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EVALUATION OF NITRIC OXIDE LEVELS AND ARGINASE ACTIVITY IN ORAL CANCER PATIENTS

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ABSTRACT

Background: Cancer of Oral cavity is an important cancer globally. It remains a major cause of mortality and morbidity throughout the world. In India, because of cultural, geographic factors and the popularity of addictive habits, the frequency of oral cancer is high. Patients with malignant tumors induce various degrees of metabolic derangements. Substantial information has been obtained in the past decades on the role of arginine in tumor growth. Arginine has several important benefits including promotion of protein/collagen synthesis, wound healing and support of the immune system. Disturbances in arginine metabolism possibly contribute to events that lead to cancer cachexia. The net beneficial or negative effect depends on how arginine is metabolized and the strength of the activities of each of these arginine-catabolizing enzymes: Nitric Oxide Synthase and Arginase. Relative changes in these enzymatic activities serve as major determinants of Nitric Oxide (NO•) production. In view of this, the present study was planned to estimate the serum levels of NO• and arginase activity in oral cancer patients and to compare them with age, sex matched healthy controls.

Material & Methods: Fifty histopathologically proved cases of oral cancer from any stage (Stage I to stage IV) in the age group of 40-75 years admitted in Sassoon Hospital, Pune and fifty age, sex matched healthy controls were recruited for this study. Intravenous blood sample was obtained to evaluate study parameters. Arginase activity was estimated by Roman and Ray method while NO• levels were measured by Cortas & Wakid method.

Results: The results of this study showed significant increase in the activity of arginase when compared with controls and activity was found to be significantly increased in stages III+IV when compared with stages I+II ($p < 0.01$). Nitric oxide level was found to be significantly increased in oral cancer condition when compared to normal individuals ($p<0.01$). Comparison of nitric oxide levels between stages I+II and stages III+IV showed further rise ($p < 0.05$).

Conclusion: We can conclude that both nitric oxide and arginase might be playing important role in carcinogenesis and tumor progression in oral cancer patients. The estimation of these two parameters can give additional insight regarding disease progression.

Key Words: Arginase, Nitric Oxide, Oral cancer

INTRODUCTION

Oral cavity cancer is an important cancer and is one of the ten most frequent cancers worldwide. In India, because of cultural, ethnic, geographic factors and the popularity of addictive habits, the frequency of oral cancer is high (1). It ranks number one in terms of incidence among men and third among women. Several factors like tobacco and tobacco related products, alcohol, genetic predisposition and hormonal factors are suspected as possible causative factors (1, 2). Chronic inflammation is a risk factor for cancer. Cancer-related alterations in metabolism of the host are an important factor in determining mortality. Evidence is accumulating that the amino acid arginine is of importance in cancer. Arginine is one of the 20 amino acids found in protein with numerous roles in cellular metabolism. Arginine has been shown to have several important benefits, including promotion of protein/collagen synthesis, wound healing and support of the immune system through nitric oxide production. Thus, both arginine and its product nitric oxide ($NO^{\bullet}$) are important mediators in the defense against tumor cells, because both influence T cell–mediated immunity (3, 4), cytokine induction, and macrophage-mediated tumor toxicity (5).

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Nitric oxide is a pleiotropic ancestral molecule, which elicits beneficial effect in many physiological settings but is also tenaciously expressed in numerous pathological conditions. NO• plays multiple roles in both intracellular and extracellular signaling mechanisms (6). This highly reactive molecule is produced in the body by the three isoenzymes of nitric oxide synthase (NOS) using L-arginine as a substrate. NO• is either cytostatic or cytotoxic, interacting with a number of molecular targets within cells. Cells within different tissues display varying responses to NO•. Chronic inflammation causes overexpression of NOS leading to genotoxicity. NO• may mediate DNA damage through the formation of carcinogenic nitrosamines, generation of RNS and inhibition of DNA damage repair mechanism. It can thus be considered as a tumor initiating agent. However, NO• may also have an impact on other stages of cancer development ranging from cellular transformation and formation of neoplastic lesions to the regulation of various other aspects of tumor biology (7). NO• plays an important role in host defense and homeostasis when generated at a low level for a brief period of time, but becomes genotoxic and mutagenic when generated at higher concentrations for prolonged periods of time. Thus, the biological outcome of the NO• mediated effects is complex and depends on the internal and external environment of the target and generation sites of the cells as well as the concentration of NO• generated (8).

Besides, various malignant tumor tissues contain considerable amount of the enzyme arginase (9), which converts arginine to ornithine and urea. L-ornithine is the precursor of polyamines, which are essential components of cell proliferation. It was recently shown that the high arginase activity of tumors is a mechanism of tumor-induced immunosuppression through depletion of arginine concentrations in the microenvironment of the tumor (10). Arginase reflects the type of inflammatory response in a specific disease process.

Arginine being the common substrate for both arginase and nitric oxide, the prevailing route determines the fate of arginine in tumor promotion or regression.

With this background the present work was undertaken to estimate serum levels of arginase and nitric oxide in oral cancer patients and to compare them with age, sex matched healthy controls.

MATERIAL AND METHODS

The study was carried out at the department of Biochemistry, B. J. Medical College after the approval from institutional ethical committee.

Study group: Included total of 50 subjects with oral cancer in the age group of 40-75 years. These patients were divided as stage I, II, III, IV. Stages were grouped as initial stage = I+II and final stage III+IV. All the cases were clinically diagnosed and histopathologically proven for oral cancer.

Control group: Fifty age, sex matched healthy adults without oral cancer were included.

Exclusion criteria: Subjects with other systemic diseases, taking chemotherapy, radiotherapy any medications/antioxidant supplementation will not be included in the study.

A detailed case history of the patient was taken, with an informed consent which was duly signed by each patient.

Collection of serum: 5ml of intravenous blood samples of the subjects was collected, centrifuged to separate the serum and stored at -80ºC till the analysis is done.

Estimation of serum arginase activity by Roman and Ray method (11)

Ninhydrin reacts with ornithine formed by arginase action in the presence of $MnCl_2$ giving a pink coloured ornithine-ninhydrin complex which is read spectrophotometrically at 530nm. Concentration was determined using standard graph.

Estimation of serum nitric oxide (NO_2 +NO_3) levels by Cortas and Wakid method (12)

Nitric oxide concentration was measured as total nitrates and nitrites (NO_2 +NO_3) by the Cortas and Wakid method. Absorbance was read at 545nm. Concentration was determined using standard graph.

Statistical analysis: Results are presented as mean ± standard deviation value and statistically analyzed by ANOVA, Dunnett t (2-sided) Post Hoc tests and Student's unpaired 't' test. A 'p' value of 0.05 or less was considered significant.

RESULT

The present study involved the estimation of arginase activity and levels of nitric oxide in oral squamous cell carcinoma patients (initial stage I + II= 25, final stage III + IV = 25) and their comparison with normal individuals (N=50). We even compared stage III+IV results with stage I+II. The results are expressed in Table-I.

In this study serum arginase activity was found to be significantly increased ($p<0.01$) in oral cancer condition (initial stage and final stage) when compared to normal individuals. When the activity in stages III+ IV is compared with stages I+II, significant rise is observed ($p<0.01$). Nitric oxide level was found to be increased significantly in oral cancer patients (initial stage and final stage) when compared to normal individuals ($p<0.01$). Comparison of nitric oxide levels between stage I+II and stage III+IV showed significant rise ($p< 0.05$).

DISCUSSION

Research over the last couple of years has convincingly demonstrated a crucial role for arginase and nitric oxide in tumour immunobiology. The balance between cell death and cell proliferation determines tumour growth rate and even a small alteration in these parameters can be important for the expansion or regression of malignant tumours.

A high arginase level in oral cancer patients compared to controls was possibly observed due to its release into the serum from tumor. Increased arginase activity (Table I, $p<0.01$) contributes to increase in polyamine production than normal through increased ornithine production. High levels of intracellular polyamines are correlated with high grades/stages of many human cancers as increased polyamine production increases rate of cell proliferation for tumor growth. Many studies have showed that the polyamines are directly linked to cellular proliferation, differentiation, and cell death (13, 14).

Our results are supported by Hegde et al (8), Choudhury B et al (15) who reported a significant rise in arginase activity in oral cancer patients.

It was shown recently by Vissers et al (9), Rodriguez et al (16) and Kaplan et al (17) that increased arginase activity in tumor induces arginine deficiency in the microenvironment of the tumor, which enables tumor to escape the immune response.

Arginase activity in stages III + IV patients is further increased when compared with stages I+II (Table I, $p<0.01$).

As the stage advances, the *n*eed for polyamine by tumor cells increases. A considerable body of research has provided convincing evidence for a role of polyamines in tumor cell growth and in the biological response of tumor promoters and growth factors. Studies have demonstrated that the biosynthesis of polyamines increase in a variety of tumors other than in normal tissues (13, 14). This is reflected by increase in arginase activity. It may be the reason for increased arginase activity with the stages.

The study carried out by Hegde et al (8) and Chaudhary et al (15) in oral cancer patients did not mention stagewise changes. Stagewise study on arginase carried out by Kaplan et al (17) in esophageal cancer patients and Mahmoud et al (18) in breast cancer patients also showed increase in arginase activity in stages III+IV than I+II.

Apart from arginase, arginine can be converted by nitric oxide synthase (NOS) to citrulline and $NO^{\bullet}$. Nitric oxide is a highly reactive molecule to which many of the functions of arginine are ascribed and has been shown to be cytostatic/cytotoxic for tumor cells.

Highly significant rise of $NO^{\bullet}$ levels in oral cancer patients compared with controls (Table I, $p<0.01$) suggest that chronic inflammation due to inflammatory molecules released by cancer cells in the blood can lead to increased $NO^{\bullet}$ synthesis from plasma arginine. Same results were obtained by Hegde et al (8), Ratajczak-Wrona et al (19) and Avci A et al (20) in oral cancer patients.

The mammalian immuno-defense network is involved in tumour suppression, and macrophages are an important part of this process because of their ability to destroy selectively a broad range of tumour types upon specific activation. Numerous studies (19, 21, 22) have shown that cytokine activated macrophages can generate large concentrations of $NO^{\bullet}$ by up-regulation of expression of the inducible nitric oxide synthase gene (iNOS). The $NO^{\bullet}$ generated by this process is capable of killing a range of tumour cells

Role of nitric oxide in cancer biology is complex. Several lines of investigation suggest that $NO^{\bullet}$ is involved in the initiation of numerous cancers. High levels of $NO^{\bullet}$ may modify DNA directly or indirectly by inhibiting DNA repair activities, modifying the proteins by the nitration of phenolic amino acids (2, 7). A body of evidence indicates a definite role for $NO^{\bullet}$ in tumor growth.

As $NO^{\bullet}$ and arginase share a common substrate, inhibition of one enzyme may augment the activity of the other enzyme. But in oral cancer patients alongwith arginase we observed significant rise of $NO^{\bullet}$ levels in stage III+IV patients compared with stages I+II (Table I, $p<0.05$).

One of the causes of high concentrations of total $NO^{\bullet}$ in the serum of patients in the advanced stage of the disease could be secretion of $NO^{\bullet}$ by cells of the immune system. Jablonska et al (22) demonstrated higher expression and concentration of inducible nitric oxide synthase (iNOS), the enzyme responsible for the generation of $NO^{\bullet}$, in polymorphonuclear cells and peripheral blood mononuclear cells in oral cancer patients. $NO^{\bullet}$ generated by NOS (located either within the tumour or in the surrounding stroma) may promote new blood vessel formation by up-regulating VEGF. This neovasculaturization not only enhances the ability of the tumour to grow, but also increases its invasiveness and metastatic ability.

CONCLUSION

In the present study, it was found that serum arginase activity and nitric oxide levels in patients with oral cancer were significantly raised than those of the control group. This finding suggests that the arginase enzyme and $NO^{\bullet}$ might be playing a role in the progression of oral cancer, and that arginase activity and $NO^{\bullet}$ levels might be used as markers for the diagnosis of oral cancer.

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Table I: Shows changes in the activity of arginase and nitric oxide levels in oral cancer patients and controls.

	Controls (n=50)	Stage I+II (n= 25)	Stage III+IV (n= 25)
Arginase (IU/L)	2.72 ± 1.35	10.97 ± 3.65*	15.69 ± 5.20*#
Nitric Oxide (µmole/L)	53.81 ± 5.75	81.75 ± 9.66*	88.77 ± 11.65*##

Comparison with control: *- p<0.01
Comparison of stage III+IV with stage I+II: #- p<0.01, ##-p<0.05