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ARTICLE

Serum Levels of the Soluble Adhesion Molecules in Patients with Malignant Melanoma

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The incidence of malignant melanoma has been steadily increasing over the past decades. CD 44 is a transmembrane glycoprotein which is implicated in a number of adhesive and migratory events. Downregulation of CD 44 is implicated in the metastatic process. P-Selectin is a member of the selectin family of cell surface molecules. The levels of P-Selectin in biological fluids may be elevated in subjects with a variety of pathological conditions. In malignant melanoma, elevation of the plasma level of soluble intercellular adhesion molecule-1 (sICAM-1) has been associated with a reduction in disease-free survival. This study was performed to investigate the differences in the serum concentrations of the adhesion molecules in patients with malignant melanoma.The study group consisted of 52 patients with malignant melanoma and 20 healthy subjects. No meaningful difference was observed for P-selectin and sICAM 1 levels. A statistically significant decrease was observed in the cancer patients for serum CD 44 levels. (Pathology Oncology Research Vol 6, No 1, 42–45, 2000)

Keywords: CD 44, adhesion molecules, malignant melanoma

Introduction

The incidence of malignant melanoma has been steadily increasing over the past decades. With an annual increase of about 5% malignant melanoma is one of the fastest growing cancer forms. Thus, a major challenge facing clinical biochemical studies is to find specific and reliable serum markers that are of value in diagnosing and monitoring progression of the disease.

Human hematopoietic CD44 is an 80-90 kD type 1 transmembrane glycoprotein that has been implicated in a number of adhesive and migratory events. The CD44 molecule is expressed on both hematopoietic and non- hematopoietic cells, including T and B cells, NK cells, granulocytes, macrophages, monocytes, fibroblasts, endothelial cells and columnar and transitional epithelium.[14,20,21] Soluble forms of CD44 have been detected in human serum and synovial fluid.[6] In addition to its interactions with the extracellular matrix, CD 44 is reported to contribute to leukocyte extravasation[7] by homotypic binding and to present cytokines to neighboring cells.[2] By these properties it can influence expression of adhesion molecules and activation of vascular endothelial cells and tumor cells. The CD 44 molecule is also expressed by highly aggressive human melanoma cell lines.[5] The melanoma cells not only express more CD44, they also shed significantly more CD 44 molecules from the cell surface.[19]

P-Selectin is a calcium-dependent cell surface glycoprotein that plays a critical role in lymphocyte migration.[10] P–Selectin is involved in the adhesion of platelets to monocytes and neutrophils.[21] The P-Selectin molecule is consitutively expressed in normal capillaries and skin cells. Endothelial P-Selectin expression is reduced in metastatic or advanced primary melanoma.[13] An association between serum P-Selectin levels and clinical outcome has been suggested in malignant melanoma.[16,17]

Cell adhesion molecules (CAMs) are cell surface proteins involved in the binding of cells, usually leukocytes, to each other, to endothelial cells, or to the extracellular matrix.[1] The Ig superfamily of adhesion molecules bind to integrins on leukocytes and mediate their flattening onto the blood vessel wall and their subsequent extravasation

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into the surrounding tissue. The soluble intercellular adhesion molecule (sICAM-1) seems to be involved in disease progression in malignant melanoma. Elevated serum levels of sICAM-1 are significantly associated with a reduction in disease-free survival.[1,4,22]

The aim of this study was to investigate the clinical significance of serum adhesion molecule levels at the time of diagnosis in patients with malignant melanoma and to evaluate the usefulness of these assays in terms of prognosis and survival.

Materials and Methods

Serum samples were taken from 52 patients (19 men, 33 women, age 51.7 ± 15.1 years) with malignant melanoma admitted to the Istanbul University Oncology Institute from April 1994 to February 1998. In all patients the diagnosis was proven by histology. Staging was performed according to the criteria of AJCC / UICC based on the TNM system (*Table 1*).

The reference group consisted of 15 healthy people (10 women and 5 men, age 34 ± 13.5 years). All sera were stored at –20°C until assayed and determined blind of clinical information.

The serum levels of soluble receptors were determined quantitatively by specific enzyme-linked immunosorbent assay (ELISA). The serum samples were dilued according to the manufacturer's instructions (R&D Systems, Abington,U.K) (1/10 for CD 44 and ICAM-1 and 1/20 for P-Selectin). Soluble receptor concentrations were calculated from standard curves generated by standard dilutions of known concentrations.

The cut-off values were calculated as the mean ± 2 SD were 880 ng/mL for CD 44, 860 ng/mL for sICAM–1 and 220 ng/mL for P–Selectin, respectively. The results were analysed by Mann-Whitney U test. Kaplan-Meier estimates were performed for survival analysis and differences in overall survival between groups were tested with the log-rank test.[9]

Table 1. **Melanoma patients and disease characteristics**

	n	*%*
Stage		
IA	3	5.9
IB	8	15.7
IIA	12	23.5
IIB	6	11.8
III	15	29.4
IV	7	13.7
Histology		
SSM	12	23.5
NM	11	21.6
ALM	4	7.8
LMM	1	1.9
Unknown	23	45.2
Localization		
Extremity	22	43.1
Trunk	17	33.3
Head&neck	8	15.8
Choroid	3	5.9
Rectum	1	1.9

SSM – superficial spreading melanoma; NM – nodular melanoma; ALM – acral lentiginous melanoma; LMM – lentigo malignant melanoma

Results

The distribution of serum levels in relation to the disease are given in *Table 2*. The serum P-Selectin and sICAM-1 concentrations in melanoma patients and the control group were similar. Likewise, no meaningful difference was observed according to the stage of the disease. On the other hand, serum CD44 levels were significantly lower in patients with malignant melanoma when compared to the controls (p = 0.004). However, a difference according to the stage of the disease was not found. Only one patient among 52 melanoma patients had a higher value than the cut- off levels for P-Selectin and CD44. Serum sICAM–1 level was elevated only in three patients.

In series of 52 patients with follow-up data at the time of analysis, fifteen patients (28.8 %) were dead. The mean survival time was 57.6 ± 2 months, and the four-year survival rate was 56.2%. The survival analyses in terms of the serum cell adhesion molecules could not be performed due to the inadequate number of the patients with elevated serum values. An association between serum adhesion molecule levels and local or advanced disease was not

Table 2. **Soluble adhesion molecules in the serum of malignant melanoma patients (overall results)**

	P-Selectin *(ng/mL) $\overline{x}$ ± SD (m)*	*sICAM-1* *(ng/mL) x ± SD (m)*	*CD 44* *(ng/mL) $\overline{x}$ ± SD (m)*
Control (n = 15)	99.3 ± 61.5 (88)	416.8 ± 221.3 (400)	468.7 ± 207.8 (370)
Malignant melanoma (n = 52)	107.81 ± 51.3 (100)	434.12 ± 279.4 (360)	327.4 ± 166.8 (289)

The mean (x), standard deviation (S.D.) and median (m) values.

Table 3. **Soluble adhesion molecules in the serum of malignant melanoma patients (stage dependency)**

Stage	*n*	*CD44 (ng/mL) $\bar{x} \pm SD$, m*	*ICAM-1 (ng/mL) $\bar{x} \pm SD$, m*	*P-Selectin (ng/mL) $\bar{x} \pm SD$ m*
I	11	305 ± 146.9, 290	462 ± 244.6, 360	105.1 ± 62.2, 78
II	19	347.5 ± 214.6, 289	417.1 ± 330.3, 310	113 ± 56.1, 110
III	16	298.8 ± 122.8, 281	408.9 ± 205, 355	93.3 ± 37.9, 89
IV	6	381.2 ± 144.1, 378	495.8 ± 389.8, 405	134.7 ± 41.9, 138

found. The data are depicted in *Table 3*. Mean serum levels were essentially the same for patients with early (stage I and II) or advanced (stage III and IV) disease.

Discussion

A number of studies examining both cultured melanoma cell lines and excised lesions of melanocytic tissue have demonstrated that sICAM–1 expression correlates with disease progression.[5] sICAM-1 expression has been associated with a reduction in disease-free interval and survival.[8] Increased serum levels of circulating sICAM-1 have been found in patients with malignant melanoma[22] and patients with elevated serum ICAM-1 levels had a significantly shorter survival.[4,8,16] Our results do not support earlier observations since we did not observe a difference between the serum levels in the patients and the control group.

We have also found that P-Selectin levels did not provide useful information in melanoma patients. The serum P-Selectin levels were within the normal range in almost all of the patients. In contrast, positive immunostaining of intratumoral vessels and statistically elevated levels of P-Selectin in serum have been reported in a few studies.[13,16,17] These data were associated with serological tumor markers and poor clinical outcome.

CD44 is remarkable for its ability to generate alternatively spliced forms which may differ in their activities. With its various possible forms, CD44 can play a significant role in many metastatic steps. Expression of CD44 has been linked to tumor progression and metastasis formation in melanoma patients.[12] It was interesting to note that CD44 levels were lower in the patients. However, no association was found between the stage of the disease and serum CD 44 levels. This finding is in contrast to the data observed in advanced colorectal and gastric cancer,[3] but in accordance with a recent study on patients with ovarian cancer[18] and the reports on the presence of low levels of soluble CD44 protein in the circulation of normal individuals.[15] Almost all of the studies concerning cell adhesion molecules have been performed immunohistochemically in melanoma tissue. Therefore, further studies are necessary to characterize the distribution and behavior of the soluble adhesion molecules in the circulation and to determine whether soluble molecules in the circulation can provide a marker for malignant melanoma.

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