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ORIGINAL ARTICLE / RESEARCH

Evaluation Of The Cellular Immune Response In Patients With Head And Neck Cancer

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ABSTRACT

Aim: The aim of the present study is to evaluate the cellular immune response in patients with head and neck cancer.

Material-Method: Twenty volunteer healthy subjects as controls and 34 patients who have been diagnosed with squamous cell carcinoma at the Department of Ear Nose Throat in Firat University Firat Medical Center from June 2002 to January 2005 were included in the study. The ages of the patients ranged between 27 and 74 years (mean 56.8+/-10.3), and the ages of the controls ranged from 21 to 69 years (mean 53.4+/-12.7). There were squamous cell carcinoma (SCC) of larynx in 30 patients, SCC of hypo pharynx in three patients, and SCC of paranasal sinus in one patient. 18 of these patients were in the early stage, four patients in the advanced stage, and 14 patients in the metastatic stage. Histopathological diagnoses were carried out by biopsies in preoperative term and pathological examination of the specimens in postoperative term. The examination of lymphocyte sub groups was done by flow-cytometiric analysis.

Results: In the flow cytometry of peripheral blood samples of head and neck cancer patients and controls, while the ratio of CD4+/CD8+ T cell significantly decreased, the ratio of B lymphocyte, NK cell and activated Tc cell increased in patients compared to the controls. When the patients were compared to each other, CD4+25+ cells were in high level in metastatic patients in comparison to patients in early stages.

Conclusion: These results indicated that especially cellular immune response was activated in head and neck cancer, but it was not enough to prevent the cancer

Key words: Immune response, head and neck cancer

Introduction

Decrease in immunity in patients with cancer generally involved the alteration of immune competent cells in circulation. Cellular immunity plays an important role in controlling tumours. T-lymphocytes take on the main role in immune response against the tumours [1]. In general, the deterioration of T-cell functions and the insufficiency of immune response against the tumour are valid in patients with cancer. The other reason of decreased immunity in these patients is the alteration of immune competent cells in circulation [2],[3],[4],[5]. The immune system plays an important role in tumour growth and

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regression. The tumours may develop more frequently when the immune system is less active [5]. Many tumours have lymphoid infiltrates. The immune system can limit the spread of tumours with different mechanisms. The aim of this study is to evaluate the cellular immune response in patients with head and neck cancer.

Materials And Methods

Patients: Twenty volunteer healthy people as control and 34 patients with diagnoses of squamous cell carcinoma at the Department of Ear Nose Throat in Firat University Firat Medicine Center from June 2002 to January 2005 were included in this study. The ages of patients ranged from 27 to 74 years (mean 56.8+/-10.3), and the ages of controls ranged from 21 to 69 years (mean 53.4+/-12.7). There were squamous cell carcinoma (SCC) of larynx in 30 patients, SCC of hypopharynx in three patients, and SCC of paranasal sinus in one patient. Sixteen of these patients were in the early stage, four patients in the advanced stage, and 14 patients in the metastatic stage.

Histopathology: Histopathological diagnosis was carried out by biopsy material in pre- and post-operatively. ***Analysis by flow cytometry:*** The examination of lymphocyte subgroups was done by flow-cytometric analysis. For immunophenotypic analysis, 2 ml of peripheral blood samples were collected in glass tubes containing ethylenediaminetetraacetic acid (EDTA) and analyzed within one hour of collection. Lymphocyte (CD3+, CD4+, CD8+, CD19+, CD23+, CD25+, CD26+, and CD30+) cell rates were calculated by using flow cytometry conducted on a Coulter EPICS XL–MCL (Beckman Coulter, USA) using fluorescence-labelled (FITC or PE) monoclonal antibodies (Coulter Immunotech, France). Erythrocytes were removed from the peripheral blood sample in the EDTA tube by using the whole blood lysis method. Following erythrocyte removal, 5000 cells were counted in the appropriate gate for each tube and analyzed. Lymphocytes reacting with each monoclonal antibody were separated according to their fluorescence properties and their numbers reported as percentage.

Statistical analysis: All the statistics were performed in SPSS version 10.01 software (SPSS, Inc., Chicago, IL, USA). Data were analyzed by using Independent- Samples *t* test, One Way analyses of ANOVA, and Tukey's HSD test as needed. $p < 0.05$ was considered to be significant.

Results

In the flow cytometry of peripheral blood samples of cancer patients and controls, while the ratio of the $CD4^+/CD8^+$ T cell significantly decreased, the ratio of B-lymphocyte, NK cell, and activated Tc cell increased in patients compared to controls [Table/Fig 1]. When the patients were compared to each other, $CD4^+25^+$ cells were observed to be in high level in patients with metastasis compared to patients in early stages [Table/Fig 2].

Table/Fig 1 Phenotypic lymphocyte analysis of the cases

Lymphocyte (%)	Patient (n:34)	Control (n:20)	p values
CD3+	63.8775	64.6672	p> 0.05
CD4+	37.2707	41.3301	p<0.05
CD8+	24.8109	21.7806	p>0.05
CD4+/CD8+	1.5	1.9	p<0.05
CD19+	14.4418	10.8392	p<0.05
CD16+56+	21.4898	16.1172	p<0.05
CD4+26+	22.2891	23.6963	p>0.05
CD4+30+	2.8227	2.0683	p>0.05
CD23+	9.3691	7.3873	p>0.05
CD25+4+	2.7976	2.7268	p>0.05
CD25+8+	0.6790	1.5657	p<0.01

Discussion

The effector mechanisms of both cell-mediated immunity and humoral immunity have been shown to kill tumour cells in vitro [6]. The T-cell response is unquestionably the most important host response for the control of growth of antigenic tumour cells. It is responsible for both the direct killing of tumour cells and the activation of other components of the immune system. T-cell immunity to tumours reflects the function of the two T-cell subsets: class II-restricted T cells that largely represent CD4 helper T (T_H) cells that mediate their effect by direct

interaction with antigen-presenting cells (APC) and by the secretion of lymphokines to activate other effector cells and induce inflammatory responses and class I-restricted T cells that largely represent CD8+ cytotoxic T (Tc) cells that can also secrete lymphokines but mediate their effect mostly by direct lyses of tumour cells [7].

Cardi *et al*. [8] observed that lymphocytes extracted from subcutaneous metastasis were characterized by a significantly reduced ratio of CD4+ to CD8+ T cells as compared to peripheral blood lymphocytes from the same patients.

Table/Fig 2: Phenotypic lymphocyte analysis of the patients with cancer

Lymphocyte(%)	Early stage n:16	Advanced stage n:4	Metastasis stage n:14	p values
CD3+	65.1489	61.9900	66.4100	p> 0.05
CD4+	39.6342	39.8550	39.4936	p> 0.05
CD8+	25.9274	23.0150	23.7864	p> 0.05
CD4+/CD8+	1.53	1.73	1.66	p> 0.05
CD19+	15.6800	12.9200	11.4064	p> 0.05
CD16+56+	19.7895	23.2575	19.8314	p> 0.05
CD4+26+	21.6864	24.6025	24.3536	p> 0.05
CD4+30+	3.6847	2.2525	2.2064	p> 0.05
CD23+	9.9457	8.6167	8.6170	p> 0.05
CD25+4+	2.2888	3.1950	3.4850	p<0.05
CD25+8+	0.6087	0.3500	0.9040	p> 0.05

Gonzalez *et al. [9]* studied the functional response and phenotypic characterization of peripheral blood T-cells and their correlation with the clinical stage of disease in 29 males with previously untreated carcinoma of the larynx and 24 healthy male controls. Peripheral blood T cells, phenotypically CD2+, CD3+, were significantly decreased in patients relative to the controls. Mandel-Brown et al [10] reported that quantification of the T-lymphocyte subpopulation in peripheral blood did not indicate the presence of metastatic diseases. Significantly higher CD4/CD8 ratio was present in lymph nodes containing metastatic disease compared to lymph nodes without metastatic disease.

Kuss *et al.*[11] determined a significantly lower absolute number of CD3+, CD4+, and CD8+ T cells in patients with head and neck squamous cell carcinoma (HNSCC) compared to normal controls (NC). However, no differences in the percentages of T-cell subsets between patients and NC were observed.

In the present study, we found a lower CD4/CD8 ratio in the study group. These findings indicate that increased CD8+ T lymphocytes (Tc) ratio has an important role in immunity developed against cancer.

NK cells can kill a wide range of tumour targets in vitro [7], especially cells that have reduced class I MHC expression and can escape killing by Tc [6]. NK cells play a role in host defense against growth of tumour cells at both primary and metastatic sites [7].

Mickel *et al*. [12] evaluated peripheral blood lymphocyte and lymph node lymphocyte natural killer (NK) cell activity in 22 patients with head and neck squamous cell carcinoma. The peripheral blood lymphocyte NK activity of cancer patients was significantly less than controls.

Kou and Oi [13] indicated that the NK cell's activity in patients with laryngocarcinoma was lower than both the normal control group and the polyp of vocal cord patients group. They found that the more severe was the degree of disease, the lower the activity of the NK cell.

According to our result, the percentage of NK cells was higher ($p<0.05$). However, there is no difference in NK cells among early, advanced, and metastatic stage patients. B-lymphocytes have a potential role on tumour immunity producing tumour-reactive antibodies. Antibodies may mediate tumour cell lyses by both complement fixing and antibody dependent cellular cytotoxicity (ADCC). ADCC is a more potent lytic mechanism than complement mediated cytotoxicity [7].

Dawson *et al*.[2] documented no significant differences in the circulating levels of total T cells, T cell subsets, B cells, monocytes, or natural killer cells when compared to age, alcohol- and tobacco-use matched controls.

In our study, the B lymphocyte (CD19+) percentage was determined to be higher in patients with cancer than healthy controls. According to our result, the B lymphocyte percentage was higher in early stage patients compared to advanced and metastatic ones. However, no statistical difference was found. CD4+ T cells play a central role in initiating and maintaining anticancer immune responses. However, regulatory CD4+CD25+ T cells that express Foxp3 have also been shown to inhibit antitumour effector T cells [1]. Schaefer *et al*. [15] determined that patients with squamous cell carcinoma of head and neck (SCCHN) have depressed antitumour immunity. The presence of CD4+CD25+ (Treg) cells in these patients might be, in part, responsible for down regulation of antitumour immune responses. Wolf *et al*.[16] reported that patients with epithelial malignancies show an increased level of CD4+CD25+ T cells in the peripheral blood together with characteristics of Tregs. This is similar to studies where increasing CD4+CD25+ (Treg) cells in patients with cancers [15],[16] have been determined. We demonstrated a significant increase, especially in the metastatic group of patients. This increase might be responsible for the regulation of antitumour immune responses and the inhibition of tumour effector T cells.

Our results indicate that especially cellular immune response is activated in patients with head and neck cancer. Nevertheless, these responses are not strong enough to prevent the development of cancer. The role of immune mechanisms in the defense of tumours should be defined in the future.

Conflict of Interest: None declared.

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