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Original Research

Antioxidants *In Vitro* is it a Need for Oral Precancerous Lesion

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Abstract:

Background: At the backdrop of the debatable role of antioxidant (AO) therapy, patients with oral premalignant lesions (OPLs) were evaluated to assess the logic behind AO t herapies.

Subjects and Methods: Oxidative stress and AO status have to be assessed in OPLs patients and compared with healthy individuals, classify into study groups which could be useful in selection of ideal patients requiring AO therapy. Total 121 subjects were included for this study *n* = 76 were with OPL, *n* = 45 were healthy controls. Serum levels of reactive oxygen species (ROS) (malondialdehyde [MDA]) and AO enzymes super oxide dismutase (SOD), catalase and glutathione peroxidase (GSH-PX) were evaluated in blood serum and grouped under levels of ROS and AO enzymes activity.

Results: In 76 OPL patients increase in the in serum (MDA) level observed along with a decrease in activity of AO enzymes, SOD, catalase, and GSH-PX in comparison to 45 healthy subjects. classification of OPL patients shown that 38.15% Group I patients did not have any significant change in oxidative stress. Group II (21.05%) patients though had enhanced stress, their AO enzymes level were normal these two groups did not require AO treatment. However, 10.5% of patients Group III had normal MDA with low AO defense and whereas 30.2% Group IV patients had an abnormality in all parameters, last two groups to be aided by AO therapy.

Conclusion: Prior valuation of the oxidative stress and AO status in the patients with OPL can be done to reduce the abuse of severe AOs medication.

Key Words: Antioxidant, glutathione peroxidase, malondialdehyde, oral premalignant lesions, reactive oxygen species, superoxide dismutase

Introduction

Oxidative stress which is considered as an imbalance of the body's ability to scavenge free radical species, (both reactive oxygen species [ROS] and reactive nitrogen species [RNS]) was found to be associated with cancer by several studies. Moreover, the development of cancer occurs through several steps involving initiation, promotion and progression. Premalignant lesions are the first clinically identified lesions of cancer. In the backdrop of increased prevalence of cancer, identification and management of premalignant stage seem to be important steps to prevent the development of cancer.

Antioxidants (AOs) are known to neutralize ROS and RNS by several mechanisms.[1,2]

Hence, AO therapies are employed as evidence base medicine to patients who have propensities to have a future risk of cancer due to oxidative stress.[3] AOs, however, are not totally risk-free carotene an AO in high doses had been reported to increase the risk of lung cancer, feeding animals with a high content of butylated hydroxytoluene a synthetic AO resulted in decrease in the α-tocopherol content of liver.[4]

The oxidative injury may involve damage to lipids, therefore, inhibiting lipid peroxidation which will not protect the tissues and also cause damage to proteins.[5] In spite of all these risks involved in AO treatment, there should be a rationale to which it has to be used. Therefore, it is very relevant to focus on the procedures like free oxygen radical test and free oxygen radical defense test,[6] laboratory tests which will help to detect invaluably the usage of AOs in specific diseases.[7]

Oral cancer is one of the ten most frequent cancers in the world and most common in India. The incidence of new cancer is shown that occur 0.8 to 2.0/1,000,000 persons annually compared to the prevalence of 5.0/1,000,000 in the same population in India.[8] A number of premalignant lesions have been identified which include leukoplakia, erythroplakia, oral submucous fibrosis, lichen planus, smokeless tobacco keratitis, all have a potential role in turning malignant.[9]

Studies were performed to assess the role of oxidative stress and AO status in a particular lesion like oral submucous fibrosis.[10] In the present study, oxidative stress and AO status were assessed in a number of oral premalignant lesions (OPLs) such as leukoplakia, erythroplakia, oral submucous fibrosis, and smokeless keratitis their comparison with hale and hearty individuals. Further, an attempt is made to classify into study

groups which could be useful in selection of ideal patients requiring AO therapy than to all affected.

Subjects and Methods

A total of 121 subjects were included for the study among which 76 (43 males and 33 females) were with OPLs and 45 (26 males, and 19 females) were normal healthy controls without any reported diseases. Patients with diabetes, hypertension, and dyslipidemia were excluded, and patients with lipid lowering drugs and with vitamin supplements also excluded.

Sample collection

Patients were randomly selected from outpatient from Department of Oral Medicine, Thai Moogambigai Dental College, Chennai, India and referred to Oral Pathology Department of same college. 12 h after fasting blood samples were collected from participants in sterile test tubes, allowed to clot and then carefully centrifuged at 3000 rpm for 10 min clear serum was collected and kept in −4°C until the test were performed.

The estimation of malondialdehyde (MDA) in serum was done by thiobarbituric acid reactive species method.[11] Enzyme activity mainly catalase and superoxide dismutase (SOD) and glutathione peroxidase (GSH-PX) was done as AO defense serum catalase activity was determined spectrophotometric assay.[12] The assay of SOD was based on the reduction of nitroblue tetrazolium to water insoluble blue formazon.[13] GSH-PX activity was estimated by direct spectrometric assay.[14]

The design of this study was approved by the institutional ethical committee of the institution (Dr. MGR University and Research Institute, Maduravoyal) and informed consent was obtained from the participants.

Statistical analysis

The data from OPL patients and controls were compared by Student's *t*-test values are expressed as mean ± standard deviation (SD). $P < 0.05$ was considered as statistically significant.

Results

Table 1 shows the distribution of different types of OPLs among 76 study patients. Baseline variables in patients and healthy controls have depicted in Table 2. Among overall study subjects, the patients with OPLs have significantly increased MDA levels (mean ± SD; 3.1 ± 0.3 mmol/L) compared to the healthy controls (mean ± SD) 1.4 ± 0.7 mmol/L and catalase (mean ± SD; 81.7 ± 5.9 U/L) were significantly lower in those patients in comparison to the healthy subjects. Table 3 shows the distributions of the study population according to oxidative stress and AO status. 37 OPL patients (48.7%) had a normal level of serum MDA in contrast to 39 (51.3%) patients having an increased level of the same. As far as AO enzyme activity is concerned, 45 OPL patients (59.2%) had normal activity of all the enzymes whereas 31 (40.8%) had somewhat decreased AO enzyme activity they are grouped as Group I ($n = 29$) having all the variables within normal range, Group II ($n = 16$) having only increased level of MDA, Group III ($n = 8$) having only decreased level of AO enzyme activity and Group IV ($n = 23$) abnormality in both the parameters the group wise distribution of all the parameters is presented in Table 4.

Discussion

OPL had long been related to oxidative stress and generation of free radicals by different study groups.[15,16] A similar finding is observed in this study (Table 2). To overcome this oxidative stress, the AO therapies and diet enriched with AOs are advised to prevent or, at least, to attenuate organic deterioration[17] several studies are in agreement with the above role of AO produced *in vivo* for body's own defense.[18]

Several other studies have raised doubtful questions about the role of AOs administered from outside.[19] In this study, the lower dose of vitamin C intake was found to be associated

Table 2: Baseline variables in patients and healthy controls (mean±SD).

Variables	Healthy controls (*n*=45)	OPL patients (*n*=76)
Age (years)	41.6±8.7	40.4±4.3
Sex (M/F)	43/33	26/19
MDA (mmol/L)	1.4±0.7	3.1±0.3*
GSH-PX (mmol/L)	117.4±6.4	84.2±9.7*
SOD (mmol/L)	76.3±3.8	40.5±7.2*
Catalase (U/I)	104.2±9.2	81.7±5.9∞

*$P<0.001$; ∞$P<0.05$; $P=0.05$ considered significant by unpaired, two-tailed, student's *t*-test. MDA: Malondialdehyde, GSH-PX: Glutathione peroxidase, SOD: Super oxide dismutase, OPL: Oral premalignant lesions

Table 3: Distributions of study population according to oxidative stress and antioxidant status.

Variables	MDA normal	MDA increased	Total (%)
Antioxidant normal	29 (Group I)	16 (Group II)	45 (59.2)
Antioxidant decreased	8 (Group III)	23 (Group IV)	31 (40.8)
Total (%)	37 (48.7)	39 (59.3)	76 (100)

MDA: Malondialdehyde

Table 1: Distributions of different types of lesions in the study subjects.

Disease group	Leukoplakia	Erythroplakia	Oral sub mucous fibrosis	Lichen planus	Smokeless tobacco keratosis
Male	3	2	21	4	13
Female	2	0	17	3	11
Total	5	2	38	7	24

Table 4: Group wise distribution of oxidative stress and antioxidant status in patients with OPLs compared to healthy controls.

Parameters	Healthy control (*n*=45)	Group I (*n*=29)	Group II (*n*=16)	Group III (*n*=08)	Group IV (*n*=23)
MDA (mmol/L)	1.4±0.7	1.6±0.8	4.8±0.4*	1.7±0.5	4.6±0.6*
GSH-PX (mmol/L)	117.4±6.4	123±14.2	114±0.5	52±0.4*	48±6.0*
SOD (mmol/L)	76.3±3.8	58±0.5	52±0.2	25±2.6*	26±7.2*
Catalase (U/I)	104.2±9.2	116±12.3	109.6±4.5	51±0.9*	49.6±8.1*

All results are expressed in mean±SD: Considered significant by unpaired, two-tailed, student's *t*-test, MDA: Malondialdehyde, GSH-PX: Glutathione peroxidase, SOD: Super oxide dismutase, *P<0.001

with increased free radical damage to DNA.[20] Paradoxically the same was observed with high dosage vitamin C.[21] Hence, there should be a rational approach to treat the patients with AOs.

Moreover, there might be other causes which contribute in the pathogenesis of OPL. As all OPL patients are not having abnormalities in oxidative stress related blood parameters (Tables 3 and 4) group wise analysis shows that the patients in Group I (*n* = 29, 38.15%) have normal MDA concentration and AO defenses comparable with the healthy controls these patients may have other etiological cause in the disease process.[22]

AO medication in them may be harmful rather than beneficial to them. Some ROS/RNS have useful roles *in vivo*.[23] Administrations of the proposed AOs may endanger these patients generating more reactive species by themselves or reducing the effect of beneficial free radicals.

The patients belonging to Group II in the current study had raised MDA concentration, but their AO enzyme levels are within the normal range. These patients may not be benefitted with an extra amount of AOs besides if the AOs acts by scavenging, the resulting AO derived radicals themselves may cause damage sulfur or oxy sulfur radicals which act as AO *in vivo* has been found to be inactivated ă-1 anti-proteinase having a protective role.[24] Animals exposed to paraquat, an herbicidal prior to administration of vitamin C was found to be protective. Those treated with vitamin C after exposure increase the herbicidal damage by interacting with the released transition metals.[25]

Patient belonging to Group III had reduced level of AOs enzymes though they had normal MDA concentration. These patients might be subjected to oxidative stress as a result of the insufficient removal of free radicals due to the deficient AO defense. These individuals are probably at high risk and one to benefit from extra AOs apparently they are the ideal candidates to be selected for AO therapy

A lot of natural and chemical compounds are available in the medical field as AOs unfortunately this usage of this compounds failed to be highly beneficial in all precancerous lesions[26] and more seriously some AOs have more harmful long-term effects.[27] Thus, this study provides further scope to evaluate therapeutic use of AOs, supplements, which should be monitored at frequent intervals to see not only the progress of disease but also to look for harmful side effects of AOs.

Conclusion

Although our study has the small sample size and confined to limited parameters which can only give an index. The prior assessment of oxidative stress and AO status are necessary in patients with OPL before administering the AOs, thus reducing the abuse of medications.

References

1. Kim YW, Byzova TV. Oxidative stress in angiogenesis and vascular disease. Blood 2014;123(5):625-31.
2. Carocho M, Ferreira IC. A review on antioxidants, prooxidants and related controversy: Natural and synthetic compounds, screening and analysis methodologies and future perspectives. Food Chem Toxicol 2013;51:15-25.
3. Tennant DA, Durán RV, Gottlieb E. Targeting metabolic transformation for cancer therapy. Nat Rev Cancer 2010;10(4):267-77.
4. Stephens NG, Parsons A, Schofield PM, Kelly F, Cheeseman K, Mitchinson MJ. Randomised controlled trial of vitamin E in patients with coronary disease: Cambridge Heart Antioxidant Study (CHAOS). Lancet 1996;347(9004):781-6.
5. Lukovic L, Petty MA, Bolkenius FN, Grisar JM, Dow J, De Jong W. Protection of infarcted, chronically reperfused hearts by an alpha-tocopherol analogue. Eur J Pharmacol 1993;233(1):63-70.
6. Buege JA, Aust SD. Microsomal lipid peroxidation. Methods Enzymol 1978;52:302-10.
7. Palmieri B, Sblendorio V. Oxidative stress tests: overview on reliability and use. Part I. Eur Rev Med Pharmacol Sci 2007;11(5):309-42.
8. Sankaranarayanan R, Ramadas K, Thomas G, Muwonge R, Thara S, Mathew B, *et al.* Effect of screening on oral cancer mortality in Kerala, India: a cluster-randomised controlled trial. Lancet 2005;365(9475):1927-33.
9. Barnes L, Eveson JW, Reichart P, Sidransky D. World Health Organization Classification of Tumours. Pathology and Genetics of Head and Neck Tumours, New Delhi, India: International Agency for Research on Cancer (IARC) IARC Press; 2005. p. 177-9.
10. Gupta S, Reddy MV, Harinath BC. Role of oxidative stress and antioxidants in aetiopathogenesis and management of oral submucous fibrosis. Indian J Clin Biochem 2004;19(1):138-41.
11. Avinash Tejasvi ML, Bangi BB, Geetha P, Anulekha

Avinash CK, Chittaranjan B, Bhayya H, *et al.* Estimation of serum superoxide dismutase and serum malondialdehyde in oral submucous fibrosis: a clinical and biochemical study. J Cancer Res Ther 2014;10(3):722-5.
12. Góth L. A simple method for determination of serum catalase activity and revision of reference range. Clin Chim Acta 1991;196(2-3):143-51.
13. Beauchamp C, Fridovich I. Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Anal Biochem 1971;44(1):276-87.
14. Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med 1967;70(1):158-69.
15. Sun Y. Free radicals, antioxidant enzymes, and carcinogenesis. Free Radic Biol Med 1990;8(6):583-99.
16. Freeman BA, Crapo JD. Biology of disease: free radicals and tissue injury. Lab Invest 1982;47(5):412-26.
17. Myung SK, Kim Y, Ju W, Choi HJ, Bae WK. Effects of antioxidant supplements on cancer prevention: meta-analysis of randomized controlled trials. Ann Oncol 2010;21(1):166-79.
18. Halliwell B, Gutteridge JM. The chemistry of oxygen radicals and other oxygen-derived species. In: Free Radicals in Biology and Medicine, New York: Oxford University Press; 1985. p. 20-64.
19. Kim MK, Sasaki S, Sasazuki S, Okubo S, Hayashi M, Tsugane S. Long-term vitamin C supplementation has no markedly favourable effect on serum lipids in middle-aged Japanese subjects. Br J Nutr 2004;91(1):81-90.
20. Taylor A, Jahngen-Hodge J, Smith DE, Palmer VJ, Dallal GE, Lipman RD, *et al.* Dietary restriction delays cataract and reduces ascorbate levels in Emory mice. Exp Eye Res 1995;61(1):55-62.
21. Priemé H, Loft S, Nyyssönen K, Salonen JT, Poulsen HE. No effect of supplementation with vitamin E, ascorbic acid, or coenzyme Q10 on oxidative DNA damage estimated by 8-oxo-7,8-dihydro-2'-deoxyguanosine excretion in smokers. Am J Clin Nutr 1997;65(2):503-7.
22. Krunkosky TM, Martin LD, Fischer BM, Voynow JA, Adler KB. Effects of TNFalpha on expression of ICAM-1 in human airway epithelial cells *in vitro*: oxidant-mediated pathways and transcription factors. Free Radic Biol Med 2003;35(9):1158-67.
23. Willson RL. From nitric oxide to desferal: nitrogen free radicals and iron in oxidative injury. Basic Life Sci 1988;49:87-99.
24. Halliwell B. Free radicals and antioxidants: updating a personal view. Nutr Rev 2012;70(5):257-65.
25. Andersson CM, Halberg A, Hogberg T. Advances in the development of pharmaceutical antioxidants. Adv Drug Res 1996;28:65-180.
26. Willett WC. Diet and health: what should we eat? Science 1994;264(5158):532-7.
27. Blair A, Shaul PW, Yuhanna IS, Conrad PA, Smart EJ. Oxidized low density lipoprotein displaces endothelial nitric-oxide synthase (eNOS) from plasmalemmal caveolae and impairs eNOS activation. J Biol Chem 1999;274(45):32512-9.