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Relative DNA concentration in thyrocytes from scintigraphically hot nodi

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The aim *of our study was to study the cytological appearance and the relative DNA content of scintigraphically hot thyroid nodi.*
Methods: *Sixty-seven patients with hyperthyroidism due to hot nodi were treated. The relative DNA content of thyrocytes in hot nodi was determined by single cell cytophotometry and compared to results of cytology, and scintigraphy. T4, T3, TSH and thyroglobulin were measured in sera of the patients as well.*
Results: *The modal value of the relative DNA concentration in thyrocytes was in 16 hot nodi diploid (Type 1), in 21 hyperdiploid (Type 2). The 12 nodi with diploid (Type 3) as well as 18 with hyperdiploid (Type 4) modal value of the relative DNA concentration had signs of increased proliferation. The thyrocytes of 4 normal controls were diploid. Cytomorphological signs of atypia and degenerative changes of thyrocytes were significantly more frequent in Types 3 and 4 than in Types 1 and 2.*
Conclusion: *Dominant scintigraphically hot thyroid nodi are diploid or hyperdiploid. Some of them are in the state of proliferation. DNA cytophotometry can be useful as an additional diagnostic method in cases with thyroid (hot) nodi of uncertain cytology, especially when therapy with low dose of radioiodine is planned.*

Key words: hyperthyroidism – radionuclide imaging; DNA; cytophotometry

Introduction

Autonomous nodi are present in about 40% of patients with endemic goiter.[1] Scintigraphy with technetium (^{99m}Tc) or iodine (^{131}J) regularly shows an autonomous nodule as a hot spot with a more or less suppressed paranodal thyroid gland. A hot spot on the scan of the thyroid can present a true adenoma, a hyperplastic clone of hyperactive autonomous thyrocytes or a highly differentiated follicular carcinoma. It is therefore desirable to differentiate among these conditions before the therapy is given, although in general the incidence of thyroid carcinoma is low.[2,3] The aim of our study was to determine a pattern of DNA distribution in hyperactive thyroid nodi.

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Materials and methods

Subjects

Sixty-seven hyperthyrotic patients with autonomous goiter, sent to our department for routine investigations before the therapy with radioiodine, were in-

cluded in the study. The inclusion criterion was the presence of a single hot node or utmost 3 hot nodi with a discernible dominant hot node. There were 57 females and 10 males, aged 43 to 89 years (mean 61.5 years).

The normal *control group* consisted of 4 females, aged 33 to 37 years, sent to our department for suspected autoimmune thyroiditis. In each of them thyroid disease was excluded by hormonal tests, negative thyroid autoantibodies and by cytological examination of the thyroid.

Methods

The final clinical diagnosis of thyroid disease was based on the disease history, the physical examination of the patients, the results of thyroid cytology, ultrasonography, scintigraphy, serum T4, T3, TSH and thyroglobulin concentration. The results of cytophotometry were compared with cytomorphology (cytology) and final diagnosis.

Scintigraphy and ultrasonography

The planar scintigraphy of the thyroid was performed with Siemens Basicam gamma camera, 20 minutes after intravenous application of 80 MBq of ^{99m}Tc-pertechnetate. Scintigram with 2 MBq of ^{131}J was accomplished 24 hours after the oral application of radiotracer. Ultrasonography (US) of the thyroid was performed by using high resolution transducer (10 MHz, Diasonics DRF 300). The diameter of dominant hot nodi was measured with US. Scintigraphically hot nodi were identified on the ultrasound scan with the help of scintigrams. Fine needle biopsy of a dominant hot node, guided by US, was done after scintigraphy. Smears for cytology and the single cell cytophotometry were prepared from each sample obtained with fine needle aspiration biopsy.

Cytomorphology

Smears were stained by the May-Gruenewald-Giemsa method. The morphological changes in thyroid cells were grouped into 6 classes:

1 normal thyrocytes
2 hyperactive thyrocytes
3 thyrocytes with degenerative changes
4 proliferation or atypical thyrocytes
5 malignancy suspected
6 definite malignancy

Cytochemical DNA assessment

The single cell cytophotometry was performed after the Feulgen staining procedure, including acid hydrolysis in 4 N HCl at 28°C for 60 min. DNA measurements were carried out on microspectrophotometer Opton USPM 30/50 at wave length of 580 nm and diaphragm 0.63. The objective's magnification was 25x or 40x. Processing was done by the computer. Hundred and fifty to 200 thyrocytes and 25-100 leucocytes were evaluated in each smear. The modal relative DNA concentration of leucocytes in the same thyroid biopsy smears served as a reference value for the normal diploid DNA concentration, the so called "L" value.[4,5] The thyrocytes with the modal relative DNA concentration were considered to be in G1 (gap1) phase of cell division cycle, G2 (gap 2) phase was double modal value. The cells with the intermediate DNA concentration were considered to be in S (synthesis) phase. According to their relative DNA content, thyrocytes from hot nodi were classified as diploid ($0.75<G1<1.25$ L) and hyperdiploid (1.25 L $<$ G1). The results were expressed as percentage of thyrocytes in each class. DNA frequency distribution histograms of thyrocytes in hot nodi were compared to the histograms of leucocytes and of normal thyrocytes in control patients.

Biochemical analyses

The analysis of serum total triiodothyronine – T3 (normal 1.09-3.12 nmol/l) was done with the RIA method (SPAC T3 – Byk-Sangtec, Dietzenbach), total thyroxine – T4 (normal 53-182 nmol/l) with SPAC T4 – Byk-Sangtec, Dietzenbach, thyrotropin – TSH (normal 0.17-4.05 mE/l) was measured with the immunoradiometric method, IRMA (Imunotech, Marseille) and thyroglobulin – Tg (normal 0-34 µg/l) with the RIA method (Henning, Berlin).

Statistical analysis

Mean, standard deviation, median and modal values and, when appropriate, Chi square test were calculated.

Results

Scintigraphy was performed in 67 patients:
37 pts had a solitary hot node,
18 pts had 2 hot nodi (with 1 dominant node),
12 pts had 3 hot nodi (with 1 dominant node),

The thyroid scintigrams performed with ^{99m}Tc-pertechnetate showed the same distribution pattern of radioactivity as the scintigrams with ^{131}J in all patients.

Results of cytology, ultrasound investigation, hormonal analyses and Tg of 67 patients with hot nodi are given in Tables 1 and 2.

Table 1. The size and cytological class of 67 scintigraphically hot nodi.

	units	mean ± sd	median	mode
radius of nodi	cm	2.7 ± 0.7	3	3
cytology	class	2.07 ± 1.1	2	1

Table 2. TSH, T4, T3 and Tg in plasma of 67 patients with scintigraphically hot nodi.

	units	mean ± sd	median
TSH	mE/l	0.08 ± 0.13	0.04
T 4	nmol/l	151.4 ± 45.5	139
T 3	nmol/l	3.47 ± 0.94	3.39
Tg	ug/l	58.7 ± 46.9	46

DNA measurements

According to the cellular DNA content in hot nodi we noticed four different types of DNA frequency distribution histograms (See also Table 3.):

Type 1- modal class was diploid (mean relative DNA concentration was 1.24 ± 0,05L),

Type 2 – modal class was hyperdiploid (mean relative DNA concentration was 1.57± 0,13 L),

Type 3 – modal class was diploid but some polyploid cells were noticed.

Type 4 – modal class was hyperdiploid and some polyploid cells were noticed.

There was no significant difference in percentage of cells in S phase between different types.

The results of cytomorphology were compared with individual types of DNA frequency distribution histograms in Table 4.

The *cytomorphology* in group of patients with DNA frequency distribution histograms of the types 1 and 2 differed significantly from the types 3 and 4 ($p<0.001$). There was no statistically significant difference in the results of cytomorphological examination between types 1 and 2, or 3 and 4.

Final diagnosis

By the clinical investigation and the hormone analysis subclinical hyperthyroidism was proved in two patients with antonomous nodi wheres overt moderate hyperthyroidism due to toxic nodular goiter was proved in 65 patients. One patient had histologically verified follicular carcinoma within the solitary hot nodule (cytologic class was 4, DNA frequency distribution histogram was type 4). One patient had oncocytic tumor within 3 hot nodi (cytological class 5, DNA frequency distribution histogram was type 3).

In the control euthyrotic group, all the results were within the normal limits.

Discussion

Two types of hormone producing scintigraphic hot nodi were found considering the modal value of the relative DNA concentration in thyrocytes: diploid and hyperdiploid. The proliferation was apparent in some of these nodi. Atypia as noted with the

Table 3. Types of DNA frequency distribution in 67 scintigraphically hot nodi compared to normal thyroid (controls) and leucocytes.

Histogram	Patients (N)	G1(%)	S (%)	G2 (%)	>G2 (%)
Type 1	16	94 ± 6	5 ± 6	0.25 ±0.5	1 ± 1.7
Type 2	21	94 ± 5	4 ± 4	0.7 ± 1.4	0.1 ± 0.4
Type 3	12	68 ± 19	5 ± 3	23.9 ± 17	2.7 ± 2.8
Type 4	18	78 ± 13	8 ± 8	14 ± 12	1.7 ± 2.9
Controls	4	96 ± 3.6	1-8	0	0
Leucocytes	67	100	0	0	0

Legend:
G1, G2, S (%) – percentage of thyrocytes in individual phases of cell division cycle
>G2 – percentage of thyrocytes with more than tetraploid DNA concentration

Table 4. The comparison of cytomorphologic results in different types of DNA frequency distribution histograms in 67 hot nodi.

Histogram	Patients (N)	Active thyrocytes (% pts)	Aniso-nucleosis (% pts)	Oncocytes (% pts)	Degenerated thyrocytes (% pts)	Microfollicles (% pts)
Type 1	16	100	0	0	0	10
Type 2	21	80	5	0	10	15
Type 3	12	83	42	8	33	25
Type 4	18	94	17	6	39	11

cytomorphology was more frequent in nodi with the signs of proliferation in the DNA frequency distribution histogram.

In a recent study the somatic mutation of TSH receptor gene was shown in the major part of autonomous nodi. The proof of this mutation might have an implication on the prognosis of thyroid adenoma.[6] According to standards somatic mutation is present in 58 % of autonomous nodi in our study. It is apparently more frequent among solitary hot nodi where the average relative DNA concentration was slightly higher than in groups with 2 or 3 hot nodi.

Several authors assume the aneuploidy characteristics of true adenomas.[7-10] Also Lukasz found predominant hyperdiploidy in thyroid adenomas.[11] According to these authors it is conceivable that among hot nodi in our patients those with the DNA frequency distribution histograms of Types 2 and 4 are true adenomas. In favour of this it is also the cytomorphology, which showed higher grades of atypia in these nodes.

Among 67 hot nodi in our patients 2 (2.8%) were malignant. Citomorphologically one patient had follicular carcinoma (solitary hot node) and the other one (3 hot nodi) oncocytoma. In both cases the single cell cytophotometry showed the DNA distribution histograms reflecting proliferation and hyperdiploidy. Since similar changes were found in some benign follicular nodi as well, such changes can not be considered a proof of malignancy. Contrary to our experience, Bengtsson et al.[12] considers the DNA cytophotometry convenient for the differentiation of malignant from benign lesions in the thyroid. Most other authors believe that DNA cytophotometry cannot reliably differentiate between thyroid cancer and benign thyroid adenoma,[7-9, 13-14]

The percentage of malignant hot nodi in our patients was small but significant. After our opinion cytomorphology is therefore mandatory in all dominant, especially solitary, hot nodi before the therapy, especially if the radioiodine therapy is considered. When cytomorphology is inconclusive, the DNA cytophotometry can be of a help in the final decision about the therapeutic plan.

Conclusion

Two types of DNA frequency distribution histograms are found in scintigraphically hot nodi of the thyroid: one with diploid and the other with the hyperdiploid modal DNA value. Increased proliferation was noted in some hot nodi. Both, benign and malignant hot nodi in the thyroid could be diploid or hyperdiploid.

The DNA cytophophotometry can be useful as an additional diagnostic tool in the assessment of hot nodi before the therapy, especially when the results of cytology are ambiguous.

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