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PATHOMORPHOLOGICAL AND IMMUNOHISTOCHEMICAL FEATURES OF LUNG CHANGES IN OBESITY

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Increased secretion of leptin in individuals with excessive amount of adipose tissue can be the basis for the development of comorbid pathology associated with obesity, including lung pathology. Aim of the study – to establish and analyze pathomorphological and immunohistochemical features of lung tissue changes in the autopsy material of deceased obese persons. The total sample of the deceased amounted to 60 deceased. Two research groups were formed: group 1 (control group, n=30) consisted of deceased persons with normal body weight and group 2 (n=30) – of deceased with obesity. The deceased were weighed to measure and evaluate body weight, and the body mass index (BMI) was calculated. The histological and histochemical examination was performed in line with the hematoxylin-eosin staining and Azan trichrome method. Leptin polyclonal antibodies (Thermo Fisher Scientific, Germany) were used to determine leptin expression. The average value (M) and the average (m) error were calculated. Differences in average values were considered significant with a probability level of at least 95% (p<0.05). The average body weight and BMI of the deceased in group 2 were significantly higher than that of the deceased in group 1. According to the results of the pathohistological, histochemical, and immunohistochemical examination of the lung tissue of the deceased in group 1, slight changes in the lung tissue were revealed. In group 2, on the other hand, pronounced pathological changes in the lungs were observed. Pathomorphological changes typical for chronic bronchitis, pulmonary emphysema, focal pneumosclerosis, and the initial manifestation of pulmonary hypertension were found in the obese deceased (group 2). The immunohistochemical study established a moderate and significant expression of leptin in the deceased of group.

Key words: *obesity, leptin, lungs, histochemistry, immunohistochemistry.*

Connection of the publication with planned research works.

The study was carried out within the framework of the complex research "Study of pathogenetic mechanisms and pathomorphological features of diseases of the endocrine, cardiovascular, respiratory, nervous, digestive, urinary and reproductive systems with the aim of improving their morphological diagnosis" (state registration number 0123U201668).

Introduction.

According to current statistical data, the number of people with excess body weight accounts for more than 2 billion today, which is about 30% of the world's population, and continues to grow steadily worldwide [1]. The World Health Organization (WHO) defines obesity as an abnormal or excessive accumulation of fat, which poses a health risk, in particular, because obesity is a risk factor for developing a number of diseases [2].

Even though adipose tissue was considered for long only as an energy depot, currently, it has been proven that it is an important endocrine organ that produces adipocytokines with hormonal activity [3]. Among adipocytokines, leptin, which acts as a pro-inflammatory cytokine, is considered the most studied and the one with the most significant effect. Increased secretion of leptin in individuals with an excessive amount of adipose tissue can have a significant effect on the function of internal organs, in particular through direct potentiation of inflammation and disruption of homeostasis through expression in target organs, and be the basis for the development of comorbid pathology associated with obesity [4]. Such diseases include arterial hypertension, coronary artery disease, heart rhythm and conduction disturbances, cerebral blood circulation disorders, venous thrombosis of various sites, metabolic syndrome and type II diabetes, osteoarthritis, non-alcoholic fatty hepatitis, gallstone disease, chronic kidney disease, diseases of the reproductive system, infertility, malignant tumors of various anatomical sites and others [5].

As for lung pathology, its association with obesity is not so obvious and has not been studied enough. Although the relationship between overweight and obesity, particularly in the development of obstructive sleep apnea and hypoventilation syndrome, is becoming increasingly evident, there are still much conflicting data on whether overweight and obesity reliably influence the development, course, and mortality of patients with chronic obstructive pulmonary disease (COPD) [6]. The potential association between obesity and COPD is currently widely investigated, but very little is known about the mechanisms underlying this association [7].

The aim of the study.

To establish and analyze the pathomorphological and immunohistochemical features of lung tissue changes in the autopsy material of deceased obese individuals.

Object and research methods.

The total sample of the deceased amounted to 60 people. Two research groups were formed:

- Group 1 (control group, n=30) consisted of deceased with normal body weight, without diagnosed diseases, which were directly or indirectly associated with pathological changes in lung tissue;

• Group 2 (n=30) – deceased with obesity, without diagnosed diseases, which were directly or indirectly associated with pathological changes in lung tissue.

To measure body weight, the deceased were weighed, and the body mass index (BMI) was calculated.

The weighing of the deceased was carried out using medical stretchers with a weighing function, which was developed by us. The medical stretcher contains a frame of two longitudinal crossbars with support legs, while the crossbars are connected to each other by transverse crossbars, which are additionally equipped with modular lamellae and four strain gauges located on the product's support legs, and one weight processor module on the front side of the stretcher. For weighing, the body of the deceased was placed on a medical stretcher and placed on any surface. Information about the load from all strain gauges was transmitted to the weighing processor module, which generated the received data into weight in kilograms and grams.

BMI was calculated according to the following formula:

$$BMI = \frac{m}{l^2},$$

where l – body length (m), m – body mass (kg).

Fixation of lung necroptates and their subsequent processing were performed according to standard methods using hematoxylin-eosin staining.

The histochemical examination was carried out in line with the Azan trichrome method (using the five-reagent set "Azan trichrome") – according to the results of nuclear staining, erythrocytes, acidophilic granules of the pituitary gland acquire a red color, neurofibrils (neuroglia) – shades of red, muscle fibers – from pink to red-pink, collagen, reticulin, membranes of basophilic cells, the stroma of renal glomeruli, basal membranes – from blue to dark blue, respectively. Elastic fibers are not stained [8].

Polyclonal antibodies against human leptin (Thermo Fisher Scientific, Germany) were used to determine leptin expression. The evaluation of the immunohistochemical reaction was carried out by a semi-quantitative method taking into account the spread of expression in the fields of vision, "-" no expression, "+" separately located expression fields, "++" – expression zones with a tendency to merge, "+++" expression zones that completely fill the field of vision.

Statistical processing of the results was carried out using the Statistica 12 software package. The average value (M) and the average (m) error were calculated. Differences in average values were considered significant with a probability level of at least 95% ($p<0.05$).

Research results and their discussion.

The average age of the deceased in group 1 was 51.6±3.3 years; in group 2, this indicator was 55.6±2.7 years. There were 15 men and 15 women in each group. The groups did not significantly differ from each other ($p>0.05$) by age and sex.

The average body weight of the deceased of group 2 was 98.6±4.3 kg and was significantly ($p<0.001$) higher than that of the deceased of group 1, where this indicator was 60.0±2.0 kg. Consistently, the average value of BMI (20.5±0.7 in group 1 and 34.7±1.3 in group 2) was also significantly ($p<0.001$) higher in the deceased of group 2 compared to those in group 1.

During the postmortem pathomorphological examination of the lungs in group 1, minor pathological changes were found. Focal desquamation of the bronchial epithelium, slight peribronchial sclerosis, and small focal peribronchial mononuclear infiltration were identified in the airways. In the respiratory departments, slight thickening of the interalveolar membranes (IAM) due to pleurisy and focal sclerosis, isolated foci of emphysema, and atelectasis were visualized. We also confirmed slight sclerosis of the walls of the pulmonary vessels and congestion of the microcirculation vessels **(fig. 1).**

Histochemical examination revealed moderate peribronchial and intravascular collagenization and single foci of collagen fibers in IAM **(fig. 2)**.

According to the results of an immunohistochemical study in the lungs of deceased persons in group 1, the presence of separately located fields of leptin expression in the airways and respiratory departments was established, which were considered insignificant – "+" **(fig. 3)**.

Changes detected and described in the control group are considered minimal. Low expression of leptin in lung tissue is expected to be found in the epithelial cells of the airways and respiratory departments, as well as in immune cells if they are present [9, 10].

According to the results of the postmortem pathomorphological examination of the lungs of the deceased of group 2, focal desquamation of the bronchial epithe-

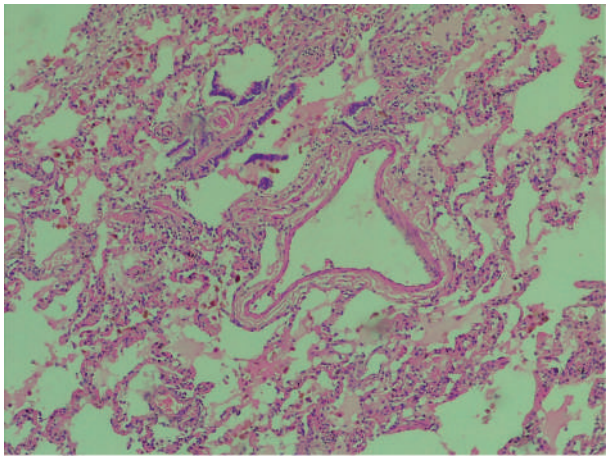

Figure 1 – Focal desquamation of the bronchial epithelium, slight peribronchial, and vascular sclerosis in lung tissue of deceased of group 1. Hematoxylin-eosin staining. Magnification: x100.

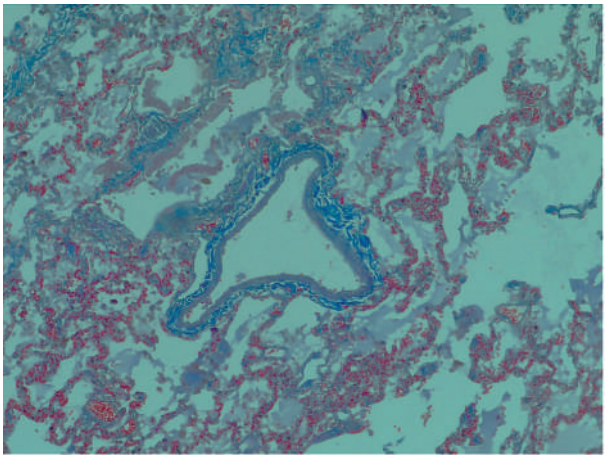

Figure 2 – Peribronchial and intravascular collagenization in lung tissue of deceased of group 1. Azan trichrome staining. Magnification: x100.

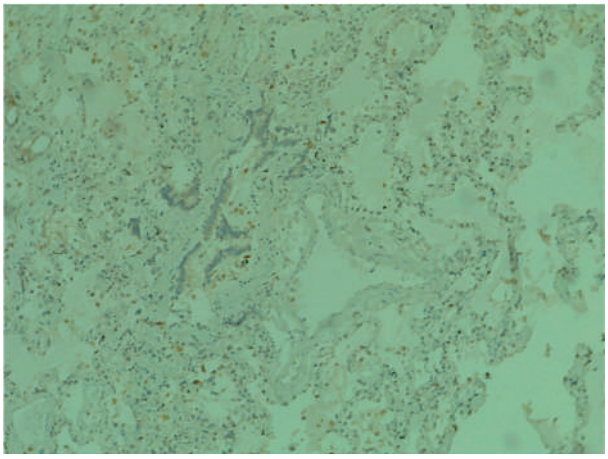

Figure 3 – Insignificant leptin expression in lung tissue of the deceased of group 1. Immunohistochemical method. Magnification: x100.

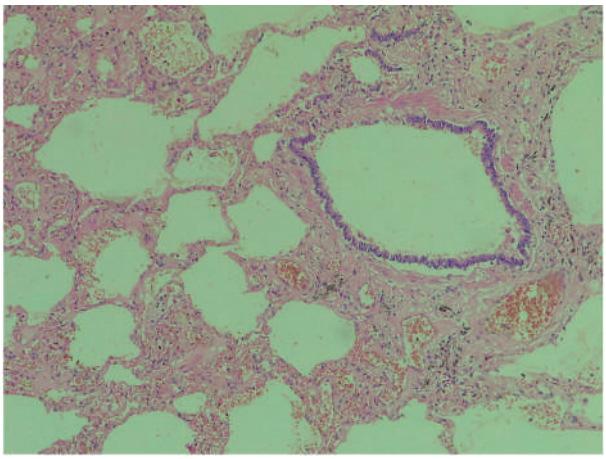

Figure 4 – Significant thickening of the IAM and peribronchial sclerosis in lung tissue of deceased group 2. Hematoxylin-eosin staining. Magnification: x100.

lium, significant sclerosis of the bronchial walls, and peribronchial sclerosis with subsequent deformation of the bronchial lumens, as well as multifocal peribronchial mononuclear infiltration were found in the airways. In the respiratory departments, significant thickening of the IAM was noted caused by severe sclerosis and pleurisy, focal emphysema, and atelectasis. Also, the pulmonary vessels were distinguished by the presence of significant sclerotic changes with stenosis and deformation of their lumens **(fig. 4).**

According to the results of the histochemical examination, significant intra – and peribronchial, as well as intravascular collagenization, was revealed. Compared with those in the deceased group 1, the changes of IAM with visualization of the diffuse arrangement of collagen fibers in most of them were significantly different **(fig. 5).**

Immunohistochemical examination of leptin expression in the lungs of deceased individuals in group 2 revealed multiple fields of expression in the airways and respiratory departments, which were graded as moderate "++" and significant "+++" **(fig. 6).**

The presence of emphysema of the lungs, pneumosclerosis, and pathological changes typical for chronic bronchitis in the deceased with obesity can mutually be considered as signs of COPD. Current research data describing the relationship between obesity and COPD is conflicting. According to the data of the study by Patel et al., an increase in BMI and overweight are associated with a significantly more severe clinical course of COPD than in patients with normal body weight; in particular, stage 3 and 4 COPD was most common in overweight and obese individuals [11]. At the same time, other population-based studies claim that the life expectancy of patients with COPD is significantly higher in overweight and obese individuals than in normal-weight patients, which is considered the "obesity paradox" [12].

Current research data on the association of pneumofibrosis with obesity is also limited and controversial. At present, there are many theories of the occurrence of pulmonary fibrosis caused by obesity. One of them considers the possible induction by a high-fat diet of the release of inflammatory mediators from adipose tissue involving adipocytes and macrophages, the effects of which are important for the development of pulmonary fibrosis. It is also believed that the accumulation of adipose tissue can mechanically disrupt lung function and cause diaphragm fibrosis, which in turn can indirectly potentiate the occurrence of inflammatory changes in the lungs with the development of pneumofibrosis [13].

Conclusions.

In the deceased of the control group, minor pathomorphological, histochemical, and immunohistochemical changes in the lung tissue were identified, which were considered to be minimal and of no meaningful pathological significance.

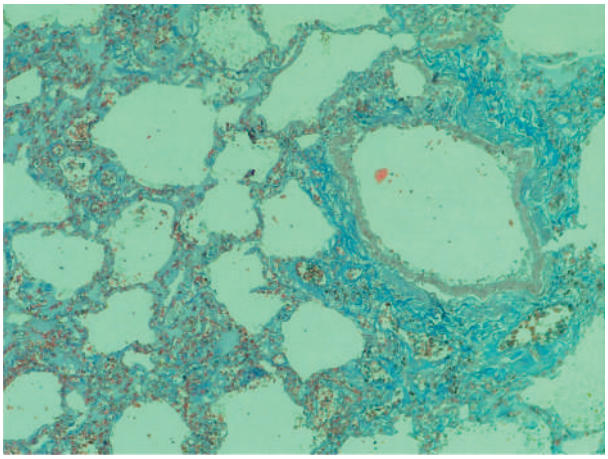

Figure 5 – Diffuse arrangement of collagen fibers in IAM and peribronchial collagenization in lung tissue of deceased group 2. Azan trichrome staining. Magnification: x100.

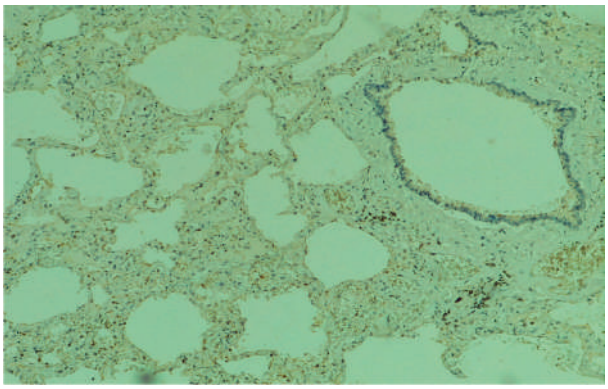

Figure 6 – Significant expression of leptin in lung tissue of deceased group 2. Immunohistochemical method. Magnification: x100.

Pathomorphological and histochemical changes typical for chronic bronchitis, pulmonary emphysema, focal pneumosclerosis, and the initial manifestation of pulmonary hypertension were found in the obese deceased (group 2). The immunohistochemical study allowed the establishment of moderate and significant leptin expression in the deceased of group 2.

Taking into account the fact that the deceased with obesity were not diagnosed with any diseases that were directly or indirectly associated with pathological changes in the lung tissue, it can be assumed that the development of a whole range of pathological changes in the airways, respiratory departments of the lungs, as well as pulmonary vessels, is associated with the obesity in this cohort of the deceased.

Prospects for further research.

It is planned to study the connection of obesity with the occurrence, development, and complications of diseases of the respiratory system, in particular, their pathomorphological, histochemical, and immunohistochemical features in people of different ages, sexes, and degrees of obesity, given the insufficient data on this problem and the controversy of the results of existing studies.

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ПАТОМОРФОЛОГІЧНІ ТА ІМУНОГІСТОХІМІЧНІ ОСОБЛИВОСТІ ЗМІН ЛЕГЕНЬ ПРИ ОЖИРІННІ

Мазур О. Ю.

Резюме. *Вступ.* Ожиріння є вкрай актуальною проблемою системи організації охорони здоров'я; частка осіб із ожирінням прогресивно зростає в усьому світі. Незважаючи на те, що жирову тканину тривало було прийнято розглядати лише як депо енергії, на сьогодні доведено, що це важливий ендокринний орган, який продукує значиму кількість адипоцитокінів, серед яких найбільш вивченим та таким, що володіє найбільш вагомим впливом, вважають лептин, який діє як прозапальний цитокін. Хоча взаємозв'язок надваги та ожиріння, зокрема в розвитку обструктивного апное сну та гіповентиляційного синдрому, стає все більш очевидним, досі є багато суперечливих даних щодо того, чи надмірна вага та ожиріння, а також підвищений рівень лептину достовірно впливають на розвиток, перебіг та смертність у пацієнтів з хронічним обструктивним захворюванням легень. *Мета.* Встановити та проаналізувати патоморфологічні та імуногістохімічні особливості змін легеневої тканини в автопсійному матеріалі померлих осіб із ожирінням.

Об'єкт і методи дослідження. Загальна вибірка померлих становила 60 осіб. Було сформовано дві дослідні групи: групу 1 (контрольна група, n=30) склали померлі з нормальною масою тіла, без діагностованих захворювань, які прямо чи опосередковано були асоційовані із патологічними змінами тканини легень; групу 2 (n=30) – померлі із ожирінням, без діагностованих захворювань, які прямо чи опосередковано були асоційовані із патологічними змінами тканини легень. Для вимірювання маси тіла здійснювали зважування померлих і розраховували індекс маси тіла (ІМТ). Гістохімічне дослідження виконувалось за методикою Азан трихром (із застосуванням п'ятиреагентного набору «Азан трихром»). Для визначення експресії лептину використовувались поліклональні антитіла проти людського лептину (Thermo Fisher Scientific, Німеччина). Розраховували середнє значення (M) і похибку середнього (m). Відмінності середніх величин вважали значущими з рівнем ймовірності не менше 95% (p<0,05).

Результати. Середня маса тіла померлих групи 2 становила 98,6±4,3 кг і виявилась достовірно (p<0,001) вищою, аніж така у померлих групи 1, де цей показник склав 60,0±2,0 кг. Закономірно, середнє значення ІМТ (20,5±0,7 у групі 1 та 34,7±1,3 у групі 2) також виявилось достовірно (p<0,001) вищим у померлих групи 2 у порівнянні із таким у групі 1. За результатами гістохімічного дослідження тканини легень померлих групи 2 виявлено значну інтра – та перебронхіальну, а також інтраваскулярну колагенізацію. У порівнянні із такими у померлих групи 1 значимо відрізнялись зміни МАП з візуалізацією дифузного розташування колагенових волокон у більшості з них. Імуногістохімічне дослідження експресії лептину в легенях померлих осіб у групі 2

виявило множинні поля експресії в повітроносних шляхах та респіраторних відділах, які були оцінені як помірні «++» та значні «+++».

Висновки. У померлих із ожирінням (група 2), виявлено патоморфологічні та гістохімічні зміни притаманні хронічному бронхіту, емфіземі легень, вогнищевому пневмосклерозу та початковому прояву легеневої гіпертензії. Імуногістохімічне дослідження дозволило встановити помірну та значну експресію лептину у померлих групи 2. Можна припустити, що розвиток цілої низки патологічних змін повітроносних шляхів, респіраторних відділів легень, а також легеневих судин асоційовані із наявністю ожиріння у даної когорти померлих.

Ключові слова: ожиріння, лептин, легені, гістохімія, імуногістохімія.

PATHOMORPHOLOGICAL AND IMMUNOHISTOCHEMICAL FEATURES OF LUNG CHANGES IN OBESITY

Mazur O. Yu.

Abstract. *Introduction.* Obesity is an extremely significant problem in the healthcare system; the proportion of people with obesity is progressively increasing worldwide. Even though adipose tissue was considered for long only as an energy depot, today it has been proven that it is an important endocrine organ producing adipocytokines, including leptin, which acts as a pro-inflammatory cytokine. Although the relationship between overweight and obesity, particularly in the development of obstructive sleep apnea and hypoventilation syndrome, is becoming increasingly evident, there is still much conflicting evidence as to whether overweight and obesity, as well as increased leptin levels, reliably influence the development, course, and mortality of patients with chronic obstructive pulmonary disease. *Aim of the study.* To establish and analyze pathomorphological, and immunohistochemical features of lung tissue changes in the autopsy material of deceased obese persons.

Object and research methods. The total sample of the deceased amounted to 60 deceased. Two research groups were formed: group 1 (control group, n=30) consisted of deceased persons with normal body weight, without diagnosed diseases, which were directly or indirectly associated with pathological changes in lung tissue; group 2 (n=30) – deceased with obesity, without diagnosed diseases, which were directly or indirectly associated with pathological changes in lung tissue. To measure body weight, the deceased were weighed, and the body mass index (BMI) was calculated. The histochemical examination was performed in line with the Azan trichrome method (using the five-reagent "Azan trichrome" kit). Polyclonal antibodies against human leptin (Thermo Fisher Scientific, Germany) were used to determine leptin expression. The average value (M) and the average (m) error were calculated. Differences in average values were considered significant with a probability level of at least 95% ($p<0.05$).

The results. The average body weight of the deceased of group 2 was 98.6±4.3 kg, which was significantly ($p<0.001$) higher than that of the deceased of group 1, where this indicator was 60.0±2.0 kg. Regularly, the average value of BMI (20.5±0.7 in group 1 and 34.7±1.3 in group 2) was also significantly ($p<0.001$) higher in the deceased of group 2 compared to those in group 1. The results of the histochemical examination of the lung tissue of the deceased of group 2 revealed significant intra – and peribronchial, as well as intravascular collagenization. Compared to those, the changes in interalveolar membranes with visualization of the diffuse location of collagen fibers in most of them differed significantly compared to the deceased of group 1. Immunohistochemical examination of leptin expression in the lungs of deceased individuals in group 2 revealed multiple fields of expression in the airways and respiratory tracts, which were graded as moderate "++" and significant "+++".

Conclusions. Pathomorphological changes typical for chronic bronchitis, pulmonary emphysema, focal pneumosclerosis, and the initial manifestation of pulmonary hypertension were found in the obese deceased (group 2). The immunohistochemical study established a moderate and significant expression of leptin in the deceased of group 2. It can be assumed that the development of a wide range of pathological changes in the airways, respiratory departments of the lungs, and pulmonary vessels is associated with obesity in this cohort of the deceased.

Key words: obesity, leptin, lungs, histochemistry, immunohistochemistry.

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