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Oxidant, vitamin A and angiogenic markers in laryngeal cancer patients

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Abstract

In this study the status of oxidant stress, vitamin A and angiotensin-converting enzyme (ACE) levels were evaluated in cases of laryngeal carcinoma patients from Northern India. In control subjects the levels of malondialdehyde (MDA), vitamin A and ACE were 0.23 ± 0.07 nmole/ml, 2515 ± 84 IU, and 1.4 ± 0.8 U/ml respectively. Thirty laryngeal cancer patients were divided into three groups according to the TNM classification (American Joint Committee on Cancers). In laryngeal cancer patients according to tumour size, MDA and ACE levels increased to 0.32 ± 0.04 nmole/ml and 4.7 ± 0.5 U/ml respectively and the effect was statistically significant ($p<0.01$). The correlation coefficient between different subgroups was also highly significant ($r = 0.96$, $p<0.01$). However, serum vitamin A levels decreased to 621 ± 20 IU and the effect was statistically significant ($p<0.01$). In another two groups of laryngeal cancer patients, a similar pattern of various markers was obtained. Thirty patients with laryngeal carcinoma were divided into four different groups according to nodal involvement and it was observed that in laryngeal cancer patients with no nodal involvement, ACE levels were low 3.6 ± 1.4 U/ml while patients with maximum nodal involvement had the highest levels of ACE 7.1 ± 0.18 U/ml. The correlation coefficient between different groups is highly statistically significant ($r = 0.95$, $p<0.01$). In patients with laryngeal cancer the serum MDA and vitamin A levels correlation coefficient between different groups was not significant. It is thus concluded that serum ACE might be a specific test marker for laryngeal cancer disease burden. The use of this marker enzyme for therapeutics is being planned.

Key words: Laryngeal Neoplasms; Malondialdehyde; Peptidyl-Dipeptidase A; Vitamin A

Introduction

Life originated as a result of free radical reaction, metabolic perturbations produced diseases. Oxidant stress and angiogenic hyperactivity are important factors in the progression of neoplastic disease. Free oxy-radicals produce lipid peroxidation in lipid bilayer membranes and thus, by a complex mechanism, changes in cellular metabolism and gene expression are modulated. Pro-oxidants modify a family of pro-oxidant genes, which are related to cell growth and differentiation. Ultimately DNA structures are modulated by an epigenetic mechanism.[1] These changes are observed during the initiation and maintenance phase of carcinogenesis. The initiation phase produces electrophilic forms that bind covalently to cellular DNA and other macromolecules. Promotion requires prolonged and multiple exposure to many toxic species to produce tumour growth. Protease inhibitors from a vegetarian diet produce tumour suppression.[2] Vitamin A and other retinols also provide protection from tumour progression.[3]

Several newer directions are being accumulated due to intensive research efforts in the area of the local renin angiotensin system (RAS). In a retrospective analysis Lever *et al.*[4] have reported on 5 207 patients attending a Glasgow blood pressure clinic and being treated with angiotensin converting enzyme (ACE) inhibitors. On a long-term basis, the risk of fatal cancers in the ACE treated group was much lower than in groups taking calcium channel blockers, β-blockers or diuretics. The presence of angiotensin II receptors have been well established in various organs including the endothelial cells of blood vessels. Activation of MAP kinase reverse pathways produces several proto-oncogenes (C-fos-C-jun) and these are thought to be associated with neoplastic growth of the larynx. It is possible that hyperreactivity of RAS may have a direct bearing on the pathological function of tumour growth. ACE inhibitors block the production of angiotensin II, and their AT_1 receptor mRNA has a relationship with coronary artery disease.[5] ACE gene polymorphism has been studied in the area of

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cardiovascular disease. Serum ACE levels are correlated with ACE gene polymorphism.[6] AT_1 receptors are present in cases of laryngeal squamous cell carcinomas.[7] Renin and angiotensin II AT_1 receptors are in higher concentrations in laryngeal carcinomas than in other carcinomas.[8-10] Laryngeal neoplastic disease might have some relationship with neovascularization and RAS proto-oncogenes. ACE activity in laryngeal carcinomas has not been worked on by any group therefore, serum ACE, serum malondialdehyde and serum vitamin A levels were measured in patients with laryngeal cancers and it was tried to correlate them with various stages of cancer of the larynx.

Materials and methods

Patients attending the out-patient department (OPD) or admitted to the surgical wards of Gandhi Memorial and Associated Hospitals attached to King George Medical College (KGMC), Lucknow were included in the study. The approval of the ethics committee of KGMC was obtained. The study subjects were randomized with the help of a random number table.

The present study included a control group (n = 10) of eight males and two females with a mean age 47 ± 6.2 years. Patients were classified using the TNM (tumour, node, metastasis) staging system based on clinical, endoscopic, and radiological examination according to the guidelines reported by the American Joint Committee on Cancers (AJCC).[11] The study group consisted of 30 patients with laryngeal carcinoma, 22 males and eight females average age 54.5 ± 9.8 years. Blood from control and laryngeal carcinoma patients was withdrawn from the antecubital vein in a sterilized test tube by a disposable syringe. Five ml of blood was centrifuged (5000 r.p.m. × 5 min) and the serum was separated and stored at –65°C for biochemical estimations in the Division of Pharmacology. Additionally in all 30 patients a punch biopsy was performed by biopsy forceps, a 2 × 2 mm piece from the laryngeal area was removed and was examined by the senior histopathologist. The original histopathological slides and other findings were reviewed by an expert pathologist, ear nose and throat surgeon and several post-graduate medical students and a diagnosis of squamous cell carcinoma of larynx was very well established. Group I included data from 10 control subjects. Groups II, III, IV were formed according to their tumour size, and nodal involvement stages of cancer respectively according to the TNM classification of the AJCC. Tumour size was approximated by endoscopic examination, followed by radiological examination (magnetic resonance imaging (MRI) in two and computed tomography (CT) in two cases) was performed. Tumour size ranged from 0.5 to 4 cm. Nodal measurements in their longest dimension were carried out using a Vernier caliper and were noted in cm. Eleven patients with laryngeal carcinoma did not show any lymph node involvement while 19 patients had differing extents of node involvement. There was a positive history of tobacco chewing, smoking or both. Mean years of usage was 10.8 ± 3.2 years with range (7.5 to 20 years). All the patients were from North India and their diet was the same. Haemoglobin, blood urea and blood sugar estimation was performed in both the controls and study group. Patients with associated illnesses such as diabetes, hypertension, myocardial ischaemia or infarction, renal disorders, hepatic disorders, pancreatic disorders or pulmonary diseases, which are known to alter free radical and ACE levels, were excluded from the study.

Serum ACE levels were estimated by using the tripeptide hippuryl-leucine-histidyl (HHL) as described by Leberman.[12] Results were expressed in U/ml of serum. Serum malondialdehyde was estimated by the method of Ohkawa *et al.*[13] in which release of thiobarbituric acid was measured using a spectrophotometer at 532 nm wavelength and values were expressed in nmol/ml. The serum vitamin A level was estimated using standard methods as described in the Indian Pharmacopoeia[14] and results were expressed in IU. All the results were expressed as the average value of mean ± S.E.M. Statistical analysis was done by the Student '*t*' test. Comparing the controls and laryngeal cancer patients a value of $p<0.05$ was considered to be statistically significant. The correlation coefficient between the various groups of laryngeal cancer patients were calculated by the Karl Pearson method.

Results

Serum ACE, serum malondialdehyde and vitamin A are shown in Table I. In 10 control subjects serum ACE levels were 1.4 ± 0.8 U/ml, while serum MDA levels were 0.23 ± 0.07 n mole/ml and serum vitamin A levels were 2515 ± 84 IU. In group II, 30 cases of laryngeal cancer were classified according to AJCC based on approximate tumour size as examined during endoscopic examination and radiological

TABLE I
SERUM ACE, MDA AND VITAMIN A LEVELS IN CONTROLS AND LARYNGEAL CARCINOMA PATIENTS

	ACE (U/ml)	r	MDA (nmol/ml)	r	Vitamin A (IU)	r
Group I	1.4 ± 0.8		0.23 ± 0.07		2515 ± 84	
Group II	4.7 ± 0.5**	0.96*	0.32 ± 0.04**	NS	1621 ± 209**	NS
Group III	5.2 ± 0.4**	0.95*	0.33 ± 0.02**	NS	1483 ± 263**	NS
Group IV	4.1 ± 0.4**	0.83*	0.34 ± 0.03**	NS	1728 ± 265**	NS

Group I = 10 Normal controls; Group II = 30 patients with laryngeal cancer according to tumour size; Group III = 30 patients with laryngeal cancer according to nodal involvement; Group IV = 30 patients with laryngeal cancer according to stages of cancer. Classification was based on TNM methodology.[11]
**$p<0.01$ compared with controls.
*r = significant correlation coefficient as calculated by the Karl Pearson method.

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examination in four patients. It was found that ACE levels were elevated to 4.7 ± 0.05 U/ml, as compared to 1.4 ± 0.8 U/ml in the control group and the difference between two groups was statistically significant ($p<0.01$). In Group III, 19 patients with significant nodal enlargement and 11 without nodal involvement were included and mean levels of ACE were 5.2 ± 0.4 U/ml. The difference from the control group was statistically significant ($p<0.01$). In Group IV, 30 patients were classified according to stages of cancer and the mean serum of ACE level was 4.1 ± 0.4 U/ml and the value was higher as compared to control and statistically significant ($p<0.01$). The serum ACE, MDA, vitamin A levels were measured in 30 patients in Group IV and according to various stages of tumour AJCC, it was observed that the correlation coefficient between the various stages in this group was also highly significant ($r = 0.83^*$ $p<0.01$). Vitamin A levels were lower in the patients with laryngeal cancer. Patients with nodal involvement had the lowest vitamin A levels 1483 ± 263 IU and difference from the controls was highly statistically significant ($p<0.01$). Serum ACE levels of all 30 patients with, or without, nodal involvement were further divided into four subgroups according to AJCC as shown in Figure 1. The ACE level increased with the severity of nodal involvement. In 11 patients with laryngeal carcinoma no regional lymph node metastasis were found and ACE levels were 3.63 ± 0.42 U/ml, while in seven patients metastasis of a single lymph node (<3 cm) was found and ACE levels were 4.0 ± 0.54 U/ml. In a third group of nine patients, metastasis of a single ipsilateral lymph node (>3 cm but <6 cm), to multiple ipsilateral lymph nodes (none >6 cm) or to bilateral or contralateral lymph nodes (none >6 cm) were found and ACE levels were 6.06 ± 0.45 U/ml. In the fourth group, metastasis of lymph node <6 cm were seen in three patients and ACE levels were 7.10 ± 0.10 U/ml and each group values were statistically significant ($p<0.01$) and the correlation coefficient between the different groups was statistically significant ($r = 0.935^*$ $p<0.01$).

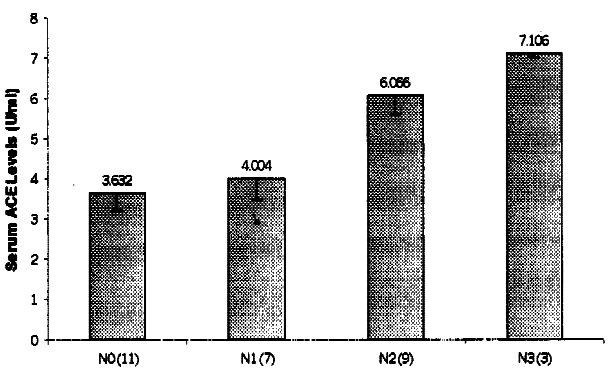

FIG. 1

Levels of ACE (U/ml) in laryngeal carcinoma patients N_0, N_1, N_2, N_3 are the stages of cancer according to nodal involvement, American Joint Committee on Cancer staging for head and neck cancer.[11] In all the four subgroups correlation coefficients between different groups (r-value 0.935 $p<0.01$) was statistically highly significant. Parenthesis denotes number of cases.

Discussion

The role of oxidant stress in several carcinomas has been worked out by several investigators.[2,15] The increased level of malondialdehyde observed in laryngeal cancer cases in our series is in agreement with the previous findings of Torun *et al.*[15] and Olemedilla *et al.*[16] However, it is interesting to note that laryngeal cancer patients were divided in different groups according to TNM classification AJCC.[11] Correlation between various groups was statistically significant. In our series vitamin A levels were lower in laryngeal cancer as compared to the controls and these findings are in agreement with several published reports[16–18] where serum retinol, tocopherol and carotenoid levels were measured and lower levels were found in cases of laryngeal cancer patients and supplementation with vitamin A by enteral formula feeding resulted in decreased oxidant stress. However, in their study Olemedilla *et al.*[16] included male patients. In our series both male and female patients were included without much difference in their oxidant stress and vitamin A level during the active disease process. The beneficial effect of a diet rich in retinol and vitamin A have been reported by Verma *et al.*[13] We are planning to enlarge our studies to study the therapeutic benefits provided by vitamin A or a retinol-rich diet on the disease process and morbidity and mortality patterns of laryngeal cancer patients.

We have observed in our patients that ACE; an important enzyme associated with cancer disease has shown increased levels in the serum. Lever *et al.*[4] and His *et al.*[19] have reported that long-term use of ACE I protects against the cancer. Our finding gives credence to the reported findings in hypertensive patients that neoplastic disease conditions are associated with neovascularization leading to increased activity of ACE. The role of AT_1 receptors in laryngeal carcinomas[7] and mitosis modulation and gene expression by ACE I in pancreatic cancer cells has been reported by Reddy *et al.*[10] These findings have relevance as the local renin angiotensin system has been reported to play an important role in the disease process. It is thus conceivable that increased activity of this enzyme might be responsible for spread of disease. Three types of ACE gene polymorphism have been reported in cases of myocardial infarction (MI) by Cambian *et al.*,[6] it is possible that laryngeal cancer patients might also have differences in ACE polymorphism. In our study the cases were divided into three groups according to TNM classification and it was observed that serum ACE levels were increased and a statistically significant correlation coefficient ($r = 0.93^*$ $p<0.01$) was observed in patients with nodal involvement. Therefore, it could be concluded that serum ACE levels could be a good marker for expressing the severity of laryngeal cancer. It is planned to start a randomized double blind clinical trial to investigate the role of ACE inhibitors on the disease process of laryngeal cancer patients.

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- **This study evaluates the status of oxidant stress, vitamin A and angiotensin converting enzyme (ACE) levels in cases of carcinoma of the larynx and control subjects**
- **The study concludes that laryngeal cancer patients have increased serum ACE levels, higher oxidant stress and lower vitamin A levels**

It is thus concluded that laryngeal cancer patients have increased serum ACE levels, higher oxidant stress and lower vitamin A levels. Additional studies from different centres will help to define further the role of RAS in the neoplastic disease process.

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