Original Article

SUPEROXIDE DISMUTASE LEVELS IN LEUKEMIAS

K. N. Pujari, S.P. Jadkar

ABSTRACT:

Superoxide dismutase is antioxidant enzyme responsible for the quenching of superoxide radicals which are released during the chemical reactions of the various metabolic pathways. It is a non-heme protein. Different iso-enzymes of superoxide dismutase are described. The mitochondrial enzyme is manganese dependent, cytoplasmic enzyme is copper-zinc dependent. These enzymes are evenly and ubiquitously distributed among aerobic organisms and tissues but are found in appreciable quantities only inside the cell. Superoxide dismutase work in conjugation with two enzymes that remove H_2O_2 in human cells: Catalase, and glutathione peroxidase.

The enzyme levels of superoxide dismutase are altered to considerable extent in various diseased states exhibiting either elevation or depletion in their activity, this phenomenon was found to be more evident in leukemias. We have determined the superoxide dismutase activity in the red blood cells of leukemic patients and these levels were significantly low as compared to control.

In respect to sex, the mean superoxide dismutase levels in males (3.268 ± 0.305) were found to more than that in females (3.165 ± 0.337) however, we found significant difference only in patients with chronic myeloid leukemia. Whereas in respect to age, the mean superoxide dismutase levels were found to elevated (in < 10 years value was 2.845 ± 0.266 whereas in age >51 years value was 3.442 ± 0.247) indicating the extent of the free radicals might have stimulated the production of the antioxidant. We observed highly significant ($p<0.001$) superoxide dismutase levels in acute leukemias with respect to age.

Our results suggest that oxidative stress in leukemia patients causes the deficiency in antioxidant enzyme superoxide dismutase, which arise as a result of enormous production of reactive oxygen species in the system.

Key words: Superoxide dismutase, Acute leukemia, Chronic leukemia.

INTRODUCTION:

Superoxide dismutase (SOD) is dimeric antioxidant enzyme responsible for the quenching of superoxide radicals which are released during the chemical reactions of the various metabolic pathways. Different iso-enzymes of SOD are described. The mitochondrial enzyme is manganese dependent, cytoplasmic enzyme is copper-zinc dependent. It is a non-heme protein. The gene coding SOD is on chromosome 21. A defect in SOD gene is seen in some patients with amyotrophic lacteral sclerosis. These enzymes are evenly & ubiquitously distributed among aerobic organisms and tissues but are found in appreciable quantities only inside the cell. That is extracellular fluids contain only traces of activity. Superoxide dismutase work in conjugation with two enzymes that remove H_2O_2 in human cells: catalase, and glutathione peroxidase (1).

There are several ways by which free radicals are formed. The most important free radicals in biological systems are radical derivatives of oxygen; these are known as reactive oxygen species (ROS). ROS, such as hydrogen peroxide, superoxide and hydroxyl radical are the products of oxygen metabolism in all aerobic organisms. ROS are generated as a result of energy production from mitochondria as part of an antimicrobial or antiviral response as well as detoxification reaction carried out by the cytochrome p-450 system. Environmental agents such as ultraviolet light, ionizing radiation, redox chemical and cigarette smoke also generate ROS (2).

Once formed, these free radicals initiate their own reactions thereby exerting potentially harmful effects on various systems of the body. Normally these ROS are converted to less reactive compounds by the use of antioxidant. In normal cell, there are appropriate pro-oxidants (free radicals): antioxidant balance. However, this balance can be

shifted toward the pro-oxidants when production of oxygen species is increased greatly (e.g. following ingestion of certain chemicals or drugs) or when levels of antioxidants are diminished (e.g. by inactivation of enzyme involved in disposal of oxygen species and by conditions that cause low levels of antioxidants). This is called as "oxidative stress" and can result in serious cell damage if the stress is massive or prolonged (3).

Oxidative stress is examined by most researchers by assessing various stress markers in blood and urine such as byproducts of lipid peroxidation, protein and DNA oxidation products, and changes in status of antioxidant compounds like glutathione, vitamin C, E and antioxidant enzyme activities (4). However, the enzymatic levels of SOD are altered to considerable extent in various diseases exhibiting either elevation or depletion in activity (5).

In this study we measured SOD activity in erythrocytes of patients with leukemia and compared with healthy individuals.

MATERIALS AND METHODS:

Present study was carried out in the Department of Biochemistry, Government Medical College, Miraj and Department of Medical Oncology, Shri Siddhivinayak Ganpati Cancer Hospital, Miraj, Maharashtra (India).

Study protocol was approved by ethical committee of Government Medical College, Miraj.

Sample Size: Study cases: The study group includes a total 193 subjects. This includes patients as well as control.

Patients: Total 133 patients with confirmed diagnosis of leukemia were selected for this study. The patients in the study were those who referred to Department of Medical Oncology, Shri Siddhivinayak Ganapati Cancer Hospital, Miraj.

Patients were grouped according to type of leukemia as;

1) AML (Acute myeloid leukemia) : 36 patients

2) ALL (Acute lymphocytic leukemia): 37 patients

3) CML (Chronic myeloid leukemia): 30 patients and

4) CLL (Chronic lymphocytic leukemia): 30 patients.

Control:

The 60 healthy control were taken in all age groups with both genders (compared to leukemia patients) attending the OPD of the Shri Siddhivinayak Ganapati Cancer Hospital and Govt Medical College and Hospital, Miraj during the same period.

Control having history of smoking, alcoholism and other diseases which induce oxidative stress such as Diabetes Mellitus, Pulmonary diseases, Respiratory diseases, Liver diseases etc are not such concurrent or past history of diseases were excluded from the study.

Collection of blood samples:

Informed consent was obtained from the participants. 1ml venous blood collected in bulb having anticoagulant (heparin) from the subjects under aseptic condition by venipuncture using 2 ml sterile disposable syringe and needle. Blood samples from heparin bulb were centrifuged and plasma was removed. Erythrocytes were washed with normal saline for three times and used for estimation of Superoxide dismutase. Enzyme activity was estimated by method described by Marklund and Marklund, modified by Nandi et al (6). The data were evaluated statistically.

RESULTS:

Mean SOD levels are increased in males (3.268 ± 0.305) as compared to females (3.165 ± 0.337) (Table No-1). We found significant decrease in SOD levels in male patients with CML as compared to female ($p<0.05$). Whereas in respect to age, the mean SOD levels are found to be elevated (in < 10 years value was 2.845 ± 0.266, whereas in age >51 years value is 3.442 ± 0.247) we observed highly significant ($p<0.001$) SOD levels in acute leukemias with respect to age (Table No-2).

Table no 3 shows Correlation between age and SOD levels in patients with leukemia, we found significant positive correlation (0.01) in all leukemic patients except CLL.

DISCUSSION:

The overall mean erythrocyte SOD activity in leukemic patients is significantly low as compared to

normal control (table no-1), the reduction being more prominent in ALL. Similar findings of depletion in antioxidant- superoxide dismutase level has been also reported earlier in other cancers and leukemia (7-13) indicating the role of antioxidants in the prevention of cancer. Senturker et al (14) studied the antioxidant enzyme levels and oxidative damage in lymphocytes of children with acute lymphoblastic leukemia (ALL) and also in disease free children and they observed a higher level of DNA base lesions and reduced antioxidant levels in former group as compared to the latter. This might indicate a possible link between decreased activities of antioxidant enzymes and increased levels of base lesion due to damaging effect of free radical reactions released during oxidative stress leading to the carcinogenesis process. An increased concentration of thiobarbituric acid reacting substances (TBARS) are found in spite of antioxidant defense activation suggesting that cellular peroxidation reactions might be occurring during the carcinogenesis process (15).

Auclair et al have shown significant SOD activity deficiency in patients with Hodgkin's disease and myeloma (16). They concluded that this erythrocyte SOD deficiency with intravascular hemolysis was induced by nitrogen mustard therapy. In their study, most patients with malignant lymphoma and acute myeloid leukemia were treated with those anticancer drugs, so the erythrocyte SOD may be influenced by them.

Deficiency of the enzyme activity of superoxide metabolic pathway will result in a net increase in the level of the superoxide free radical so that the erythrocyte will therefore always be susceptible to damage. So far, the mechanism of the cause of deficiencies of erythrocyte SOD is uncommon (17).

In general, activities of antioxidant enzymes in tumor cells have been found to be lower than in normal cells. In fact, in tumor cells, MnSOD activity is always low. Catalase activity is almost always low and Cu,Zn-SOD activity is usually low. On the other hand, levels of GPx are found to be highly variable in some tumor cell lines and higher in others. Decreased levels of GPx, SOD and catalase may cause the accumulation of O_2^- and H_2O_2 in tumor cells (14).

The significant sex difference in SOD levels are observed in CML and CLL patients, males shows higher level of enzyme activity as compared to females (table no. 1). This might explain the significant sex difference found in SOD activity in present study in case of chronic leukemias (CML and CLL), whereas in AML and ALL there is no significant sex difference in SOD activity as compared to control indicating failure of antioxidant mechanism to counteract the extensive oxidative damage resulting in leukemia which progresses aggressively in short time. In case of chronic leukemias, SOD levels are higher than that of acute leukemia which supports our previous contention of defective antioxidant mechanism and fast progression of disease in case of acute leukemia. Our results are similar with the study done by Poongothai et al (18).

Table no. 2 shows the variation of SOD levels in the leukemia patients with different age groups. In age group less than 10 SOD level is low as compared to other age group (that is greater than 11), as the age increases SOD activity goes on increasing in ALL and CML. Similarly, we observed increase in results with increase in age in AML and CLL with exception in age group 31 to 40. Poongothai et al (18) showed the relation between age and SOD activity in patients with leukemia, they are observed similar results. Kesavulu et al (19) showed the excessive production of free radicals in the organism and the imbalance between the concentration of these and the antioxidant defenses was related to the processes such as aging and the development of several diseases such as cancer.

Table no 3 shows Correlation between age and SOD levels in patients with leukemia, we found significant positive correlation (0.01) in all leukemic patients except CLL.

Our results suggest that oxidative stress in leukemia patients causes the deficiency in antioxidant enzyme SOD, which arise as a result of enormous production of ROS in the system. These findings may also indicate a possible link between decreased antioxidants and increased levels of cells alterations due to oxidative damage, supporting the idea that there is a persistent oxidative stress in leukemia.

		AML	ALL	CML	CLL	Pooled	Control
Males	**N**	19	27	21	14	81	30
	MEAN	3.116	3.142	3.390	3.335	3.268	5.626
	SD	0.327	0.258	0.277	0.289	0.305	0.324
Females	**N**	17	10	9	16	52	30
	MEAN	3.005	3.120	3.077	3.186	3.165	5.263
	SD	0.383	0.293	0.438	0.050	0.337	0.625
Total	**N**	36	37	30	30	133	60
	MEAN	3.051	3.154	3.293	3.256	3.228	5.445
	SD	0.335	0.275	0.360	0.211	0.321	0.894
TEST STATISTICS Student – t test		**p>0.05**	**p>0.05**	**p<0.05**	**p>0.05**	**p>0.05**	**p<0.05**

Table No 1: SOD levels in males and females with leukemias

	AML			ALL			CML			CLL			Pooled		
AGE	N	MEAN	SD	N	MEAN	SD	N	MEAN	SD	N	MEAN	SD	N	MEAN	SD
<10	4	2.425	0.095	16	2.937	0.196	0	-	-	-	-	-	20	2.845	0.266
11-20	10	2.840	0.195	9	3.200	0.173	1	2.600	-	-	-	-	20	3.090	0.258
21-30	5	3.100	0.187	6	3.266	0.103	5	2.940	0.194	1	3.200	-.	17	3.114	0.203
31-40	4	2.975	0.095	1	3.400	-	4	3.100	0.408	3	3.133	0.057	12	3.166	0.269
41-50	4	3.400	0.182	1	3.600	-	6	3.316	0.075	4	3.200	0.081	15	3.330	0.221
>51	9	3.400	0.200	4	3.575	0.095	14	3.514	0.228	22	3.277	0.242	49	3.442	0.247
TOTAL	36	3.047	0.362	37	3.154	0.274	30	3.293	0.337	30	3.250	0.214	133	3.245	0.329
TEST STATISTICS		**F = 5.132** P = 0.001**		**F = 21.362** P = 0.001**			**F = 1.74 P = 1.75NS**			**F = 0.326 P = 0.981 NS**			**F = 2.219 ** P = 0.001**		

Table No. 2: SOD levels in leukemic patients with respect the different age groups

		AML	ALL	CML	CLL	Pooled
	Pearson Correlation	0.809**	0.771**	0.763**	0.212	0.610**
	Sig. (2-tailed)	0.01	0.01	0.01	0.260	0.01
	N	36	37	30	30	133

Table No. 3: Correlation between age and SOD levels in patients with leukemia

** Correlation is significant at the 0.01 level (2-tailed).

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