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Main Article

Significance of free radical injury in laryngeal and hypopharyngeal cancers

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Abstract

Background and objectives: Laryngeal and hypopharyngeal cancer is a multifactorial disease caused by various carcinogens such as tobacco, alcohol and viruses. Likewise, oxidative stress is known to cause aberrations in the cell membrane and DNA, leading to cancer. We conducted this prospective study in order to evaluate the level of oxidative stress in laryngeal and hypopharyngeal cancer patients.

Methods: Fifty patients with laryngeal and hypopharyngeal cancer and 40 control subjects were selected. Serum malondialdehyde concentrations and ferric reducing antioxidant power were assessed, in order to evaluate oxidative stress. Results were analysed by Student's *t*-test.

Results: Malondialdehyde levels were significantly higher and ferric reducing antioxidant power lower in the cancer patients, compared with the controls, indicating higher oxidative stress in the former. There was no statistically significant difference in malondialdehyde concentration or ferric reducing antioxidant power, comparing patients with versus without neck secondaries, and patients with early stage versus late stage tumours.

Conclusion: Oxidative stress may have a role to play in the initiation of laryngeal and hypopharyngeal cancers, especially in patients with other risk factors such as tobacco and alcohol use.

Key words: Larynx; Hypopharynx; Cancer; Oxidative Stress

Introduction

Malignancies of the larynx and the hypopharynx are among the commonest cancers affecting humanity, and contribute significantly to the morbidity and mortality of those afflicted. It has now been proven that these cancers have a genetic component, with DNA mutations being the primary initiating event.[1] Such mutations can be caused by tobacco, alcohol, certain viruses, and a diet lacking micronutrients and antioxidants. Antioxidants are required to neutralise the harmful effects of free oxygen radicals, which are capable of altering cell homeostasis.

Many studies have demonstrated an association between oxygen free radicals and various cancers.[2–5] However, there are limited studies investigating the relationship between laryngeal and hypopharyngeal cancers and, firstly, increased free radicals, and, secondly, decreased total antioxidant capacity.

In this study, we attempted to evaluate the relationship between these reactive oxygen species and laryngeal and hypopharyngeal malignancies. We hoped that this research might improve understanding of the pathogenesis of these diseases, reveal new therapeutic possibilities, and contribute to a reduction in the incidence of such illness. Laryngeal and hypopharyngeal malignancies were investigated together, as they share a common anatomical site and behave similarly with respect to aetiology, clinical features and local spread.

Materials and methods

All patients with laryngeal or hypopharyngeal malignancies attending the ENT out-patient department at a tertiary care centre were included in the study, regardless of their operative feasibility.

The project was approved by the medical college ethics committee.

Our study group comprised 50 patients with laryngeal or hypopharyngeal malignancy, confirmed by direct laryngoscopy and biopsy with histopathological examination. A control group of 40 subjects was selected from individuals aged over 40 years with a history of smoking, who were free of malignancy. Patients with other malignancies or concurrent illness known independently to increase oxidative stress (e.g. diabetes or heart disease) were excluded from the study. All patients were classified by tumour–node–metastasis (TNM) staging, according

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to the cancer staging system of the American Joint Committee on Cancer.[6]

Under strict, aseptic conditions, venous blood was drawn from each patient and centrifuged at 1500 rpm to separate the serum. Lipid peroxidation was assessed by measuring serum malondialdehyde concentration, according to the colorimetric method of Satoh.[7] Total antioxidant capacity was measured by serum ferric reducing antioxidant power assay, according to the method of Benzie and Strain.[8]

Results were expressed as mean ± standard deviation. Patients' results were compared with those of controls. Results were also compared for patients with versus without nodal metastasis, and for patients with early stage (i.e. T_1 or T_2) versus late stage (i.e. T_3 or T_4) tumours, as these are the major factors determining survival.[9] Statistical analysis was performed using Student's *t*-test. Statistical significance was set at a p value of <0.05.

Results

The study included 50 patients with laryngeal or hypopharyngeal cancer (mean age 55 years) and 40 control subjects (mean age 52 years). All the study patients abused tobacco in some form (the women chewed tobacco while the men smoked it). Thirty-two study patients also abused alcohol. The control subjects used tobacco in a similar fashion. All patients and controls were from a low socioeconomic background, lived in rural areas, and had very little knowledge of the importance of diet in health.

Control subjects had a mean malondialdehyde concentration of 0.965 nmol/ml with a standard deviation (SD) of 0.208 (95 per cent confidence interval (CI) = 0.898 to 1.032), while study patients had a mean malondialdehyde concentration of 3.584 nmol/ml with a SD of 1.941 (95 per cent CI = 3.032 to 4.136); this level was approximately three times greater.

Controls had a mean ferric reducing antioxidant power of 1069 μmol/l with a SD of 143.88 (95 per cent CI = 1022.984 to 1115.015), while study patients had a mean ferric reducing antioxidant power of 526.8 μmol/l with a SD of 112.117 (95 per cent CI = 494.937 to 558.663); this level was approximately halved, that of controls (Table I).

There was no statistically significant difference in the mean malondialdehyde concentration or ferric reducing antioxidant power of patients with versus without nodal metastasis ($p > 0.5$) (Table II). Likewise, there was no statistically significant difference in either of these two parameters for patients with early stage versus advanced tumours (Table III).

TABLE I

MALONDIALDEHYDE AND FERRIC REDUCING ANTIOXIDANT POWER IN CONTROLS AND PATIENTS

Statistic	MDA (nmol/ml)		FRAP (μmol/l)	
	Controls	Pts	Controls	Pts
Mean	0.965	3.584	1069	526.8
SD	0.208	1.941	143.88	112.117
SEM	0.03289	0.2745	22.74943	15.85574
n	40	50	40	50

MDA = malondialdehyde; FRAP = ferric reducing antioxidant power; pts = patients; SD = standard deviation; SEM = standard error of the mean

TABLE II

MALONDIALDEHYDE AND FERRIC REDUCING ANTIOXIDANT POWER IN PATIENTS WITH AND WITHOUT NODAL METASTASES

Statistic	MDA (nmol/ml)		FRAP (μmol/l)	
	N_0	N_+	N_0	N_+
Mean	3.689	3.519	532.6316	523.2258
SD	1.537	2.173	131.4805	129.7019
SEM	0.35261	0.39028	30.1637	23.29515
n	19	31	19	31

MDA = malondialdehyde; FRAP = ferric reducing antioxidant power; N_0 = no nodal metastases; N_1 = nodal metastases; SD = standard deviation; SEM = standard error of the mean

Discussion

All mammals produce oxygen radicals as part of the oxidative phosphorylation process within mitochondria. These oxygen radicals, also known as reactive oxygen species, are powerful oxidising agents and induce lipid peroxidation, leading to loss of cell homeostasis through modification of the structure and functions of the cell membrane. Most importantly, lipid peroxidation results in significant damage to DNA.[10,11] This DNA damage can cause mutations that can initiate tumour formation and progression.[12,13]

In order to neutralise the deleterious effects of reactive oxygen species, mammalian cells possess elaborate antioxidant defence mechanisms.[14,15] These antioxidant mechanisms are present in the extracellular fluids, and may be from endogenous or exogenous sources; together, they are referred to as the total antioxidant capacity.

Oxidative stress is defined as an imbalance between reactive oxygen species and antioxidant defence mechanisms. There is extensive evidence of an association between oxidative stress and many cancers, such as breast cancer. In order to counteract these reactive oxygen species, individuals should consume at least five portions of fruit or vegetables each day.[5]

TABLE III

MALONDIALDEHYDE AND FERRIC REDUCING ANTIOXIDANT POWER IN PATIENTS WITH EARLY AND LATE STAGE TUMOURS

Statistic	MDA (nmol/ml)		FRAP (μmol/l)	
	Early	Late	Early	Late
Mean	4.05	3.321	545.5556	516.25
SD	2.28	1.7	170.6121	100.3462
SEM	0.5374	0.30052	40.21366	17.73887
n	18	32	18	32

MDA = malondialdehyde; FRAP = ferric reducing antioxidant power; early = tumour (T) stage T_1 or T_2; late = T_3 or T_4; SD = standard deviation; SEM = standard error of the mean

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Oxidative stress is assessed in biological samples by monitoring malondialdehyde concentration, a measure of lipid peroxidation.[16,17] Ferric reducing antioxidant power has been reported as a novel indicator of total antioxidant capacity, and is considered a useful measure of the body's ability to regulate the damage caused by reactive oxygen species.[18]

In the current study, we found that cancer patients' malondialdehyde concentrations were approximately three times greater, and their ferric reducing antioxidant power was approximately halved, compared with controls. Similar results were obtained by Dwivedi *et al.*[2] This indicates that oxidative stress may be involved in the development of these cancers.

- **Oxidative stress influences the initiation of laryngeal and hypopharyngeal malignancies in patients with other risk factors such as smoking and alcohol**
- **This study assessed serum malondialdehyde concentration and ferric reducing antioxidant power to evaluate oxidative stress**
- **The role of oxidative stress in the initiation and progression of these cancers needs to be evaluated by larger cohort studies and animal models**

However, we found no statistically significant difference in malondialdehyde concentration or ferric reducing antioxidant power, comparing patients with and without nodal metastasis. There were also no differences in either of these two parameters, comparing patients with early and late stage tumours (i.e. T_1 or T_2 versus T_3 or T_4). This may be because further progression of the cancer was independent of oxidative stress. This may also be due to the smaller sample size in our study.

Conclusion

Oxidative stress influences the initiation of laryngeal and hypopharyngeal malignancies in patients with other, well proven risk factors such as smoking and alcohol. However, the role of oxidative stress in the initiation and progression of these cancers needs to be evaluated using larger cohort studies and animal models. In addition, the role of oxidative stress in later stage tumour progression and tumour metastasis requires further evaluation. Better understanding of these interactions will help us to further establish the importance of dietary antioxidant factors in cancer protection.

References

1 Loyo M, Pai SI. The molecular genetics of laryngeal cancer. *Otolaryngol Clin North Am* 2008;**41**:657–72
2 Dwivedi RC, Raturi DP, Kandpal N, Dwivedi RC, Singh RP, Puri VN. Oxidative stress in patients with laryngeal carcinoma. *Indian J Cancer* 2008;**45**:97–9
3 Caliskan-Can E, Firat H, Ardic S, Bolkan S, Torun M, Yardin-Akaydin S. Increased levels of 8-hydroxydeoxyguanosine and its relationship with lipid peroxidation and antioxidant vitamins in lung cancer. *Clin Chem Lab Med* 2008;**46**:107–12
4 Ernst P. The role of inflammation in the pathogenesis of gastric cancer. *Alimentary Pharmacology & Therapeutics* 1999;**13**:13–18
5 Brown NS, Bicknell R. Hypoxia and oxidative stress in breast cancer: oxidative stress – its effects on the growth, metastatic potential and response to therapy of breast cancer. *Breast Cancer Res* 2001;**3**:323–7
6 Greene FL, Page DL, Fleming ID, Fritz AG, Balch CM, Haller DG, eds. *AJCC Cancer Staging Manual*, 6th edn. New York: Springer, 2002;17–88
7 Satoh K. Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. *Clinica Chemica Acta* 1978;**90**:37–43
8 Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: the FRAP assay. *Analytical Biochemistry* 1996;**239**:70–6
9 Watkinson JC, Gaze MN, Wilson JA, eds. Tumours of the larynx. In: *Stell and Maran's Head and Neck Surgery*, 4th edn. Oxford: Butterworth-Heinemann, 2000;233–74
10 Abidi S, Ali A. Role of oxygen free radicals in the pathogenesis and etiology of cancer. *Cancer Lett* 1999;**142**:1–9
11 Van-Ginkel G, Sevanian A. Lipid peroxidation-induced membrane structural alterations. *Methods Enzymol* 1994; **233**:273–88
12 Wiseman H, Halliwell B. Damage to DNA by reactive oxygen and nitrogen species: role in inflammatory disease and progression to cancer. *Biochem J* 1996;**313**:17–29
13 Suresh DR, Silvia WD, Agarwal R. Lipid peroxidation and total antioxidant capacity in patients with chronic renal failure. *Asian J Biochem* 2008;**3**:315–18
14 Klaunig JE, Xu Y, Isenberg JS, Bachowski S, Kolaja KL, Jiang J *et al.* The role of oxidative stress in chemical carcinogenesis. *Environ Health Perspect* 1998;**106**:289–95
15 Barber DA, Harris SR. Oxygen free radicals and antioxidants: a review. *Am Pharm* 1994;**34**:26–35
16 Llesuy S, Evelson P, Campos AM, Lissi E. Methodologies for evaluation of total antioxidant activities in complex mixtures: a critical review. *Biol Res* 2001;**34**:51–73
17 McCall MR, Frei B. Can antioxidant vitamins materially reduce oxidative damage in humans? *Free Radical Biol Med* 1999;**26**:1034–53
18 Pstova J, Zahalkova J, Hrbac J, Simanek V, Bartek JJ. Determination of total antioxidant capacity (TAC) in plasma by cyclic voltammetry. *Biomed Pap* 2001;**145**: 81–3

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