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Effect of Treatment on Immune Status of Patients with Head and Neck Cancer

KEYWORDS: Head and Neck Cancer, Biomarkers, Immunity, Malnutrition

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ABSTRACT *Malnutrition, head and neck cancer, and surgery are all associated with impaired immune function, especially with regard to cellular immunity. The present study was initiated to assess the effect of treatment on immune status of patients with head and neck cancer. For this study 15 head and neck cancer patients with the group ranging from 30 to 60 years of both the sex were selected from department of Radiotherapy, Government General Hospital, Guntur, India. The blood samples were analyzed for Biomarkers (CD3, CD4, CD45, CD3% & CD4%) before and after treatment. There is no difference between before and after treatment in immune status of head and neck cancer cases. In the present study the investigator unable to show any noticeable immunologic differences between before and after treatment. Along with treatment immune boosters (Nutritional supplements) may be benefit via stimulating components of immune function, nitrogen balance and wound healing. A large patient cohort with a longer follow up period for therapeutic response studies may yield more significant.*

Introduction:
Malnutrition, head and neck cancer, and surgery are all reported to be associated with impaired immune function, especially with regard to cellular immunity. (Shears, 1999; Lichtenstein, Zieghelboim et al.1980; Van Bokhorstde, Van der Schueren et al.1998). However, the immuno competent patients with reasonable performance status, radiotherapy is an established treatment option in the definitive management of head and neck cancer. (Emily., Klein., et al. 2001). The decrease in immunity among cancer patients generally involves in the alteration of immune competent cells in circulation. Cellular immunity plays an important role in controlling tumours. Among immune related cells, T-lymphocytes take the lead role in immune response against the tumours (Badoual, Hans's., et al. 2006). Possibly, the origin of head and neck cancer is linked to environmental carcinogens namely tobacco, alcohol, etc., whereas tumor progression could be linked to a failure of host immunomodulation as head and neck cancer could also corrupt the antitumor response via several mechanisms (Whiteside, 2005). The present study was initiated to assess the effect of treatment on immune status of patients with head and neck cancer.

Methods and Materials:
For this study 15 head and neck cancer subjects with the group ranging from 30 to 60 years were selected from the Department of Radiotherapy, Government General Hospital, Guntur, India. Most of the patients were with rural background involved in farming.

Sample Collection & Preparation:
BD Tritest™ CD3 fluorescein isothiocyanate (FITC), CD4 phycoerythrin PE and CD45 peridinin chlorophyll protein*(PerCP) is a three color direct immuno fluorescence reagent for use with a suitably equipped flow cytometer to identify and determine the percentages and absolute counts of mature human T lymphocytes (CD3+), helper T lymphocyte (CD4+) and T-cell activator (CD45+) receptors on T-cells and leukocytes obtained from erythrocyte-lysed whole blood.

With the consent of the patients, two ml blood was drawn aseptically by venipuncture into a sterile EDTA (ethylenediamine-tetraacetic acid) BD Vacutainer™ blood collection tube. BD Tritest CD3/CD4/CD45 reagent and BD Trucount tubes were validated with both liquid and dry formulations of EDTA. A minimum of 100µl of whole blood was required for this procedure. The collection tube manufacturer's guidelines for the minimum volume of blood to be collected to ensure proper specimen dilution, especially when determining absolute counts with BD Trucount beads. Obtained white blood cell (WBC) count and differential white cell count from the same whole blood sample before staining and ensured that the WBC count was within the linear range.Anticoagulated blood stored at room temperature (20° to 25°C) was stained within 72 hours of draw and then analyzed within 6 hours of staining. (BD Tritest, 2007)

Statistical analysis:
Statistical analysis was carried out by student's t-test. A value of $P<0.05$ was considered as statistically significant. Results are presented as mean ± SDs.

Results and Discussion:
Table 1 shows the mean CD3, CD4, CD45, CD3% and CD4% values of head and neck cancer subjects before & after treatment. Before treatment CD3 counts were found to be 1448±539 and after treatment 1286±369. Before treatment CD4 counts were 408±194 and after treatment 323±225. Before treatment CD45 values were 1957±651 and after treatment 1774±825. Before treatment CD3% values were 69.4±10.5 and after treatment 64.9±16.5. Before treatment CD4% values were 20.87±8.74 and after treatment 16.40±7.87 respectively.

As head and neck cancer progresses, it produces a catabolic state and increased susceptibility to other infections; when this is compounded by a lack of food, progressive malnutrition ensues. In patients with head and neck cancer, weight loss and low BMI, especially associated with low CD4 cell count, may be etiological predictors of survival in head and neck cancer.

There is no significant difference in counts and percentages of CD3, CD4, CD45, CD3%, CD4% between cancer subjects before and after treatment.

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Iris Kuss, Bridget Hathaway et al. (2004) reported that patients with active disease had significantly lower. CD3+ and CD4+ T-cell counts than those with NED (No evidence of Disease). Patients who had NED after surgery and radiotherapy had the lowest T-cell counts among the NED cohort.

F Mozaffari, C Lindemalm et al (2007) studied on NK cell and T-cell functions in patients with breast cancer: effects of surgery and adjuvant chemo- and radiotherapy. Both radiotherapy & Chemo had a profound effect in decreasing the numbers of circulating T cells with CD4 + cells being affected to a great extent than CD8+ cells. They subsequently analyzed the Treg sub in the patients before and after therapy in comparison to normal donors and also examined the activation markers on T cells. Tregs were measured as frequently of CD3+ CD4+ $CD25^{hi}$ cells. The frequency of CD4+CD25hi as well as CD8+CD25 T cells was significantly reduced in pretreatment patients ($P<0.05$). However, patients who had received therapy as in particular the RT + CT group had a significantly reduced number of CD4+CD28+ T cells. The frequency of IL-4 producing CD4 cells was significantly higher in patients before treatment compared to healthy volunteers ($P<0.001$) and patients who received RT ($P<0.05$).

In the present study the investigators were unable to show any noticeable immunologic differences between before and after treatment among the studied samples of head and neck cancer subjects. The degree of immunologic impairment induced by trauma or surgery is assumed to be related to the severity of tissue injury. (Kloosterman T, Von Blomnerg ME et al.1994). Therefore, in our study, the extent of the trauma might have been at low incidence and hematopoietic microenvironment of ther subjects under study must not have been invaded by metastasis and thus immune function remained undisturbed and the same protects the subjects from various secondary infections. Specific nutrients have been reported to improve immune responsibilities after experimental injuries. (Ganotti , Alexander et al.1993; Kinsella , Lokesh et al.1990; Pizzini , Kumar et al.1990; Daly , Reynolds et al.1988). Arginine is one such nutrient that is of benefit via its purported role in stimulating components of immune function, nitrogen balance and wound healing.

Conclusion:

Present study primarily focused on the effect of treatment on immune status of patients with head and neck cancer. The results showed least noticeable immunologic differences between before and after treatment. However, the negative effects of radiation and/or chemotherapy were noticed on the cell counts as revealed by the values of CD3, CD4, and CD45 in head and neck cancer. Along with treatment, immune boosters (Nutritional supplements) may stimulate components of immune function, nitrogen balance and wound healing. The studies including a large patient cohort with a longer follow up period for therapeutic response may yield more observable and significant cues. .

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Table I- Mean Biomarker values of Head and Neck Cancer Patients in Plasma Before and After Treatment:

Biomarkers	Before Treatment (µl)	After Treatment (µl)
CD3	1448±539	1286±369
t value	0.96	
CD4	408±194	323±225
t value	1.12	
CD45	1957±651	1774±825
t value	0.67	
CD3%	69.4±10.5	64.9±16.5
t value	0.89	
CD4%	20.87±8.74	16.40±7.87
t value	1.47	
* Significant at 0.05% level.		

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