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Article

Immunohistochemical Characterization of Apoptotic and Macrophage Reactions in The Thyroid Gland Under Microelement Deficiency

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Abstract: The study evaluates apoptotic activity and macrophage response in thyroid tissue under combined microelement deficiency (Mg, Fe, Se, Zn) using CD95 and CD163 markers in white outbred rats. Microelement deficiency was associated with increased CD95 expression, indicating enhanced apoptosis in thyrocytes, and elevated CD163 expression, reflecting activation of M2 macrophages and tissue remodeling. These changes demonstrate structural and functional alterations in the thyroid gland linked to increased cell death and immune reorganization.

Keywords: apoptosis, CD95, CD163, thyroid gland, microelement deficiency, immunohistochemistry, macrophages, thyrocytes

Introduction

In recent decades, the problem of microelement deficiency has become increasingly important in modern medicine, which is associated with its high prevalence and variety of clinical manifestations. According to the World Health Organization, deficiency of vital micronutrients such as iron, zinc and selenium affects a significant part of the world's population and has a pronounced impact on the functioning of various organs and systems [1].

The thyroid gland is one of the most sensitive organs to micronutrient imbalances, since the processes of thyroid hormone synthesis directly depend on sufficient levels of selenium, iron, and other cofactors of enzymatic reactions (Zimmermann et al., 2015). Violation of microelement homeostasis can lead to the development of structural changes, disruption of hormonal function and the formation of pathological conditions of the thyroid gland [2].

In recent years, special attention has been paid to the study of cellular mechanisms of tissue damage, including apoptosis and immune reactions. Apoptosis plays a key role in maintaining tissue homeostasis, but its excessive activation leads to the loss of functionally active cells. In this context, the CD95 marker is considered as one of the main indicators of activation of the receptor-dependent pathway of apoptosis [3].

An equally important component of the tissue response is the macrophage reaction. As shown by the studies of Gordon and Martinez (2010), M2-phenotype macrophages are involved in the regulation of inflammation, tissue repair and the development of fibrosis. The CD163 marker is widely used to identify these cells and assess the extent of their activity in tissues [4].

Despite a significant number of studies on the role of trace elements in the functioning of the thyroid gland, the relationship between apoptosis and macrophage activity in their deficiency remains insufficiently studied. In this regard, immunohistochemical analysis using the CD95 and CD163 markers is an up-to-date area that allows to deepen the understanding of the pathogenetic mechanisms of thyroid damage in microelement deficiency [5].

The aim of this study was to assess the immunohistochemical activity and macrophage response in thyroid tissue in combined microelement deficiency (Mg, Fe, Se, Zn) using CD95 and CD163 markers.

Materials and Methods

The material of the study was micropreparations of thyroid tissue of 6-month-old white mongrel rats, divided into two groups: control group (intact animals), an experimental group with a combined deficiency of trace elements (Mg, Fe, Se, Zn) [6].

The object of the study was thyroid tissue, and the subject was morphological, morphometric and immunohistochemical changes in microelement deficiency.

To achieve this goal, a set of morphological and immunohistochemical research methods was used.

Immunohistochemical study was carried out using the markers CD95 (marker of apoptosis) and CD163 (marker of macrophages of the M2 phenotype), which made it possible to assess the degree of apoptotic activity and the severity of the macrophage response in the thyroid tissue [7].

Staining of slides was performed by chromogenic method using diaminobenzidine (DAB). The analysis was carried out at a magnification of ×200 followed by digital image processing. Scanning of specimens and quantification of marker expression were carried out using QuPath 0.4.0 software [8].

In the course of the analysis, the following were determined:

total number of cells,

the number of positive and negative cells,

percentage of positive expression,

localization of expression cells.

Morphometric assessment was carried out on specified areas (px^2) with the calculation of relative expression indicators [9].

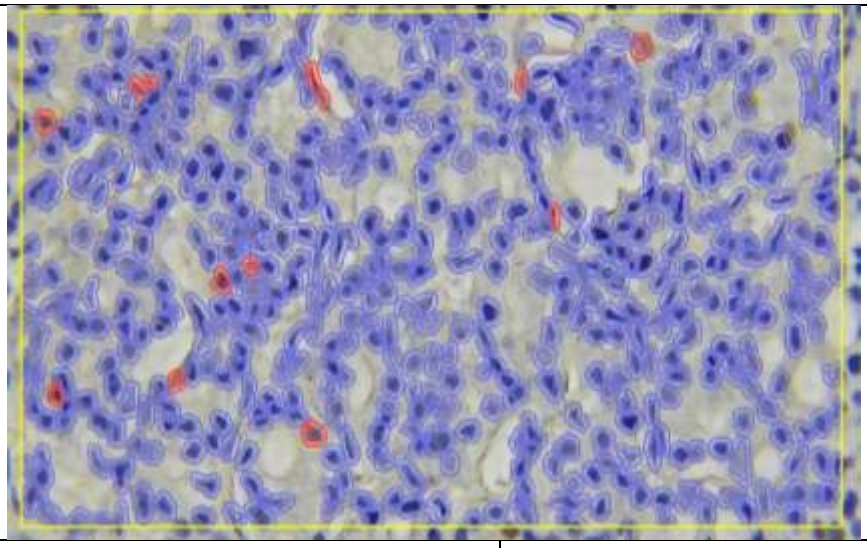
Results and Discussion

In order to confirm changes in morphological and morphometric parameters during the study, immunohistochemical analysis of the expression of CD95 and CD163 markers in the thyroid tissue of rats of the control group and the group with combined micronutrient deficiency was carried out.

CD95 (Fas, APO-1) is a receptor that triggers the process of apoptosis (programmed cell death) and is one of the key markers of the receptor-dependent (external) pathway of apoptosis. In general, CD95 is seen as an indicator of a cell's readiness for apoptosis. When CD95 binds to its ligand (Fas-L), caspase-8 is activated, a caspase cascade is triggered, as a result of which the cell undergoes controlled apoptotic death [10].

CD163 is a marker of monocyte macrophages with an anti-inflammatory (M2 phenotype) type of activation. Its expression does not indicate an inflammatory reaction, but the predominance of tissue restructuring and fibrosis processes. Macrophages expressing CD163 are involved in the capture of hemoglobin-haptoglobin complexes, reducing oxidative stress, suppressing inflammation, as well as in the processes of tissue remodeling and regeneration. In addition, they contribute to the development of fibrosis and stroma growth. In this regard, CD163 is considered as a marker of "repair-type" macrophages [11].

In the course of a study of thyroid tissue slides obtained from 6-month-old white outbred rats of the control group, the expression of the CD95 marker using DAB (diaminobenzidine reaction) and at a magnification of 200 times was carried out to quantify positive and negative cells. It was found that in the thyroid tissue of the control rats, the expression of CD95 was low. Thus, in the 360135 px² area, the total number of cells was 561, of which 549 cells were negative and 12 were positive, with a positive expression rate of 2.14% (Fig. 1) [12].

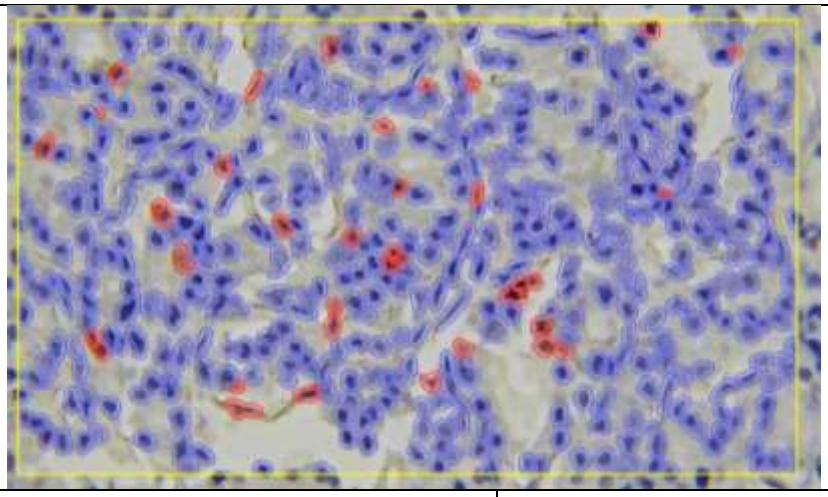


Obshchee kolichestvo vyyavlennyx kletok	561
Positive cells	12
Negative cells	549
Positive expression	2,14%
Obshchaya ploshchad	360135 px^2

Figure 1. Expression of the CD95 marker in the thyroid tissue of 6-month-old white outbred rats of the control group. Staining was performed by the DAB chromogenic method (diaminobenzidine). Increase ×200. The image was scanned in QuPath 0.4.0. The level of expression is quantified. Positive cells are marked in red.

Positive cells were marked in red, and it was found that they are mainly localized in the cells of the follicular epithelium. It is known that follicular cells, having passed through the stages of growth and development and having fulfilled their morphofunctional tasks, undergo physiological death, that is, apoptosis [13].

In the conditions of combined deficiency of trace elements (Mg, Fe, Se and Zn), the chromogenic method of DAB staining (diaminobenzidine reaction) with a magnification of 200-fold was also used in the study of the expression of the CD95 marker in microslides of the thyroid tissue of white outbred rats. At the same time, the number and localization of positive and negative cells were determined. Analysis of several microslides examined in the 403744 px² area showed that the total number of cells was 519, of which 482 cells were negative and 37 were positive. The level of positive expression was 7.13%, which was assessed as a low level of expression. Positive cells, as in the control group, were mainly localized in the follicular epithelium (Fig. 2). The data obtained indicate that in this group of rats, under the influence of a combined deficiency of trace elements, there is an increase in apoptotic processes in thyrocytes [14].

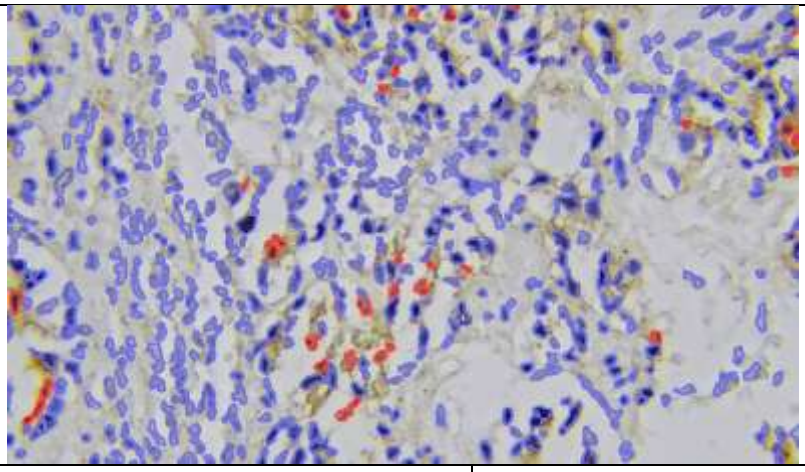


Obshchee kolichestvo vyyavlennyx kletok	519
Positive cells	37
Negative cells	482
Positive expression	7,13%
Obshchaya ploshchad	403744 px^2

Figure 2. Morphological and morphometric parameters of the thyroid gland of white rats with combined deficiency of trace elements (Mg, Fe, Se, Zn). Expression of the CD95 marker. Staining was performed by the DAB chromogenic method (diaminobenzidine). Increase ×200. The image was scanned in QuPath 0.4.0. The level of expression is quantified. Positive cells are marked in red.

In the course of a study of the expression of the CD163 marker in thyroid tissue slides of 6-month-old white outbred rats of the control group, stained by the DAB method and studied at a magnification of 200 times, the quantitative determination of positive and negative cells was carried out. It was found that the expression of CD163 in the thyroid tissue of this group was low. Thus, in the area of 439668 px², the total number of cells was 728, of which 691 cells were negative and 37 were positive, while the level of positive expression was 5.08%.

Positive cells were marked in red; It has been established that they are mainly localized on the inner surface of the follicle wall (Fig. 3). To ensure the growth and development of follicular cells (thyrocytes), an alternative activation of the monocyte population occurs, aimed at purifying the blood of harmful substances and filtering them, accompanied by physiological phagocytosis. This process is reflected in the expression of the CD163 receptor, which is considered as a "cleansing" marker.



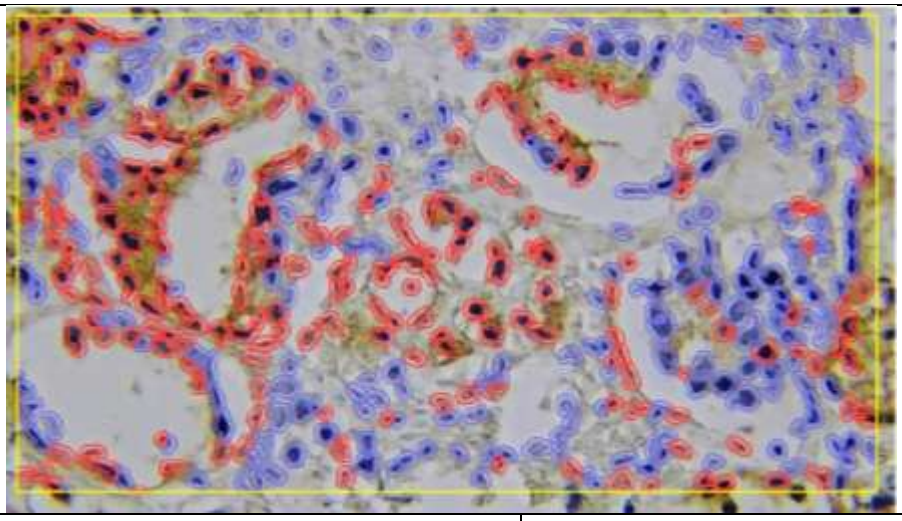
Obshchee kolichestvo vyyavlennyx kletok	728
Positive cells	37
Negative cells	691

Positive expression	5,08%
Obshchaya ploshchad	439668 px^2

Figure 3. Morphological and morphometric parameters of the thyroid gland of 6-month-old white mongrel rats of the control group. Expression of the CD163 marker. Staining was performed by the DAB chromogenic method (diaminobenzidine). Increase ×200. The image was scanned in QuPath 0.4.0. The level of expression is quantified. Positive cells are indicated in red.

Under conditions of combined deficiency of trace elements (Mg, Fe, Se and Zn), the number and localization of positive and negative cells were determined when studying the expression of the CD163 marker in microslides of the thyroid tissue of white mongrel rats stained by the DAB chromogenic method (diaminobenzidine reaction) and examined at a magnification of 200 times. An analysis of several microslides studied in the 377482 px² area showed that the total number of cells was 483, of which 250 cells were negative and 233 were positive. The level of positive expression was 48.3%, which was regarded as an average level of expression. Positive cells have been found to be predominantly localized in the follicular epithelium (Fig. 4).

Thus, an increase in the expression of the CD163 marker in this group indicates that in the thyroid tissue of white outbred rats with a combined deficiency of trace elements, the effect of intoxication factors increases, which leads to alternative activation of the monocyte-macrophage system and the formation of phagocytic activity of medium severity [15].



Obshchee kolichestvo vyyavlennyx kletok	483
Positive cells	233
Negative cells	250
Positive expression	48,3%
Obshchaya ploshchad	377482 px^2

Figure 4. Morphological and morphometric parameters of the thyroid gland of white rats with combined deficiency of trace elements (Mg, Fe, Se, Zn). Expression of the CD163 marker. Staining was carried out by the DAB chromogenic method (diaminobenzidine). Increase ×200. The image was obtained using the QuPath 0.4.0 program, the expression was quantified. Positive cells are visualized in red.

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Conclusion

Thus, low expression of the CD95 marker (2.14%) was found in the thyroid tissue of white outbred rats of the control group, while positive cells were evenly localized mainly in thyrocytes. The preservation of stromal elements and the uniform distribution of expression indicate the stability of the tissue structure, the active course of metabolic processes and the physiological balance between the

processes of regeneration and apoptosis.In conditions of combined micronutrient deficiency (Mg, Fe, Se and Zn), the expression of the CD95 marker in the thyroid tissue of white outbred rats increased to 7.13%, indicating a 3.08-fold increase in apoptotic death of follicular cells (thyrocytes) compared to the control group. An increase in the expression of the CD163 marker to 48.3% in this group indicates that the combined deficiency of trace elements in the thyroid tissue increases the impact of intoxicating factors. This, in turn, leads to alternative activation of the monocyte-macrophage population of moderate severity, which reflects a change in immune reactions and the formation of a pathological process in the tissue.

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