Serum Zinc Level and Cell Mediated Immunity among Patients with Oral Squamous Cell Carcinoma

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Introduction

It has been documented that zinc has a role on cellular immunity (1). Some recent studies revealed a zinc dependency of Thymic dependent cells (i.e., T-cells) with special reference to T-helper cells, T-effector cells and T-natural killer cells (2,3).

Zinc deficient animals and children have atrophy of the thymolymphatic system, depressed cell-mediated immunity (CMI), and increased susceptibility to infection and malignancies (4-6). The in-vivo and in-vitro observations stated that among the patients of different graded malignancies, there lies impaired cellular immunity, and it has been shown to be related to the stages of the disease and the prognosis of the disease (7-9). The properties of T-cells and delayed hypersensitivity have been widely used to test the cellular immune competence of the cancer patients, but still there exist several unresolved discrepancies (10,11).

Moreover, severe liver disease, alcoholism, nutritional status, and a number of disease states may affact plasma zinc levels (12-14). Investigators have suggested that zinc deficiency alters the taste acuity among oral cancer patients especialy who are treated with radiation therapy (15-17), conversely, improvement of taste acuity with zinc supplementation has been reported (18-20).

However, the present study has been carried out to investigate the interelationship between serum concentration of zinc and cell-mediated immunity (CMI) among the patients of oral squamous cell carcinoma with different histologic grades. The study was carried out also to investigate the effect of zinc supplementation on CMI in the patients group who had lowered level of serum zinc and depressed CMI specially after radiation treatment.

Materials and Methods

Patients

Biopsy-proven one hundred and ninety-five patients with oral squamous cell carcinoma were studied. Among the total of 195 patients the sex ratio was 3.1:1 between male and female. Their age ranging from 38 to 83 with a mean

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age of 60.5 years. There were 53 patients in grade I, 43 grade II, 49 in grade III, and 50 in grade IV. Gradation was considered on the basis of cell differentiation by histopathological examinations. According to the proposed design of the present study two more separate groups were formed by the number of patients who had lower level of serum zinc and depressed CMI. They were 46 and 56 in number in the new two groups and patients were from grade II and IV. These two new groups were formed one for radiation therapy and the other for combined therapy of radiation and zinc sulphate supplementation. All the patients had no prior history of cancer. Care was taken that the patients entered in the study were not currently taking or had not recently taken medications that might possibly affact the cell mediated immunity and the zinc level in serum. The patients were also freed from any infection.

The patients who were given radiation therapy were observed carefully. They were treated by radiation to a tumour dose of 5,000 to 7,200 rads to the primary lesion. A daily dose of 160 to 200 rads sometimes at one day interval was given for six weeks.

After getting the informed consent from the patients and the attending physician, an administration of zinc sulphate, USP Capsule ($ZnSO_4$, $7H_2O$), by oral route was performed in order to maintain the equivalent supply of an additional 150 mg of elemental zinc daily. One capsule containing 220 mg was given three times a day for six weeks. The patients were examined at three days intervals. The patients were requested to take a capsule three times a day just after meal.

Blood sample

The blood was drawn from the patients in early moring after an overnight fast and a basal 5 ml venous blood was collected from the anticubital vein of each subjects using disposable syringe. The blood was divided in two separate test tubes, one for T-cell another for serum zinc. The serum was collected maintaining 4^0C. All operations were performed in ion free condition.

Serum zinc

Serum zinc was determined from all patients prior to any zinc supplementation. The zinc was estimated from serum by a direct measurement method using atomic absorption spectro photometer. Care was taken to avoid contamination by using all plastic polyethylene syringes and stainless steel needles with polypropylene hubs. Details of the method of the preparation of the sample, and analytical techniques have been followed according to Smith J.C. Jr. et al. (21).

T-Lymphocytes

T-lymphocytes of human form rosette with antigenic red cells of sheep. Spontaneous formation of rosettes of sheep RBC (SRBC) with human T-lymphocytes was used as a

marker of thymus derived lymphocytes (22). One ml of inactivated rabbit serum was absorbed with 0.1 ml of packed SRBC for one hour at 37^0C and clumped cells were removed by centrifugation. Lyhphocytes used from human blood were adjusted to $5x10^6$ Cells/ml in Hank's Balanced Salt Solution (HBSS). To O.1 ml of lymphocyte suspension, 0.1 ml of absorbed serum was added and incubated at 37^0C for 15 minutes. The mixture was washed with HBSS, briefly centrifused to pellet the cells and kept at 4^0 C for 3-4 hours. At this stage an aliquot was removed for counting the rosettes and the rest of the cells left overnight at 4^0C. Supernatant was removed gently with a pasteur pipette and the cells were resuspended in 0.5 ml of HBSS. The rosettes were counted.

Delayed Type

Hypersensitivity (DTH) reaction test:

PPD Reactivity: Purified Protein Derivative (PPD) of tuberculim (PPD-5 TU/0.1 ml, i.e., one TU of PPD (0.02) each in 0.1 ml solution, Conaught lab.,) were applied by intradermal inoculation. We observed the reaction after 48 hours. Induration greater than 5 mm or more was considered a positive reaction for PPD. The method was followed according to Das O.H et al. (23).

DNCB Reactivity: The DNCB (2,4 Dinitrochlorobenzine, Sigma Chemicals, USA) test was adapted from Catalona and Chretien (24). Reactions at +3 or +4 were interpreted as positive and normal, while reactions below these values interpreted as negative and abnormal, with no further testing.

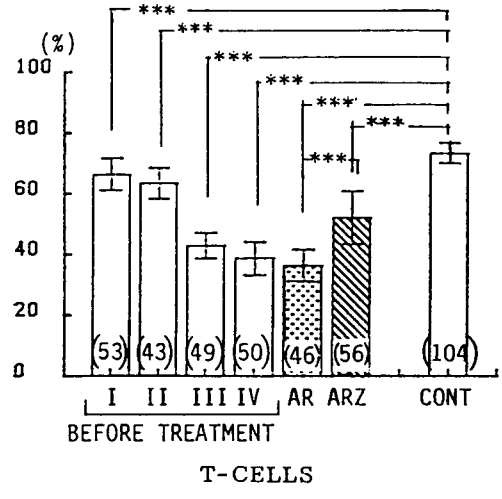

FIG. I. Analytical Results of Mean Values for T-Cells in different Patient Groups with Oral SCC and Healthy Controls.

*, **, ***: P <0.05, p <0.01, P < 0.001.

NS: Not Significant at 0.05 Level.

I, II, III, IV: Histopathological Grades.

AR: After Radiation, ARZ: After Radiation and Zinc Supplementation. CONT: Control Subjects.

Results

Serum Zinc

The patients with grade III and IV had decreased level of serum Zinc i.e. 66.10±9.02 µg/dl and 60.80±7.30 µg/dl respectively. The patients who were being irradiated had more lower level of of serum zinc, i.e., 55.12±8.15 µg/dl, but after zinc supplementation the level significantly increased,

91.2±14.18 µg/dl. In grade I and II the serum zinc value was 109±12.18 µg/dl and 107.4±6.58 µg/dl respectively. In normal healthy subjects the serum level of zinc was increased highly significantly (P <0.001).

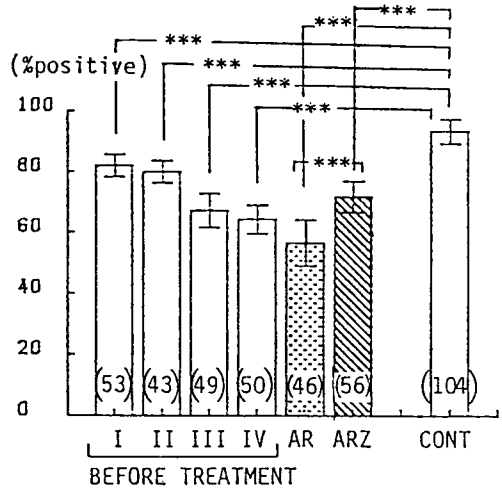

FIG.2. Analytical Results of Man Values for DNCB Reactivity in Different Patient Groups with Oral SCC and Healthy Controls.

*, **, ***: P <0.05, p <0.01, P < 0.001.

NS: Not Significant at 0.05 Levels.

I, II, III, IV: Histopathological Grades.

AR: After Radiation, ARZ: After Radiatin and Zinc Supplementation., CONT: Control Subjects.

T-Lymphocytes

The mean value of %T-cells in normal healthy controls and in patients with grade I, II 66.3±5.3, 63.3±5.1 and 73.0±3.3 repectiviely. In grade III and IV the mean value was 42.9±4.2 and 38.6±5.5 respectively. Again, after radiation therapy the value for T-cell percentage was decreased more and which became 36.2±5.2. But after zinc sulphate supplementation the value raised significantly and fairly which became 52±8.2. (Fig.1) (P <0.001).

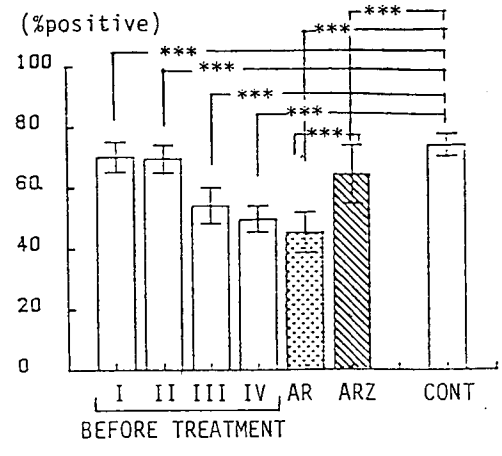

FIG.3. Analytical Results of Man Values for PPD Reactivity in Different Patient Groups with Oral SCC and Healthy Controls.

*, **, ***: P <0.05, p <0.01, P < 0.001.

NS: Not Significant at 0.05 Levels.

I, II, III, IV: Histopathological Grades.

AR: After Radiation, ARZ: After Radiatin and Zinc Supplementation., CONT: Control Subjects.

DTH Reactions

1) PPD Reactivity: In grade III and IV the value was 54.1±5.8 and 49.6±4.3, comparing the value in normal control, 73.8±3.7. Irradiated patients had very low value, i.e., 45.3±6.5 which became improved after zinc

sulphate supplementation, that raised to 64.1±9.6 (P<0.001). In grade I and II it was 69.7±4.9 and 69.4±4.6 respectively which was relatively than grade III and IV (Fig.3).

2) DNCB Reactivity: In grade III and IV the values were low, i.e., 67.1±5.6, and 64.4±4.6, comparing the control of 93.6±4.1. In grade I and II the value was 81.6±3.7 and 79.9±3.7 which are greater than grade III, IV. The irradiated patients had the reactivity of 56.6±7.5, but after zinc supplementation it became raised to 72.0±5.0 (P <0.001), (Fig.2),

Discussion

General depression of cell mediated immunity in vivo is often associated with tumour-bearing state in human (25). In general, specially in advanced stages of all malignancies the cell mediated immunity becomes depressed (26). It becomes more severe in cases of Hodgkin's disease and in head neck malignancies (27,18). Catalona et al. 1973 and Lichtenstein et al. 1980 shown earlier that oral cancer patients showed depressed CMI in comparision with other solid tumours. The results of the present study has in vitro correlation of DTH responses which included the DNCB and PPD reactivity. It showed a depressed DTH responses in advanced cases of oral squamous cell carcinoma i.e., the patients suffering with grade III and IV. Besides, %T-cells was also significantly lower comparing the patients of grade I and II, and the normal healthy controls. A similar data was revealed by Dellon et al (29). Since T-lymphocytes are considered to be responsible for cell mediated immunity (30) and their function in patients with squamous cell carcinoma, as studied in vivo and in vitro is impaired (31), the depression of cellular immuno-competence in patients with squamous cell carcinoma must be strongly suspected. Despite a profound decrease of 5%T-cells, PPD reactivity and DNCB reactivity, a significant observation was noted that after radiation therapy the values still continued to be depressed, but, after zinc therapy in irradiated patients the values raised.

Even though, patients in this study experienced decreases in numbers and responses of lymphocytes during radiation treatment, a deficiency noticed among patients followed up for six weeks after treatment. This study revealed that radiotherapy has an adverse effect to natural tumour immunity and results in worsened prognosis which can only be obtained by correlation of clinical progression of neoplasia with in vitro and in vivo assessment of cellular immunity in these patients, as measured by T-cell% counting and DTH reaction.

In this study when we consider the immunologic aspects of zinc we can correlate in the means that severe zinc deficiency after radiation therapy among the depleted group of advanced oral SCC cases might have an association with atrophy and decreased cellularity of thymus,

spleen, bursa or orther lymphoid tissues which has been stated elsewhere (32). It is obvious from this study that zinc has a role to improve the cellular immunity that confirms the evidence once more during radiation therapy among zinc depleted irrradiated cases of oral SCC with grade III and IV.

However, we suggest that zinc may be involved in the occurance of depressed CMI among the patients may be defective liver function, is the reason for depressed CMI due to impaired utilization of zinc in advanced oral SCC cases, but not among grade I and II cases, still many things remain to investigate.

Summary

One hundred and ninety-five patients of Oral Squamous Cell Carcinoma (SCC) of different histologic grades were studied to assess the relationship between the serum level of zinc and Cell Mediated Immunity before and after radiation treatment, and also after combined treatment of radiation therapy with zinc sulphate supplementation by oral route. The findings were composed with 104 normal healthy controls matched with age and sex. Patients of grade III and IV showed lower level of serum zinc and depressed cellular immunity i.e., Delayed Type of Hypersensitivity (DTH) reaction and low percentage of T-lymphocytes as compared to the patiensts of grade I, II and also to control subjects ($p < 0.001$). Forty-six patients identified from grade III and IV showed severe depletion of serum zinc concentration and more impaired CMI. Another forty-one cases from grade III and IV who were given combined treatment of radiation therapy and oral zinc supplementation ($ZNSO_4$, tHO_2) at a dose of 220 mg three times a day for six weeks, showed an elevated concentration of serum zinc and improved CMI ($P<0.001$).

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