Leucocyte Migration Inhibition Assay in Oral Cancer Patients

V. K. **Sasidharan**, *Lecturer in Biochemistry, Department of Life Sciences, University of Calicut, Kerala, India*
P. Remani, *Regional Cancer Centre, Trivandrum*
D. M. Vasudevan, *Medical College, Kottayam—8*
T. Vijayakumar, *Regional Cancer Centre, Trivandrum*

Introduction

Cell mediated immune responses are reported to be the major defense against tumour both in animals and in human.[1] Halliday[1] reported that leucocyte migration inhibition assay (LMIA) can detect cell mediated immune reactivity against tumour associated antigens in human cancers. LMIA was devised by Soborg and Bendixen[3] from macrophage inhibition techniques of George and Vaughan.[4] The LMIA is widely being used for the detection of antigen associated with neoplastic cells.[5] Cell mediated immunity to tumour associated antigens has been described in patients with breast cancer, [6,7] prostatic carcinoma,[8] leukaemia[9] and in stomach. colon and lung cancers.[10] Even though oral cancer constitute more than 25% of the total cancer incidence in Kerala,[11] studies on the immunological aspcets of this cancers are scandy or over contradictory.[12–14] So the present study was conducted to find the correlation of LMI with clinical stages in ornal cancer and to see whether LMIA can be used as a marker for the diagnosis and/or prognosis of oral cancer

Material and Methods

Two hundred and twenty oral cancer patients and 120 normal controls were selected for the study. None of the subjects had any infection and none of them were on any treatment at the time ef the study.

Blood was collected from the peripheral veins into heparinized tubes. The leucocytes were separated by the method of Fujesawa *et al*[15] Tumour extracts of oral cancer tissue was prepared by 3M Kcl extraction technique of Meltzer *et al.*[16] The leucocyte migration inhibition assay described by McCoy *et al*[17] was employed. The leucocyte migration inhibition index (MI) was calculated using following formula.

$$MI = \frac{\text{Area of the macrophage fan obtained presence of Ag}}{\text{Area of the macrophage fan obtained in the absence of Ag}} \times 100$$

The tests were done in triplicate and the mean value were taken for calculation.

Results

The mean migration indices are given in Table — 1. The mean migrating index decreased significantly in patients as compared with normal controls ($p<0.005$). It was found that the LMI is positive in 57.3% of the oral cancer patients whereas only 11.7% positivity was observed in control subjects. The difference in leucocyte migration with progression of clinical stages of oral cancer are given in Table — II. There was a significant depression in the leucocyte migration as the clinical stage advanced.

Table—1

Mean migration index and percentage of positive leucocyte migration inhibition reaction in oral cancer patients and in control subjects.

Group	Mean migration index	Percentage of positive ractions
Oral cancer (n=220)	0.79 ± 0.05	57.30
Control (n=120)	1.01 ± 0·06*	11.70

Values are Mean ± S.E.
* $P<0.005$

Address for Communication : T. Vijayakumar, Scientific, Officer Dept. of Science, Technology & Environment near A. K. G. Centre, General Hospital Road, Trivandrum—695 037

Table—2

Changes in Leucocyte migration index of patients with progression of clinical stages

STAGE	I (n=28)	20	91.4%
STAGE	II (n=88)	60	68.2%
STAGE	III (n=74)	42	56.8%
STAGE	IV (n=30)	4	13.3%

A positive leucocyte migration inhibition reaction was defined as migration index <0.80.

Discussion

The LMI assay has been extensively used for evaluation of cell mediated immunity.[3,18] This technique was used for the detection of antigenecity of Leukaemic cells[9] and melanoma cells.[19] This assay with tumour extract has frequently been used for the study of cellular immunity in patients with breast cancer[7], prostatic carcinoma[8] and in thyroid cancer.[20] These studies showed that there is stronger inhibition of leucocyte migration in neoplasia than control subjects.

The present result suggests that carcinomas of the oral cavity possess a common tumour associated antigen and this antigen elicit cell mediated immune responses in the patients. The observed inhibition appears to be directed against oral cancer associated antigens and not due to the toxicity of the tumour extracts. One of the mechanisms of depressed response to antigen was reported to be due to the presence of suppressor T—lymphocytes.

Correlation of leucocyte migration inhibition with clinical stage, tumour recurrence or magnitude of tumour burden and survival has not been analysed by most of the previous investigators. It was reported that with advancing disease, presumably due to increased tumour burden, there is reduction in leucocyte migration against histologically related tumour extract.[22,23] In this study it was observed that the LMI increased with progression of the clinical stages.

Most investigations have found a relatively high incidence of LMI in preoperative cancer patients.[24] Several studies have demonstrated persistent elevation in LMI in breast cancer patients for up to one year or more when the tumour burden was presumably small or absent[25,26] In contrast, several investigators have also reported a decreasing incidence of reactivity during the immediate post operative period.[27] In our study there is a significant depression in LMI as the oral cancer progress from Stage I to Stage IV. This finding may be explained on the basis of the fact that there is a depression in the cell mediated immunity with the progression of the clinical stage. This is in agreement with the findings of Vijayakumar and Vasudevan[13] and Vijayakumar. *et al*[14] From this study it is reasonable to assume that LMIA can be of use in the diagnosis and prognosis of oral cancer along with the enumeration total and high affinity rosette forming cells and serum immunoglobulin levels.

Summary

Tumour directed cellular immune response of 220 oral cancer patients were assessed by leucocyte migration inhibition assay and compared with that of 120 normal healthy adult controls. The mean migratory index was found to be decreased significantly in oral cancer patients as compared to normal controls. There was a significant depression in leucocyte migration with progression of clinical stages of the disease.

Key words

Oral cancer, cell mediated immunity, leucocyte migration inhibition.

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