A Comparative Study of Antioxidant Status in Carcinoma Breast Patients and Controls

Deshmukh Pratibha R[a], Garkal K D[b], Shete Anjali N[c]

[a] Assistant Professor and [c] Associate professor, Department of Physiology, Government Medical College, Aurangabad, Maharashtra, India
[b] Associate Professor in Biophysics and Registrar, MUHS, Nashik, Maharashtra, India.]

Corresponding author: Deshmukh Pratibha R

Abstract

As cancers are now ubiquitous, an attempt was made to evaluate the role of antioxidant status in breast cancer. For that purpose, 40 carcinoma breast cases and 40 age matched controls were evaluated for plasma ascorbic acid (vitamin C) levels as antioxidant status. The observations revealed that the mean plasma ascorbic acid levels in cases was (0.3313±0.104) mg/dl while in controls it was (1.117±0.336) mg/dl. The decrease was statistically highly significant (P<0.0001). The low levels of ascorbic acid indicate that in carcinoma breast the antioxidant system is insufficient to counter the increased oxidative stress.

KEYWORDS: Ascorbic acid, antioxidant status, oxidative stress

Introduction:

Breast cancer is the commonest cause of death in middle aged women in the western world. The incidence is increasing in India also. The etiological factors are, increasing age, genetic abnormality (short arm of chromosome 17), diet rich in saturated fatty acids and low in vitamin C, hormonal causes, sedentary lifestyle, etc. Of these, oxidative stress and low levels of vitamin C play a very important role in the etiology of breast cancer.

Free radicals are highly reactive species containing unpaired electron. If two radicals react, both are eliminated. But if a radical reacts with a non-radical, another free radical is produced. E.g. radiation causes splitting of water and $H^{\cdot}$ and $OH^{\cdot}$ radicals are produced. The $OH^{\cdot}$ radical propagates the chain reaction. It attacks DNA and imperfect repair of DNA damage causes oncogene activation and carcinogenesis. The hydroxyl radical can attack fatty acid side chains of membrane phospholipids (lipid peroxidation). The membrane structure is damaged and the free radicals or reactive oxygen species are released in the circulation and their blood levels increase. This phenomenon is called as oxidative stress.

The free radicals are normally also generated in small amounts and are useful sometimes like in phagocytosis but if their levels increase, it is damaging to the body.

Our body normally has a defense against the free radicals in the form of vitamin C, vitamin E, β- carotene and there are enzymes like superoxide dismutase, catalase and glutathione peroxidase. These are called as antioxidants. They are substances which when present in low concentration significantly delay or inhibit oxidation of a substrate. Antioxidants could act against oxidative stress by

1) Decreasing localized oxygen concentration e.g. combining with oxygen and displacing it
2) Preventing initiation of peroxidation by scavenging species capable of extracting hydrogen atoms like $OH^{\cdot}$
3) Scavenging singlet oxygen, which can react with membrane lipids to produce peroxides
4) Binding metal ions in forms that will not generate reactive species
5) Removing peroxides by converting them to non-radical products such as alcohol e.g. glutathione peroxidase
6) Chain breaking, so preventing continued hydrogen extraction from fatty acid side chains

Antioxidants in mechanisms 1 to 4 are called preventive antioxidants. Antioxidants acting by mechanism number 4 are not consumed during the reaction. Type 5 are also preventive antioxidants but may or may not be consumed during the reactions depending on their chemical behavior e.g. glutathione peroxidase acts by this mechanism and being an enzyme acts as a catalyst and is not consumed. Chain breaking antioxidants act by combining with chain propagating radicals and are consumed during the reaction and also antioxidants acting by scavenging singlet oxygen. Many antioxidants have more than one mechanism of action. Rapid repair of oxidative damage to DNA or to protein may be equally or more important than damage to lipids as a mechanism for mediating against oxidative stress.

In severe oxidative stress, antioxidant defense is insufficient and leads to cell injury and cell death.

Dr. William McCormick was the first to recognize that the generalized stromal changes of scurvy are identical with the local stromal changes observed in the immediate vicinity of invading neoplastic cells and the evidence that cancer patients are invariably ascorbate depleted supported his view later. In this study, to assess the antioxidant status in carcinoma breast cases, plasma ascorbate levels were assessed.

Methods:

The study was carried out in the Department of Physiology, Government Medical College, Aurangabad. 40 females having carcinoma breast stage IV, aging between 40-50 years were studied along with 40 age matched controls. Both groups were having a mixed diet and Diabetes mellitus and hypertension were excluded from cases as well as controls. All the cases and controls were studied for assay of plasma Ascorbic Acid levels (vitamin C) by Ayekyaw (1978) method.

Fasting blood is used. A test tube containing 2 ml plasma and another 'blank' (2ml distilled water) is taken and 2 ml colour reagent (acid phosphotungstate) is added to both tubes. The solution is mixed and allowed to stand for 30 minutes at room temperature and then centrifuged at 3000 rpm for 10 minutes. The resultant clear blue supernatant is taken in the cuvette and absorbance is read at 700 nm against blank.

1 mg of L- ascorbic acid is dissolved in 100 ml 0.5% oxalic acid and used as a standard solution.

The ascorbate levels in plasma are found as:

$$\frac{\text{O.D. of test}}{\text{O.D. of standard}} \times \text{Concentration of standard (mg/dl)} = \text{mg of ascorbic acid/dl}$$

Normal Range: 0.7-1.7 mg/dl

Results:

The plasma ascorbic acid levels showed a highly significant decrease in cases as compared to control group.

Refer table on page 4

Plasma Ascorbic Acid levels in mg/dl

	Mean	S.D.	Mean± S.D.	'T' Value	'P' Value
Controls	1.1170	0.336	1.1170±0.336	14.13	<0.0001
cases	0.3313	0.104	0.3313±0.104		

Discussion:

To assess the status of antioxidants in carcinoma breast, we have measured plasma vitamin C levels and found that there was a highly significant decrease in vitamin C levels in cases as compared to controls. The reasons may be, consumption of vitamin C by growing cancer tissue as it is a requirement for growth and consumption of vitamin C as an antioxidant in breaking the free radical chain reactions[1]. When acting as an antioxidant, tocopherols (vitamin E) transfer a phenolic hydrogen to a peroxyl free radical of a peroxidized PUFA. The resultant phenoxy free radical can react with vitamin C to regenerate tocopherol. Thus vitamin C spares vitamin E and is necessary for the action of vitamin E[1]. Also, vitamin E must be present in biological membrane for vitamin C to protect it from peroxidation[2]. Also, many factors involved in host resistance against cancer are significantly dependant on availability of ascorbate[3]. Similar studies were carried out by other scientists and the results are comparable with our study[4,5]. Kumara guruparan et al[6] showed significantly depressed levels of ascorbic acid in patients of adenocarcinoma of breast. From many other studies it is shown that high dietary intake of vitamin C can protect against cancers[7,8,9,10,11,12]. The reverse is also true[4]. Antioxidants can be given along with chemotherapy to protect from cytotoxic effects of chemotherapeutic agents[13,14]. Also, upgrading the overall antioxidant status of the patient

rather than providing a single antioxidant may be helpful in preventing as well as treating cancers. Synergistic interactions of all antioxidants are essential for preventing cancer as it is a multistage multifactorial process[15,16].

Of course, this is a preliminary study and more work should be done in his field e.g. assess tissue levels of oxidants/ antioxidants in tumor itself and to bring to light new preventive and therapeutic methods for cancers.

References:

1) Murray R K, D K Grammer, P A Mayes, V W Rodwell Harper's Biochemistry 25th Edi. 1988. Appleton and Lange publications.
2) Sies H Oxidative stress: from basic research to clinical application. The Am J Med 1991; 91(3c)
3) Cameron E, L Pauling and B Leibovitz, Ascorbic Acid and Cancer: A Review, Cancer Research 1979; 39 (3): 663-81
4) Byers T and Perry G, Dietary carotenes, vitamin C and vitamin E as protective antioxidants in human cancers, Ann. Rev. Nutrition 1992; 12: 139-59.
5) Halliwell B, Reaction oxygen species in living systems: source, biochemistry, and role in human disease. The Am J Med 1991 sep 91(3c)
6) Kumaraguruparan R, Antioxidant profile in the circulation of patients with fibroadenoma and adenocarcimona of the breast. Clin Biochem 2002 Jun; 35(4): 275-9
7) Carr AC, Frie B, Toward a new recommended dietary allowance for vitamin C based on antioxidant and health effects in humans. American Journal of clinical Nutrition 1999;69 (6): 1086-1107.
8) Fairfield K M, Fletcher RH, Vitamins for chronic disease prevention in adults. JAMA 2002 Oct 1/10:41-51
9) Grobstein C, Cancer Research 1983 Jun; 43: 3018-23.
10) Hinds MW, Laurence C, Jean Hankin, James Lee, Dietary vitamin A, Carotene, vitamin C and risk of lung cancer in Hawaii, Am J Epidemiol 1984 Feb 119/2: 227-36
11) Maxwell SRJ, Prospects for the use of antioxidant therapies, Drugs 1995; 49(3):345-61.
12) Troll W and R Weisner, The role of oxygen radicals as a possible mechanism of tumor promotion, Ann Rev Pharmacol Toxicol. 1985; 25: 509-28
13) Prasad K N, Kumar A, Kochupillai V, Cole W C, High doses of multiple antioxidant vitamins: Essential ingredients in improving the efficacy of standard cancer therapy. J Am Col Nutr 1999; 18(1): 13-25
14) Rifici V A, Khachadurian A K, Dietary supplementation with vitamins C and E inhibits in vitro oxidation of lipoproteins, Journal of Am Col Nutr 1993; 12(6): 631-7
15) Gey K F, Prospects for the prevention of free radical disease, regarding cancer and cardiovascular disease. British Medical Bulletin 1993; 49(3): 679-99
16) Lasheras C, Huerta J M, Gonzalez S, Brana A F, Patterson A M, Fernandez S, Independent and interactive association of blood antioxidants and oxidative damage in elderly people. Free Radic Res 2002; 36(8): 875-82