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Sodium Iodide Symporter Protein Expression and Clinicopathological Variables in Pure Testicular Seminomas

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BACKGROUND
Testis carcinomas cause cancer-related morbidity and mortality in adults. Radioiodine I-131 (RAI) therapy may be an alternative for the treatment. The aim of this study is to investigate the presence and localization of sodium iodide symporter (NIS) expression in pure testicular seminomas, two lymph nodes with seminoma metastasis, and two embryonal carcinomas by immunohistochemically methods.

MATERIAL and METHODS
The localization of NIS expression was defined as positive according to >10% membranous staining. Cytoplasmic staining was noted as present/absent. NIS expression was compared to the invasions.

RESULTS
There was no rete testis invasion in 66.7% (26/37) and epididymis invasion in 84.6% (33/37) of the cases. Lymphovascular invasion was seen in 64.1% (25/37) of the cases. There was no membranous staining in pure seminomas, but it was present in two embryonal carcinomas. Cytoplasmic staining was present in 41% (16/34) of the tumors. Cytoplasmic NIS expression was present in 72.7% (p=0.135) and 75% (p=0.6) of the rete testis and epididymis invasions, respectively. There was no statistically important relationship with the cytoplasmic expression, lymphovascular invasion, and the other parameters (p>0.05).

CONCLUSION
Research of NIS expression in pure seminomas with larger series may clear the option of RAI treatment in seminomas. Also, evidence of different expression profiles in non-seminomatous testicular tumors was determined.

Keywords: Adult, male, seminoma, testicular neoplasms, sodium iodide symporter expression

INTRODUCTION

According to Globocan 2012 data, the incidence and mortality of testis carcinomas in males are 0.7% and 0.2%, respectively. The five-year prevalence is 1.4% in adults (1). The incidences of germ cell testis tumors (seminoma/non-seminoma) were doubled every 30 years (2). Testis carcinoma cause cancer-related mortality and morbidity in adult males. Standard treatments of seminomas are radical orchiectomy, follow-up, adjuvant radiotherapy or chemotherapy (2). Although the treatment response of early-stage testis tumors especially seminomas is usually good, resistance may develop in a limited number of patients.

Although radioiodine I-131 (RAI) is primarily used for the treatment of thyroidal cancer, it is able to concentrate in extra-thyroidal organs like the breast, salivary glands, and the gastric mucosa (3). Also, a limited sodium iodide symporter (NIS) expression was determined in normal epididymis, testis, and prostate tissues (4). Therefore, RAI is considered an alternative method for the treatment of extra-thyroidal cancers. There are a few studies about NIS expression in normal testis tissue and testis tumors (5, 6).

Sodium ioidide symporter glycoprotein is necessary for the efficiency of RAI treatment as it is responsible for the iodine transport across the thyrocyte basal membrane. It is mentioned that NIS protein must be expressed in basolateral membranes of thyroid follicular epithelium in order to function (7, 8).

The aims of this study are to investigate the presence and localization of NIS expression in pure testicular seminomas, lymph nodes with seminoma metastasis, and embryonal carcinomas by immunohistochemical methods and to discuss RAI treatment as an alternative therapy.

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MATERIALS and METHODS

Patients

The study materials were 37 pure seminomas, two lymph nodes with seminoma metastasis, and two embryonal carcinomas from the patients who had radical orchiectomy and/or lymph node dissection between 2006 and 2011. Approval was obtained from the ethics committee of our hospital before the study. Informed consent was implied because of the retrospective design of this study.

Immunohistochemical Studies and Grading

Whole sections of the formalin-fixed paraffin-embedded tissues of the cases were stained by the avidin-biotin peroxidase complex method using anti-NIS antibody [SPM186] (Abcam, Cambridge, USA; dilution 1:100) by automatized staining system (Leica, Weitzlar, Germany). Hyperplastic thyroid tissue was used as a positive control. Sections of formalin-fixed paraffin-embedded tissues 4 μm in thickness were fixed on slides covered with poly-L-lysine. After one-night incubation at 37-40°C, dewaxing of the 4 μm sections was completed by another 45 minutes of incubation at 65°C and immersion in xylol for 20 minutes. Slides were rehydrated in alcohol and hydrated in distilled water. For regaining the antigen, the slides were boiled in water buffered with ethylenediaminetetraacetic acid (EDTA; pH: 9.0; Leica, Weitzlar, Germany) and buffer/citrate (pH: 6.0) at 95-99°C for 10 minutes. After the slides were cooled at room temperature for 15-20 minutes, washed with distilled water, and applied with 3% hydrogen peroxide at room temperature for 15 minutes for blocking endogenous peroxidase activity, they were then immerged into distilled water. After washing with phosphate-buffered saline (PBS; 0.01 M), Superblock was applied to the sections and waited for 3-5 minutes. Then the primer antibody was dripped and waited for 30-45 minutes. After washing with PBS, the slides were incubated with a biotin-antibody complex at room temperature for 20 minutes and washed again with PBS for 5 minutes. Slides were incubated at room temperature for 10 minutes with conjugate streptavidin enzyme and washed with PBS. Then 3.3'-diaminobenzidine (Leica, Weitzlar, Germany) chromogen was applied and washed with distilled water. The slides were counterstained with Harris hematoxylin for 10 seconds, then washed with distilled water and dried in alcohol. The slides were mounted with balsam before being examined.

Sodium ioidide symporter expression was evaluated immunohistochemically from whole surface sections of 34 out of 37 pure testicular seminomas that had paraffin-embedded blocks, two lymph nodes with seminoma metastasis, and a separate group of two embryonal carcinomas. Localization of NIS expression was scored as positive according to >10% cytoplasmic membranous staining and no cytoplasmic membranous staining, respectively. Purely cytoplasmic staining was noted as present or absent. Measured diameters of the tumors were recorded in centimeters during macroscopic examination. The presence of multifocal tumors was noted. After verification of the diagnosis of the cases by examining the hematoxylin-eosin stained sections with a light microscope, hilar tissue, tunica vaginalis, lymphovascular tissues, rete testis, and epididymis were reexamined for the invasions. The invasions were compared to NIS expression. The computerized tomography scans and serum markers of 29 follow-up cases (17-101 months) were used for clinical relapse diagnosis.

Statistical Analysis

The Statistical Package for Social Sciences version 18.0 (IBM Corp.; Armonk, NY, USA) program carried out the statistical analysis of the results. A value of $p<0.05$ was accepted as statistically significant.

RESULTS

The mean age of the patients was (34.4±7.4). The tumors were mostly unifocal (89.2%, 33/37). Rete testis invasion was detected in 28.2% (11/37) of the cases while epididymis invasion was found only in 10.1% (4/37). Lymphovascular invasion was seen in 64.1% (25/37) of the tumors. Relapse was seen in three out of 29 follow-up patients. There was no membranous staining in pure seminomas. Both membranous and cytoplasmic staining were not observed in two lymph nodes with seminoma metastasis. Membranous expression was present in two embryonal carcinomas (Figure 1). Cytoplasmic staining was present in 41%

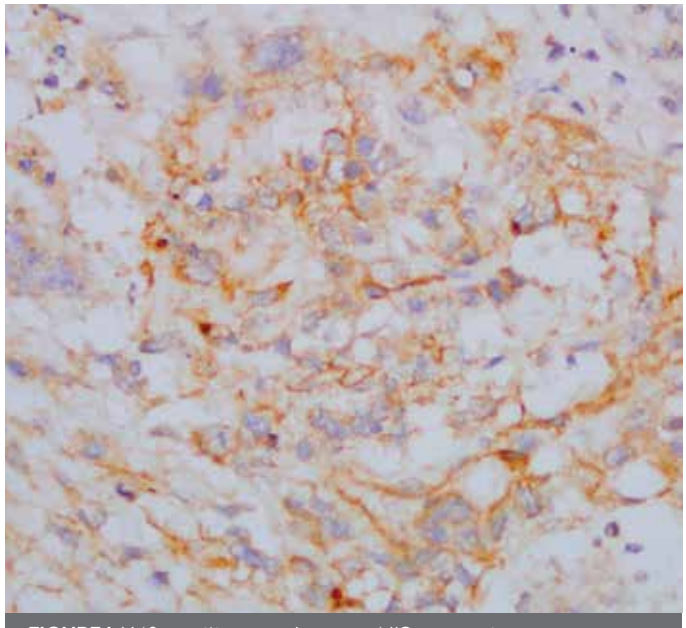

FIGURE 1. X40; positive membranous NIS expression
NIS: sodium ioidide symporter

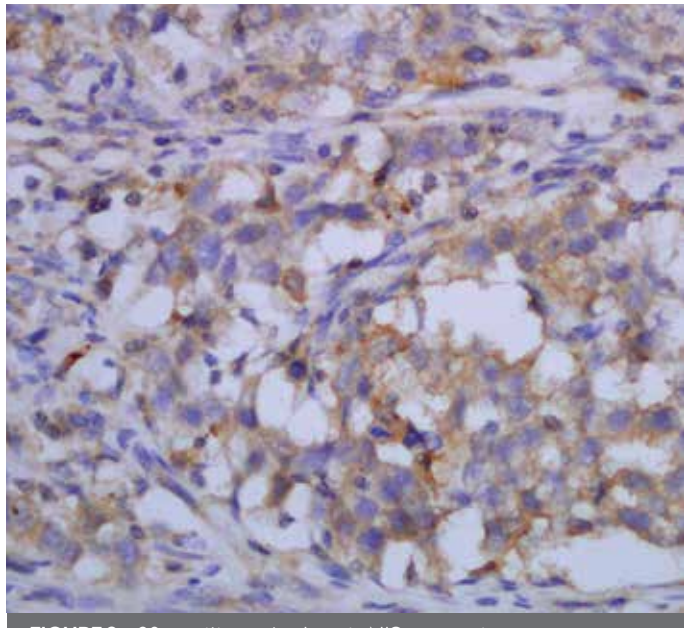

FIGURE 2. x20; positive cytoplasmic NIS expression
NIS: sodium ioidide symporter

TABLE I. NIS expression in testicular seminomas and the correlation of clinic-pathological parameters

	Cytoplasmic NIS Expression		
	Positive Number (%)	Negative Number (%)	p
Age (mean±SD)	37.13±8.14	32±7.27	0.94
Lymphovascular invasion			
Present	11 (50.0)	5 (50.0)	>0.005
Absent	11 (50.0)	5 (50.0)	
Rete testis invasion			
Present	8 (72.7)	3 (27.3)	0.135
Absent	8 (38.1)	13 (61.9)	
Epididymis invasion			
Present	3 (75)	1 (25)	0.6
Absent	13 (46.4)	15 (53.6)	

NIS: sodium ioidide symporter

(16/34) of the tumors (Figure 2). The mean ages of the patients with tumors with and without cytoplasmic staining were 37.1±8.1 and 32±7.3, respectively, (p=0.094). Rete testis and epididymis invasions were present in 72.7% and 75% of the cases with cytoplasmic NIS expression, respectively (p=0.135 and p=0.6). There was no statistically important relationship with the cytoplasmic expression of NIS, lymphovascular invasion, and the other parameters (p>0.05) (Table I).

DISCUSSION

In our study, cytoplasmic NIS expression was observed in pure seminomas. There was neither membranous nor cytoplasmic NIS expression in two retroperitoneal lymph nodes with metastasis. Membranous expression was determined in two embryonal carcinomas.

However, Micali et al. (5) observed >50% high intensity of membranous staining in 64 seminomas and five embryonal carcinomas. Usage of different clones of the monoclonal NIS antibody may be the reason of the different results, as a similar method was used in our study as in Micali et al. (5). Evaluations with NIS antibodies of different clones in a larger series of testis tumors will provide the opportunity for comparison. The investigation of NIS mRNA and protein levels by other methods like western blotting or polymerase chain reaction will be helpful to understand the difference.

Micali et al. (5) determined NIS mRNA expression in five out of eight seminoma cases, but in one of the positive cases, NIS staining was weak with the immunohistochemical method. Micali et al. (5) did not include lymph nodes in their research. There is a need for more studies about metastatic lymph nodes.

In our study, cytoplasmic staining of NIS expression was determined more in rete testis and epididymis invasions. The meaning of cytoplasmic expression is still unclear. The results of Peyrotties et al. (9) show that the intracellular expression of NIS relates to a non-specific signal. Rete testis invasion is thought to be a marker of aggressive behavior of testis tumors (10). Tumor size and rete testis invasion were additional important factors to guide treatment of malignant germ cell testis tumors (11). On the other hand, contrary to our study, Micali et al. (5) found a relationship between membranous expression and lymphovascular invasion. More prospective studies should be done about these parameters for prognostic information.

Standard treatments of seminomas are radical orchiectomy, paraaortic and pelvic lymph node dissection, adjuvant radiotherapy, and chemotherapy. Although seminomas are usually early-stage tumors and curable with standard treatments, chemotherapy after surgery is preferred for non-seminomatous tumors. Güden et al. (12) reported that five-year survival and five-year disease-free survival rates were 98.6% and 90.54%, respectively, in 74 stage I seminomas. According to the World Health Organization toxicity scale, grade I and II enteritis were reported as 9.4% and 5.4% while grade I and II nausea and vomiting were observed in 36.4% and 5.4% of the patients, respectively (12). Radioiodine treatment is worth investigating as an alternative therapy for treatment-resistant tumors or for patients who cannot have radiotherapy or chemotherapy because of severe side effects. Radioiodine is commonly used for the ablation of the residual thyroidal tissue after surgery in thyroid cancers. Side effects like temporary dysfunction in organs like the salivary gland and testis are reported during radioiodine therapy (6).

These observations create the possibility of RAI concentration in other tissues as well. The expression of NIS protein in membranes of tissues like salivary glands, the gastric mucosa, and the lactational breast was reported in several studies (4). An intense expression of NIS was observed especially in the endometrium, urinary bladder, kidney, and bile ducts in the study by Wapnir at al. (4). The researchers also reported a weak expression in testicular tissue (4). NIS mRNA expression was found in the testis nine times less than adult thyroid tissue as determined by Russo et al. (6). NIS expression was localized in the lumen side of the seminiferous ducts and Leydig cells but not observed in Sertoli cells with immunohistochemical examination (6). Interestingly, Sodré et al. (13) reported low NIS mRNA levels in malignant thyroid nodules, and also it was expressed intracellularly. The weak expression of NIS mRNA in testicular tissue may be reason of less concentrated radioiodine. The main regulator of NIS protein is TSH in thyroidal tissue. TSH stimulates NIS transcription, and its translocation to the cell membrane and extends NIS protein half-life (3). However, TSH does not play the same role in breast tissue. The absence of a regulator factor in testicular tissue may reduce the membranous localization of NIS protein. Histone deacetylase inhibitors have shown the capacity to stimulate NIS expression in an in vitro model of Leyding cell carcinoma. This report shows the presence of epigenetic control of NIS expression in Leydig cell tumors (14). Xing and Liu demonstrated that suppressing MAP kinase and PI3/Akt pathways and histone deacetylase could stimulate NIS expression and RAI uptake in non-thyroid human cancer cells (15).

CONCLUSION

In our study, cytoplasmic NIS expression was found in nearly half of the patients with pure seminomas. Cytoplasmic NIS expression was found more in patients with rete testis or epididymis invasion. The clinical importance of cytoplasmic NIS expression in pure seminomas is not clear. More researches about NIS

expression with larger series in pure seminomas and other germ cell tumors are needed to clear the option of RAI treatment.

Also, the finding of evidence about different expression profiles in non-seminomatous testicular tumors should be investigated.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Ankara Atatürk Training and Research Hospital (Decision No: 10715/2015-166).

Informed Consent: Informed consent is not necessary due to the retrospective nature of this study.

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