CELLULAR IMMUNE STATUS IN PATIENTS WITH CANCER OF THE ORAL CAVITY

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SUMMARY

Cellular Immune Status was observed in 70 patients of squamous cell carcinoma of the oral cavity by in vitro observation of absolute lymphocyte counts, T-cell counts and lymphocytes blastogenesis to PHA. The results were compared with 40 age matched normal controls. Absolute lymphocyte counts, absolute T-cell, T-rosette forming cells and blastogenic index was significantly lower in oral cancer ($P < .001$) as compared to normal controls and further showed a gradual decrease with increasing clinical stage, Well differentiated tumours were associated with higher T-cell counts and blastogenic index than the poorly differentiated tumours. The observations implies that the cellular immune response is invariably impaired in oral cancer patients and is related to the malignant process in the host. The significant depression of absolute lymphocyte counts, T-cell counts and blastogenic index suggest that the existence of tumours mass itself may play an active role in subverting the host immune competence.

INTRODUCTION

One of the important immunologic defect in cancer patients has been the cell mediated Immunity which is more profoundly impaired in oral cancer patients than those with other malignancies (1-4). Oral cancer patients generally have lower T-cell counts and impaired T-cell function as reflected by poor lymphoproliferative response to various antigens and mitogens (5-8) which may be related with tumour load and other clinical parameters. However, little attention has been paid to corelate the lymphocyte populations and function with the clinical parameters. Some have shown a correlation between impaired cell mediated immunity with clinical stage (9) whereas others (10) have found none (11). Therefore, present observation has been done to observe the lymphocyte populations and function in oral cancer patients and their correlation with the clinical parameters.

MATERIALS AND METHODS

This study was done on 70 proved cases of oral cancer and 40 age matched normal controls. The age of the patients were ranging from 31 to 70 years. Staging of the oral cancer was done according to TNM classification. The blood samples were drawn before treatment, None of the patients had recieved immunotherapy or immunosuppressive drugs before the investigation.

T-Cell Counts :

T-lymphocyte counts was done by the spontaneous rosette formation with sheep RBC by the modified method of Jondal

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et al (11). Absolute T-cell count was derived from the E-rosette percentage and absolute lymphocyte counts as follows :—

$$\text{Absolute T-cells} = \frac{\text{E-rosettes (\%)} \times \text{Absolute lymphocyte counts}}{100}$$

Lymphocyte Blastogenesis to PHA :

Lymphocytes were separated by Ficoll-hypaque gradient method (11) and washed in Normal saline. A suspension of 1×10^6 cells per ml. was made in the medium RPMI containing 10% Foetal Calf serum and antibiotics.

The cell suspension was divided into two aliquots. In one aliquot 0.05 ml PHA (Phytohaemagglutinin, Sigma USA) was added and another was left as such for control. The cells were harvested at 37°C for 48 hours and labelled with tritiated thymidine (^{3}H, BARC, Bombay) and cultured for another 24 hours. The thymidine incorporation was measured by liquid Scintillation counter (ECIL-ISS-34) and stimulation index was calculated as the ratio of counts per minute (cpm) in PHA treated vials and untreated controls.

Student's 't' test was applied for statistical significance of the data.

OBSERVATIONS

Absolute lymphocyte counts, absolute T-cells, E-rosettes and stimulation index is significantly decreased ($P < .001$) in patients with oral cancer as compared to normal controls (table 1). Absolute lymphocyte counts, E-rosette and stimulation index further shows gradual decrease with advancing clinical stage (table 2). Regional lymphnode involvement was associated with significantly decreased ($P < .001$) E-rosettes and stimulation index as compared to those with uninvolved node whereas tumours size did not show significant correlation (table 3). Histological differentiation of the tumour also shows significant correlation with the Tlymphocyte population and function which is significantly impaired in poorly differentiated tumours (table 4) as compared to the well differentiated tumours.

Table-1

Lymphocyte studies in oral cancer as compared to normal controls. (values in mean ± SD).

Lymphocyte studies	Oral cancer	Normal controls	P-Value
No of cases	70	40	
Absolute lymphocyte counts/mm^3	2591.3 ±900.0	4202.4 ±997.0	<.001
Absolute T-cell/mm^3	1021.0 ±464.0	2756.0 ±468.5	<.001
E-rosette (%)	38.5 ±5.6	65.8 ±3.3	<.001
Stimulation index	2.5 ±1.8	4.5 ±1.0	<.001

Table-2

Lymphocyte studies in different stages of Oral Cancer.

Lymphocyte Studies	Normal Controls	Stage I & II	Stage III	Stage IV
No. of Cases	40	8	27	35
Absolute Lymphocyte counts/mm^3	4202.4 ±997.0	3200.0* ±430.0	3018.7* ±845.7	2556.5† ±985.7
Absolute T-Cell counts/mm^3	2756.0 ±468.5	1428.5† ±352.4	959.0† ±392.0	972.3† ±484.4
E-rosettes (%)	65.8 ±3.3	48.2† ±5.5	37.5† ±3.2	36.8† ±5.2
Stimulation Index	4.5 ±1.0	4.0 ±0.5	2.6† ±0.5	2.0† ±1.5

*P/<.05 ; †P/<.001

Table-3

Lymphocytes Studies in Oral Cancer in relation to tumour size and lymphnode status

Lymphocyte Studies	Tumour Size			Lymphnode Status	
	T_1	T_2	T_3	N_0	N_+
No. of Cases	7	16	47	23	47
Absolute lymphocyte counts/mm^3	2313.0 ±628.7	2715.0 ±989.8	2489.7 ±884.4	2593.0 ±796.3	2583.5 ±947.5
Absolute T-Cell counts/mm^3	876.7 ±302.7	1158.8 ±559.0	925.6 ±433.0	1100.6 ±448.7	991.0 ±463.8
E-rosettes (%)	37.2 ±3.4	40.8 ±7.6	36.4 ±8.5	41.0 ±6.6	37.2* ±4.8
Stimulation Index	3.1 ±1.0	2.6 ±1.0	2.0* ±0.5	3.2 ±1.0	2.0* ±1.5

*P/<.001

Table-4

Lymphocyte Studies in Oral Cancer in relation to Histological Differentiation

Lymphocyte Studies	Well differentiated	Poorly differentiated	P-value
No. of Cases	30	40	
Absolute lymphocyte counts/mm^3	2757.8 ±797.7	2672.2 ±944.3	NS
Absolute T-Cells/mm^3	1136.4 ±431.0	1054.82 ±478.41	NS
E-rosettes	40.3 ±5.6	38.5 ±5.4	<.05
Stimulation Index	3.0 ±1.0	2.2 ±0.7	<.05

Discussion

The studies of immune response in cancer patient has proved that the immune system plays an important role in the development of the tumour but it is still not clear whether tumour develops due to the failure of the recognition mechanism or due to the impairment of the immune response. Immune response in fact, is a complex phenomena which is evoked in different steps. It is initiated when antigen is processed by the macrophage system which is followed by an interaction with the lymphoid system that leads to the proliferation and differentiation of lymphocytes to become sensitized cells capable of expressing cellular immune response (T-cells) or differentiation of plasma cells (B-cells) capable of secreting specific antibody. Therefore any defect either in immune recognition and antigen processing or differentiation of lymphocytes may lead to immunodeficiency.

The enumeration of peripharal lymphocyte populations and T-cell population has been very useful in assessing the cellular immune responses in cancer patients. Similarly, the capacity of lymphocytes to be stimulated by antigen and mitogens and to undergo transformation in vitro had been used to assess the immunological competence of these cells. A decreased capacity of lymphocytes to transform has been associated with a decrease in the general immune responsiveness of the patients.

Impairment of cell mediated immunity has been well documented in patients with diffcrent malignancies (12, 13) . The degree of impairment as well as its frequency seems to be related to the intrinsic factor such as tumour histologic type and stage[1] as well as other less charecterized like exogenous factors (14) like tobacco and alcohol abuse and malnutrition (15, 16). Inwidely disseminated cancer the impairment of cell mediated immunity could be explained on the nutritional basis alone, however, most results cannot substantiate if these defects are due to general debility of cancer patient or a consequence of advancing malignancy. Little evidence is available on the correlation between the immune competence of oral cancer patients and the extent of disease or survival.

In the present observation absolute lymphocyte counts, T-cell counts and stimulation index is significantly impaired in oral cancer patients as compared to normal controls which further shows a decreasing tendency with increasing clinical stage of the disease similar to the previous reports (6, 9, 17). It is interesting to note that the absolute lymphocyte counts and T-cell counts are impaired even in the primary stage of oral cancer whereas stimulation index is impaired in the advanced stage. Therefore, it seems that the lymphocyte populations may be impaired in the primary stage, however, its functional capacity is retained which is impaired only when the tumour advances beyond the local confines. The decreased T-cell function in advanced stage as evidenced by lower stimulation index may be due to impaired function of some T-cell subset. The significant impairment of T-cell and stimulation index in patient with lymph-node involvement further shows that the

impairment of T-cell population and funct.on is related to the anatomical spread of the oral cancer. Poorly differentiated tumours showed lower stimulaton index and T-cell counts whereas absolute lymphocyte population do not show any significant correlation. Similar observation was reported by earlier workers (10, 16).

The reason for depressed cellular immune response in oral cancer patients is unknown but it may probably be due to the production of some humoral factors capable of interacting at the lymphocyte surface. It might be possible that certain tumour specific antigen and immune complexes may alter the ability of the lymphoid cells to respond to other exogenous factors.

Our studies as well as that of others points to a persistent impairment of cell mediated immunity in oral cancer patients. The significantly impaired T-lymphocyte population and stimulation index suggest that either existence of tumour or its dissemination may play an active role in subverting the host cellular immune competence.

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