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ORIGINAL ARTICLE

Serum total protein, albumin and advanced oxidation protein products (AOPP) - implications in oral squamous cell carcinoma

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Abstract

Background: The role of oxygen free radicals in the initiation, promotion and progression of carcinogenesis and the protective role of anti-oxidant defenses have been the subject of much speculation in the recent past with conflicting reports in the literature. *Objectives:* The aim of this study was to measure the concentration/levels of serum total proteins, albumin and advanced oxidation protein products as markers of oxidative stress in sera of patients with an oral pre-cancerous lesion and frank oral cancer. *Materials and methods:* The study consisted of sera analysis of 30 new patients of histologically proven well-differentiated, oral squamous cell carcinoma and 10 patients, clinically diagnosed with a potentially malignant epithelial lesion, speckled leukoplakia, aged between 40 to 60 years, in addition to 25 healthy controls. One way analyses of variance were used to test the difference between groups. The normality of data was checked before the statistical analysis was performed. *Results:* The study revealed variations in sera levels of albumin and advanced oxidation protein products to be statistically significant ($p < 0.001$). *Conclusion:* The results obtained emphasize the need for more studies with larger sample sizes to be conducted before a conclusive role could be drawn in favour of sera levels of total protein, albumin and advanced oxidation protein products as markers of diagnostic significance and of the transition from the various oral pre-cancerous lesions and conditions into frank oral cancers.

Key words: Oral squamous cell carcinoma, reactive oxygen species, transformation, pre-cancerous, serum albumin.

INTRODUCTION

Despite tremendous advances in the diagnosis and management of oral cancers, the diagnostic adjuncts which are used to aid an early diagnosis of oral cancers either suffer from a lack of sensitivity in the initial stages of the processes leading to frank oral cancers or from a setback of not being so cost effective. In addition, biopsy, which is considered the gold standard in the diagnosis of oral cancers, suffers from the reliability of an appropriate site for the obtainment of specimens to be conclusive. The introduction of the concept of field of cancerization has further questioned the significance of biopsy results in the approval or rejection of the reports that come out to be confirmative of either dysplastic or frank cancerous changes seen in the tissue. The role of biochemical markers, on the other hand, comes out to be a convincing enough evidence of the changes taking place in the body at a time when tissue and cell level changes are not obvious to be taken as an evidence of frank malignant degenerations. Hence, the present study was planned to assess the levels of serum total protein and albumin as plasma's potent anti-oxidant defenses and advanced oxidation protein products, the markers of oxidant mediated protein damage, as reliable markers of oxidative stress in the body that could be helpful in the early identification and even more significantly, in determining the pre-disposition of the various oral pre-cancerous lesions and conditions, into their transformation to frank oral cancers.

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MATERIALS AND METHODS

Source of data

The study was conducted in the Department of Oral Medicine and Radiology, Government Dental College and Research Institute, Bangalore for a period of 3 months from Jan 2010 to March 2010. The study consisted of 30 new cases of clinically-diagnosed and histologically-proven well-differentiated, oral squamous cell carcinoma and 10 patients with speckled leukoplakia, aged between 40-60 years, in addition to 25 healthy controls.

Ethical clearance for the study was obtained from the institutional ethics committee and clearance was also obtained from the Bangalore Medical College and Research Institute and Associated Hospitals.

Collection of data

None of the patients were on any treatment modality prior to inclusion in the study or suffering from any systemic condition, especially hepatic or renal disorders that could have affected the sera albumin levels. Also, controls as well as the patients who were chronic alcoholics were excluded from the study to rule out the probability of derangement of liver functions that could have contributed towards the variations in sera levels of albumin.

Selected patients were explained in detail about the planned study and written informed consents were obtained. These patients were subjected to a detailed history and a thorough clinical examination using a specially prepared proforma.

Collection of blood and serum separation

Following an overnight fast, 5 ml of venous blood was taken from selected patients between 8 A.M. and 10 A.M. The samples were allowed to clot and serum was immediately separated by ultracentrifugation taking full precautions to prevent hemolysis. The supernatant was discarded and the rest of the sample was stored at -20 degrees Celsius.

Assessment of serum total protein, albumin and advanced oxidation protein products

Biochemical analysis of serum total protein, albumin and advanced oxidation protein products was done in the Department of Clinical Biochemistry, Bangalore Medical College and Research Institute and associated Hospitals, Bangalore.

Assessment of serum total protein and albumin was done using the Biuret method with absorption at 540 nm. Serum total protein and albumin were expressed as g/dL. Advanced oxidation protein products were measured by spectrophotometry. The assay was calibrated using chloramine-T and the absorbance was read at 340 nm on a microplate reader. Advanced oxidation protein products' concentration was expressed as micromol/L of chloramine-T equivalents. Current AOPP methods suffer from poor reproducibility and accuracy due to precipitation of lipids in plasma samples. Solubilization of plasma lipids was therefore carried-out before spectrophotometric analysis of AOPP levels. It was done to prevent both loss of lipoproteins due to precipitation and overestimation as a result of light scattering.

Statistical analysis

The results were averaged (mean +/- standard deviation) for continuous data and number and percentage for dichotomous data were presented in the Tables and Graphs. One way analyses of variance (Anova) were used to test the difference between groups.

P values less than 0.05 were taken to be statistically significant. The data was analyzed using SPSS (version 10.5). The normality of data was checked using Kolmogorov-Smirnov and Shapiro-Wilk tests for significance before the statistical analysis was performed (Table 1).

RESULTS

While the mean values of serum total protein were much the same in controls (8.236 +/- 1.5025 g/dL) as against cases diagnosed with speckled leukoplakia (9.85 +/- 3.6788 g/dL) and well-differentiated, oral squamous cell carcinoma (7.8 +/- 3.1500 g/dL) (Table 2), there were observed great variations in the minimum (1.6 g/dL) to the maximum values (18.2 g/dL) for well-differentiated, oral squamous cell carcinoma. The p value however came out to be statistically insignificant implying the role of various other factors in protein metabolism in cancer patients (Table 2).

Serum albumin levels, however, came out to be statistically significant ($p<0.001$) (Table 2) with serum albumin levels as low as 1.7 g/dL in frank oral squamous cell carcinoma as against a minimum of 3 g/dL in the control group. The mean value of serum albumin was 4.956 +/–1.0579 g/dL in the control group.

TABLE 1 Table depicting tests of normality of data

Serum Analyte	Group	Kolmogorov-Smirnov			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
Total protein	Control	0.133	25	0.200	0.961	25	0.427
	Speckled leukoplakia	0.327	10	0.003	0.744	10	0.003
	Well-differentiated, oral squamous cell carcinoma	0.123	30	0.200	0.932	30	0.055
Albumin	Control	0.092	25	0.200	0.969	25	0.611
	Speckled leukoplakia	0.139	10	0.200	0.962	10	0.804
	Well-differentiated, oral squamous cell carcinoma	0.109	30	0.200	0.953	30	0.198
Advanced oxidation protein products	Control	0.166	25	0.075	0.901	25	0.019
	Speckled leukoplakia	0.188	10	0.200	0.930	10	0.447
	Well-differentiated, oral squamous cell carcinoma	0.147	30	0.098	0.841	30	0.000

Advanced oxidation protein products also showed marked variations in controls and patients diagnosed with frank squamous cell carcinomas (0.42563 +/- 0.2010 micromol/L) with $p<0.001$ (Table 2). The mean value of serum advanced oxidation protein products was 0.0788 +/- 0.0279 micromol/L in the control group (Table 2). Sera levels of advanced oxidation protein products rose to 0.918 micromol/L in the frank oral squamous cell carcinoma group as against a minimum of 0.041 micromol/L in the control group. The mean value of serum advanced oxidation protein products in patients diagnosed with speckled leukoplakia was also significantly higher (0.368 +/- 0.0978 micromol/L) (Table 2).

DISCUSSION

Most free radicals are said to be highly reactive and short lived.[1,2] It has been proposed that free radicals are involved in both the initiation and promotion stages of carcinogenesis.[3] These free

TABLE 2 Mean serum total protein, albumin and advanced oxidation protein products in study groups with standard deviation and F and P values

Parameter	Group	N	Mean	SD	Min.	Max.	F-value	p-value
Serum total protein (g/dL)	Control	25	8.236	1.502	5.50	10.70	2.113	0.130
	Speckled leukoplakia	10	9.850	3.678	6.00	19.30		
	Well-differentiated, oral squamous cell carcinoma	30	7.800	3.150	1.60	18.20		
Serum albumin (g/dL)	Control	25	4.956	1.057	3.00	7.80	9.427	<0.001
	Speckled leukoplakia	10	3.790	0.940	2.00	5.10		
	Well-differentiated, oral squamous cell carcinoma	30	3.693	1.217	1.70	7.20		
Serum advanced oxidation protein products (micromol/L)	Control	25	0.078	0.027	0.041	0.146	41.91	<0.001
	Speckled leukoplakia	10	0.368	0.097	0.242	0.534		
	Well-differentiated, oral squamous cell carcinoma	30	0.425	0.200	0.202	0.918		

radicals have been shown to cause DNA damage, activate pro-carcinogens and alter the cellular anti-oxidant defense mechanisms.[2,4-13]

Plasma is known to contain a wide range of important antioxidants too, including albumin, ascorbic acid and uric acid. In contrast, concentrations of enzymes such as super-oxide dismutase, reduced glutathione and catalase, all of which are known to be important intracellular antioxidants, are low in plasma. While ascorbate is an important extra-cellular antioxidant, albumin via its thiol groups, provides quantitatively almost ten folds greater antioxidant protection against the various reactive oxygen and nitrogen species held responsible for the genetic damage eventually leading to the development of cancers.[1,13-16]

The analysis of changes in serum total protein in malignancy is in itself a means of studying abnormality in the protein metabolism in this condition. Until recently, radical induced damage to proteins was considered to be mainly a chain-terminating process. It was thought that the products of damage produced on the protein, as a result of protein scission, cross-linking, chemical modification of side chains, were relatively inert with the intermediaries subsequently degraded by intra-and extra-cellular enzymes. It has recently been demonstrated, however, that these intermediaries are capable of initiating further chemical reactions thereby leading to the depletion of important cellular reductants such as ascorbates and glutathione via redox reactions.[16-18] Serum total protein in our study came out to be statistically insignificant implying the role of the several complex factors that may play a role in protein metabolism in cancer patients as held by the numerous other studies conducted earlier in this regard.[19]

In humans, albumin is the most abundant plasma protein accounting for about 55-60% of the measured serum proteins. It consists of a single polypeptide chain of 585 amino acids with a molecular weight of around 66,500 Da. The mature, circulating molecule is arranged in a series of alpha-helices, folded and held by 17 disulphide bridges.[20]Albumin synthesis takes place only in the liver and it is secreted into the portal circulation as soon as it is formed. The rate of synthesis varies with nutritional and disease states.[19-21] Amongst the numerous plasma proteins that possess anti-oxidant properties owing to their rich concentrations of free thiol groups, albumin is unusual in having a free sulfhydryl group in addition.[20]

With normal concentrations lying between 3.5-5.5 g/dL, the serum level of albumin is related mainly to its synthesis and catabolism. In fact, only a small number of factors are known to result in variation in serum albumin. In addition, it has been reported that serum albumin decreases with age and cigarette smoking with the usual half-life of albumin being 20 days. [20, 22, 23]

Several lines of evidence suggest strongly that a reduced serum albumin concentration, although within the normal range, is associated with increased mortality risk.[20,24] From studies performed with healthy subjects and patients, it has been reported that the estimated increase in the odds of death ranges from 24 to 56 % for each 2.5 g/L decrement in serum albumin concentration. The serum albumin level thus appears to be an independent predictor of mortality risk with a direct protective effect of the albumin molecule being suggested by the persistence of the association after adjustment for other risk factors. Albumin may thus represent quantitatively the most important component that plays a determinant role in the efficient antioxidant defense, organisms have developed to protect against oxidative attack.[20,22-5]

Albumin in our study came out to be statistically significant with values varying from a minimum of 2 g/dL to 5.1 g/dL in patients diagnosed with speckled leukoplakia to as low as 1.7 g/dL in patients afflicted with frank oral squamous cell carcinoma. There is, however, a lack of consistent studies in relation to the exact role serum albumin plays in patients of oral squamous cell carcinoma or, for that matter, patients suffering from the various oral pre-cancerous lesions and conditions gradually progressing towards frank malignant degenerations. The exact role of albumin as a diagnostic marker of significance or, in the pathogenesis or, in assessing the prognosis is, therefore, warranted by larger, follow-up studies correlating the level of serum albumin in these groups of patients with the overall 5-year survival rates.

Also, cells can generally remove oxidized proteins by proteolysis. This in only when the normal physiology of the cells is disturbed that the products of oxidative metabolism of proteins are retained and lead to damaging actions of oxidized proteins during aging and sometimes culminating into a number of oxidative stress mediated pathologies including cancers.[19-21]

Advanced oxidant protein products, first described by Witko-Sarsat *et al*. in 1996,[26]

further have been hypothesized to activate the endothelial cells and to a lesser extent, fibroblasts to generate reactive oxygen species. Furthermore, advanced oxidation protein products generated by different oxidation patterns lead to the production of either NO or, H_2O_2 suggesting their role in the generation of different types of reactive oxygen species that set a cascade of reactions with a potential to damage cellular micro-molecules eventually turning out into frank oral squamous cell carcinoma.[16,17,26-32]

The level of advanced oxidation protein products in our study ranged from a minimum 0.242 micromol/L to 0.534 micromol/L in patients diagnosed with speckled leukoplakia to as high as 0.918 micromol/L in patients diagnosed with histologically proven well-differentiated, oral squamous cell carcinoma. The results obtained, however, could not be compared with the observations of other studies as the AOPP levels have been assessed in relation to other cancers of the body in the studies conducted in the past. This study deserves the credit of being the first of its kind assessing the sera levels of AOPP as one of the important diagnostic marker in patients diagnosed with oral cancers.

CONCLUSION

Reactive oxygen and nitrogen stresses have long been implicated in the genesis of oral cancers. There is enough literature available that shows convincing evidence in the use of anti-oxidants as chemo-preventive agents to halt the transformation of various oral pre-cancerous lesions and conditions into frank oral cancers. The results obtained emphasize the need for more studies to be conducted in this regard for the assessment of sera levels of total protein, albumin and advanced oxidation protein products to accept their utility and to assess their role in the pathogenesis and their impact on the prognosis of oral cancers providing a scientific ground for the use of diverse chemo-preventive strategies in controlling damage at genetic and molecular levels to prevent the ongoing transition of various oral pre-cancerous lesions and conditions into frank malignant degenerations .

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REFERENCES

1. Kolanjiappan K, Ramachandran CR, Manoharan S. Biochemical changes in tumor tissues of oral cancer patients. Clin Biochem. 2003;36(1):61-5.
2. Hershkovich O, Shafat I, Nagler RM. Age-related changes in salivary antioxidant profile: possible implications for oral cancer. J Gerontol A Biol Sci Med Sci. 2007;62(4):361-6.
3. Storz P. Reactive oxygen species in tumor progression. Front Biosci. 2005;10:1881-96.
4. Evans MD, Dizdaroglu M, Cooke MS. Oxidative DNA damage and disease: Induction, repair and significance. Mutat Res. 2004;567(1):1-61.
5. von Sonntag C. Free radical-induced DNA damage and its repair: A chemical perspective. Basle: Springer; 2006.
6. Halliwell B, Gutteridge J. Free radicals in biology and medicine. 4th ed. Oxford: Clarendon Press; 2007.
7. Szatrowski TP, Nathan CF. Production of large amounts of hydrogen peroxide by human tumor cells. Cancer Res. 1991;51(3):794-8.
8. Toyokuni S, Okamoto K, Yodoi J, Hiai H. Persistent oxidative stress in cancer. FEBS Lett. 1995;358(1):1-3.
9. Oberley TD. Oxidative damage and cancer. Am J Pathol. 2002;160(2):403-8.
10. Klaunig JE, Kamendulis LM. The role of oxidative stress in carcinogenesis. Annu Rev Pharmacol Toxicol. 2004;44:239-67.
11. Valko M, Rhodes CJ, Moncol J, Izakovic M, Mazur M. Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chem Biol Interact. 2006;160(1):1-40.
12. Pelicano H, Carney D, Huang P. ROS stress in cancer cells and therapeutic implications. Drug Resist Updat. 2004;7(2):97-110.
13. Elango N, Samuel S, Chinnakkannu P. Enzymatic and non-enzymatic antioxidant status in stage (III) human oral squamous cell carcinoma and treated with radical radio therapy: Influence of selenium supplementation. Clin Chim Acta. 2006;373(1-2):92-8.
14. Bahar G, Feinmesser R, Shpitzer T, Popovtzer A, Nagler RM. Salivary analysis in oral cancer patients: DNA and protein oxidation, reactive nitrogen species, and antioxidant profile. Cancer. 2007;109(1):54-9.
15. Engel RH, Evens AM. Oxidative stress and apoptosis: A new treatment paradigm in cancer. Front Biosci. 2006;11:300-12.
16. Iwao Y, Anraku M, Hiraike M, *et al*. The structural and pharmacokinetic properties of oxidized human serum albumin, advanced oxidation protein products (AOPP). Drug Metab Pharmacokinet. 2006;21(2):140-6.
17. Demirbilek ME, Kilic N, Komurcu HF, Akin KO. Advanced oxidant protein products in aged with dementia. Am J Immunol. 2007;3(2):52-5.
18. Ihara H, Hashizume N, Hasegawa T, Yoshida M. Antioxidant capacities of ascorbic acid, uric acid, alpha-tocopherol, and bilirubin can be measured in the presence of another antioxidant, serum albumin.

J Clin Lab Anal. 2004;18(1):45-9.
19. Barle H, Hammarqvist F, Westman B, *et al*. Synthesis rates of total liver protein and albumin are both increased in patients with an acute inflammatory response. Clin Sci (Lond). 2006;110(1):93-9.
20. Nicholson JP, Wolmarans MR, Park GR. The role of albumin in critical illness. Br J Anaesth. 2000;85(4):599-610.
21. Zoellner H, Hofler M, Beckmann R, *et al*. Serum albumin is a specific inhibitor of apoptosis in human endothelial cells. J Cell Sci. 1996;109 (Pt 10):2571-80.
22. Kouoh F, Gressier B, Luyckx M, *et al*. Antioxidant properties of albumin: Effect on oxidative metabolism of human neutrophil granulocytes. Farmaco. 1999;54(10):695-9.
23. James WP, Hay AM. Albumin metabolism: Effect of the nutritional state and the dietary protein intake. J Clin Invest. 1968;47(9):1958-72.
24. Soejima A, Matsuzawa N, Hayashi T, *et al*. Alteration of redox state of human serum albumin before and after hemodialysis. Blood Purif. 2004;22(6):525-9.
25. Himmelfarb J, McMonagle E. Albumin is the major plasma protein target of oxidant stress in uremia. Kidney Int. 2001;60(1):358-63.
26. Witko-Sarsat V, Nguyen-Khoa T, Jungers P, Drueke TB, Descamps-Latscha B. Advanced oxidation protein products as a novel molecular basis of oxidative stress in uraemia. Nephrol Dial Transplant. 1999;14 Suppl 1:76-8.
27. Selmeci L, Seres L, Antal M, Lukacs J, Regoly-Merei A, Acsady G. Advanced oxidation protein products (AOPP) for monitoring oxidative stress in critically ill patients: A simple, fast and inexpensive automated technique. Clin Chem Lab Med. 2005;43(3):294-7.
28. Liu SX, Hou FF, Guo ZJ, *et al*. Advanced oxidation protein products accelerate atherosclerosis through promoting oxidative stress and inflammation. Arterioscler Thromb Vasc Biol. 2006;26(5):1156-62.
29. Servettaz A, Guilpain P, Goulvestre C, *et al*. Radical oxygen species production induced by advanced oxidation protein products predicts clinical evolution and response to treatment in systemic sclerosis. Ann Rheum Dis. 2007;66(9):1202-9.
30. Baskol M, Baskol G, Kocer D, Ozbakir O, Yucesoy M. Advanced oxidation protein products: A novel marker of oxidative stress in ulcerative colitis. J Clin Gastroenterol. 2008;42(6):68
31. Shi XY, Hou FF, Niu HX, *et al*. Advanced oxidation protein products promote inflammation in diabetic kidney through activation of renal nicotinamide adenine dinucleotide phosphate oxidase. Endocrinology. 2008;149(4):1829-39.
32. Alev K, Aysun T, Hatice S, *et al*. Advanced glycation end-products and advanced oxidation protein products in patients with insulin dependent diabetes mellitus and first degree relatives. Bakırköy Tıp Dergisi. 2011;7:130-5.