Original Article / Çalışma - Araştırma

Alterations of NIS expression in functioning thyroid nodules

Fonksiyone tiroid nodüllerinde NIS ekspresyonundaki değişiklikler

Hülya Ilıksu Gözü, M.D.,[1] Dilek Yavuzer, M.D.,[2] Handan Kaya, M.D.,[3] Selahattin Vural, M.D.,[4] Haluk Sargın, M.D.,[1] Cem Gezen, M.D.,[4] Mehmet Sargın, M.D.,[1] Sema Akalın, M.D.[5]

[1]Departments of Endocrinology and Metabolism, [2]Pathology, [4]General Surgery, Dr. Lütfi Kırdar Kartal Training and Research Hospital, İstanbul, Turkey

Departments of [3]Pathology, [5]Endocrinology and Metabolism, Medicine Faculty of Marmara University, İstanbul, Turkey

Objectives: This study aimed to analyze both the level and the cell site of the sodium-iodide symporter (NIS) protein expression in autonomously functioning thyroid nodules (AFTNs) and extranodular thyroid tissues. In addition, this study sought to compare the clinical results of patients with the levels of human NIS (hNIS) protein expression.

Patients and Methods: The histological slides consisted of 36 AFTNs and 31 extranodular thyroid tissues from 28 patients (5 males, 23 females; mean age 54.5±11.0 years; range 37 to 72 years) who underwent surgery for toxic multinodular goitre. The expression of NIS protein was investigated by immunohistochemistry in paraffin-embedded tissue sections using anti-hNIS monoclonal antibody by the labeled streptavidin-biotin method.

Results: The percentage of hNIS positive follicular cells was significantly higher in the AFTNs (13.33±12.09) than in the extranodular thyroid tissues (1.35±3.03). Staining for hNIS was mostly confined to the cell membrane in the AFTNs (88.9%) and in the extranodular thyroid tissues (54.5%). The clinical parameters and nodule volume did not establish any correlation with hNIS immunoreactivity.

Conclusion: Our data indicate that functioning nodules express higher amounts of NIS protein than the extranodular thyroid tissue, but the level of hNIS immunoreactivity was lower than had been reported in the previous literature. This result may be due to interindividual variability between different populations, and iodine status. Furthermore, the localization of the NIS protein might not give an indication of its functional status.

Key Words: Genetic transcription; sodium-iodide symporter; thyroid gland/surgery; thyroid neoplasms.

Amaç: Bu çalışmada, otonom olarak fonksiyone tiroid nodüllerinde (FTN) ve ekstranodüler tiroid dokusunda sodyum-iyot simporter (NIS) protein ekspresyonunun seviyesi ve hücredeki yerleşim yeri araştırıldı. Bunun yanı sıra bu çalışmada hastaların klinik bulgularıyla human NIS (hNIS) protein ekspresyonu seviyeleri karşılaştırıldı.

Hastalar ve Yöntemler: Histolojik kesitler, toksik multinodüler guatr nedeniyle ameliyat olmuş 28 hastaya (5 erkek, 23 kadın; ort. yaş 54.5±11.0 yıl; dağılım 37-72 yıl) ait 36 otonom FTN ve 31 ekstranodüler tiroid dokusundan oluşmaktaydı. Parafine gömülmüş bu doku kesitlerinde, NIS protein ekspresyonu, labeled streptavidin-biotin yöntemi ile anti-hNIS monoklonal antikoru kullanılarak immünohistokimyasal olarak araştırıldı.

Bulgular: Otonom fonksiyone tiroid nodüllerindeki hNIS pozitif hücrelerin oranı (13.33±12.09) ekstranodüler tiroid dokusuna (1.35±3.03) göre anlamlı olarak daha yüksekti. hNIS boyanması genellikle otonom FTN'lerde (%88.9) ve ekstranodüler tiroid dokusunda (%54.5) hücre zarı ile sınırlı idi. Klinik parametreler ve nodül volümü ile hNIS immünoreaktivitesi ilişkili bulunmadı.

Sonuç: Bizim verilerimiz, fonksiyone nodüllerde ekstranodüler tiroid dokusuna göre daha fazla miktarda NIS protein ekspresyonu olduğunu gösterdi, fakat, hNIS immünoreaktivitesi literatürde daha önce bildirilenlere göre daha düşük seviyelerde bulundu. Bu durum değişik toplumlardaki kişisel farklılıklara ve mevcut iyodin durumuna bağlı olabilir. Ayrıca NIS proteininin hücredeki yerleşim yeri NIS'nin fonksiyonunun göstergesi olmayabilir.

Anahtar Sözcükler: Genetik transkripsiyon; sodyum-iyot simporter; tiroid bezi/cerrahi; tiroid tümörleri.

Received / *Geliş tarihi:* July 30, 2010 Accepted / *Kabul tarihi:* September 15, 2010

Correspondence / İletişim adresi: Dilek Yavuzer, M.D. Dr. Lütfi Kırdar Kartal Eğitim ve Araştırma Hastanesi Patoloji Kliniği 34865, Cevizli, İstanbul, Turkey. Tel: +90 216 - 441 39 00 / 1051 Fax *(Faks):* +90 216 - 457 54 74 e-mail *(e-posta):* dilekyavuzer@yahoo.com

Iodide uptake is the first step in thyroid hormone production by thyrocytes.[1-5] The thyroid gland is capable of concentrating iodide by a factor of 20-40 with respect to concentration of the anion in the plasma under physiological conditions.[1,2] The active transport of iodide into the thyroid is mediated by the sodium-iodide symporter (NIS) which is an intrinsic membrane glycoprotein and is localized on the basolateral membrane of the thyroid follicular cells.[2-6] Sodium-iodide symporter-mediated transport of iodide is driven by the electrochemical sodium gradient generated by the Na^+/K^+-ATPase.[1,3] Thyrotropin (TSH) and iodide regulate iodide accumulation in thyroid gland by modulating NIS activity via transcriptional and posttranscriptional mechanisms.[1-3] Since the isolation of a cDNA (Complementary deoxyribonucleic acid) encoding rat NIS in 1996, a number of studies for investigation of the putative role of this protein in different types of thyroid pathologies have been accumulated.[5,7,8]

Autonomously functioning thyroid nodules (AFTNs) are characterized by high levels of iodide trapping that correspond to high levels of NIS gene expression. Their increased iodide transport is the main cause of the hot character of these nodules.[9]

The technique of thyroid scintigraphy using iodine radioisotopes depends on the ability of normally functioning thyroid follicular cells to concentrate iodide.[7] Cold thyroid nodules, most thyroid cancers and some forms of thyroiditis are characterised by low or absent radioiodine uptake. On the other hand several forms of hyperthyroidism, such as Graves' disease and AFTNs revealed increased levels of radioiodine uptake.[7,10,11] Alterations of NIS expression or function in different thyroid pathologies might be responsible for these differencies in radioiodine uptake.[7] The iodide concentrating ability of thyroid follicular cells has allowed the use of radioiodine for diagnosis and therapeutic management of benign thyroid diseases and thyroid cancer.[12,13] Consequently NIS should be important in the characterization and the treatment of autonomously functioning thyroid nodules.

In the literature, high levels of hNIS protein expression have been detected in functioning thyroid nodules with respect to normal thyroid tissues. The cell site of expression was most often confined to the cell membrane.[14-17] The aim of this study was to examine both the level and the cell site of hNIS protein expression in AFTNs and extranodular thyroid tissues, and to compare the clinical characteristics of the patients (sex, age, TSH, FT3, FT4 level, and nodule volume) with the levels of hNIS protein expression.

PATIENTS AND METHODS

Patients and nodules

A total of 28 patients (5 males, 23 females; mean age 54.5±11.0 years; range 37 to 72 years) who underwent near-total thyroidectomy for toxic multinodular goiter (TMNG) were included in this study. Twenty patients had only one hyperfunctioning thyroid nodule, while eight patients had two hyperfunctioning thyroid nodules.

The diagnosis of TMNG was based on the findings of thyroid function tests (high free T_4 and/or free T_3 and supressed thyroid stimulating hormone; TSH), thyroid ultrasonography, thyroid scintigraphy and histopathological examination. One patient had subclinical hyperthyroidism, two patients were euthyroid, 20 patients were clinically thyrotoxic and five patients had already been treated with proplythiouracil or methimazole at the time of diagnosis. Near-total thyroidectomy was performed and histopathology showed nodular hyperplasia in all of the patients.

The nodules which matched with the hyperfunctioning thyroid nodules were identified by scintigraphy, and their surrounding (extranodular) thyroid tissues were dissected and these histological slides were used for determination of NIS protein expression by immunohistochemistry.

Laboratory methods

Free T_3 (FT3), free T_4 (FT4) and TSH were measured by competitive analog immunoassay and immunometric assay (DPC, USA), respectively (Normal values; TSH: 0.27-4.20 μIU/ml, FT3: 2.8-7.1 pmol/L and FT4: 12-22 pmol/L).

The thyroids were examined by using a real time B mode, high resolution, General Electric Ultrasound (US), with a 7.5 MHz probe (Logiq 9). Thyroid and nodule volumes were calculated using the formula (height x length x thickness x $\pi/6$).[18]

Immunohistochemistry

Immunohistochemistry was performed on 10% formalin-fixed, paraffin-embedded tissue sections from 36 AFTNs and 31 extranodular thyroid tissues. One group of sections was stained with hematoxylin-eosin for histological evaluation. Additional

3 μm. sections were used for immunohistochemistry. First, adhesive-coated slides were deparaffinized (incubation at 37 °C overnight and then xylene for 2x20 min.) and rehydrated in alcohol. Tissue sections were subjected to antigen retrieval in 10 mM citrate buffer (pH 6.0) in a microwave oven for 20 min. The endogenous peroxidase activity was blocked by incubating the slides in 3% hydrogen peroxidase solution for 10 min. After that, slides were treated with solution of the anti- hNIS antibody (1:50 dilution) at room temperature for 45 min. Subsequently sections were incubated with a biotin-labelled secondary antibody and avitin-biotin-complex for 20 min, respectively. 3,3'-Diaminobenzidine tetrahydrochloride was used as chromogen. Finally, the sections were counterstained with Mayer's hematoxylin, dehydrated and mounted.

The evaluation was done as follows:[16,17] *(i)* the level of hNIS expression was assessed. If the percentage of follicular cells showing a positive staining for hNIS was 1-10%, it was graded as (+); 11-29%, (++); and more than 30%, (+++); *(ii)* the cell site of hNIS expression (cell membrane or cytoplasm) was established.

Statistical analysis

Computer-assisted data analysis was performed using SPSS (SPSS Inc., Chicago, Illinois, USA) for Windows 10.0 program. In addition to descriptive statistical methods (mean and standard deviation), the Kruskal-Wallis test was used to compare more than two groups and the Mann-Whitney U-test was used to compare two groups. The relationships between the parameters were evaluated by Spearman's correlation analysis. The results were evaluated at 95% confidence interval and $p<0.05$ was considered as significant.

RESULTS

Demographic characteristics of the patients (and nodules)

The characteristics of the patients and their nodules were described in table 1. Mean serum levels of TSH, FT4 and FT3 were measured as 0.14±0.46 μIU/ml (0.005-1.84), 45.65±30.20 pmol/l (4.92-100) and 30.20 pmol/l (5.13-47.61), respectively. The mean volume of thyroid nodules was 15.39 ml (0.20-100).

Immunohistochemistry

The level of hNIS positivity was mild (+) in 15 (41.7%), moderate (++) in 12 (33.3%) and high (+++) in five nodules (13.9%) (Figure 1). Human NIS immunostaining was negative in four out of 36 nodules (11.1%). On the other hand, hNIS immunostaining was shown in only 11 (35.5%) extranodular thyroid tissues. Twenty (64.5%) extranodular thyroid tissues did not show any immunostaining for hNIS. The mean percentage of NIS immunostaining was found significantly higher in AFTNs [13.33±12.09%; (0-40%)] than in extranodular thyroid tissues [1.35±3.03%; (0-15%)], ($z=-4.3$, $p=0.0001$).

Staining for hNIS was confined to the cell membrane in 28 out of 32 nodules (88.9%; Figure 2). It was confined to the cell membrane in six (54.5%) specimens of extranodular thyroid tissues. In the rest of the cases the staining was detected in the cytoplasm (Figure 3). Distribution of the level and localization of immunostaining for hNIS in AFTNs and extranodular thyroid tissues is shown in table 2.

Comparison of the clinical parameters at different levels of hNIS immunostaining

No significant correlation was found between age and the level of hNIS immunostaining in AFTNs ($r=-0.186$, $p=0.278$). The mean percentage of hNIS immonostaining in AFTNs was higher in the female group (14.4±12.8%) than in the male group (9.5±8.5%) but it was not statistically significant ($z=-0.592$, $p=0.554$).

Serum levels of TSH, FT4, and FT3 and nodule volumes were not significantly different between nodules with different levels of hNIS immunostaining. Similarly no significant difference was documented in the serum levels of TSH, TF4 and TF3 in patients with different levels of hNIS immunostaining in extranodular thyroid tissues (Table 3).

DISCUSSION

Various thyroid pathologies are characterized by an altered hNIS protein expression.[10,14-17,19-24] In differentiated thyroid carcinomas, some investigators found 61.8% and 77.5% of NIS positivity rate using immunohistochemistry. They also found less NIS immunopositivity rate in poorly differantiated thyroid carcinomas (33.3% and 25%; respectively).[22,24] But, in other studies, the authors detected either a small amount or absence of hNIS immonostaining in thyroid carcinomas.[10,14,20] The same authors found that hNIS protein expression in normal thyroid tissue was heterogeneous and limited to a minority of follicular cells. In contrast, in Graves' disease the majority of follicular cells

Table 1. Clinical characteristics of the patients (nodules) and hNIS immunostaining in the AFTNs and their surrounding (extranodular) thyroid tissues

No	Age/gender	TSH	FT4	FT3	Nodule volume (ml)	hNIS nodule			hNIS extranodular tissue		
						Level	Positive cells (%)	Cell site	Level	Positive cells (%)	Cell site
1	65/F	0.005	28.56	11.62	10.90	+	2	M	0	0	–
2	72/F	0.013	54.20	20.66	16.26	++	15	M	++	15	M
3	62/M	0.005	38.75	14.01	20.70	+	3	M	0	0	–
3	–	–	–	–	0.20	+	2	C	0	0	–
4	43/F	0.005	29.50	10.00	4.40	+	7	M	+	2	M
5	54/F	1.840	17.64	5.13	0.20	++	25	C	–	–	–
5	–	–	–	–	2.10	+++	30	C	+	1	C
6	58/M	–	–	–	0.90	++	20	M	0	0	–
6	–	–	–	–	9.20	++	25	M	0	0	–
7	54/F	0.005	38.71	9.88	20.21	0	0	–	+	2	C
8	71/F	0.005	35.26	10.08	23.20	0	0	–	+	2	C
9	45/F	0.005	52.25	19.52	19.70	+++	40	M	+	7	M
10	52/M	0.005	100.00	28.00	34.00	+	10	M	0	0	–
10	–	–	–	–	25.53	+	5	M	0	0	–
11	41/F	0.208	4.92	5.30	5.60	++	25	M	+	5	C
13	45/F	0.005	86.23	29.07	39.00	+++	30	M	–	–	–
13	–	–	–	–	6.00	+	2	M	0	0	–
14	50/F	0.005	45.76	47.61	109.00	+	2	M	0	0	–
15	54/F	–	–	–	11.10	++	20	M	0	0	–
16	48/F	0.012	38.30	14.40	–	++	12	M	0	0	–
17	38/F	–	–	–	8.80	+	5	M	0	0	–
18	66/M	0.005	24.03	10.31	12.00	+	8	M	–	–	–
18	–	–	–	–	0.70	+	3	M	–	–	–
19	72/F	0.015	100.00	41.80	9.50	++	20	M	0	0	–
19	–	–	–	–	1.80	+	4	C	0	0	–
20	43/F	–	–	–	17.00	++	25	M	+	1	M
21	37/F	0.005	18.00	6.15	11.30	+++	30	M	–	–	–
22	54/F	0.021	27.80	8.71	4.20	+	3	M	0	0	–
23	62/F	0.009	23.09	14.90	9.20	+++	40	M	0	0	–
23	–	–	–	–	0.70	+	3	M	0	0	–
24	53/F	0.008	26.70	6.72	13.40	++	12	M	0	0	–
25	43/F	–	–	–	13.32	0	0	–	0	0	–
26	60/F	0.035	27.80	9.27	2.30	++	25	M	0	0	–
27	47/F	0.046	25.67	11.59	0.50	0	0	–	+	4	M
28	72/F	0.081	36.70	11.00	4.10	+	7	M	+	1	M

Normal values; TSH: 0.27-4.20 μIU/ml, FT3: 2.8-7.1 pmol/L and FT4: 12-22 pmol/L. M: Males; F: Females; Thyroid function tests were not considered in the patients who had already been treated by antithyroid drugs at the time of diagnosis. The localization of staining was abbreviated as M for membranous and C for cytoplasmic staining; NIS: Sodium-iodide symporter; AFTNs: Autonomously functioning thyroid nodules.

expressed the hNIS protein.[10,20] In some studies, a high level of hNIS expression in functioning (hot) thyroid nodules of toxic multinodular goiter (TMNG), with respect to the nonfunctioning (cold) nodules and normal surrounding thyroid tissues was demonstrated.[15-17,19] Tonacchera et al.[16] detected a high level of hNIS protein expression in six out of eight functioning thyroid nodules (between 40%-80% of follicular cells). The normal extranodular parenchyma showed 1-10% of hNIS

positive cells. The same investigators, in a different study found that 16 AFTNs and 12 toxic adenomas showed a high level of hNIS protein expression (20-80%, 30-90%; respectively) in comparison to normal tissue (1-10%). The mean percentage of hNIS protein expression was measured as 54.8% in hyperplastic nodules, but as 2.3% in extranodular tissue.[17] In cold nodules, hNIS expression was found to be low or similar to normal thyroid tissue.[15,17,19] Moreover nodules from iodine-sufficent area exhibited weak or absent hNIS immunostaining whereas almost all nodules from the iodine-deficient area were hNIS positive.[23]

In the present study, contrary to the literature, the level of hNIS immunostaining was most commonly found to be mild (+) in 41.7% of AFTNs or moderate (++) in 33.3% of AFTNs, while high level immunostaining (+++) was observed in only 13.9% of the AFTNs. Besides, when we evaluated the mean level of hNIS expression, it was found as 13.33±12.09% which is also lower than the literature.

When we think over the discrepancy between our results and the previously published data, we speculate that the interindividual variability between different populations and the iodine supply might be the reason of these results.

One of the main factors regulating the accumulation of iodide in the thyroid and, thus NIS activity, has been considered to be iodide itself. Iodine supply influences the expression and localization of hNIS. Exposure of thyroid cells to high concentrations of iodine in vivo and in vitro results in the reduction of iodide transport and its organification into proteins, the so called acute Wolff-Chaikoff effect. The acute Wolff-Chaikoff effect is transient and, after some days of exposure, an escape or adaptation from effects of iodine occur, through the so called ''escape phenomenon'', the organification of iodide is restored and normal hormone synthesis resumes. In consistent with this, some in vivo studies suggested that the escape from the acute Wolff-Chaikoff effect was associated with a decrease in NIS expression.[12,13]

Iodine deficiency was an important public health problem in Turkey. Goiter prevalence was reported as high as 30.5% in 1988 and as 31.8% in 2002. Legislation for the mandatory iodization of household salt was passed in 1999 and strictly enforced in 2000.[25]

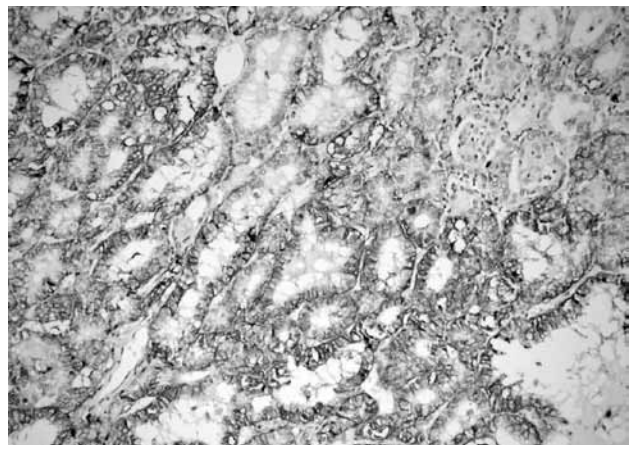

Figure 1. *A hot nodule showing high (+++) immunoreactivity for hNIS (Immunohistohemistry x 200).*

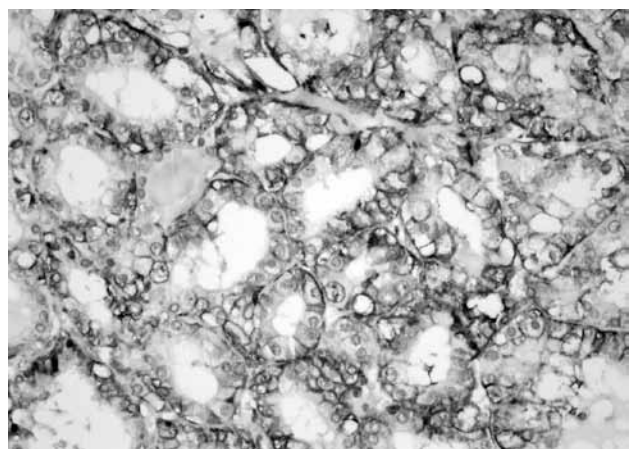

Figure 2. *Membranous staining of hNIS (Immunohistochemistry x 400).*

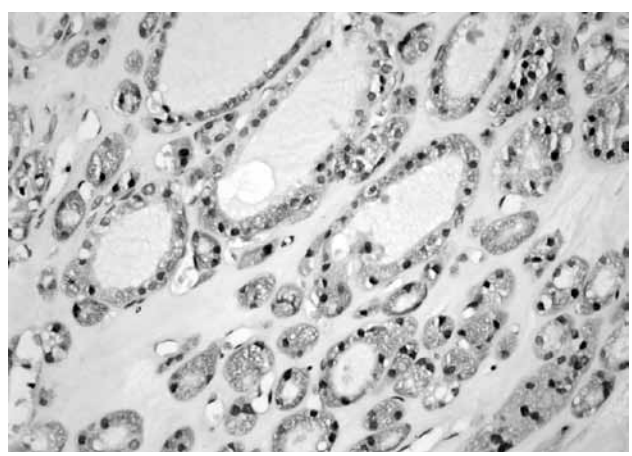

Figure 3. *Cytoplasmic staining of hNIS (Immunohistochemitry x 400).*

In this study the level of hNIS immunoreactivity was lower than had been reported in the previous literature. We speculate that the thyroid tissues tried to regulate continuous excessive

Table 2. The level and localization of hNIS protein immunostaining in AFTNs and the extranodular tissues

	hNIS immunostaining in the nodules		hNIS immunostaining in extranodular tissues	
	n	(%)	n	(%)
No staining	4	11.1	20	64.5
(+)	15	41.7	10	32.3
(++)	12	33.3	1	3.2
(+++)	5	13.9	–	–
Total	36	100	31	100
	The site of hNIS immunostaining in the nodules		The site of hNIS immunostaining in extranodular tissues	
	n	(%)	n	(%)
No staining	4	11.1	20	55.6
Membrane	28	77.8	6	16.7
Cytoplasmic	4	11.1	5	13.9
Total	36	100	31	100

hNIS: Human sodium-iodide symporter; AFTNs: Autonomously functioning thyroid nodules.

exposure to iodide by decreasing NIS expression in follicular cells due to a high iodide intake of the population, at the time of our patient recruitment. This is in consistent with the findings of the study reported by Scipioni et al.[23] They found that nodules from the iodine-sufficient region exhibited weak or absent hNIS immunostaining whereas almost all nodules from the iodine-deficient region were hNIS positive. This finding suggests that iodine supply may exert

Table 3. Comparison of clinical parameters at different levels of hNIS immunostaining in AFTNs and surrounding (extranodular) thyroid tissues

AFTNs	TSH (0.27-4.20 μIU/ml)		FT4 (12-22 pmol/L)		FT3 (2.8-7.1 pmol/L)		Nodule volume (ml)	
	Mean±SD	Median	Mean±SD	Median	Mean±SD	Median	Mean±SD	Median
No staining	0.01±0.02	0.005	33.21±6.7	35.26	10.51± 0.93	10.08	14.30±10.09	16.76
(+)	0.01±0.02	0.005	50.22±31.26	37.72	19.95±12.74	14.01	16.24±27.51	6.70
(++)	0.26±0.63	0.01	46.19±36.16	33.05	17.03±13.80	11.83	14.22±19.70	9.50
(+++)	0.3±0.82	0.005	39.44±29.81	23.09	14.95±9.92	14.90	16.26±14.17	11.30
Total	0.14±0.46	0.005	45.66±30.26	35.98	17.39±11.90	11.90	15.39±21.43	9.50
Test values	KW: 6.1, p=0.105		KW: 1.690 p=0.639		KW: 2.206 p=0.531		KW: 1.144 p=0.766	
Extranodular tissue	TSH (0.27-4.20 μIU/ml)		FT4 (12-22 pmol/L)		FT3 (2.8-7.1 pmol/L)		Nodule volume (ml)	
	Mean±SD	Median	Mean±SD	Median	Mean±SD	Median	Mean±SD	Median
No staining	0.0106±0.008	0.008	53.65±32.68	38.7	21.65±13.43	14.9	15.33±24.32	9.20
(+)	0.030±0.032	0.030	39.66±13.01	36.7	14.55±5.10	11.6	10.32±8.22	10.3
(++)	0.412±0.802	0.005	39.30±36.57	35.3	12.67±11.6	9.88	24.42±27.57	20.2
(+++)	–	–	–	–	–	–	–	–
Total	0.094±0.366	0.008	47.98±30.40	38.3	18.43±12.2	14.01	15.85±22.42	9.35
Test values	KW: 0.718; p=0.698		KW: 1.103; p=0.603		KW: 3.668; p=0.160		KW: 1.079; p=0.583	

NIS: Sodium-iodide symporter; AFTNs: Autonomously functioning thyroid nodule; SD: Standart deviation; KW: Kruskal-Wallis test.

a modulating effect on the regulation of hNIS expression.[23]

In the normal thyroid tissues, hNIS localization was observed in the cell membrane.[16,17] In hyperfunctioning thyroid nodules, the localization of hNIS expression was reported to be confined generally to the cell membrane.[15-17] However, Dohan et al.[26] showed that 70% of differentiated thyroid carcinomas (DTC) exhibited increased hNIS protein expression with respect to the normal surrounding thyroid tissue and significantly, NIS was localized mainly intracellularly in most of the cases. In addition, Tonacchera et al.[16] reported that 54% of benign nonfunctional thyroid nodules overexpressed hNIS protein, as compared to normal surrounding tissue, and NIS was located mainly at the cytoplasmic site in these nodules as well. The authors concluded that these findings strongly suggested that the defective iodide trapping of the most thyroid carcinomas and hypofunctioning nodules is not only due to low NIS expression but also defective targetting of hNIS to the cell membrane.[5,16]

On the other hand, a low level of NIS expression was observed in cold nodules and the localization of NIS protein was in the cell cytoplasm in 40.7% of them. However, in the same study, 30% of the hot nodules showed intracellular localization of NIS.[15] The authors suggested that the decreased iodine trapping, characteristic for cold nodules, might be partly explained by the low level of NIS expression and its cytoplasmic localization. Furthermore, cytoplasmic NIS occurrence in hot nodules are characterized by excessive iodide trapping, indicating that the intracellular localization of NIS does not determine loss of its activity.[15]

Our findings are concordant with the literature as we observed membranous localization in the majority of the nodules (22 out of the 28 functioning nodules; 88.8%). The cytoplasmic localization in 12.2% of the functioning nodules supports the literature suggesting that cytoplasmic localization should not be regarded as a predictor of a loss in NIS activity. In our study, membranous and cytoplasmic localization was almost equal in the extranodular thyroid tissue. We speculate that NIS localization is not a factor to be evaluated to decide on the functional status of the thyroid tissue.

There is limited data on the relation between NIS expression and the clinical parameters in the literature. In one of those studies, Goto et al.[27] detected significant correlations between the NIS messenger ribonucleic acid (RNA) level and the levels of FT3 and FT4, and between the thyroglobulin (Tg) messenger RNA level and goiter weight in a study with Graves' disease patients. However, the authors couldn't find a significant correlation between TSH level and NIS expression. In another study performed on patients with nodular goiter, a significant inverse correlation between the level of NIS expression and the level of FT3 was reported in serum samples. In the same study, the authors examined the patients with hot nodules and also revealed a significant inverse relationship between the level of NIS expression and serum concentration of TSH-R antibodies. Besides, in a group of patients with nontoxic nodular goiter, they found a significant inverse correlation between the level of NIS expression and thyroid volume.[28]

In our study we couldn't find any correlation between the level of hNIS expression and the serum levels of TSH, FT4, and FT3. Similarly, no significant correlation was found between the level of hNIS expression and the nodule volumes.

In conclusion, our study demonstrates that hNIS expression is significantly higher in AFTNs than the surrounding extranodular thyroid tissues. However, the percentage of positive cells were found to be lower in this study compared to some others in the literature. This might be due to inter-individual variability between different populations, iodine supply or other undetermined factors. The result of this study suggest that the localization of the NIS protein does not give an indication on its functional status. Absence of a correlation between clinical parameters and hNIS expression warrants further evaluation.

Acknowledgements

We wish to thank Dr. S. Costagliola from Institut de Recherche Interdisciplinaire en Biologie Humaine et Nucleaire (Bruxelles, Belgium) for the kind gift of anti-human NIS monoclonal antibodies.

REFERENCES

1. Carrasco N. Iodide transport in the thyroid gland. Biochim Biophys Acta 1993;1154:65-82.
2. Dohán O, De la Vieja A, Paroder V, Riedel C, Artani M, Reed M, et al. The sodium/iodide Symporter (NIS): characterization, regulation, and medical significance. Endocr Rev 2003;24:48-77.
3. Bizhanova A, Kopp P. Minireview: The sodium-iodide symporter NIS and pendrin in iodide homeostasis of

the thyroid. Endocrinology 2009;150:1084-90.
4. Nilsson M. Iodide handling by the thyroid epithelial cell. Exp Clin Endocrinol Diabetes 2001;109:13-7.
5. Dohán O, Carrasco N. Advances in Na(+)/I(-) symporter (NIS) research in the thyroid and beyond. Mol Cell Endocrinol 2003;213:59-70.
6. Eskandari S, Loo DD, Dai G, Levy O, Wright EM, Carrasco N. Thyroid Na+/I- symporter. Mechanism, stoichiometry, and specificity. J Biol Chem 1997; 272:27230-8.
7. Joba W, Spitzweg C, Schriever K, Heufelder AE. Analysis of human sodium/iodide symporter, thyroid transcription factor-1, and paired-box-protein-8 gene expression in benign thyroid diseases. Thyroid 1999;9:455-66.
8. Dai G, Levy O, Carrasco N. Cloning and characterization of the thyroid iodide transporter. Nature 1996;379:458-60.
9. Deleu S, Allory Y, Radulescu A, Pirson I, Carrasco N, Corvilain B, et al. Characterization of autonomous thyroid adenoma: metabolism, gene expression, and pathology. Thyroid 2000;10:131-40.
10. Caillou B, Troalen F, Baudin E, Talbot M, Filetti S, Schlumberger M, et al. Na+/I- symporter distribution in human thyroid tissues: an immunohistochemical study. J Clin Endocrinol Metab 1998;83:4102-6.
11. McDougall IR. In vivo radionuclide tests and imaging. In: Braverman LE, Utiger RD, editors. Werner and Ingbar's The Thyroid. 9th ed. Philadelphia: Lippincott Williams & Wilkins; 2005. p. 309-28.
12. Riesco-Eizaguirre G, Santisteban P. A perspective view of sodium iodide symporter research and its clinical implications. Eur J Endocrinol 2006;155:495-512.
13. Carvalho DP, Ferreira AC. The importance of sodium/iodide symporter (NIS) for thyroid cancer management. Arq Bras Endocrinol Metabol 2007;51:672-82.
14. Faggiano A, Caillou B, Lacroix L, Talbot M, Filetti S, Bidart JM, et al. Functional characterization of human thyroid tissue with immunohistochemistry. Thyroid 2007;17:203-11.
15. Syrenicz A, Wolny M, Kram A, Sworczak K, Syrenicz M, Garanty-Bogacka B, et al. Analysis of the sodium iodide symporter expression in histological slides from a nodular goiter. Arch Med Res 2007;38:219-26.
16. Tonacchera M, Viacava P, Agretti P, de Marco G, Perri A, di Cosmo C, et al. Benign nonfunctioning thyroid adenomas are characterized by a defective targeting to cell membrane or a reduced expression of the sodium iodide symporter protein. J Clin Endocrinol Metab 2002;87:352-7.
17. Tonacchera M, Viacava P, Fanelli G, Agretti P, De Marco G, De Servi M, et al. The sodium-iodide symporter protein is always present at a low expression and confined to the cell membrane in nonfunctioning nonadenomatous nodules of toxic nodular goitre. Clin Endocrinol (Oxf) 2004;61:40-5.
18. Ueda D. Sonographic measurement of the volume of the thyroid gland in healthy children. Acta Paediatr Jpn 1989;31:352-4.
19. Russo D, Bulotta S, Bruno R, Arturi F, Giannasio P, Derwahl M, et al. Sodium/iodide symporter (NIS) and pendrin are expressed differently in hot and cold nodules of thyroid toxic multinodular goiter. Eur J Endocrinol 2001;145:591-7.
20. Jhiang SM, Cho JY, Ryu KY, DeYoung BR, Smanik PA, McGaughy VR, et al. An immunohistochemical study of Na+/I- symporter in human thyroid tissues and salivary gland tissues. Endocrinology 1998;139:4416-9.
21. Castro MR, Bergert ER, Beito TG, McIver B, Goellner JR, Morris JC. Development of monoclonal antibodies against the human sodium iodide symporter: immunohistochemical characterization of this protein in thyroid cells. J Clin Endocrinol Metab 1999;84:2957-62.
22. Mishra A, Pal L, Mishra SK. Distribution of Na+/I-symporter in thyroid cancers in an iodine-deficient population: an immunohistochemical study. World J Surg 2007;31:1737-42.
23. Scipioni A, Ferretti E, Soda G, Tosi E, Bruno R, Costante G, et al. hNIS protein in thyroid: the iodine supply influences its expression and localization. Thyroid 2007;17:613-8.
24. Wapnir IL, van de Rijn M, Nowels K, Amenta PS, Walton K, Montgomery K, et al. Immunohistochemical profile of the sodium/iodide symporter in thyroid, breast, and other carcinomas using high density tissue microarrays and conventional sections. J Clin Endocrinol Metab 2003;88:1880-8.
25. Erdoğan G, Erdogan MF, Emral R, Baştemir M, Sav H, Haznedaroğlu D, et al. Iodine status and goiter prevalence in Turkey before mandatory iodization. J Endocrinol Invest 2002;25:224-8.
26. Dohán O, Baloch Z, Bánrévi Z, Livolsi V, Carrasco N. Rapid communication: predominant intracellular overexpression of the Na(+)/I(-) symporter (NIS) in a large sampling of thyroid cancer cases. J Clin Endocrinol Metab 2001;86:2697-700.
27. Goto A, Uchino S, Noguchi S, Wakiya S, Watanabe Y, Murakami T, et al. NIS mRNA expression level in resected thyroid tissue as a marker of postoperative hypothyroidism after subtotal thyroidectomy in patients with Graves' disease. Endocr J 2008;55:73-81.
28. Syrenicz A, Wolny M, Kram A, Sworczak K, Syrenicz M, Garanty-Bogacka B, et al. Correlation between level of sodium/iodide symporter expression in tissue sections and some clinical parameters in patients with nodular goiter. Arch Med Sci 2006;2:240-6.