Research Article

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ROLE OF PURINE METABOLIC ENZYMES AND VITAMIN A IN PATIENTS WITH ORAL CANCER

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ABSTRACT:

Aims: - Oral cancers account for 9.6 % of all head and neck cancers in India. The disease process has better prognosis if diagnosed in its early stages. In view of this the aim of the study was to assess the reliability of purine metabolic enzymes adenosine deaminase, 5'- nucleotidase and non-enzymatic antioxidant vitamin A in patients with oral cancer as supportive parameters for diagnostic purpose. Materials and Methods: - 25 clinically and histopathologically confirmed patients of oral cancer in age group of 45-75 years were included in the study and 25 healthy subjects with in the same age group served as controls. Results: -The levels of serum ADA and 5'-NT were significantly elevated in oral cancer patients in comparison to controls whereas vitamin A levels were significantly decreased. Conclusion: -These biochemical parameters may be used as supportive parameters for diagnostic purpose and may add further for prognostic information. Further studies are required on a larger sample size to correlate between oxidative stress and release of membrane bound enzymes ADA and 5'-NT in circulation along with strategies by which normal levels of anti-oxidants can be maintained by incorporation of a vitamin A rich diet to reduce morbidity and mortality due to cancer.

KEYWORDS:

Adenosine deaminase, 5'-nucleotidase, oral cancer, vitamin A, anti-oxidants

INTRODUCTION

Carcinoma of the oral cavity has been described as early as 600 B.C. by the ancient Indian physician Sushruta. Its frequency seems to be on an increase in the recent years. It is the fifth most common cancer worldwide and in India it makes up 9.4% of all head and neck cancers [1]. Its high prevalence may be due to the use of tobacco in various forms, consumption of alcohol and low socioeconomic condition related to poor hygiene, poor diet or infections of viral origin. Despite therapeutic and diagnostic progress in oncology over the last few decades, the prognosis of intraoral squamous cell carcinoma still remains poor. Latest serum markers emerging for detection of patients with squamous cell carcinoma of various head and neck cancers is SCC antigen[2] and Cyfra 21-1[3]. Other markers include

EGFR, p53, NSE, ERA (MOC-31), Ki-67, Cyclin D1, bcl-2 these may help differentiate squamous from other tumours and also be useful in prognostication [4]. The analytical methods involved for these are however tedious and unapproachable for the general population since they are available only at sophisticated, well equipped centres and are expensive. Therefore there is need for simple biochemical investigations which can be easily assayed are less expensive and can detect metastasis. Adenosine deaminase is involved in the catabolism of purine bases and catalyses the deamination and hydrolytic cleavage of adenosine irreversibly converting it into inosine and ammonia [5]. It is widely distributed in human tissues, highest being in the lymphoid tissue. The activity of the ADA enzyme is subjected to changes, depending upon the degree of activity of the cell. Increased ADA activity during rapid and stimulated growth of normal tissues is of importance in making a fully functional purine salvage pathway possible [6]. An increased serum ADA level is associated with various tumours like liver cancer [7], breast cancer [8], colorectal cancer [9], gastric cancer [10], and ovarian cancer [11] etc. ADA is also considered as a sensitive marker for tuberculosis [12]. 5'-NT is an ecto-enzyme in many tissues and is concerned with the conversion of AMP into adenosine. This activity is probably part of a metabolic pathway for removing extracellular adenine nucleotides released during processes such as neuro-transmission, strenuous exercise, platelet thrombus formation and shock [13]. 5′-NT is an important enzyme participating in the purine and DNA metabolism. Its levels are found to increase in hepato-biliary disorders [14]. 5′-NT activities have found to be increased (Ozturk et al), unchanged (Durak et al) or decreased (Camici et al) in some cancerous tissues and cell systems. Decreased activity was evaluated as an attempt of the cancer cell to preserve the mononucleotide pool, and an increased activity was mostly evaluated as an attempt to supply salvage pathway activity. Reactive oxygen species have been implicated in progression of neoplastic disease. Oxidative stress occurs when there is an imbalance between the production of reactive oxygen species and a cell's oxidant capacity or when there is a decrease in this capacity. This stress may cause mutagenesis, cyto-toxicity and changes in gene expression that initiate or promote carcinogenesis. Protease inhibitors from a vegetarian diet produce tumour suppression. Vitamin A also plays a protective role against cancer by scavenging free radicals and reducing nitrate. In a study conducted by Ramaswamy PG and his co-workers, vitamin A levels were estimated in various epithelial cancers. In cancer of the oral cavity, the males showed significantly lower levels of vitamin A compared with their female counterparts [15]. In view of this the present study was undertaken to assess the clinical utility of enzyme markers namely adenosine deaminase and 5′-nucleotidase and non-enzymatic antioxidant vitamin A in oral cancer patients.

MATERIALS AND METHODS

The present cross-sectional study was conducted on patients with oral cancer attending the outpatient department of Otorhinolaryngology and Head and Neck Surgical Oncology of KLES Dr. Prabhakar Kore Hospital and Medical Research Center, Belgaum during a period of one year. Ethical clearance was obtained from the ethical clearance committee of Jawaharlal Nehru Medical College, Belgaum. Twenty five cases of oral carcinoma in the age group of 45-75 years were compared with twenty five healthy controls of the same age group. Routine clinical and laboratory examination was done and diagnosis confirmed histopathologically, with staging as per TNM classification. Patients with acute or chronic infections, cancers of other parts of the body or those who had received any previous treatment for malignancy were excluded from the study. Written informed consent was obtained from the participants in the study. 5ml of venous blood was collected from the antecubital vein under aseptic precautionary measures using disposable syringe. The whole blood was allowed to clot and serum was separated by centrifugation and stored at 4°C. The estimation of serum adenosine deaminase, 5'-nucleotidase and vitamin A was carried out within 48 hours. Serum adenosine deaminase was estimated by method of Galanti and Giusti [16], 5'-nucleotidase by method of Campbell [17] and vitamin A by method of Bessey OA et al [18].

STATISTICAL ANALYSIS

Statistical analysis of the obtained parameters in patients with oral cancer and control groups was done using student's unpaired t test. The mean and standard deviation (S.D) for each of the outcome was computed. The comparison between controls and various stages of laryngeal cancer subjects was done by analysis of variance (ANOVA) followed by Bonferrine multiple comparison tests. A p value of < 0.05 was considered as statistically significant.

RESULTS AND DISCUSSION

The mean (±SD) age of cases and controls was 56.0 ± 8.35 years and 54.52 ± 8.48 years with in the range of 45-75 years respectively. In the present study out of the 25 cases of oral cancer, 22 (88%) were males and 3 (12%) were females. This is in consistence with findings of Neville [19] who stated that males have a higher

incidence rate than females at all age levels. High proportion of cases among males may be due to high prevalence of tobacco and alcohol consumption habits among males. Moreover, tobacco is consumed in both chewing and smoking form in males whereas in our society females indulge lesser in tobacco and alcohol consumption. Variable distribution of cancer at various sites in different populations suggests differences in habits and exposure to the risk factors. In the present study out of 25 cases of oral cancer, 8 cases (32%) had lesions on the buccal mucosa (**Table No.1**). Most of these patients had combined habits (**Table No.2**). Carcinoma of the buccal mucosa, lateral border of the tongue and vestibule are frequently seen in betel quid chewers because the quid is placed in the mouth compressed against the buccal mucosa or tongue. According to the staging of oral cancer done by considering the tumour status and nodal status, 5 of the patients were in stage IV, 8 in stage III, 11 in stage II, and 1 were in stage I. The majority of the patients studied, were in stage II (**Table No.4**). **Table No.3** shows mean and SD of serum ADA, 5'-NT and vitamin A in control and oral cancer patients. Mean ADA and 5'-NT levels were significantly increased in patients with oral cancer whereas vitamin A levels were significantly decreased. Serum ADA levels were estimated in various stages of oral cancer and compared with controls. There was a statistically significant increase in serum ADA levels as disease progressed from stage II to stage IV (**Table No.4**). An inter-stage comparison of serum ADA level was done by Bonferrine multiple comparison test which showed a significant increase in levels of ADA when compared between stage II and stage III with p value of 0.001, between stage II and stage IV with p value of 0.000 and between stage III and IV with a p value of 0.000 (Table No.5). A significant increase in serum 5′-NT was also observed in Stage II, III and IV when compared to controls with p value < 0.000 (**TableNo.4**). An inter-stage comparison of serum 5′-NT levels showed a significant increase in levels of 5′-NT when compared between stage II and stage III with p value of 0.004, stage II and stage IV with p value of 0.000 and between stage III and IV a p value of 0.001(**Table No.6**). Major function of extracellular enzymes related to nucleotide metabolism appears to be termination of the physiological action of nucleotides released from cells. Nucleotides represent the ubiquitous class of cell to cell signalling substances eliciting physiological response in every tissue. ATP, ADP exerts their function via surface located receptors and are involved in a large variety of physiological and path physiological functions [20]. Studies on ADA the crucial enzyme involved in adenosine degradation reveal that its activity differ from tumour to tumour as well as tumour and pre-tumour normal tissue [21]. Poorly differentiated cancers with elevated ADA activity grew faster than well differentiated ones with low ADA activity [22]. The results of our work indicate an increase in activity of purine salvage enzymes ADA and 5'-NT in oral cancer patients compared to controls. Our results are in accordance with the findings of Borzenko BG [23], Harbans Lal [24], Ashok KJ [25], R. Mishra [26], Orhan Canbolatal [27] and Kalcioğlu MT [28]. 5'-NT has been regarded as linked to maturation and its determination in different types of leukaemia leads to its use in typing cells, early diagnosis, monitoring of the disease and chemotherapy as its potential clinical application [29]. Elevation of ADA and 5'-NT activities together was suggested, as a physiological attempt of cancer cells to provide more substrate needed by them to accelerate the salvage pathway activity. Adenosine is produced at increased levels in the tissues of solid cancers as a result of local hypoxia. Adenosine inhibits the cell-mediated anti-tumour immune response, promotes tumour cell migration and angiogenesis and stimulates the proliferation of tumour cells. As a result activities of serum ADA and 5'-NT were increased to detoxify high amounts of toxic adenosine and deoxyadenosine substrates produced from accelerated purine metabolism in the cancerous tissues. A significant decrease in serum retinol was observed in StageII, III and IV when compared to controls with p value < 0.000 (**Table No.4**). An inter-stage comparison of serum retinol levels showed a significant decrease in levels of retinol when compared between stage II and stage III with p value of 0.006, between stage II and IV with p value of 0.000 and between stage III and IV the p value of 0.056 (Table No.7). These observations were in accordance with findings of Pillai R [30] and Khanna R [31] who stated that decrease in vitamin A levels in cancer could be due to the increased utilization by the affected tissues or in combating the excessive oxidative stress in circulation as a result of cancer. Geert van Poppel et al [32] concluded that smoking reduced β-carotene concentrations. In a study conducted by Nicholas Wald [33] it was suggested that serum retinol levels in man have predictive value for subsequent cancer, low levels being most clearly associated with an increased risk of lung cancer and gastro intestinal tract cancers. The results of the study conducted by Manoharan S and his co-researchers showed increased lipid peroxidation in oral cancer patients as a consequence of free radical generation [34]. By products of lipid peroxidation cause marked alteration in the structural integrity and function of cell membranes. Pratibha K [35] and co-investigators studied the increased activity of ADA in acute infective hepatitis

and concluded that lipid per-oxidation is followed by loss of structural integrity of plasma membrane. As a result, there occurs a release of membrane associated enzymes ADA and 5'-NT into the circulation. Enhanced lipid per-oxidation with decline in antioxidants has been reported in the venous blood of oral cancer patients. The extent of oxidative damage caused by ROS can be exacerbated by a decreased efficiency of antioxidant defence mechanisms of the body. Measurement of malondialdehyde (MDA) in this study therefore would have been useful to state lipid peroxidation as one of the possible causes of oral cancer progression associated with decreased antioxidant levels and to correlate it with the release of membrane associated enzymes ADA and 5'-NT into the circulation.

Table No. 1:- SITE OF LESION IN ORAL CANCER PATIENTS

Site	No. of cases	Percentage
Buccal mucosa	8	32
Tongue	7	28
Hard palate	4	16
Lip	3	12
Floor of mouth	2	8
Alveolus	1	4
Total	25	100

Table No. 2:- TYPES OF HABITS FOUND IN ORAL CANCER PATIENTS

Habits	No. of Cases	Percentage
Tobacco and betel quid chewing	7	28
Smoking	4	16
Alcohol	0	0
Combined	14	56
No habits	0	0
Total	25	100

Table No. 3:- SERUM ENZYMES AND VITAMIN A LEVELS IN CONTROL AND ORAL CANCER PATIENTS

GROUPS	ADA (U/L)	5′-NT (U/L)	Vitamin A (µg/dl)
Control (n = 25)	19.8 ± 4.25	8.7 ± 4.15	41.1 ± 9.37
Oral cancer cases (n = 25)	41.1 ± 8.77*	49 ± 10.45*	22.3 ± 5.81*

*p-value < 0.0001

Table No.4:- SERUM ENZYMES AND VITAMIN A LEVELS IN CONTROLS AND DIFFERENT STAGES OF ORAL CANCER

Enzymes and Vitamin A	Control		Stage II (n = 11)		Stage III (n = 8)		Stage IV (n = 5)	
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
ADA(U/L)	19.8	4.25	34.8*	3.31	42.8*	4.61	54.4*	4.87
5'NT(U/L)	8.7	4.15	41.7*	3.32	51.0*	7.69	64.6*	4.27
Vitamin A (µg/dl)	41.1	9.37	25.7*	3.76	20.3*	3.25	15.4*	2.31

*Only one case of oral cancer was found in Stage I of the disease due to which it was not included in the statistical analysis. *p < 0.000 statistically significant

Table No.5:- COMPARISON OF ADA BETWEEN DIFFERENT STAGES OF ORAL CANCER.

STAGE (I)	STAGE (J)	MEAN DIFFERENCE (I-J)	t-value	p-value
II	III	-8.0	4.118	P = 0.001
	IV	-19.6	8.691	P = 0.000
III	IV	-11.6	4.867	P = 0.000

Table No.6:- COMPARISON OF 5'-NT BETWEEN DIFFERENT STAGES OF ORAL CANCER

STAGE (I)	STAGE (J)	MEAN DIFFERENCE (I-J)	t-value	p-value
II	III	-9.3	4.182	p = 0.004
	IV	-22.9	8.872	p = 0.000
III	IV	-13.6	4.985	p = 0.001

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Table No.7:- COMPARISON OF VITAMIN A BETWEEN DIFFERENT STAGES OF ORAL CANCER

STAGE (I)	STAGE (J)	MEAN DIFFERENCE (I-J)	t-value	p-value
II	III	5.4	1.610	P > 0.05
	IV	10.3	2.646	P = 0.000
III	IV	4.9	1.191	P > 0.05

CONCLUSION

In conclusion the present study shows a significant increase in serum levels of purine metabolic enzymes ADA and 5'-NT and decrease in levels of non-enzymatic anti-oxidant vitamin A in patients with oral cancer. These biochemical parameters are inexpensive, rapid and can easily be analyzed in smaller laboratories not exposed to sophisticated technology. Further studies on oxidative stress are required for correlating with release of membrane associated enzymes like ADA and 5'-NT into the circulation along with strategies by which normal levels of anti-oxidants can be maintained by a vitamin a rich diet which may play a significant role in reducing the morbidity and mortality due to cancer.

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