Expression of Ki-67, PCNA and MPM2 antigens in follicular cells of the thyroid gland after iodotherapy

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Abstract

Hyperfunctional nodular goitre is the most common thyroid non-neoplastic condition in endemic areas. Iodotherapy is the basic method in thyroid gland hyperfunction treatment. The aim of the study was to evaluate proliferation of thyroid follicular cells in nodular goitre after iodotherapy. The study was carried out on 32 women, 30-76 years old. Cytological and immunohistochemical evaluations were based on the material, obtained by Fine-Needle Aspiration Biopsy (FNAB). Proliferative activity was immunohistochemically assessed. The influence of radioiodine on thyroid follicular cells was evaluated as a difference between the proliferation of follicular cells before and after its application. It was concluded that the proliferative activity of thyroid follicular cells decreased considerably after radioiodine therapy.

Key words: hyperthyroidism, iodotherapy, proliferative activity, PCNA, Ki-67, MPM2.

Introduction

One of the most common thyroid gland conditions is the diseases that constitute the complex of symptoms, called hyperfunction. The most frequent illnesses with symptoms of hyperthyroidism include: Graves' disease, multinodular goitre, hyperfunctional autonomous thyroid nodule [1]. The north-east region of Poland is the area of iodine deficiency in which a predominant number of thyroid hyperfunction cases (over 60%) is connected with the presence of autonomous tissue [2, 3, 4]. The environmental iodine deficiency triggers the pathomechanism of thyroid hyperfunction with point mutations, related to TSH receptor, which stimulates thyroid gland growth and the excessive synthesis of its hormones [3, 5]. Iodine deficiency induces the activity of topical growth factors, which results in a decrease of antiproliferative TGF-ß value and an increase of IGF-1 and EGF concentrations [3]. It makes the thyroid tissue more sensitive to TSH activity, what also induces hyperplasia of thyreocytes.

The main lines of treatment of hyperthyroidism include pharmacotherapy (thyreostatics, inhibitors of ß-adrenergic receptors, tranquillisers), radioiodine treatment and surgical treatment [1, 6, 7, 8, 9]. Pharmacotherapy, especially in patients with nodular goitre, should be an introduction to a more radical treatment (thyroid gland surgery or radioiodine therapy) [1, 7, 9]. It has been found that therapeutic doses of iodine have no teratogenic effect and do not influence the human genome. It is considered to be a method of choice in the treatment of hyperthyroidism in all age groups [1, 6, 10].

The aim of the study was to evaluate proliferative activity of thyroid follicular cells in patients with nodular goitre after iodotherapy.

Materials and methods

The study was carried out on 32 women, aged 30 - 76 years. The cytological and immunohistochemical evaluation was performed on material, obtained by US-guided Fine-Needle Aspiration Biopsy (FNAB) and fixed by Cytofix. Proliferative activity was immunohistochemically assessed with the use of antibodies against Proliferating Cell Nuclear Antigen (PCNA), Ki-67 Antigen and Mitotic Proteins (MPM-2) (DAKO) by determination of the percentage of follicular cells, showing a positive reaction to the given antibody [11, 12]. The influence

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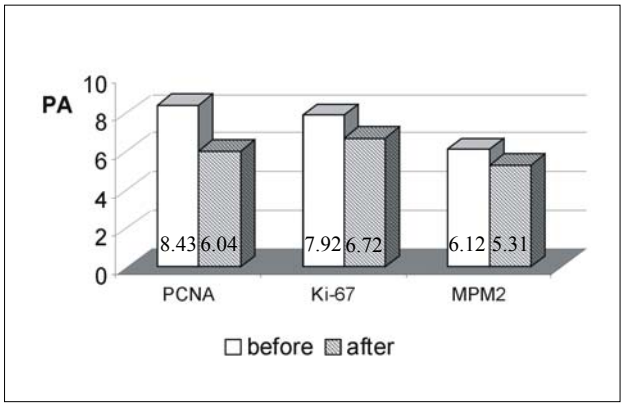
Figure 1. Proliferative activity of thyroid cells before and after radioiodine therapy. PA is numerically equal to the mean percentage of cells positive for the respective antigens (PCNA, Ki-67 or MPM2)



of radioiodine on thyroid follicular cells was evaluated as a difference between proliferative activity of follicular cells before and after iodotherapy. The results were statistically analysed with the Statistica Pl computer software. Student's t test was used for statistical analysis. The difference was considered as significant for $p < 0.05$.

Results

There were no essential morphological differences in the microscopical views of thyroid follicular cells before and after iodotherapy. The proliferative activity of follicular cells, referred to as PCNA before the radioiodine therapy, ranged from 6.1 to 15.4 - the mean value: 8.43, for Ki-67 from 6.39 to 15.61 - the mean value: 7.92 and for MPM-2 from 3.26 to 13.85 - the mean value: 6.12. The obtained values changed under the influence of I^{131} and amounted to: for PCNA: from 3.14 to 10.75 - the mean value: 6.04, for Ki-67 from 2.54 to 11.23 - the mean value: 6.72 and for MPM2 from 1.92 to 9.12 - the mean value: 5.31. Statistically significant differences between the means were found for PCNA, Ki-67 and MPM2, before and after the therapy. (Fig.1)

Discussion and conclusions

Therapeutic effect of I^{131}, mediated by beta radiation, emitted from I^{131} in the tissue. This radiation induced the formation of radioiodine free radicals which damage DNA structure. It results either in cell death or in loss of cell ability to grow and proliferate. Histological alterations include interstitial oedema, cell necrosis, lymphocyte infiltration and fibrosis [10]. Sporadically, drug-induced complications may appear after the treatment. In the early period after the isotope application, the complications appear in the form of an increased level of hormones, released from damaged follicular cells, hypermetabolic crisis or thyroid inflammation or ophtalmopathy; later on, they are manifested by thyroid hypofunction or, rarely, are observed as parathyroid hypofunction [1, 7, 9, 10, 13]. Nevertheless, this kind of treatment has certain advantages, including good therapeutic effects, scarce recurrences, comfort and comparatively high safety, low costs and the possibility of application to elderly patients [1, 8, 9, 13]. It is worth underlining that the application of radioiodine has been considered to be the best method of treatment of autonomous nodules and hyperfunction of multinodular goitre [6, 9, 13].

Basing on the obtained results, it has been concluded that the proliferative activity of thyroid follicular cells decreased considerably after radioiodine therapy, what indicates the necessity of constant monitoring of patients treated with iodotherapy.

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Morphometrical analysis of immunohistochemical reaction of inflammatory infiltrate in chronic thyroiditis

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Abstract

The aim of the study was to quantitatively evaluate B and T lymphocytes and macrophages, based on immunohistochemical investigations (CD43, CD20, CD8 and CD68) of chronic focal and Hashimoto thyroiditis. A new method of image analysis was applied, based on spatial visualization of the antigens reactivity. The obtained results indicated that the numbers of lymphocytes, in particular of cytotoxic T lymphocytes, and of macrophages increased with the progress of inflammatory process. Quantitative measurements of the markers made the results more objective and supported pathomorphological diagnosis.

Keywords: chronic thyroiditis, inflammatory infiltrate, spatial image analysis, measurements.

Introduction

Chronic thyroiditis is a heterogeneous group of diseases, regarding morphology and prognosis. Upon pathomorphological diagnosis of case with thyroiditis, immunohistochemical analysis is used to distinguish chronic focal thyroiditis from Hashimoto thyroiditis. The interpretation of results, obtained from routine visual evaluation in light microscopy and semi-quantitative assessment of immunohistochemical reaction strength, is highly subjective, in particular, in cases suspected to be progressing into Hashimoto thyroiditis. This may be a source of inconsistencies in the diagnosis, caused by diffusion of antigen, non-homogeneous expression of colour reaction in the measurement field and background staining. Therefore, the aim of our study was a quantitative assessment of the elements of inflammatory infiltrate: B and T lymphocytes and macrophages in chronic focal thyroiditis and Hashimoto cases by using a new method of digital image analysis, based on the spatial visualization technique.

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Material and Methods

Material for our study was obtained after thyroidectomy in patients with chronic focal thyroiditis and Hashimoto thyroiditis and used for immunohistochemical study. Sections were cut from a formalin-fixed and paraffin embedded archival tissue, stained with HE and then immunostained. The immunohistochemical stains were performed for the following antigens: CD20 (DAKO, dilution 1/100) present on B-lymphocytes, CD43 (Dako, dilution 1/100) expressed on the surface of T-lymphocytes, CD8 (Novocastra, dilution 1/40) found on the cytotoxic subset of human T-lymphocytes, CD68 (Dako, dilution 1/100) present on the surface of macrophages. Microscopy images, of 640x480 pixels each, were acquired by using a digital light microscope, running under Motic Images v. 1.2 software for Windows (Micro Optic Industrial Group Co) at 400x magnification. The obtained images were extended to their spatial representation by introducing image brightness as the third dimension. The colour immunohistochemical reaction was exposed on a three-dimensional view by reducing the scenery behind to the background. Then, filters of brightness and saturation were fixed for image series, acquired from each specimen, and colours, representing the immunohistochemical reaction, were extracted. The area and intensity of reaction in three-dimensional space were determined by using a computer program, programmed in C++ by Strzelczyk [1]. The results, obtained for chronic focal thyroiditis and Hashimoto thyroiditis, were compared by using the Mann-Whitney test.