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.

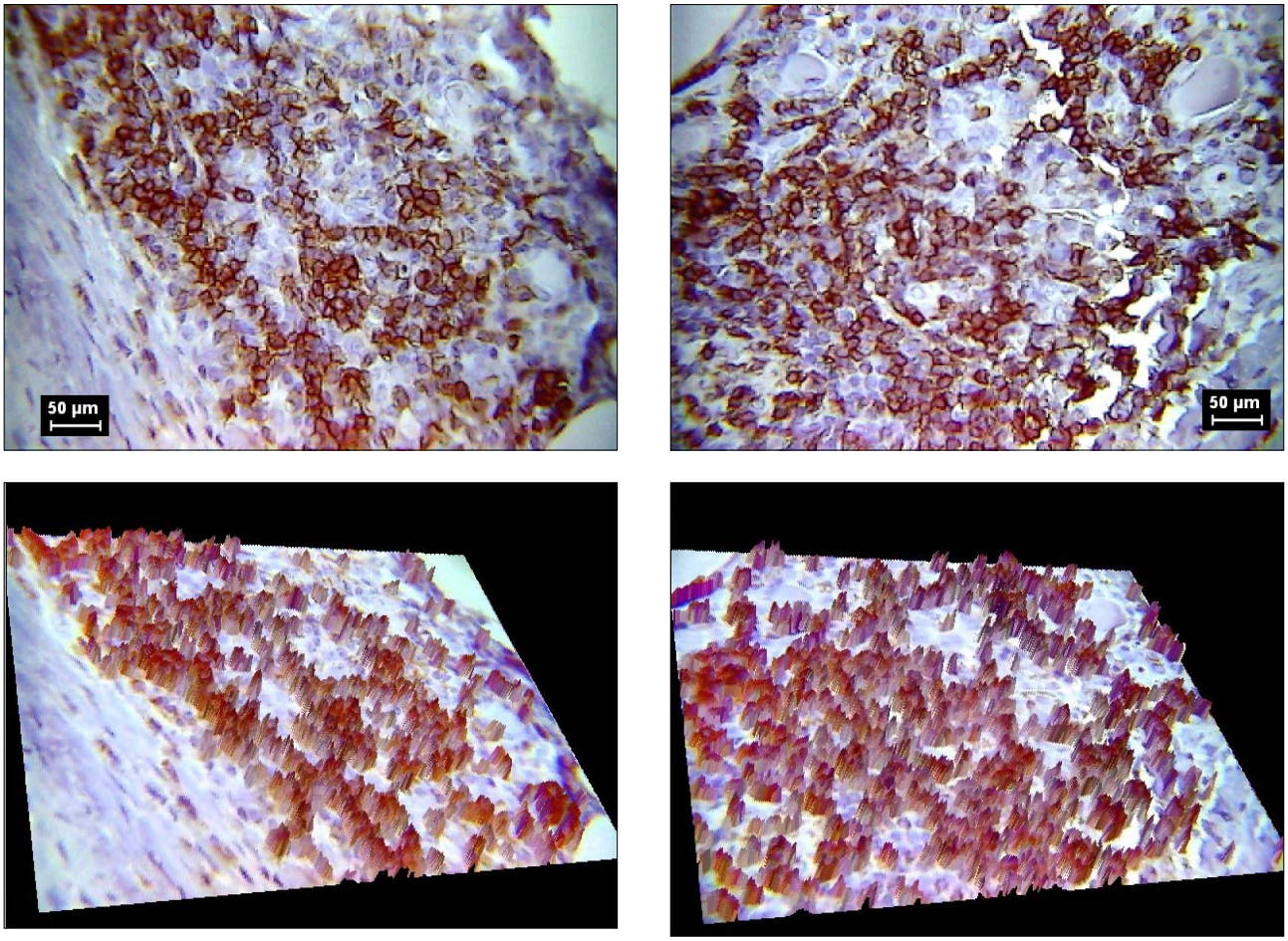
Table 1. Area of positive reaction of the analyzed markers (per μm2).

Markers	Chronic Focal Thyroiditis	Hashimoto Thyroiditis	Significance level (p)
	Mean ± SD	Mean ± SD	
CD 20	0.206 ± 0.282	0.380 ± 0.154	P<0.001
CD 43	0.204 ± 0.236	0.369 ±0.301	P<0.005
CD 8	0.014 ± 0.028	0.836 ± 0.098	P<0.001

Table 2. Intensity of immunohistochemical stains.

Markers	Chronic Focal Thyroiditis	Hashimoto Thyroiditis	Significance level (p)
	Mean ± SD	Mean ± SD	
CD 20	122 ± 29	97 ± 12	P<0.001
CD 43	125 ± 18	101 ± 14	P<0.001
CD 8	144 ± 18	120 ± 12	P<0.001

Figure 1. CD43 in chronic focal thyroiditis (left) and in Hashimoto thyroiditis (right). The lower row shows the spatial representation of original images from the upper row.

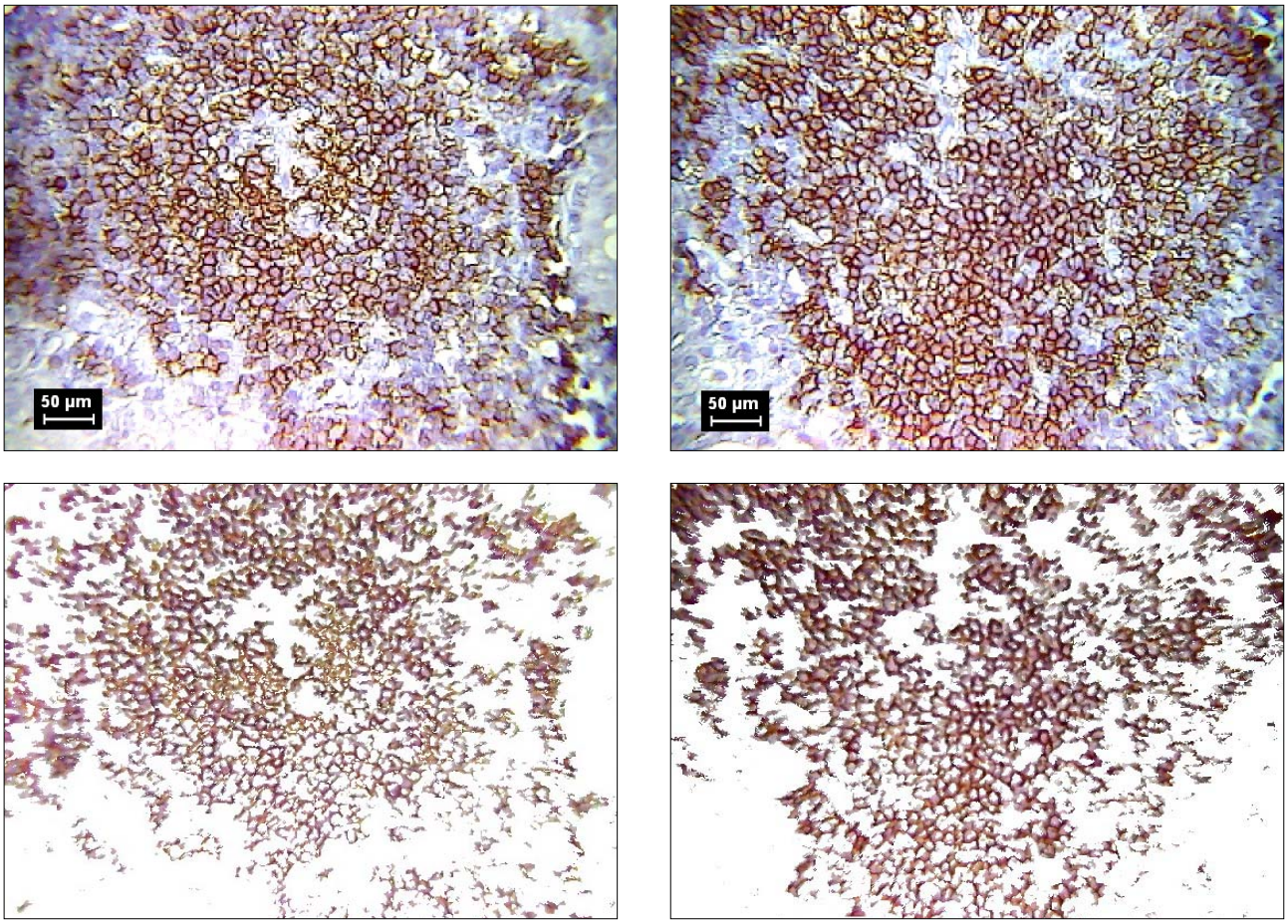


Results

The reactivity of CD43, CD20 and CD8 was significantly lower in chronic focal thyroiditis than in Hashimoto disease (Tab. 1). In particular, numerous cytotoxic T lymphocytes occurred in Hashimoto cases, while in focal thyroiditis, they were less frequent. The average colour intensity of CD43+, CD20+ and CD8+ markers was significantly higher in chronic focal thyroiditis than in Hashimoto disease (Tab. 2). Inflammatory foci areas became larger and larger in the course of disease progress and were occupied by dense clusters of lymphocytes (Figs. 1-2).

The number of macrophages, visualized with CD68 marker, significantly increased in Hashimoto cases (25 ± 11 per image), comparing to respective values in chronic focal thyroiditis (10 ± 8 per image).

Figure 2. CD20 in chronic focal thyroiditis (left) and in Hashimoto thyroiditis (right). The lower row shows the extracted reaction from the original images (upper row).



Final remarks

At present, measurements of immunohistochemical reactions in raw colour images make a potential benefit in quantitative studies [2, 3]. The method, based on three-dimensional visualization of the immunohistochemical markers and used in this study, permitted to obtain objective results and indicate extremely significant differences between morphological features of inflammatory foci in chronic thyroiditis and large inflammatory areas in Hashimoto cases. The results indicated that lymphocytes, in particular cytotoxic T lymphocytes, and macrophages increased with the progress of inflammatory process. Lymphocytes were visualized with the markers in light brown colours with dark brown contours (Figs. 1-2). Lower average colour intensity of CD43+, CD20+ and CD8+, observed in chronic focal thyroiditis than that, found in Hashimoto disease (Tab. 2), can be explained by the increasing number of large inflammatory foci in the course of progressing disease. Therefore, the average results of colour intensities were lower. Concluding, quantitative measurements of inflammatory process markers made the results more objective and supported the pathomorphological diagnosis.

References

1. Nieruchalska E, Strzelczyk R, Woźniak A, Żurawski J, Kaczmarek E, Salwa-Żurawska W. A quantitative analysis of the expression of a-smooth muscle action in mesangioproliferative (GnMes) glomerulonephritis. Folia Morph, 2003; 62: 451-3.

2. Matkowskyj K, Schonfeld D, Benya R. Quantitative immunohistochemistry by measuring cumulative signal strength using commercially available software Photoshop and Matlab J Histochem Cytochem, 2000; 48: 163-74.

3. Matkowskyj KA, Cox R, Jensen RT, Benya R. Quantitative immunohistochemistry by measuring cumulative signal strength accurately measures receptor number. J Histochem Cytochem, 2003; 51: 205-14.