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Plasma human leukocyte antigen-G (HLA-G) in patients with thyroid cancer

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Background/aim: A number of tumor markers detected in the serum or pathological specimens using immunohistochemical methods are used for early detection of malignancies and postoperative follow-up. Human leukocyte antigen-G (HLA-G) is a nonclassic HLA class I molecule. Recent studies suggested a relationship between HLA-G positivity and the stage or the phenotype of the malignancy. In this study, the relationship between serum HLA-G positivity and thyroid cancer was investigated.

Materials and methods: Fifty patients with thyroid cancer and 45 healthy volunteers were included in this study. Serum HLA-G levels were measured using ELISA.

Results: HLA-G was positive in only 3 out of 50 patients with thyroid cancer (2 papillary, 1 follicular type). On the other hand, HLA-G was positive in 20 out of 45 healthy subjects ($P < 0.001$). The prevalence of detectable levels of serum HLA-G was independent of sex and age in the whole study population. No correlation was found between serum HLA-G value and thyroid hormone profile, neither in papillary thyroid cancer nor follicular thyroid cancer patients.

Conclusion: In this study, serum HLA-G was significantly less common in patients with thyroid cancer than in healthy controls.

Key words: Thyroid cancer, tumor markers, HLA-G, preoperative diagnosis

1. Introduction

The most important surgical indication for a thyroid nodule is its potential risk of cancer. Though many diagnostic methods and treatment algorithms have been developed, it is not always possible to diagnose thyroid cancers in the preoperative period. The most commonly used method for preoperative diagnosis in thyroid cancers is fine-needle aspiration biopsy (FNAB), which has a range of accuracy of 60%–85%. Diagnosis by FNAB depends on individual factors. First of all, it is necessary to take the biopsy directly from the nodule rather than the neighboring thyroid tissue. For this reason, biopsies taken in the presence of ultrasonography are preferred instead of blind biopsies in many centers. However, if more than one nodule is present or if the biopsy-intensive radiologist experience is inadequate, the accuracy of this method is reduced. Once a biopsy has been taken, it must be evaluated by an experienced cytologist. Therefore, there is a need for nonperson-dependent methods to help identify thyroid cancers preoperatively. For many types of cancer, immunohistochemical staining methods as well as many markers are used as early signs of a possible cancer or in the posttreatment period are investigated in serum and pathology preparations (1)

Human leukocyte antigen-G (HLA-G) is a nonclassic HLA class I molecule. It is now clear that HLA-G positivity is associated with the stages of the disease in breast cancer as in lung cancer, renal cell carcinoma, and malignant melanoma, also indicating different expressions among lobular and ductal subtypes (2).

There are insufficient data on the possible role of serum HLA-G in thyroid cancer patients. In this study, the relationship between thyroid cancer and serum HLA-G was investigated.

2. Materials and methods

The study consists of two groups: 50 patients diagnosed with thyroid cancer and 45 healthy control group subjects. Serum was obtained from blood samples taken from both groups and stored at –80 °C for measurement of the HLA-G level. Patients receiving immunosuppressive treatment, those detected to have another malignancy, and those with pregnancy were excluded from the study.

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2.1. Serum HLA-G measurement method

A commercial ELISA kit was used for the measurement of serum HLA-G (HLA-G ELISA, BioVendor Research and Diagnostic Products, Czech Republic, Catalog No. RD194070100R). Following the addition of 100 µL of serum samples to monoclonal anti-sHLA-G antibody-coated wells, the plate was incubated for 16 hours at 4 °C. At the end of this period, 100 µL/well conjugate solution (monoclonal anti-human β2-microglobulin antibody) was added to wells washed five times in the automated ELISA washer with the prepared washing solution.

The plate, which was incubated for 1 h at room temperature, was finally washed five times in the automatic washer and the substrate was added to make 100 µL/well. After 25 min of incubation at room temperature and in darkness, the reaction was stopped by adding stopper solution (phosphoric acid, 1 M) in such a fashion that it could create 100 µL/well and optical densities due to color changes were spectrophotometrically evaluated in an automated ELISA reader using a 450/620 nm filter.

Microstat, a computer statistical program, was used to calculate the HLA-G concentrations of the samples. Taking advantage of the OD values obtained from known standard concentrations, the OD values of the samples were subjected to a regression-correlation analysis and the concentrations in the samples were calculated. The results were evaluated as HLA-G-positive or HLA-G-negative.

2.2. Statistical analysis

The chi-square test was used for statistical analysis. $P < 0.05$ was considered as significant.

3. Results

Clinical and histological characteristics of the study subjects are summarized in the Table. Patients and controls were matched for age and sex. According to the sixth TNM staging system, patient distribution was as follows: 28 patients in stage I, 10 in stage II, and 2 in stage III for papillary thyroid carcinoma (PTC). Nine patients were in stage I and 1 in stage II for follicular thyroid carcinoma (FTC).

The HLA-G measurement in 47 out of 50 patients with thyroid cancer was found to be negative (blue column) by the ELISA method and positive in three of the cases. On the other hand, the HLA-G measurement was found to be negative (blue column) in 25 out of 45 healthy subjects and positive in 20 by means of ELISA (Figure). Serum HLA-G positivity was found in lower levels in patients with thyroid cancer than in healthy subjects ($P < 0.001$). Among thyroid carcinoma subtypes, serum HLA-G positivity was seen for PTC in 2 patients and for FTC in 1 patient. However, the number of patients was inadequate to obtain significant statistical data. The prevalence of detectable levels of serum HLA-G was independent of sex and age in the whole study population. No correlation was found between serum HLA-G values and thyroid hormone profiles, neither in PTC nor FTC patients.

4. Discussion

Thyroid nodules affect approximately 50%–70% of the adult population. The vast majority of thyroid nodules are benign. However, thyroid cancer is found in 5%–10% of the cases, and it is the most common malignant neoplasia of the endocrine system. PTC and FTC are classified as differentiated tumors, represent 90% of the cases, and are derived from follicular cells (3). Early diagnosis and treatment of thyroid cancer is crucial for improved prognosis and a better survival rate. The characterization of new biomarkers has proved useful, not only for the

Table. Clinical and pathological characteristics of patients with PTC and FTC as well as demographic features of healthy controls

	Controls	PTC patients	FTC patients
	(n: 47)	(n: 40)	(n: 10)
Age (years)	39 (25–49)	40 (26–51)	41 (33–53)
Sex	33/14	30/10	8/2
(female/male)			
TNM			
Stage I		28	9
Stage II		10	1
Stage III		2	-
Stage IV		-	-

Age is reported as median (range). TNM, Sixth TNM tumor staging system. PTC, Papillary thyroid carcinoma. FTC, Follicular thyroid carcinoma.

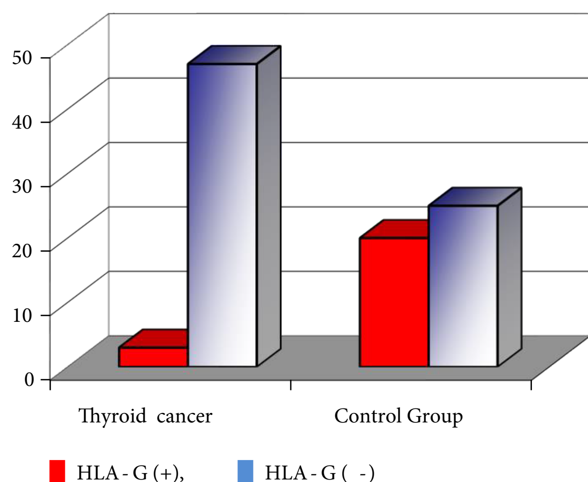

Figure. HLA-G positivity in thyroid cancer patients and healthy subjects.

early detection of thyroid cancer but also for detecting recurrent and persistent diseases and for predicting the effectiveness of surgical removal, radioiodine ablation, and chemotherapy (4).

HLA-G is a nonclassic HLA class I molecule. Compared to classic HLA class I molecules, it exhibits less polymorphism and there are seven different isoforms with 15 different alleles, one of which is null. HLA-G was initially described as the only HLA molecule expressed in some trophoblastic cells, but as a result of studies it has been found that continuous expression is maintained in the immunosuppressed regions (such as the fetomaternal junction, thymus, and cornea), and that the soluble forms are present in amniotic fluid, placental macrophages, cord blood, and peripheral maternal. To date, it has been shown in many studies that HLA-G is a molecule that causes inhibition in a variety of forms (5). In addition, as in other class I molecules, it has also been shown that when HLA-G binds to CD8-bearing cells, it induces apoptosis of CD8+ T cells and NK cells by a Fas-Fas ligand-dependent mechanism and that if HLA-G expression is present on the surface of cells targeted by these cells, cytolytic functions are directly inhibited (6,7). It is thought that the HLA-G molecule may play a key role in the suppression of the expected immune response to the fetus by the mother due to its expression in some trophoblastic cells during the first defined period. Detection of the sustained expression in regions protected from the immune system has led to an emphasis on the immunoinhibitory effect (8).

Recent studies have shown that HLA-G protects muscle cells from lysis in an autoimmune myopathy model (9), suggesting a relationship with a higher level of plasma HLA-G in patients with lung cancer (10). In a study on liver transplantation, it was shown that liver function tests were normal in patients with high levels of plasma HLA-G after transplantation, while the same tests were impaired in patients with low levels of plasma HLA-G, and a correlation between these two parameters was shown. It was also reported that there might be a relationship between plasma HLA-G levels and tissue rejection (11). Expression of HLA-G in malignant diseases including hematological and solid tumors has been extensively investigated, and its relevance to clinical parameters and potential significance in diagnosis, prognosis, and immune target therapy has been postulated (3). Since the first description of HLA-G expression, its association with malignant lesions has been intensively studied. There is evidence that HLA-G is induced in various cancers, including ovarian, gastric, endometrial, breast, renal cell, and lung carcinomas; coetaneous melanoma; hematopoietic tumors; mesothelioma; and trophoblastic tumors. Several studies have demonstrated a correlation between HLA-G expression and the patient's clinical outcome, including overall survival and the risk of developing metastatic diseases (4,12–15). However, the data are still conflicting. To the best of our knowledge, scientific data indicate that HLA-G can serve as a diagnostic and prognostic tumor marker, but data about the possible role of HLA-G in thyroid cancer growth and progression are obscure.

In the literature, there are few publications questioning the relationship between thyroid diseases and HLA-G expression. Kemp et al., evaluating autoimmune thyroid disease, revealed that HLA-G is not expressed in either thyrocytes or thyroid tissue (16). On the other hand, Dardano et al. showed significantly higher plasma HLA-G levels in PTC patients and Hashimoto's thyroiditis when compared to healthy individuals (17). Nunes et al. reported HLA-G expression in 44% of PTC cases, for which the HLA-G expression was correlated with a higher frequency of lymph node metastasis. However, the expression of HLA-G in distinct types of benign and malignant thyroid tumors is not well known (3,4). In these studies, HLA-G was highly expressed in thyroid cancer. In our study, on the contrary, we found it low compared to the control group. In the mentioned studies (3,4), HLA-G was observed in the thyroid tissue, while we studied it in the serum. Measuring HLA-G in the serum with ELISA might not reflect HLA-G expression in the thyroid tissue. To test this hypothesis, it would be wise to determine HLA-G presence in both the serum and the tissue in the same patients. Our results suggest that measuring serum HLA-G may not be a good marker for screening for thyroid cancer.

In conclusion, we found low HLA-G levels in the sera of patients with thyroid cancer, suggesting that measuring serum HLA G may not be a good marker for screening for thyroid cancer. Measurement of HLA-G in both serum and tumor tissue in a larger group of patients with thyroid

cancer, and conducting subgroup analyses of PTC, FTC, and anaplastic thyroid patients, may probably give more accurate information. The lack of evaluation of HLA-G protein expression in both normal thyroid and cancer tissue was the major drawback of our study. The integrated use of serum HLA-G measurement and HLA-G tissue expression might contribute to a better understanding of the actual role of the HLA-G complex in thyroid tumor growth and progression. Moreover, prospective studies are necessary to confirm the actual role and clinical relevance of the HLA-G complex in the development and progression of thyroid cancers.

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