STRUCTURAL AND FUNCTIONAL RESPONSES OF THE THYROID GLAND TO CONCURRENT DEFICIENCY OF ESSENTIAL MICROELEMENTS (MG, FE, SE, ZN): A COMPARATIVE STUDY

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Abstract. *This study presents a comparative morphological and morphometric analysis of the thyroid gland in white rats under combined deficiency of microelements (Mg, Fe, Se, Zn). Structural organization of thyroid follicles, colloid composition, stromal elements, and microcirculatory features were examined. The results indicate that microelement imbalance leads to decreased secretory activity of thyrocytes and induces compensatory-adaptive reactions within the stromal and vascular systems, reflecting the gland's response to metabolic stress.*

Keywords: *thyroid gland, microelement deficiency, morphology, morphometry, microcirculation, follicle, thyrocyte, stroma, adaptation.*

The aim of the study was to comparatively assess the structural–functional, morphometric, and immunohistochemical alterations of the thyroid gland in young outbred white rats under combined microelement deficiency (Mg, Fe, Se, Zn), and to determine the impact of this condition on the activity of the microcirculatory system.

Material and methods. The study was conducted in the laboratory of the Department of Morphology, Bukhara State Medical Institute. Three-month-old male white outbred rats (180–200 g) were used. The animals were divided into two groups:

Group 1 (control): rats fed a standard diet;

Group 2 (experimental): rats maintained on a special diet deprived of Mg, Fe, Se, and Zn microelements.

The experimental period lasted 60 days. All animals were kept under identical conditions (22–24 °C, 60–70% humidity, 12 h light/dark cycle). After completion, the rats were euthanized under ether anesthesia, and thyroid glands were collected for analysis.

Thyroid tissues were fixed in 10% neutral formalin, embedded in paraffin, and sectioned at 5 µm. Sections were stained with hematoxylin–eosin. Morphometric measurements were performed using a Leica DM2500 microscope and ImageJ 1.54

software. The following parameters were evaluated: follicle diameter, thyrocyte height, follicular area, relative proportion of colloid, stroma, and epithelium, as well as capillary diameter, density, and relative vascular area.

Immunohistochemical staining was performed using the DAB chromogen system. Sections were incubated with CD95 (apoptosis marker) and CD163 (macrophage activation marker) antibodies. Reactions were evaluated at ×200 magnification, counting 500–600 cells per slide. Positive expression (%) was calculated.

Statistical analysis was carried out in GraphPad Prism 9.5. Data were presented as M ± m, and differences between groups were assessed using the Student's t-test. Differences were considered significant at $p < 0.05$. The study complied with all bioethical requirements and was approved by the Ethics Committee under the Ministry of Health of the Republic of Uzbekistan (Protocol No. 4/2025).

Results and Discussion. In 3-month-old white outbred rats, the thyroid gland is located in the anterior neck region, adjacent to the trachea and larynx, and is anchored to surrounding structures by connective tissue. Macroscopically, it consists of right and left lobes connected by an isthmus and has a butterfly-like shape. The right lobe is usually slightly larger than the left, which represents physiological asymmetry in young animals. The gland is surrounded by a thin fibrous capsule that sends delicate trabeculae into the parenchyma, subdividing it into small lobules and carrying blood vessels, lymphatic capillaries and autonomic nerve fibers.

Histologically, the parenchyma is formed by follicles of predominantly spherical or oval shape. Follicles are lined by a single layer of follicular epithelial cells (thyrocytes), which in 3-month-old rats are mainly cuboidal, with occasional low-columnar cells, reflecting high physiological secretory activity. The follicular lumen is filled with homogeneous, slightly fluid colloid that appears light pink in hematoxylin–eosin staining and shows moderate density with small central retraction spaces. Thyrocytes have eosinophilic cytoplasm and centrally located round or oval nuclei with fine chromatin. Parafollicular (C) cells are observed at the periphery of follicles and are responsible for calcitonin secretion. Morphometric analysis showed that the mean thyroid mass was 4.2 ± 0.3 mg, with lobe length 1.5–2.0 mm, width 0.8–1.0 mm and thickness 0.4–0.6 mm. Capillary diameter and density, as well as the relative vascular area, were higher in the central region than in the periphery, indicating more intensive microcirculation. Follicle diameter and area were slightly larger in the peripheral zones, whereas the relative proportion of epithelium and stroma was higher centrally and the colloid fraction was higher peripherally, which corresponds to active hormonogenesis with simultaneous colloid storage in the young gland.

Immunohistochemically, CD95 (Fas/APO-1), a membrane glycoprotein mediating apoptotic cell death, and CD163, a marker of activated macrophages and scavenger

function, were evaluated. In the control group, CD95 expression in thyroid tissue was low: in an area of 360135 px², 561 cells were counted, of which only 12 were CD95-positive, corresponding to 2.14% positive cells, mainly localized in follicular epithelium. This reflects the presence of physiological apoptosis accompanying normal turnover of functionally exhausted thyrocytes. Under conditions of combined deficiency of Mg, Fe, Se and Zn, the proportion of CD95-positive thyrocytes increased to approximately 7.1–7.6%, indicating an enhancement of apoptosis and stress-induced cell loss in the thyroid parenchyma.

In the control group, CD163 expression was also low: in an area of 439668 px², 727 cells were counted, of which 37 were positive, corresponding to 5.08%. CD163-positive cells were mainly located along the inner aspect of the follicular wall, indicating physiological activation of monocyte–macrophage populations and basal clearance of metabolic by-products. In rats with combined Mg, Fe, Se and Zn deficiency, CD163 expression increased markedly: in areas ranging from 377482 to 441715 px², 44.8–48.3% of cells were CD163-positive. This moderate-to-high level of expression reflects enhanced macrophage activation and phagocytic activity in response to increased toxic and oxidative load associated with microelement deficiency.

In summary, in 3-month-old rats the thyroid gland under control conditions is morphologically and functionally normal, with well-organized follicles, adequate colloid, active epithelium and intensive microcirculation. Against the background of combined microelement (Mg, Fe, Se, Zn) deficiency, the increase in CD95-mediated apoptosis and the sharp rise in CD163-associated macrophage clearance indicate stress-induced tissue remodeling, intensified cell loss and activation of compensatory–adaptive mechanisms, which together represent the morphological substrate of metabolic imbalance threatening the stability of thyroid hormonogenesis.