxidative DNA lesions (Dreher & Junod 1996).

After treatment, patients showed a significant increase in blood GSH level, as compared with the control group, but there was no significant difference between patients either before or after treatment regarding their blood GSH level.

In our study, the haemolysate G6PD activity in leukaemic patients before treatment was significantly lower than that of the control group. This agreed with the findings of Lin et al (1997) in CML patients and Au et al (2002) in CML patients. On the other hand, the studies of Kumerova et al (1995) in CML patients and Kumerova et al (1998) in CML and CLL patients demonstrated an elevated level of G6PD in leukaemic patients before treatment.

After treatment, the haemolysate G6PD activity in leukaemic patients was still lower than that of the control group, but significantly higher than that of patients before treatment. No previous studies have ever measured haemolysate G6PD activity after treatment in leukaemic patients.

The catalase enzyme may also be released into the extracellular environment in which it has the potential to function as a potent antioxidant, and thereby regulate cell survival (Sandstrom & Buttke 1993). In light of these considerations, it seems plausible that extracellular catalase might function as an important autocrine antioxidant and survival factor (Moran et al 2002).

There was a significant increase in blood catalase activity of leukaemic patients as compared with the control group. Hileman et al (2004) found an elevation of catalase activity in tissues of leukaemia patients, which may be attributed to the oxidative stress of H_2O_2 formed by SOD. Scavenging systems such as glutathione, catalase and glutathione peroxidase correlate with intracellular ROS levels (Maeda et al 2004). This can be explained by the fact that intrinsic oxidative stress in cancer cells was associated with the upregulation of SOD and catalase protein expression, likely as a mechanism to tolerate increased ROS stress (Hileman et al 2004).

However, studies of Oltra et al (2001) in CLL patients demonstrated a significant decrease in lymphocyte content of catalase. Possible causes of this discrepancy could be the fact that the greater activity of catalase is present in erythrocytes, while these studies measured it in lymphocytes instead (Young & Woodside 2001) and catalase is released by leukaemic cells into the extracellular environment, leading to a reduced catalase level inside lymphocytes (Sandstrom & Buttke 1993).

Leukaemic patients, after treatment, showed a significant increase in blood catalase activity as compared with the control group and the same group of patients before treatment. The increase in catalase level after treatment may result

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from the chemotherapy. Hydroxyurea, *N*-acetylcysteine and 2-methoxyestradiol (2ME) cause DNA damage and this damage was almost entirely inhibited by increasing catalase production (Hileman et al 2004).

Superoxide dismutases are a family of metaloenzymes that play a key role in defending the cell against oxygen free radical toxicity by catalysing the dismutation of toxic $O_2^{\bullet -}$ to O_2 and H_2O_2. There was a significant increase in serum SOD activity in leukaemia patients as compared with the control group. This was in line with the findings of Devi et al (2000) in CML patients.

$O_2^{\bullet -}$ produced by tumour cells is able to cross the erythrocyte membrane, travelling through the anion channels, enhancing erythrocyte SOD activity, which specifically detoxifies $O_2^{\bullet -}$. So, in this study, serum SOD activity in leukaemic patients showed a significant positive correlation with serum MDA level, as $O_2^{\bullet -}$ destruction will cause SOD activation and increase MDA formed from membrane destruction.

After treatment, leukaemic patients showed a significant increase in serum SOD activity as compared with control subjects or patients before treatment. This increase in serum SOD activity could be explained by the increase in free radical production induced by chemotherapy. This increase in free radicals triggers a cascade of events that leads to increased antioxidants, such as SOD. SOD counteracts free radicals' mutagenic and carcinogenic effects (Kim et al 2003).

Crucial properties of carcinogens are their capacity to cause permanent structural changes in DNA as base-pair mutations, deletions, insertions, rearrangements, and sequence amplification, to activate cytoplasmic and nuclear signal transduction pathways, and to modulate the activity of stress proteins and stress genes that regulate effector genes related to growth, differentiation and cell death. Oxy-radicals possess all these properties (Covacci et al 2001).

Damage to DNA by oxygen free radicals is frequently postulated to cause mutations that are associated with the initiation and progression of human cancers. Upon reaction with DNA, oxygen radicals produce more than 30 different adducts and this number excludes protein and lipid addition products as well as inter and intra strand cross-links. Thus, there are potentially hundreds of different types of chemical changes in DNA resulting from oxygen free radicals that could be mutagenic lesions involved in the aetiology of cancer (Feig et al 1994).

Human cancer cells have been shown to produce excess amounts of H_2O_2. Since H_2O_2 itself is not reactive, it may pass through cellular membranes and reach any cellular compartment, including the nucleus and DNA. This may result in greater DNA damage in tumour cells than in normal cells by production of $^{\bullet}OH$ from reactions of H_2O_2 with DNA-bound metal ions. Higher levels of oxidative DNA base damage as observed in leukaemic patients and earlier in other cancers support the hypothesis that free radical reactions may be increased in malignant cells, concurrently with decreased levels of antioxidant enzymes (Senturker et al 1997).

One of the permanent structural changes in DNA is a single-strand break. As a result, this study tried to find a possible cause that might lead to this break. So, serum autoantibodies to native double-strand deoxyribonucleic acid (anti-dsDNA) were determined by measuring IgG antibodies directed against double-stranded DNA. The use of IgG antibody, rather than IgM, was due to its higher affinity. It is directed against the phosphate units of DNA (Egner 2000).

No previous study has measured the serum concentration of anti-dsDNA as a cause of double-strand break in leukaemia patients. In our study, there was a significant increase in serum anti-dsDNA concentration in leukaemic patients, either before or after treatment, as compared with the control group. Also, there was no significant difference in serum anti-dsDNA in leukaemic patients after treatment. This indicated that anti-dsDNA is a marker in leukaemia patients and does not depend on the stage of the disease.

Conclusion

In this study, the best results among the antioxidants were observed in blood catalase and serum SOD activity as they increase in response to oxidative stress and exhibit higher response after treatment in response to both ROS and chemotherapy. In addition, leucocyte H_2O_2, serum MDA level and antioxidant enzymatic and non-enzymatic activity were almost the same in CLL and CML patients. As a result, these changes are not specific for the type of leukaemia but may be dependent on the stage of the disease. Also, anti-dsDNA may be one of the mechanisms that cause DNA damage and double-strand breaks in leukaemic patients, which happened independently of the course of treatment in these patients.

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