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QUANTITATIVE VARIATION OF VITAMIN E LEVELS IN LEUKEMIAS

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ABSTRACT

Vitamin E is the major lipid-soluble antioxidant in the cell antioxidant defence system and is exclusively obtained from the diet. The major biologic role of vitamin E is to protect PUFAs and other components of cell membranes and low-density lipoprotein (LDL) from oxidation by free radicals. Vitamin E is located primarily within the phospholipid bilayer of cell membranes. It is particularly effective in preventing lipid peroxidation, a series of chemical reactions involving the oxidative deterioration of PUFAs. Elevated levels of lipid peroxidation products are associated with numerous diseases and clinical conditions. Vitamin E also may block the formation of nitrosamines, which are carcinogens formed in the stomach from nitrites consumed in the diet. It also may protect against the development of cancers by enhancing immune function.

We have estimated the vitamin E levels in the serum of the leukemic patients. The overall mean vitamin E levels in the leukemic patients were significantly low (3.41 ± 0.33) as compared to that of the normal control (7.5 ± 2.32). In respect to sex, the mean vitamin E levels in males (3.44 ± 0.35) were found to slightly higher than that in females (3.32 ± 0.29) however, we found significant difference only in patients with myeloid leukemias. Whereas in respect to age, we found lowest mean vitamin E levels in the age group 41 – 50 and above 51 years (3.24 ± 0.46 and 3.28 ± 0.34). We found highly significant ($p<0.001$) trend in vitamin E levels with respect to age in all leukemic patients (AML, ALL, CML, and CLL). Our results suggest the deficiency in antioxidant vitamin E in leukemic patients.

KEYWORDS

Leukemia, AML, ALL, CML, Vitamin E.

INTRODUCTION

Vitamin E is the major lipid-soluble antioxidant in the cell antioxidant defence system and is exclusively obtained from the diet. The term "vitamin E" refers to a family of eight naturally occurring homologues that are synthesized by plants from homogentisic acid. All are derivatives of 6-chromanol and differ in the number and position of methyl groups on the ring structure. The four tocopherol homologues have a saturated 16-carbon phytyl side chain, whereas the tocotrienols homologues have three double bonds on the side chain. There is also a widely available synthetic form, dl-α-tocopherol, prepared by coupling trimethylhydroquinone with isophytol. This consists of a mixture of eight stereoisomers in approximately equal amounts; these isomers are differentiated by rotations of the phytyl chain in various directions that do not occur naturally [1, 2].

Vitamin E is an example of a phenolic antioxidant. Such molecules readily donate the hydrogen from the hydroxyl (-OH) group on the ring structure to free radicals, which then become unreactive. On donating the hydrogen, the phenolic compound itself becomes a relatively unreactive free radical because the unpaired electron on the oxygen atom is usually delocalized into the aromatic ring structure thereby increasing its stability [3].

The major biologic role of vitamin E is to protect PUFAs and other components of cell membranes

and low-density lipoprotein (LDL) from oxidation by free radicals. Vitamin E is located primarily within the phospholipid bilayer of cell membranes. It is particularly effective in preventing lipid peroxidation, a series of chemical reactions involving the oxidative deterioration of PUFAs. Elevated levels of lipid peroxidation products are associated with numerous diseases and clinical conditions [4]. Although vitamin E is primarily located in cell and organelle membranes where it can exert its maximum protective effect, its concentration may only be one molecule for every 2000 phospholipid molecules. This suggests that after its reaction with free radicals it is rapidly regenerated, possibly by other antioxidants [5].

Vitamin E is believed to help protect cell membranes against the damaging effects of free radicals, which may contribute to the development of chronic diseases such as cancer [6]. Vitamin E also may block the formation of nitrosamines, which are carcinogens formed in the stomach from nitrites consumed in the diet. It also may protect against the development of cancers by enhancing immune function [7]. In the present study we have examined the serum vitamin E levels in patients with leukemia.

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 191 subjects. This includes patients as well as control.

Patients: The patients in the study were those who referred to Department of Medical Oncology, Shri Siddhivinayak Ganapati Cancer Hospital, Miraj. The clinical presentation varied, however, the symptoms of lassitude, anorexia, acute or recurrent infections and abnormal bleeding were common. Gross pallor, lymph node enlargement, patechiae, easy bruishing and splenomegaly were observed in examination. Routine hematological examination, among other things, aspiration and biopsy confirmed the diagnosis of one or the other type of leukemia in all cases. Further cytogenetic studies were performed in 131 patients and the diagnosis and type of leukemia was confirmed. Phildelphia chromosome was tested in 30 CML cases and is found to be positive in all patients. Age group of present study was 5 years to 65 years.

Patients were grouped according to type of leukemia as

1. AML (Acute myeloid leukemia) : 36 patients
2. ALL (Acute lymphoblastic leukemia): 37 patients
3. CML (Chronic myeloid leukemia): 28 patients and
4. CLL (Chronic lymphoblastic leukemia): 30 patients.

Control:

Sixty healthy control were taken in all age groups and both genders (compared to leukemia patients) Government Medical College and Hospital, Miraj during the same period. The patients and healthy controls having history of smoking, alcoholism and other diseases which alters serum vitamin E concentration such as cancers etc no such concurrent or past history of diseases were excluded from the study.

Collection of blood samples:

Informed consent was obtained from the participants. Blood samples were collected from 131 patients with confirmed diagnosis of leukemia, attending Medical Oncology Department of Shri Siddhivinayak Ganpati Cancer Hospital. The patient population is representative of the general population in terms of socioeconomic condition and ethnic diversity. In addition blood samples were also obtained from 60 healthy age and sex matched randomly selected individuals from general population to serve as controls.

However the variations in vitamin E level of leukemic patients with respect to type of leukemia were analysed. The clinical findings (Diagnosis, complete blood picture, type of disease) were recorded with the help of medical oncologist. In addition blood samples were also obtained from 60 healthy age and sex matched randomly selected individuals.

Vitamin E levels were estimated by method described by Baker and Frank [8], and levels were expressed as mg/L. The data were evaluated

statistically by using student't' and 'F' test, 'F' value was calculated by Minitab and SPSS software.

RESULTS

We investigated the levels of vitamin E in plasma of leukemic patients and healthy control and are given in **Table 1** and level of it in all leukemic patients (AML, ALL, CML and CLL) are found to be significantly decreased ($p<0.001$) as compared to the control. Lowest decrease is found in ALL patients (**Table 1**)

Table 2 shows the vitamin E levels in males and females with leukemias. The significant sex difference in serum vitamin E levels are observed only in myeloid leukemias (AML and CML).

Table 3 shows the variation in serum vitamin E levels in leukemic patients with respect the different age groups. In the age group 41 – 50 and above 51 years, we found lowest mean vitamin E levels in pooled patients (3.24 ± 0.46 and 3.28 ± 0.34). We found highly significant ($p<0.001$) trend in vitamin E levels with respect to age in all leukemic patients (AML, ALL, CML, and CLL).

Table 1: Serum vitamin E levels in control and leukemic patients

Subjects	N	Vitamin E (mg/L)
Control	60	7.5 ± 2.32
Leukemia	131	3.41 ± 0.33*
AML	36	3.28 ± 0.39*
ALL	37	3.10 ± 0.22*
CML	28	3.62 ± 0.25*
CLL	30	3.14 ± 0.16*

The values are Mean ± SD
* $p<0.001$(highly significant)

Table 2: Vitamin E (mg/L) levels in males and females with leukemias

		AML	ALL	CML	CLL	Pooled	Control
Males	**N**	18	27	19	14	81	30
	MEAN	3.16	3.56	3.72	3.15	3.44	7.54
	SD	0.40	0.25	0.12	0.16	0.35	2.86
Females	**N**	18	10	9	16	50	30
	MEAN	3.44	3.41	3.45	3.06	3.32	7.11
	SD	0.32	0.03	0.35	0.14	0.29	2.32
TEST STATISTICS Student – t test		**-2.319** **0.027***	**1.876** **0.069NS**	**3.056** **0.005****	**1.644** **0.111 NS**	**2.031** **0.044***	**0.64** **0.525 NS**

NS= not significant, * significant and ** highly significant

Table 3: Variation in the serum vitamin E (mg/L) levels in leukemic patients with respect the different age groups

	AML			ALL			CML			CLL			Pooled		
AGE	N	MEAN	SD	N	MEAN	SD	N	MEAN	SD	N	MEAN	SD	N	MEAN	SD
<10	4	3.57	0.35	16	3.53	0.18	0	-	-	-	-	-	20	3.54	0.21
11-20	10	3.44	0.36	9	3.52	0.19	1	3.20	-	-	-	-	20	3.43	0.25
21-30	5	3.30	0.30	6	3.50	0.24	5	3.74	0.30	1	3.40	.	17	3.50	0.30
31-40	4	3.30	0.35	1	4.10		5	3.57	0.25	3	3.23	0.20	13	3.44	0.35
41-50	4	2.97	0.68	1	3.40		2	3.45	0.35	4	3.12	0.15	11	3.24	0.46
>51	9	3.25	0.37	4	3.40	0.37	15	3.65	0.20	22	3.06	0.12	50	3.28	0.34
TOTAL	36	3.30	0.38	37	3.52	0.23	28	3.63	0.24	30	3.10	0.15	131	3.39	0.33
TEST STATISTICS		F = 69.50 P<0.001**			F=24.66 P<0.001**			F=201.81 P<0.001**			F=389.84 P<0.001**			F=271.13 P<0.001**	

**** Highly significant**

DISCUSSION

Vitamin E is a powerful antioxidant and the primary defence against potentially harmful oxidations that cause disease and aging, protecting unsaturated lipids from peroxidation. The role of vitamin E in protecting the erythrocyte membrane from oxidative stress is presently the major documented role of vitamin E in human physiology.

In present study serum vitamin E levels were decreased in leukemic patients than controls. The proposed mechanism for low vitamin E level is that there may be comparable levels of lipid peroxidation in untreated leukemia and control.

Increased lipid peroxidation process is caused an enhanced free radical formation together with a higher supply of substrate and by an insufficient defence by antioxidants as well. According to Krajcovicova et al [9] the deficiency in two antioxidants i.e. vitamin C and vitamin E for lipid peroxidation inhibition means the insufficient defence against free radicals and the increased lipid peroxidation.

Malnutrition had been previously identified as an adverse prognostic factor in patients with solid tumors [10, 11]. Dewys et al have shown shortened survival of low weight patients with different types of malignant diseases that included non-lymphoblastic leukemias[12]. In patients with cancer, the antineoplastic treatment turns malnutrition into an adverse prognostic factor because of increased haemopoietic alterations[11]. It has been shown that undernourished individuals have impaired haemopoietic and immune functions [13]. Diminished growth of haemopoietic colonies has been shown in animals with protein-calorie malnutrition[14]. In humans, under-nourishment leads to mucopolysaccharide deposition in the bone marrow, anemia, and decreased number of myeloid precursors and granulocytic reserve [15, 16].

Knekt et al was reported vitamin E as anticancer, effects as a lipid antioxidant which prevents lipid peroxidation and act as free radical scavenger. Free radicals by interacting with lipids, proteins and DNA may increase membrane permeability, inhibit catonic pumps, deplets ATP and break DNA strands leading to chain of events causing mutagenecity, cytotoxicity and changes in gene expression finally initiating and propagating carcinogenesis [17].

Vitamin E is a chain breaking, free radical trapping antioxidant in cell membranes and plasma lipoproteins. It reacts with the lipid peroxide radicals formed by peroxidation of polyunsaturated fatty acids before they can establish a chain reaction. The tocopheroxyl free radical product is relatively unreactive and ultimately forms nonradical compounds. Commonly, the

tocopheroxyl radical is reduced back to tocopherol by reaction requiring vitamin C from plasma or serum. The resultant monodehydroascorbate free radical then undergoes enzymatic or nonenzymatic reaction to yield ascorbate and dehdroascorbate, neither of which is a free radical [18]. The tendency of leukemic cells to proliferate is restrained by highly viscous intracellular glycosaminoglycans and to overcome the above situation cells release enzyme hyaluronidase. For the synthesis of hyaluronidase vitamin C may be utilized and hence total vitamin C level is decreased. The total volume of white blood cells is heavily increased in leukemias as compared to normal subjects; hence demand of ascorbic acid or vitamin C is increased [19, 20]. The available vitamin C in plasma or serum may be consumed by these white blood cells; hence vitamin C level in plasma is decreased [20]. Low levels of Vitamin C may results in accumulation of tocopheroxyl radical which is not regenerated back to tocopherol. Thus vitamin E levels may be decreased in patients with leukemia.

The significant sex difference in vitamin E levels are observed only in AML and CML patients (**Table 2**). Earlier, it was observed that total antioxidant status (TAS) was lower in females than in males; however, there was significant decrease in TAS levels with age in male but not in female indicating the genetic difference found in management of oxidative status [21]. This might explain the significant sex difference found in vitamin E levels in present study in the case of AML and CML. Both types of lymphoblastic leukemias (ALL and CLL) have exhibited reduced vitamin E levels, though sex difference was not apparent. This indicates failure of antioxidant mechanism to counteract the extensive oxidative damage resulting in leukemia.

Table 3 shows the variation of vitamin E levels in the leukemic patients with different age groups. In the age group between 41- 50 years and above 50 years there is a sudden decrease in the mean vitamin E levels of pooled patients. Whereas in the age less than 10 years there is sudden rise in vitamin E levels. Various studies have reported the process of aging associated with the degree of the antioxidant activity. Casado et al [22] reported the antioxidant enzyme were increased in the diseases of the aged individuals such as cardiovascular diseases, myomas, chronic obstructive pulmonary disease and acute cerebral accident, but the antioxidant enzyme SOD levels were seen to be decreasing with the process of aging. Whereas in another report[23] has been shown that the excessive production of free radicals in the organism and the imbalance between the concentrations of these and the antioxidant defenses was related to the processes such as aging and the development of several diseases such as cancer.

In our previous study the levels of oxidative stress markers Malonyldialdehyde (MDA), SOD, catalase and vitamin C in patients with leukemia and we found increased MDA and decreased SOD and catalase levels [24, 25, and 26]. In this way Vitamin E is utilized by proliferating malignant cells and also neutralizing oxidative stress markers and resulting in decrease levels of it.

CONCLUSION

Our results suggest that oxidative stress in leukemia patients causes the deficiency in antioxidant vitamin E, 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.

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