Correlation between level of sodium/iodide symporter expression in tissue sections and some clinical parameters in patients with nodular goiter

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Abstract

Introduction: The aim of the study was to correlate the level of NIS expression in hot, warm and cold nodules with some clinical parameters such as TSH, FT3, FT4, TG, TPO-Ab, TG-Ab, TSH-R-Ab in serum samples, iodine in morning urine samples and thyroid volume. Based on these correlations we tried to find clinical factors that interact with NIS expression in benign nodules and at the same time indicators of NIS expression.
Material and methods: The study population consisted of 97 people who underwent surgery for toxic nodular goiter (26 people) or non-toxic nodular goiter (71 people). NIS expression was detected by immunohistochemistry on tissue sections of benign nodules and extranodular parenchyma. The level of NIS expression was estimated objectively using a computer program.
Results: The study results demonstrated a significant inverse correlation between the level of NIS expression and FT3 in serum samples in the whole study population and in the group of patients with warm nodules who were not treated with thyreostatics. Furthermore, we revealed a significant inverse correlation between the level of NIS expression and serum concentration of TSH-R antibodies in patients with hot nodules. Additionally, a significant inverse correlation between the level of NIS expression and thyroid volume was investigated in the group of patients with nontoxic nodular goiter.
Conclusions: Our data indicate the autoregulatory effect of FT3 on NIS expression. Furthermore, it seems that enlargement of thyroid volume in nontoxic nodular goiter coexists with decreased level of NIS expression.

Key words: NIS protein, immunohistochemistry, iodide trapping.

Introduction

The sodium/iodide (Na^+/I^-) symporter (NIS) is a glycoprotein located at the basolateral plasma membrane of the thyroid follicular cells, which is responsible for active iodide uptake into thyrocytes [1-3]. The transport and iodide concentration in thyroid follicular cells represents the first step in the production of thyroid hormones [4]. NIS discovery in 1996 [5] contributed to broadening the knowledge on thyroid pathophysiology. Since thyroid tissue is heterogeneous in a nodular goiter with places of excessive, normal or decreased iodide uptake according to scintiscan, any changes in NIS expression could explain the variable functional status of benign nodules and might be suggestive for nodular goiter

aetiopathogenesis [6-10]. There are also some practical implications of NIS expression studies in a nodular goiter. The high level of NIS in a benign thyroid nodule may be taken as an indication of high efficiency of radioactive iodide therapy in hyperthyroidism treatment in the course of a nodular goiter. In addition, high activity of the sodium iodide symporter in a nontoxic nodular goiter appears to indicate the efficiency of I^{131} therapy in diminishing nodular size.

Studies of the level of NIS expression are not attainable in clinical practice and they are performed postoperatively. Therefore, based on the correlation between NIS expression in thyroid nodules and most often examined clinical parameters, some indicators of NIS expression might be found that could be essential for diagnosis and therapy of a nodular goiter. Additionally, these correlations may reveal clinical factors interacting with NIS expression in a nodular goiter.

In the present study we showed, using immunohistochemistry, the level of NIS expression and its cellular location in thyroid nodules and extranodular parenchyma in tissue sections of patients who underwent surgery for a nodular goiter. The level of NIS expression was estimated objectively using a computer program. Our aim was to correlate separately two quantitative indicators of the level of NIS expression with some clinical parameters: thyreotropin (TSH), triiodothyronine (FT3), thyroxine (FT4), thyroglobulin (TG), thyroid antibodies (ATA – antiperoxidase antibodies, ATG – antithyreoglobulin antibodies, TRAK – antithyreotropin antibodies) in serum samples, iodide in morning urine samples and volume of thyroid.

Material and methods

Patients

The study included 97 patients (81 females and 16 males) who underwent surgery for toxic nodular goiter (26 people: 21 females, 5 males) or nontoxic nodular goiter (71 people: 60 females, 11 males). Thyroid glands of patients were studied by physical examination, thyroid ultrasound, FNAB (fine-needle aspiration biopsy), scintiscan imaging and histology. All scintigraphic research was performed using pertechnetate (Tc99m). Thyroid nodules were benign in cytology from FNAB and the cytological diagnosis in each case was verified by postoperative histopathologic investigation.

According to scintigraphy we divided nodules into three groups: hot, warm and cold ones. Among patients with a toxic nodular goiter 18 people had hot nodules and 8 people had warm nodules in the scintiscan.

Among patients with a nontoxic nodular goiter 10 people had hot nodules, 34 people had warm nodules, 23 people had cold nodules and 4 patients had two kinds of nodules in scintiscan: warm and cold in two cases, hot and cold in two other cases. Based on ultrasonography thyroid volumes were calculated by the spherical ellipsoid formula (volume (ml) = $1/6\pi$ x AP (cm) x length (cm) x width (cm), where AP is anteroposterior diameter; volume of thyroid = volume of left thyroid lobe + volume of right thyroid lobe).

Blood samples were collected to examine the serum concentration of thyroid hormones, thyroid antibodies, thyreoglobulin and morning urine samples to assess the concentration of iodine. Patients with a toxic nodular goiter were prepared for surgery with antithyroid drugs and almost all of them were in euthyreosis (94%) or in slight hypothyreosis on the day of surgery. Therefore laboratory results of FT3 and FT4, taken in statistical evaluation, were in the normal or almost normal range. Exceptionally, TSH results of patients with thyrotoxicosis, taken into account in statistical measurements, dated before antithyroid treatment and they were diminished (TSH<0.4 uIU/ml).

This study was approved by the local ethical committee.

Laboratory measurements

TSH was measured by a radioimmunometric assay (TSH-IRMA, BioSource Europe S.A., Belgium). FT3, FT4, TPO-Ab, TG-Ab were assessed by radioimmunoassay (RIA-gnost FT3, RIA-gnost FT4, Schering S.A. France; RIAZENco TPO Ab, RIAZENco TG Ab, ZenTech S.A., Belgium). Thyroglobulin was measured by immunometric assay (IMMULITE-Thyroglobulin, EURO/DPC, Italy). TSH-receptor antibodies were searched for using radioreceptor assay (RIAZENco TSH-R Ab, ZenTech SA, Belgium). Iodine concentration in morning urine samples was assessed based on Sandell-Kolthoff reaction.

Immunohistochemistry

Immunohistochemistry was performed with 198 histological slides (study slides: 30 slides of hot nodules, 44 slides of warm nodules, 27 slides of cold nodules and 97 control slides of the non-nodular tissue of each patient).

All histological slides were stained using monoclonal anti-NIS antibodies by the labelled streptavidin-biotin method. Tissues were fixed in 10% formalin and embedded in paraffin. One group of sections was stained with haematoxilin-eosin for histological evaluation. Additional sections were used for the immunohistochemistry.

At first, sections were deparaffinized (incubation at 57°C for 16-18 hours and then in xylene for 2 x 20 min.) and rehydrated in alcohol. Then slides were incubated at 99°C-100°C in Target Retrieval Solution for 20 min. After cooling down, endogenous peroxidase activity was blocked by 3% hydrogen peroxide solution. After that, slides were incubated with a solution of monoclonal hNIS antibodies (10 µg/ml) at room temperature for 30 min. Afterwards, antibodies marked with biotin and then streptavidin tied with peroxidase were used (DAKO LSAB+ Kit, incubation with each

reagent for 20 min at room temperature). EAC chromogen – a substrate of reaction with peroxidase (incubation for 5 min at room temperature) – was used to obtain a red colour product, which was estimated in the optical microscope. Furthermore, slides were stained in haematoxylin for 1 min and rinsed using running water for 10 min. The subjective estimation of immunohistochemical expression consisted of initial selection of slides for positive and negative ones according to presence or absence of cytoplasmic and/or plasma membrane immunohistochemical staining. The areas with the highest expression of hNIS were selected for further computerized analysis.

The 2-3 areas consisted of in total approximately 200 thyreocytes with the highest expression of hNIS being selected for further computerized analysis. The immunohistochemical level of NIS expression was estimated objectively using the computerized image analysis system Quantimet 600S (Leica, Cambridge, UK) with the software adaptation done by one of us (AK). To estimate results comparatively, two indicators were arbitrarily chosen and labelled as IMM1CELL (positive immunostained area in 1 cell in relation to the positive immunostained area in 1 cell of the model slide) and IMMIOD1CELL (integrated optical density of positive immunostained area in 1 cell in relation to integrated optical density of positive immunostained area in 1 cell of the model slide), described in formulae as below. The first parameter reflected positive area of staining, the latter reflected intensity of staining. The model slide was selected from all other cases as the one with the highest immunohistochemical expression of hNIS and with the correct localization in thyroid cells. This case was used as a positive control slide in every set of immunohistochemical stainings and as the model slide in the computer estimation of hNIS immunohistochemical expression. In each immunostained slide we estimated approximately 200 thyroid cells.

$$IMM1CELL = \frac{\text{area of immunostaining/number of positive cells}}{IMM1CELL.TEST}$$

$$IMM1CELL.TEST = \frac{\text{area of immunostaining in model slide}}{\text{number of cells in model slide}}$$

$$IMMIOD1CELL = \frac{\text{IOD of immunostaining/number of positive cells}}{IMMIOD1CELL.TEST}$$

$$IMMIOD1CELL.TEST = \frac{\text{IOD of immunostaining in model slide}}{\text{number of cells in model slide}}$$

IOD – Integrated Optical Density

Statistical methods

Levels of NIS expression detected in each scintigraphically-described group of nodules were compared with its expression in the surrounding extranodular parenchyma using Wilcoxon test. The correlation analysis between level of NIS expression and laboratory results and thyroid volume was studied using Spearman's rank order correlation coefficient. The level of significance was taken as $p<0.05$.

Results

General characteristic of laboratory results in study population

The general characteristics of some clinical parameters are shown in Table I. The thyreotropin

Table I. Characteristics of laboratory results and thyroid volume with regard to study population division according to scintigraphically-described kinds of nodules in thyroid

Clinical parameter	Values of normal range	Below normal range			Under normal range		
		number and (%) of patients with			number and (%) of patients with		
		hot nodules	warm nodules	cold nodules	hot nodules	warm nodules	cold nodules
TSH	0.4-4.0 uIU/ml	11 (36.7%)	9 (20.4%)	0 (0%)	0 (0%)	1 (2.3%)	0 (0%)
FT3	3.07-6.53 pmol/L	3 (10%)	0 (0%)	0 (0%)	3 (10%)	1 (2.3%)	1 (3.7%)
FT4	9.01-23.17 pmol/L	2 (6.67%)	2 (4.55%)	0 (0%)	2 (6.67%)	2 (4.55%)	1 (3.7%)
TPO Ab	< or = 30 IU/ml	x	x	x	11 (36.7%)	19 (43.2%)	10 (37%)
TG Ab	< or = 30 IU/ml	x	x	x	6 (20%)	6 (13.6%)	7 (25.9%)
TSH-R Ab	< or = 14 IU/ml	x	x	x	1 (3.3%)	1 (2.3%)	0 (0%)
TG	1.7-55.6 ng/ml	1 (3.3%)	1 (2.3%)	0 (0%)	20 (66.7%)	28 (63.6%)	17 (62.9%)
Iodine	> or = 100 ug/L	28 (93.3%)	35 (79.5%)	20 (74.1%)	x	x	x
volume of thyroid	< or = 18 ml (women)	x	x	x	23 (76.7%)	27 (61.4%)	21 (77.8%)
	< or = 25 ml (men)	x	x	x	2 (6.67%)	9 (20.4%)	2 (7.4%)

Table II. Characteristics of IMM1CELL and IMMIOD1CELL from all nodules (study slides) and surrounding parenchyma (control slides). Comparison analysis of the level of NIS expression between nodules and surrounding parenchyma

Indicator of staining	IMM1CELL		IMMIOD1CELL	
slides	study	control	study	control
number of slides	101	101	101	101
min-max	0-3.00	0-1.240	0-1.160	0-1.160
Q_1-Q_3	0.045-0.270	0.023-0.140	0.025-0.180	0.011-0.100
median value	0.12	0.074	0.074	0.054
p (Wilcoxon test)	<0.0001		0.002	

min –minimum value
max – maximum value
Q_1 – quartile 1
Q_3 – quartile 3
p – level of significance

IMM1CELL – positive immunostained area in 1 cell in relation to the positive immunostained area in 1 cell of the model slide
IMMIOD1CELL – integrated optical density of positive immunostained area in 1 cell in relation to integrated optical density of positive immunostained area in 1 cell of the model slide

(TSH) results were taken into account dated before antithyroid treatment in patients with a toxic nodular goiter. However, the triiodothyronine (FT3) and thyroxine (FT4) results were measured after treatment of patients with thyreotoxicosis.

Level of NIS protein expression in all studied slides and in groups of hot, warm and cold nodules with respect to the surrounding parenchyma. Comparison analysis between scintigraphically-described groups of nodules

The general characteristics of the level of NIS expression are shown in Table II. In the whole study population the level of NIS expression was significantly higher in study slides from nodules than in control slides from the collateral tissue (Table II).

Correlation analysis between level of NIS expression and laboratory results and thyroid volume

The statistical analysis of correlation between the level of NIS protein and laboratory results revealed an inverse, significant correlation between triiodothyronine and two indicators of the level of NIS expression in the whole study population. A correlation between FT3 and IMM1CELL was shown also in the group of patients with warm nodules and in the smaller group of patients with warm nodules who were not treated with thyreostatics. We also revealed a significant inverse correlation between IMM1CELL and serum concentration of TSH-R antibodies in patients with hot nodules. Furthermore, a significant inverse correlation between the level of NIS expression and thyroid volume was investigated in the group of patients with a nontoxic nodular goiter (Table III).

Discussion

In the present study we showed a significant inverse correlation between triiodothyronine in serum samples and two indicators of the level of NIS expression in thyroid nodules of all the study population. The same correlation, but concerning only one indicator of immunostaining (IMM1CELL), was revealed in the group of patients with warm nodules that were not treated with antithyroid drugs at all. This data may indicate that triiodothyronine decreases the level of NIS expression in warm thyroid nodules. A similar result was observed in the study performed on Fisher Rat Thyroid Line 5 (FRTL-5). In this study incubation of FRTL-5 cells with T3 decreased iodide accumulation by up to 40% and resulted in an up to 80% decrease of NIS RNA steady-state level in a concentration-dependent manner. In addition, T3-treated FRTL-5 cells revealed a 60% decrease in NIS protein expression as compared to untreated cells [11]. NIS protein participation in thyroid hormone biosynthesis suggests autoregulatory feedback inhibition of T3 on the sodium/iodide symporter at the level of the thyrocytes [11].

Our data demonstrate a significant inverse correlation between the level of anti-receptor TSH antibodies (TSH-R Ab) in serum samples and immunostained area (IMM1CELL) in the group of hot nodules. TSH-R antibodies may stimulate or inhibit TSH receptor. Antibodies that stimulate TSH receptor theoretically may cause an increase of NIS protein biosynthesis according to the proven stimulatory effect of thyreotropin on NIS activity [12, 13]. On the other hand, the activity of inhibiting antibodies theoretically results in a decrease of NIS biosynthesis and might explain the inverse correlation between the serum level of TSH-R antibodies and one

Table III. Statistically significant correlations between IMM1CELL, IMMIOD1CELL and some clinical parameters in the whole study population and in groups of patients with some kinds of nodules and with a nontoxic nodular goiter

Group of nodules	All (n=101)	Warm (n=44)	Warm no treated (n=36)	Hot (n=30)	From nontoxic goiter (n=75)
clinical parameter	FT3	FT3	FT3	TSH-R Ab	thyroid volume
IMM1CELL	r_S= -0.226, p<0.03	r_S= -0.297, p<0.03	r_S= -0.332, p<0.05	r_S= -0.381, p<0.04	r_S= -0.246, p<0.04
IMMIOD1CELL	r_S= -0.204, p<0.05	r_S= -0.221, p>0.14	r_S= -0.284, p>0.09	r_S= -0.354, p>0.054	r_S= -0.254, p<0.03

n – quantity of nodules
r_S – Spearman's rank order correlation coefficient
p – level of significance

IMM1CELL – positive immunostained area in 1 cell in relation to the positive immunostained area in 1 cell of the model slide
IMMIOD1CELL – integrated optical density of positive immunostained area in 1 cell in relation to integrated optical density of positive immunostained area in 1 cell of the model slide

quantitative indicator of NIS expression (IMM1CELL). However, because of the autonomy of hot nodules, the contribution of TSH-R antibodies in regulation of NIS expression seems to be questionable. In hitherto-published studies there is no news concerning interaction of TSH-R Ab and NIS expression and this correlation should be confirmed in other studies.

We also revealed a significant inverse correlation between the level of NIS expression and thyroid volume in the group of patients with a nontoxic nodular goiter (tantamount to "patients were not treated with thyreostatics"). This interrelationship indicates lower NIS expression in high volume nontoxic nodular goiter.

What is more, regarding the positive correlation between the volume of nontoxic nodular goiter and quantity of thyroid nodules [17], this correlation may suggest that the level of NIS expression is related to morphological changes in the thyroid. Specifically, enlargement of the thyroid gland and increase of quantity of nodules in the course of nontoxic nodular goiter coexist with diminished iodine trapping by the sodium/iodide symporter, whereas the lack of correlation between the level of NIS protein expression and the volume of toxic nodular goiter confirms that NIS is overexpressed in hot and warm nodules independently of progress in morphological changes of thyroid.

In healthy thyroid tissue iodide uptake is primarily regulated by thyreotropin. TSH upregulates NIS gene expression and NIS protein abundance, and influences NIS cellular distribution [12, 13]. However, in thyroid nodules, as our data show, there is dissociation between the serum concentration of TSH and the level of NIS expression. In addition there is no significant difference in TSH concentration in serum samples of patients with membranous localisation of NIS protein and its cytoplasmatic distribution in thyrocytes of thyroid nodules (data not shown). It seems that TSH regulates synthesis and cellular distribution of NIS in a minor degree or these processes are completely independent of thyreotropin in thyroid nodules.

The high level of NIS expression in hot and warm nodules from toxic nodular goiters in patients with low serum concentration of TSH and significantly lower level of NIS expression in cold nodules in patients with normal serum concentration of TSH confirm this theory.

The development of functional autonomy is one of the characteristics of a nodular goiter. Some normal thyroid follicular cells take up and organify iodine in the absence of TSH. During goitrogenesis, the number of cells with functional autonomy increases, especially when the cells also have a high replicating capacity. The increase in the total mass of follicular cells with autonomous iodine metabolism during goiter growth explains why patients with a nontoxic goiter can eventually develop subclinical and then overt thyreotoxicosis [15]. Additionally, the inverse correlation observed between TSH and thyroid volume [14] does not confirm an important contribution of TSH in nodular goiter growth.

The study evaluated in rats showed that modulation of the thyroid NIS by TSH depends primarily on thyroid iodine content. Rats with high TSH concentration (after treatment with methimazol) showed a decrease in NIS activity when their thyroid pool of organic iodine increased. In conclusion, regulation of NIS activity by iodide can be more important than regulation by TSH [16]. Since the iodine excess causes a decrease of NIS protein expression [16, 17], the iodine deficiency results in stimulation of the iodine trapping by increased secretion of TSH or independently of TSH action [18]. The mechanisms of adaptation to iodine deficiency change and within the age lower TSH serum concentration and reduced iodide trapping in endemic goiter are observed [18]. In the present study the low iodine supply was investigated in most of the study population (iodine concentration in morning urine sample was below normal range

in 82.5% of patients). No significant correlation was shown between the level of NIS expression and iodide concentration in urine samples.

In addition no relationship was investigated between iodide urine concentration in patients with hot, warm and cold nodules (data not shown). Thus, it seems that the degree of iodine supply does not influence the level of NIS expression in thyroid benign nodules and does not participate in the development of nodular goiter heterogeneity. This conclusion confirms actual knowledge about iodine participation in nodular goiter aetiopathogenesis. It is generally accepted that chronic iodide deficiency initially leads to the development of a diffuse homogeneous goiter, which may later be transformed into a multinodular goiter. However, low iodide intake by itself does not explain the pathogenesis of nodularity [19]. Low iodine content in thyroid tissue observed either in endemic goiter or in sporadic goiter is the consequence rather than the cause of nodule formation and results from development of thyroid follicles with decreased iodine uptake [20].

Follicular thyreoglobulin suppresses iodide uptake by suppressing expression of NIS protein [21]. In the present study only the serum concentration of thyroglobulin was measured in the study population. 75.3% of patients had increased concentration of thyreoglobulin concentration. No correlation between the level of NIS expression in nodules and thyreoglobulin concentration in serum samples was observed. It may seem that regulation of NIS expression is not influenced by thyreoglobulin or the effect of thyreoglobulin is weaker in benign nodules. However, regarding the unknown follicular thyroglobulin content in study thyroid tissues this result should be considered with caution.

Conclusions

In summary, the inverse correlation between the level of NIS expression in nodules (especially IMM1CELL in warm nodules) and serum concentration of triiodothyronine in patients not treated with thyreostatics indicates the autoregulatory effect of this hormone on NIS expression in thyroid nodules. The level of NIS expression is inversely correlated with thyroid volume, which may indicate that sodium/iodide symporter expression is related to the pathological changes in morphology of a nontoxic nodular goiter. However, in a toxic nodular goiter the level of NIS expression is high independently of any morphological changes of thyroid. The TSH-R antibodies may influence NIS expression, but this interrelationship should be confirmed in further studies. The lack of correlation between the level of NIS expression and TSH may indicate that this main stimulator of NIS activity in normal thyroid tissue does not influence NIS expression in benign nodules.

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