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Comparison of Polymorphonuclear Leukocyte Functions in Oral Carcinoma Patients and Healthy Controls

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ABSTRACT

To compare the phagocytic activity and intracellular killing activity of Polymorphonuclear Leukocytes in peripheral blood of patients with Oral Squamous Cell Carcinoma and healthy controls. Study comprised of thirty patients with histologically proven squamous cell carcinoma of the oral cavity and ten controls that were age - and sex - matched healthy non - hospitalized patients that included five smokers and five non - smokers. Phagocytosis and intracellular killing activity were assayed. Student's t- test (SPSS version 11.0) and Anova were used to compare the healthy patients and oral carcinoma patients. Present study demonstrated suppression in both phagocytic as well as intracellular killing activity of PMNs in patients with oral squamous cell carcinoma, when compared to that of healthy controls. A significant suppression of both phagocytosis and intracellular killing of candidal species by neutrophils was observed in the patients with Oral cancer. The relatively low candidacidal activity of neutrophils is suggestive of cellular immunosuppression which is often induced in various malignancies.

Keywords: Squamous cell carcinoma, Polymorphonuclear leukocytes, Phagocytosis, Intracellular killing activity.

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INTRODUCTION

Tumours of the head and neck comprise an important group of neoplasia, the incidence of which is increasing in many parts of the world. This increase remains high, despite all the advances in modern medicine. Squamous cell carcinoma of the orofacial region is a potentially life-threatening malignant epithelial neoplasm representing more than 90% of all head and neck cancers. [1, 2]

Immune mechanisms have often been implicated as possible factors in the restriction of neoplastic proliferation and there is as an impressive accumulation of evidence to support the concept of immunological surveillance in neoplasia. It has been found that patients with reduced immunological competence (either congenital or iatrogenic) show an increased incidence of neoplasms.[3]

Patients with cancer often have accompanying defective cellular and humoral immunity. In patients with malignant neoplasm's, lymphocyte responsiveness is impaired not only against the tumour cells, but also to non-specific mitogens. Monocyte (MN) functions show decreased chemotaxis in most of the patients with cancer. [4, 5]

An immunodeficiency may be quantitative as seen when there is inadequate production of neutrophils. Alternatively, immunodeficiency may be qualitative as seen when there are normal numbers of neutrophils but they are defective in function. Immunodeficiency may be inherited, in which case it is categorized as a 'primary immunodeficiency'.

Many investigations have revealed that neutrophil phagocytic ability and intracellular killing capacity are impaired in oral malignancies. Many investigators believe that malignancies of the oral cavity may be associated with a defect in peripheral blood as well as salivary neutrophils. These defects may either be inherent prior to the development of the malignancy or may be acquired following radiotherapy for the malignancy in question.

The immune system appears to play a significant role in the pathogenesis of cancer and host response to cancer. The objective of this present study was to estimate the function of cells of the immune system, specifically with regard to phagocytosis and intracellular killing activity by peripheral blood neutrophils in patients with Oral Squamous Cell Carcinoma. The validity of the defense function of neutrophils as an indicator of the immune status in oral cancer is considered.

MATERIALS AND METHODS

The present study of a "Comparison of Polymorphonuclear Leukocyte Functions in Oral Carcinoma Patients and Healthy Controls" was carried out in the Department of Oral Pathology and Microbiology, Manipal College of Dental Sciences, Mangalore. This study was been performed after obtaining approval from the Institutional Ethics Committee.

The clinical material for this study comprised of 30 patients with histologically proven squamous cell carcinoma of the oral cavity, who were not subjected to previous treatment. Controls included ten subjects who were age- and sex - matched healthy, non - hospitalized patients of whom five were smokers and five were non - smokers. Patients with severe systemic diseases like Diabetes Mellitus, Coronary heart disease, Tuberculosis, patients who had undergone radiation therapy and /or patients who had chemotherapy were excluded from the study.

An informed consent was obtained from the study subjects following an interview conducted by the investigator. Following selection of the study sample, data was recorded on a structured proforma designed specifically for the study.

Phagocytic function of Neutrophils was evaluated by following the uptake of *Candida albicans*. This was done by incubating the cell suspension with *Candida albicans* at 37°C for 20-25 minutes followed by counting the number of *Candida albicans* ingested by each neutrophil as visualized by Gram stain.

Preparation of *Candida albicans* suspension:

The *Candida albicans* was grown on Sabouraud's 2% dextrose agar and subcultured on glucose broth for 48 hours at 37°C to obtain organisms in the yeast phase only. The cultures were washed thrice using phosphate buffered saline (PBS) by spinning at 1500 rpm for 10 minutes in a centrifuge.

Isolation of Polymorphonuclear Leukocytes [6]

10 ml of peripheral venous blood was drawn from the cubital vein and transferred into a Heparinized Vacuotainer tube. 5 ml of dextran- saline solution was added to 10 ml blood, mixed gently, and erythrocytes were allowed to sediment at room temperature for 45-60 minutes. The supernatant plasma layer was removed and transferred to a sterile test tube and centrifuged at 500 rpm for 10 minutes to obtain a cell pellet. The cell pellet was suspended in Hank's balanced salt solution. The WBC's were washed thrice in sterile Hank's balanced salt solution. 2 ml of leukocyte cell suspension was mixed with 2ml candidal suspension (maintaining an optimal cell: candidal ratio of 1:1) and the mixture was incubated in plastic-capped test tubes in a Shaker water bath at 37 °C for 15 minutes. A drop of this mixture was placed on a slide and a smear was made and stained with Gram's stain.

100 neutrophils were examined for phagocytosis and intracellular killing activity; both dark blue staining (living) organisms and pale pink (degraded/dead) organisms were counted.

Calculation of Phagocytic index

$$\frac{\text{Number of PMNs containing Candida}}{\text{Total number of PMNs counted}} \times 100$$

Calculation of intracellular killing

$$\frac{\text{Number of PMNs containing dead organisms}}{\text{Total number of PMNs counted}} \times 100$$

RESULTS

The study compared the phagocytic activity and intracellular killing activity of polymorphonuclear leukocytes in peripheral blood of patients with Oral Squamous Cell Carcinoma with that of healthy non-smokers and smokers.

Table 1: Phagocytic activity among study group versus controls

Subjects	Phagocytic Activity					
	Mild		Moderate		High	
	No:	%	No:	%	No:	%
Study group (Patients with SCC)	15	50	14	46.7	1	3.3
Control (Smokers)	0	0	3	60	2	40
Control (Non-Smokers)	0	0	0	0	5	100

Chi square = 28.775 df = 4 p = 0.001



Figure 1: Photomicrograph showing the candida ingested by phagocyte (—→) in peripheral blood [(Gram stain)100 x]

Table 1 depicts the phagocytic activity (by neutrophils of candidal organisms) (Figure1) among the study group and controls. 50% of the study group demonstrated mild phagocytic activity (less than 30%). 60% of the smokers demonstrated moderate phagocytic activity (30-60%) whereas non-smokers demonstrated a high (100%) phagocytic activity which was statistically significant ($p < 0.05$).

Table 2: Intracellular killing activity among study group versus controls

Subjects	Intracellular killing					
	Mild		Moderate		High	
	No:	%	No:	%	No:	%
Study group (Patients with OSCC)	28	93.3	2	6.7	0	0
Control (Smokers)	1	20	4	80	0	0
Control (Non-Smokers)	0	0	0	0	5	100

Chi square = 58.544 df = 4 p = 0.001



Figure 2: Photomicrograph showing the ingested viable candida phagocyte (—▶) in three phagocytes and ingested dead candida (◄—) in one phagocyte [evidence of intracellular killing in peripheral smear] 100 x]

Table 2 shows intracellular killing of candidial species by neutrophils in the study group and controls (Figure 2). 93.3% of the oral squamous cell carcinoma cases had suppressed / mild grades (less than 30%) of intracellular killing whereas 6.7% of cases belonging to the same study group demonstrated moderate grades (30-60%) of intracellular killing activity. The control group who were smokers showed moderate grades(30 -60%) of intracellular killing.80% of the control group who were known smokers showed high grades (100%) of intracellular killing activity, which was statistically significant ($p < 0.05$).

Table 3: Comparison of phagocytic activity and intracellular killing activity among study group and controls

Groups		Phagocytic activity			
		Mean	Standard Deviation	F value	P value
Study group(Patients with OSCC)		30.70	13.66	46.18	< 0.001
Control	Smokers	56.40	11.26		
	Non-smokers	87.20	5.02		

Groups		Intracellular activity			
		Mean	Std Deviation	F value	P value
Cases(Patients with OSCC)		16.37	11.15	76.95	< 0.001
Control	Smokers	41.00	8.21		
	Non smokers	76.00	3.80		

Table 3 shows the comparison of phagocytic activity and intracellular killing among the study group and controls demonstrated a mean phagocytic activity of 30.70 in the study group with squamous cell carcinoma. A mean phagocytic activity of 56.40 was observed amongst the group of smokers which was significantly lower as compared to non-smokers who demonstrated significantly higher phagocytic activity. The mean intracellular killing activity among the study group with squamous cell carcinoma was 16.37 and the mean intracellular killing activity among smokers was 41.0. The mean intracellular killing activity among non smokers was significantly higher as compared to the study group.

DISCUSSION

Squamous Cell Carcinoma represents more than 90 percent of all head and neck cancers. In India, head and neck cancers (HNCA) account for 30-40% of cancers at all sites, of which 9.4% are oral cancers. [7]

It is well known that cellular immunity is suppressed in cancer patients. Neutrophils form the first line of defense of the host immune response mechanism to antigenic insult. Neutrophil functions such as chemotaxis, phagocytosis and intracellular killing may be affected in various immunosuppressive conditions such as viral infections and autoimmune diseases. Malignant tumours have also shown to suppress PMN functions and make host susceptible to infectious diseases,[8] however this aspect of immunosuppression has not been sufficiently investigated in head and neck malignancies.

In the present study, 50% of the study group with oral squamous cell carcinoma demonstrated significantly mild/suppressed phagocytic activity, whereas, 46.7% and 3.3% demonstrated moderate and high phagocytic activity respectively. The control group of smokers, 60% demonstrated moderate phagocytic activity, 40% demonstrated high phagocytic activity, and the controls who were non-smokers demonstrated high (100%) phagocytic activity.

These results indicate that not only immunosuppressive infections, but malignant tumours also suppress PMN phagocytosis and make the host susceptible to secondary infections. Since bacterial and fungal infections constitute the majority of secondary infections in oral cancer patients, assessing PMNL functions could be used as a predictive parameter of host susceptibility to such infections.

The present study supports the results of studies done by Szucs F et al, who monitored the functional state of circulating neutrophils in a rat model with mesoblastic nephroma, during tumour progression. They observed a suppression of PMNL functions that accompanied tumour progression and an increased number of neutrophils in the peripheral blood. The reduction in superoxide generation and phagocytosis by PMNLs were observed in close association with tumour growth, thus they could be considered as indicators of tumor progression. [9]

Seres T, Knickvlbein R G, et al [10]suggested that oxidative stress may be responsible for the impaired functions of phagocytic cells. The level of percentage killing of bacteria inside the

phagocytes was considerably low, indicating defects in either the oxygen-dependent or oxygen-independent pathway of killing the microorganisms. The results of the present study with regard to intracellular killing activity of candidal species by neutrophils in the study group with squamous cell carcinoma demonstrated that 93.3% of the subjects had suppressed/mild grades of intracellular killing, whereas 6.7% of subjects demonstrated moderate grades of intracellular killing activity. The relatively low candidacidal activity of neutrophils is suggestive of cellular immunosuppression which is often induced in various malignancies.

The results were similar to the observations of Takao Saito et al who observed that the average level of intracellular killing was significantly depressed in patients with esophageal cancer or with gastric cancer. Therefore, a depression of intracellular killing of PMNs may serve to predict complications of infection in patients with cancer.[11]

Seres T and Knickvlbein RG noted that the enzyme myeloperoxidase is a vital factor in the microbial killing process inside phagocytes. Myeloperoxidase activity has been reported to be decreased during oxidative stress that is evident with various malignancies. The observed decrease in bactericidal activity of PMNL's might be due to insufficient myeloperoxidase activity to kill the pathogens by an oxygen-dependent mechanism inside the phagocytes.[10]

The oxidative stress may be further triggered by malnutrition, which is normally associated with malignancies. Studies have supported the relationship of malnutrition and abnormalities in neutrophil bactericidal activities in patients with cancer (Yuji Shigemitsu, Takao Taito et al) [10]. Deficiencies of specific micronutrients are associated with an impaired immune response and an increased susceptibility to infectious disease.

In the present study, 20% of the control group (smokers) demonstrated mild grades of intracellular killing, whereas, 80% demonstrated moderate grades of intracellular killing. Among the control group (non-smokers), all subjects demonstrated high grades of intracellular killing.

The present study supports the results of the studies done by Darius Zsimauskas which proves that smokers have significantly higher numbers of neutrophils in the peripheral circulation, but their functions are impaired. Neutrophils from smokers have shown decreased chemotaxis, phagocytosis and adherence as cigarette smokers demonstrate increased reactive oxygen species release from neutrophils. [12]

Ueta E et al [8] found suppression of intracellular killing activity in oral cancer patient as compared to healthy controls. However, they did not find significant suppression of phagocytic activity in the same study by Ueta. PMN functions were suppressed by tumour burden and tumour treatment brings additional tumour suppression and associated secondary infections.

Thus, the results of the present study demonstrate suppression in both phagocytic as well as intracellular killing activity of PMNs in patients with oral squamous cell carcinoma. The above findings suggest an association between oral squamous cell carcinoma and immunosuppression, especially pertaining to cell-mediated immunity. The consequent role of

malnutrition as an associated feature of oral squamous cell carcinoma and its effect on neutrophil function needs further investigation.

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