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Effect of Antioxidants on Level of Catalase and Glutathione in Oral Cancer Patients

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Abstract:
The present study reveals certain aspects of free radicals and antioxidants in the oral cancer patients. The production of free radicals is of course through tobacco chewing, smoking and other similar practices in the population. The body tries its best to come back the effect created by free radicals and in certain cases to be the genesis of free radicals, inhibited by the antioxidants. This study will add to the knowledge about origin and spread of oral cancer and its possible therapy by antioxidant, which may help the human population tremendously. Catalase (CAT) is present in all major body organs, being especially concentrated in the erythrocytes at the subcellular level. CAT is found mostly in peroxisomes (80%) and also in cytosol(20%). The gene coding human CAT is found on chromosomes. Catalase enzymes are responsible for the breakdown of hydrogen peroxide to water and oxygen. Glutathione is found in vivo as GSH, rather than GSSG, but up to 1/3rdof the total cellular glutathione may be present as mixed disulphides with other compounds that contains-SHgroup, such as cysteine, coenzyme A and the –SH of cysteine residues of several proteins. Glutathioneperoxidase catalyses the oxidations of GSH to GSSG at the expense of hydrogen peroxide.

Keywords: *Oral cancer, Catalase, glutathione peroxidase*

1. Introduction

Catalase is one of the most toughly investigated mammalian enzymes .It catalyzes the decomposition of hydrogen peroxide to water with the liberation of oxygen .

$2H_2O_2 \rightarrow 2H_2O + O_2\uparrow$

The enzyme was first crystallized from beef liver by Summner*etal. (1940).* It has since been crystallized from liver and erythrocytes of a number of animal species including humans.

Catalase is able to catalyse the decomposition of hydrogen peroxide by two separate type of reaction, depending upon the conditions. Both reactionsbegin with the formation of primary complex between hydrogen peroxide and the iron of the hemi prosthetic group (Chance, 1949).

$$\mathbf{Enz\text{-}Fe^{3+} - OH + H_2O_2 \rightarrow Enz - Fe^{3+} - OOH + H_2O}$$

This is called primary complex reacts with a second molecule of hydrogen peroxide for its catalytic destruction as follows:

$$Enz - Fe^{3+} - OOH + H_2O_2 \rightarrow Enz - Fe^{3+} - OH + H_2O + O_2\downarrow$$

Catalase a maker enzyme of peroxisomes reacts either catalytically or peroxidetically depending on the micro environment of cell. It plays a protective role against oxygen toxicity by degradation of hydrogen peroxide produced in several metabolic reactions. Hydrogen peroxide is a normal product of metabolism in the cell (Chanc,1949). It is produced by all organisms. It can be formed by a number of reactions occurring in living organisms and is formed by oxidases, superoxide dismutase and auto oxidation reactions. It is readily permitted by biological membrane and is believed to diffuse from one cell compartment to another such as interstitial fluid and in the circulation. If not eliminated, it may react with the superoxide radical ($O^{-\bullet}$) in the presence of Fe to produce the highly reactive hydroxyl radical.

$H_2O_2 + O_2^{-\bullet} \rightarrow O_2 + OH^- + {}^{\bullet}OH$

The hydroxyl radical potentially causes a variety of deleterious changes in biological system (Fong *et al*.,1976; Frindovich,1976 and halliwell,1981). To remove effectively both H_2O_2 and O_2^- a system must consisting of SOD, catalase and peroxidase.

Glutathione peroxidase is enzymes whose role is safely decomposed peroxides. It is mainly located in peroxisomes and acts upon hydrogen peroxide.

$2H_2O_2 \rightarrow$ Catalase $\rightarrow$ $2H_2O + O_2$

ROOH + 2GSH $\rightarrow$ GSH peroxide $\rightarrow$ ROH + GSSG

Followingcomposition of antioxidants (Enzymatic and nonenzymatic) was given to the patients in the form of capsule (1 capsule daily after meal for Premalignant cases and 2 capsule daily after malignant cases). This was a complete nutritional supplement with seven antioxidants and seven water soluble vitamins.

Water soluble vitamins : Vit.B_1 10 mg, Vit.B_2 10 mg, Vit. B_3 3 mg, Vit. B_{12}15 mcg , Folic acid 1 mg , Capanthothenate 12 mg, Niacinamide 50 mg.

Antioxidants:Vit. E 25 mg, Vit. A 5000 Iu, Vit.C 150 mg, Zn 15 mg, Copper 1.5 mg, Manganese 3 mg, Selenium 100 mcg, Chromium picolinate 200 mcg.

2. Material and Methods

A complete working proforma with routine investigation was followed. The subject taken for experiments were grouped as under:

- I.Control Group: It comprises of healthy normal volunteers of either sex , preferably between 22 to 40 years of age, including staffmembers and their families, residing at last three year.
- II. Study Group:The study group is comprises of various oral cancer patients. *i.e*. with Premalignant lesions and malignant lesions at J.K. Cancer Institute, Kanpur.
  All the control and study group patients were subjected to the following biochemical Estimations in blood/serum.
- Catalase (Hydrogen peroxide: Hydrogen peroxide oxidoreductase, EC 1.11.1.6) Estimation : The activity of Catalase in haemolysate was assayed by the method of Aeby and Berk(1998). The assay system in a final volume of 3.063 ml ,consisted of 3 ml of 50mM phosphate buffer pH6.8) 0.038 ml of 0.97 M H_2O_2 (Final concentration 12.36 m mole assay system) and 0.025 ml of diluted haemolysate. The contents were mixed carefully and the change in OD was recorded at 15 sec interval, for 2 min at 240 nm. Enzyme activity was expressed as µ moles of H_2O_2. O. D. recorded on systronic spectrophotometer.
- Glutathione Peroxidase (NADPH : Oxidized glutathione oxidoreductase, E.C. 1.11.1.9) Estimation: Using the Rensel reagent kit manufactured by Randox Laboratories Ltd. On autoanalysor RA-50, the method followed was of Paglion and Valentine (1967).
- Sample Preparation : Diluted o.05 ml hemarinized, whole blood with 1ml diluting agent and incubated it, for 5mins and then added 1ml of double strength Drabkin's reagent. Mixed well and assayed in the normal manner. Samples were assayed within 20mins of adding the Drabkin's reagent.
- Procedure:Mixed 0.02 ml diluted sample, 1.0 ml reagent (glutathione 4 m mole/1, GR 0.5U/1 and 0.28 m mole/1NADPH) and 0.04 ml of 0.18 m mole /1 cumenehydroperoxide. The content were mixed carefully and read initial absorbance of sample and reagent blank, after one min and again after 1 and 2 at 340 nm, the values were expressed as U/g Hb.

3. Results and Discussion

Catalase is present in all major body organs, being especially concentrated in liver and erythrocytes at the subcellular level. CAT is found mostly in peroxisomes (80%) and cytosol (20%). The gene encoding human CAT is found on chromosome 11p 13. Catalase enzymes causes the breakdown of peroxide to water and hydrogen.

$H_2O_2 \rightarrow 2H_2O + O_2$

It is tetrahemin enzyme studied by Kirkman*etal.* (1987) have reveals that each tetrameric molecule of human and bovine catalase has four molecules of tightly bound NADPH in CNS H_2O_2, a known cytotoxin, is proposed during amine metabolism. Catalase reduces H_2O_2 and thus serves a

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protective role. A protective role for catalase in GIT is the removal of H2O2, which can induce damage of tissue constituting by oxidizing enzyme of membrane sulphydryl groups or by initiating lipid peroxidation. It has been suggested that catalase prevents ascorbate somatic mutation(Rosin *et al.*,1980). Lipid peroxidation (Koster and Slee, 1983), free radical induced aldehydes formation (Sinha and Patterson, 1983) and DNA damage was causes by H2O2 (Chilou, 1983). In many system, CAT and SOD work together to eliminate the toxic precursor of free radicles(Frindovinch, 1981).

In this study we observed that the catalase level (Table 1.) in healthy persons (769.3072µ molesH_2O_2 reduced/mg protein) was higher than that was in premalignant persons (321.3310µ moles H_2O_2 reduced/mg protein).The significance of the difference between the two average levels of catalase has been tested by t- test. The calculated value of t was found to be highly significant at 0.1% level of significance. This indicates that the average catalase level (µ mole H_2O_2 reduced/mg protein) in premalignant persons was in general significantly less than that in healthy persons.

It was also observed that the average catalase level (Table 2.) of premalignant persons (after antioxidants therapy) was 633.1519 less than that in healthy persons, but higher than that in same group without antioxidant therapy. This indicates that the average level of

catalase in premalignant persons becomes higher than that of the persons without antioxidant therapy. In this way it is concluded that the antioxidants therapy increases the average level of catalase.

It was also noticed that the catalase level (Table 3.) in healthy person (769.3072µ moles H_2O_2reduced/mg protein) was higher than that in malignant person (277.2894µ moleH_2O_2reduced/mg protein). The significance of the difference between the two average levels of catalase has been tested by t-test. The calculated value of t was found to be highly significant at 0.1% level of significance. This indicates that the catalase level in malignant persons is in general less than in healthy persons.

Parameters	Control Group	Study Group
Size	10	20
Mean	769.3072	321.33.10
S.D.	0.4165	0.1514

Table 1: The level of Catalase (µ moles H_2O_2 reduced/mg protein) in Premalignant person.
*Diff. of Means =447.9762, S.E. of Diff. = 0.134,t-value = 4331.4828***, D.F. = 28 ***Significant at 0.1% level of significance.*

3.2. Level of Catalase and Glutathione Peroxidase in Oral Cancer Patients

Parameters	Control Group	Study Group
Size	10	20
Mean	769.3072	633.1519
S.D.	0.4165	18.7374

Table 2: The level of Catalase (µ moles H_2O_2 reduced/mg protein) in Premalignant person after Antioxidant therapy.
*Diff. of Mean = 136.1553, S.E. of Diff. = 5.9780, t-value = 22.7760***, D.F. = 28***Significant at 0.1% level of significance.*

Parameters	Control Group	Study Group
Size	10	20
Mean	769.3072	277.2894
S.D.	0.4165	0.5952

Table 3: The level of Catalase (µ moles H_2O_2reduced /mg protein) in Malignant persons.
*Diff. of Mean = 492.0178, S.E. of Diff. =0.2108, t-value =2334.4358***, D.F. = 28***Significant at 0.1% level of significance.*

Parameters	Control Group	Study Group
Size	10	20
Mean	769.3072	367.4960
S.D.	0.4165	3.5845

Table 4: The level of Catalase (µ mole H_2O_2 reduced/mg protein) in Malignant persons after Antioxidant therapy.
*Diff. of Mean = 401.8112, S.E. =1.1472, t-value = 350.2442***, D.F. =28*** Significant at 0.1% level of significance.*

In our study, we found that the average catalase (µ mole H_2O_2 reduced/mg protein) level in malignant persons was less than that in healthy persons. Average catalase level (Table4)of malignant persons (after antioxidant therapy) was 367.4960 which was less than in healthy persons but higher than that in same group without antioxidant therapy. This indicates that the average level of catalase in malignant persons becomes higher than that of persons without antioxidant therapy. In this way it is concluded that the antioxidant therapy increases the average level of catalase.

It was found that the glutathione peroxidase level (Table 5) in healthy person (30.7730 U/g Hb) . The significance of the difference between in the two average level of glutathione peroxidase has been tested by t-test. The calculated value of t was found to be highly significant at 0.1% level of significance. This indicate that the glutathione peroxide level in premalignant persons was in general significantly less than that in healthy persons.

It was also observed that the average Glutathione peroxidase level (Table 6) of premalignant persons (after antioxidant therapy) was 24.4306 is less than that in healthy persons, but higher than that in same group without antioxidant therapy. This indicates that the average level of glutathione peroxidase in premalignant persons, becomes higher than that of the persons

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Without antioxidant therapy. In this way it is concluded that the antioxidant therapy increases the average level of Glutathione peroxidase.

In this study we found that the Glutathione peroxidase level (Table7) in healthy persons (30.7730U/gHb) was higher than that in malignant persons (14.4060 U/g Hb). The significance of the difference between the two average level of Glutathione peroxidase has been tested by t-test. The calculated value of t was found to be highly significant at 0.1% level of significance. This indicates that the Glutathione peroxidase level in malignant persons is in general less than that in healthy persons.

Parameters	Control Group	Study Group
Size	10	20
Mean	30.77730	18.2674
S.D.	0.0264	0.0163

Table 5: The level of Glutathione Peroxidase (U/g Hb) in Premallignant persons
*Diff. of Mean = 12.5056, S.E. of Diff. =0.0078, t-value =1607.0990***, D.F. = 28 ***Significant at 0.1% level of significance.*

Parameters	Control Group	Study Group
Size	10	20
Mean	30.7730	24.4306
S.D.	0.0264	2.1362

Table 6: The level of Glutathione Peroxidase (U/g Hb) in Premalignant personsafter Antioxidant therapy.
*Diff. of Mean = 6.3424, S.E. of Diff. =0.6816, t-value =9.3057***, D.F. = 28 ***Significant at 0.1% level of significance.*

Parameters	Control Group	Study Group
Size	10	20
Mean	30.7730	14.4060
S.D.	0.0264	0.0476

Table 7: The level of Glutathione Peroxidase (U/g Hb) in Malignant persons.
*Diff. of Mean = 16.3670, S.E. of Diff. =00162, t-value =1007.3456***, D.F. = 28 ***Significant at 0.1% level of significance.*

Parameters	Control Group	Study Group
Size	10	20
Mean	30.7730	21.0638
S.D.	0.0264	0.8804

Table 8: The level of Glutathione Peroxidase (U/g Hb) in Malignant persons after Antioxidant therapy.
*Diff. of Mean = 0.7092, S.E. of Diff. =0.2809, t-value =34.5597***, D.F. = 28 ***Significant at 0.1% level of significance.*

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It is also observe in our study that the average glutathione peroxidase level(Table.8) of malignant persons (after antioxidant therapy) is 21.0638 is less than that in healthy persons, but higher than that in same group without antioxidant therapy. This suggests that the average level of glutathione peroxidase in malignant persons becomes higher than that of persons without antioxidant therapy. In this way, it is concluded that the antioxidant therapy increase the average level of glutathione peroxidase.

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