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RESEARCH ARTICLE

Estimation of level of MDA and SOD in oral cancer patients and their suppression by antioxidants.

R.S. Singh1*, Anupam Porwal1, S.K. Awasthi2, M.P. Mishra3 and Mahendra yadv4
1.Department of Biotechnology, Brahmanand PG college, Kanpur.
2.Institute of Life Sciences, CSJM University Kanpur.
3. J.K. Cancer Research Institute and Hospital, Kanpur.
4. Research Scholar of Singhania University, Pacheri Bari, Jhunjhunu(Raj.), India.

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****Corresponding Author***

Ajai Kumar Singh

Abstract

In the last decade there has been a rapid progress in the understanding of the actual nature and chemistry of reactive oxygen species (ROS) at the cellular and molecular level. Free radicals have been implicated to play a pivotal role in genesis of various oral cancers. All the major classes of biomolecules may be attacked by free radicals but lipids are most susceptible. Celll membranes are rich source of polyunsaturated fatty acid (PUFAs) which are readily attacked by Oxidized radicals. The free radicals cause oxidative destruction of polyunsaturated fatty acids Known as lipid peroxidation. It is particularly damaging because it proceeds as self perpetuating chain reaction, which may result in more free radical generation. Free radicals are indirectly measured by the level of MDA (malony dialdehyde). Keeping in view such consideration, this study has been designed to the role and activities of ROS and some significant stress markers such as MDA and SOD/ Catalase as enzymatic, antioxidants in oral cancer patients.

INTRODUCTION

It is indeed paradox that oxygen, which is essential for the source of living cells, may itself cause significant damage. Oxygen is usually involved for the oxidation of carbohydrate, fats and proteins and releases energy. During this biological process, there may be formation of oxygen free radicals. Free radicals are very reactive chemical species and are produced continuously in cells during metabolism or in the form its accidental by products. These radicals are recovered as one of the most potent causative agent for various diseases including oral cancer. Oral cancer in human beings is a very common type of cancer in our country. It has been attributed to various oral habitism in the form of chewing of Betel nut, Tobacco, Pan Masala and Smoking which are very common in our society. the toxic chemical liberated by the chewing of theses substances or smoking has greatly been found responsible for increased liberation of free radicals in cells as a result of altered metabolism leading to cell damage and finally carcinogenic changes in the tissues (Burton, K.P. 1988)

A free radical can be defined as a chemical species possessing an unpaired electron. that can be considered as fragment of molecules which are generally very reactive Free radicals my be formed in any of the following three ways.

1. By the hemolytic fission of a covalent bond of a normal molecule, with each fragment remaining one of the paired electrons.

2. By loss of a single electron from a normal molecule.
3. By the addition of a single electron to a normal molecule.

Oxygen free radical and reactive oxygen species: the most important free radical in biological system are radical derivatives of oxygen Reduction of Oxygen by the transfer to it of a single electron will produce the superoxide free radical anion (Superoxide) (Nishikmi and Yogi. 1977).

$$O_2 + e^- \longrightarrow O_2^{-\bullet}$$

A two electron reduction of oxygen would yield hydrogen peroxide.

$$O_2 + 2e^- + 2H^+ \longrightarrow 2H_2O_2$$

Hydrogen peroxide is often generated in biological system via the production of superoxide (Wassermann and Murray, 1979), Two superoxide molecule can react together to form hydrogen peroxide and oxygen.

$$O_2^{-\bullet} + 2H^+ \longrightarrow 2H_2O_2 + O_2$$

Because the free radical reactants produce non radical products, this is known as dismutase reaction. It can take place spontaneously or can be catelysed by the enzyme superoxide dismutase. Hydrogen Peroxide is an important compound in free radical biochemistry because it can rather easily break down in presence of transition metal ions to produce the most reactive and damaging of the oxygen free radicals, the hydroxyl (OH):

$$H_2O_2 + Fe^{2+} \longrightarrow OH + OH^- + Fe^{3+}$$

The iron source (or copper)_ catalysed reaction dependent on superoxide as both the source of the hydrogen peroxide and as the reductant of the transition metal ion (Helliwel, 1981):

$$O_2^{-\bullet} + Fe^{3+} \longrightarrow Fe^{2+} + O_2$$

$$O_2^{-\bullet} + Cu^{2+} \longrightarrow Cu^{+} + O_2$$

Ferrous (Fe^{2+}) iron and cuprous (Cu^{+}) Copper are much more reactive with hydrogen peroxides than their oxidized counterparts Ferric (Fe^{3+}) and Cupric (Cu^{2+}) respectively.

The autoxidation of reduced transition metal iron also generates Superoxides.

$$Fe^{2+} + O_2 \longrightarrow Fe3+ + O_2^{-\bullet}$$

$$Cu^{+} + O_2 \longrightarrow Cu_2^{+} + + O_2^{-\bullet}$$

The reaction of transition metal ions with oxygen can be considered reversible redox reaction and are extremely important in the promotion of free radical reaction.

Superoxide is a free radical which is mostly reactive in nature and its main significance is probably as a source of hydrogen peroxide and as a reductant of transition metal ions. At a low pH value superoxide will protonate to form the perhydroxyle radical (H_2O). A reactive oxidizing species but at physiological pH less than 1% will be in the protonated form.

Hydrogen Peroxide is an oxidizing agent but not especially reactive and its main significant lies in it being a source of hydroxyl radical in the presence of reactive transition metal ion. In the absence of metal catalyst, superoxide and hydrogen peroxide are radially removed and are Virtually harmless (Fridovich 1976).

Materials and Methods

The study was carried out at Institute of Life Sciences, C. S. J. M. University, Kanpur and J. K. Cancer Institute, G.S.V.M. Medical College, Kanpur. A complete working Performa with routine investigation was followed. The subject taken for experiments were grouped as under.

Control group: It comprises of healthy normal volunteers of either sex, preferably between 22 to 40 years of age and include staff members and their families residing at Kanpur since last three year.

Study group: The study group is comprised of various oral cancer patients i.e. with Premalignant lesions and malignant lesions available at J.K. Cancer Institute Kanpur.

Collection of blood: Blood samples were collected at J.K. Cancer Institute Kanpur from healthy persons(controls) and patients with Premalignant and malignant lesion, who are not suffering from any organic disease and have not taken any medicine for the previous 10days.Different parameters were measured spectrophotometrically.

Preparation of Plasma and RBC hemolysate and platelets: Venus blood (10ml) was collected in sterilized vials containing sodium citrate as anticoagulant and plasma was separated by centrifuging the citrated blood at 800xg in cold. RBC hemolystate was prepared by washing the packed RBCs with normal centrifuging saline and then adding distilled water 1:3 v/v ratio to the packed RBCs and further centrifuging at 100xg in cold to remove the plasmamembrane.

Preparation of platelets: The platelets were prepared according to Muenzer et al. (1975) with minor modifications. Venous blood (9.0 ml) was drawn using polypropylene syringe and collected in polypropylene tubes containing 1.0 ml of 3.8% sodium citrate pH 7.2-7.8. the tubes containing blood were centrifuged at 800 xg for 15 min. at 4°C. the pellets were taken up in 5.0 ml of buffer containing 140 mM NaCl, 25 mM Tris-HCl and 0.3 mM EDTA(pH 7.4). The suspension was centrifuged at 120xg for 20 min. the supernatant was then centrifuged at 4,000xg for 20 min. The platelet pellet, thus obtained, was taken up in 5.0 ml deionized water After keeping for 60 min. in cold, the suspension was centrifuged at 4,000 xg for 15 min. The supernatant so obtained, constituted the initial extract.

Biochemical investigation: All the control and study group patients were subjected to following biochemical estimations in blood/serum.

Lipid Peroxidation (LPO): The level of malonyldialdehyde (MDA) as a measure of LPO was estimated in blood according to the method of Ohkawa et at., (1979). 1ml whole blood collected in EDTA (1mg/ml blood) was incubated in presence of O_2 in a 25 ml conical flask at 37^0C for 1 hr. In a metabolic shaker, 1 ml of TCA (20%) was added and mixed properly and the mixture was kept at 0^0C for 1hr. for protein precipitation . After centrifugation 1ml supernatant was taken in a boiling water bath. The tubes cooled and the pink color thus developed was read at 532 nm against reagent blank. The amount of MDA was calculated by applying molar extinction coefficient of MDA-TBA complex (532nm-$1.56X10^5 cm^{-1}$). The rate of lipid peroxidation was expressed in terms of n moles MDA formed/ml blood.

Superoxide Dismutase (Superoxide: superoxide Oxidoreductase EC 1.15.1.1) Estimation: Using the ransod reagent kit manufactured by Randox Laboratories Ltd. on autoanalyser RA-50. The method was based on protocol given by Wooliams et at.,(1983).

Mixed, 0.05 ml of diluted hemolysate mixed with 1.7 ml of substrate (xanthine 0.05 m mole/l and 1.N.T.0.025 m mole/l), again added 0.25 ml of 0.94m mole/l xanthine oxidase. Mixed well and read initial absorbance A_1 after 30 sec. and started timer simultaneously read final absorbance A_2 after 3min. at 505 nm. SOD value was calculated by using standard calibration curve.

Antioxidant Therapy: Following composition 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 meal for malignant cases). This was a complete nutritional supplement with 7 antioxidants and 7 water soluble vitamins.

Water soluble vitamins Vit. $B_1$10mg, Vit. $B_2$10mg, Vit. B_6 3mg, Vit. B_{12}15mg, Folic acid 1mg, Ca panthothenate 12mg, Niacinamide 50mg.

Antioxidants: Vit. E 25 mg, Vit. A5000lu, VitC 150mg, Zn 15mg, copper 1.5mg, Manganese 3mg, Selenium 100mcg, Chromium picolinate 200mcg.

Results and discussion

Reduction-oxidation(redox)Reactions generate reactive oxygen species, the free radical which act as intercellur and intracellular mediators of signal transduction in physiologic and pathologic process. In present study, the author has tried to study the behaviour of important free radical scavenging enzymes of human blood platelets; superoxide dismutase, Catalase, reduced glutathione content, glutathione peroxidase, glutathione reeducates and MDA contents, total protein, albumin, A/G ratio and uric acid in normal healthy persons and the patients suffering from oral premalignant and malignant lesions.

There are so many pathophysiological conditions which lead to augmented production of reducing equivalents, for example, breakdown of ATP to hypoxanthine, electron transport chain disruption, autooxidation of catecholamines, activation of.

Table-1: Show the level of MDA(nmoles/mlBlood) in Premalignant Persons

Para-meters	Control Group	Study Group
Size	10	20
Mean	1.6426	1.6884
S.D.	0.0150	0.0048

Diff of Means=-0.0458;
S.E.of Diff.= 0.0036;
t-Value=-12.6317***; D.F. =28' ***Significant at 0.1%level

Table-2: Show the level of SOD (U/g Hb) in Premalignant Persons

Para-meters	Control Group	Study Group
Size	10	20
Mean	488.750	177.055
S.D.	0.0750	8.03270

Diff of Means=311.6954;
S.E.of Diff.= 2.5628;
t-Value=121.6238***; D.F. =28
***Significant at 0.1%level

Table-3: Show the level of MDA(nmoles/mlBlood) in Premalignant Persons after Antioxidant therapy

Para-meters	Control Group	Study Group
Size	10	20
Mean	1.6426	1.6650
S.D.	0.0150	0.0049

Diff of Means=-0.0224;
S.E.of Diff.= 0.0036;
t-Value=-6.1600***; D.F. =28;
***Significant at 0.1%level

Table-4: Show the level of SOD (U/g Hb) in Premalignant Persons after Antioxidant therapy

Para-meters	Control Group	Study Group
Size	10	20
Mean	488.750	377.805
S.D.	0.0750	25.1117

Diff of Means=110.9454;
S.E.of Diff.= 8.0116;
t-Value=13.8481***; D.F. =28
***Significant at 0.1%level

Table-5: Show the level of MDA (n moles/ml Blood) in Malignant Persons

Para-meters	Control Group	Study Group
Size	10	20
Mean	1.6426	2.5718
S.D.	0.0150	0.0780

Diff of Means=-0.9292;
S.E.of Diff.= 0.0251;
t-Value=-37.0299***; D.F. =28;
***Significant at 0.1%level

Table-6: Show the level of SOD (U/g Hb) in Malignant Persons

Para-meters	Control Group	Study Group
Size	10	20
Mean	488.750	149.053
S.D.	0.0750	0.72890

Diff of Means=339.6971;
S.E.of Diff.= 0.2331;
t-Value=1457.0779***; D.F. =28
***Significant at 0.1% level

Table-7: Show the level of MDA (n moles/ml Blood) in Malignant Persons after Antioxidant therapy

Para-meters	Control Group	Study Group
Size	10	20
Mean	1.6426	1.8583
S.D.	0.0150	0.0780

Diff of Means=-0.2156; 0.0729
S.E.of Diff.= 0.0235;
t-Value=-9.1836***; D.F. =28;
***Significant at 0.1%level

Table-8: Show the level of SOD (U/g Hb) in Malignant Persons after Antioxidant therapy

Para-meters	Control Group	Study Group
Size	10	20
Mean	488.750	192.200
S.D.	0.0750	10.1908

Diff of Means=296.5504;
S.E.of Diff.= 3.2513;
t-Value=91.209***; D.F. =28
***Significant at 0.1% level

arachidonate cascade and activation and accumulation of cellular components of the blood become operative under these conditions (Henary et at., 1990)

Free radicals have been implicated to play pivotal role in the genesis of various oral cancers. They are generated deliberately **b**y animal cells in certain special circumstances because these are useful entities if constrained and targated. Some enzymes require a free radical at their active site in the process of catalysis, for example, ribnucleotide reductase (Richard and Ehrenberg 1983; Stubbe1990). The free radicals cause oxidative destruction of polyunsaturated fatty acid (PUFAs) known as lipid peroxidation. It is particularly damaging because it proceeds as a self-perpetuating chain reaction; which may result in more free radical generation (Cheesemanet al.,1985, 1989, 1990).

In our study we observed that the MDA level (Table-1) in healthy persons (1.6426 n moles/ml Blood) is less than that in premalignant persons (1.6884 moles/ml Blood). The significance of the difference between the two average levels of MDA 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 MDA level in premalignant persons is in general significantly higher than that in healthy persons.

It was also observed that the average MDA level in premalignant persons is higher than that in healthy persons. It is also observed that the average MDA level of premalignant persons (after antioxidant therapy) is 1.6650 n moles/ml Blood (Table-3) is higher than that in healthy persons but less than that in same group without antioxidant therapy. This indicates that the average level of MDA in premalignant persons becomes lesser than that of the persons without antioxidant therapy In this way it is concluded that the antioxidant therapy reduces the average level of MDA.

In this study we found that the MDA level (Table-5) in healthy persons (1.6426 n moles/ml Blood) is less than that in malignant persons (2.5718 moles/ml Blood). The significance of the difference between the two average levels of MDA 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 MDA level in malignant persons is in general higher than that in healthy persons.

In the present investigation, it is observed that the average MDA level in malignant persons is higher than that in healthy persons. It is also observed that the average MDA level (Table-7) of malignant persons (after antioxidant therapy) is 1.8583 n moles/ml Blood(Table-7) is higher than that in healthy persons but less than that in same group without antioxidant therapy. This indicates that the average level of MDA in malignant persons becomes lesser than that of the persons without antioxidant therapy In this way it is concluded that the antioxidant therapy reduces the average level of MDA.

Various research workers have reported superoxide dismutase in context of oxygen toxicity as a tool in biochemical, pharmaceutical and clinical context including population genetics, trisomy 21,development and senescence and degenerative diseases, radiation damage and malignancy (Bannister et al1987).these radicals are capable of damaging cell membranes and macromolecules (Fridovich, 1974,1975) and since there is no or little SOD in plasma and extracellular fluids (Beckman et al., 1973; Mc Cord and Salin, 1975), presence of SOD in platelets may have significant implication in various diseases.

Reduction of oxygen by transfer of single electron will produce the superoxide free radical anion (superoxide).

$$O_2 + e^- \longrightarrow O_2^-$$

A two electron reduction of oxygen would yield hydrogen peroxide :

$$O_2 + 2e^- + 2H \longrightarrow H_2O_2$$

Hydrogen peroxide is often generated in biological system via the production of superoxide; two superoxide molecules can react together to form hydrogen and oxygen.

$O_2 + 2H \longrightarrow H_2O_2 + O_2$

Because the free radical reactants produce non-radical products this is known as a dismutation reaction. It can take place spontaneously or **can be catalyzed by the enzyme superoxide dismutase (SOD). Hydrogen** peroxide is not a free radical but falls in the category of "reactive oxygen species" (ROS) that includes not only oxygen free radicals but also non-radical oxygen derivatives that are involved in oxygen radical production.

Thus superoxide dismutase, dismutase the superoxide anion into hydrogen peroxide which itself is very damaging for cellular components. Hydrogen peroxide toxicity is removed by Catalase by degrading H2O2 into water and oxygen. The reduction in SOD activity could result in an impaired production against the toxic effects of O2-and thus might lead to serve cellular damage(Vanella and Villa,1989; Benzi et al., 1988; Tayaramin and Clanze,1989). Oxidative and chemical stress inhibit SOD activity. They suggested that stress directly and indirectly through inhibition of SOD increases lipid peroxidation in cell membranes and this produce damage to the associated physiological functions.

It was revealed in the present study that the SOD level(Table-2) in healthy persons (488.7504 U/g Hb) is higher than that in premalignant persons(177.0550 U/g Hb). The significance of the difference between the two average levels of SOD 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 SOD level in premalignant persons is in general significantly less than that in healthy persons.

In our investigation, it was revealed that the average SOD(U/g Hb) level (Table-4) in Premalignant persons is less than that in healthy persons. It is also observed that the average SOD level of premalignant persons (after antioxidant therapy) is 377.8050 is les than that in healthy persons but higher than that in same group. without antioxidant therapy. This indicates that the average level of SOD in premalignant persons becomes higher than that of the persons without antioxidant therapy. In this way it is concluded that the antioxidant therapy increases the average level of SOD.

It was also observed that the SOD level (Table-6) in healthy persons (488.7504 U/g Hb) is higher than that in malignant persons(149.0533 U/g Hb). The significance of the difference between the two average levels of SOD 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 SOD level in malignant persons is in general less than that in healthy persons.

It was revealed in the present study that the average SOD(U/g Hb) level (Table-8) in malignant persons is less than that in healthy persons. It is also observe that the average SOD level of malignant persons (after antioxidant therapy) is 192.2000 is less than that in healthy persons but higher than that in same group without antioxidant therapy. This indicates that the average level of SOD in malignant persons becomes higher than that of the persons without antioxidant therapy. In this way it is concluded that the antioxidant therapy increases the average level of SOD

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