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IMMUNOHISTOCHEMICAL EVALUATION OF PULMONARY MICROVASCULAR ENDOTHELIUM IN ALLOXAN-INDUCED DIABETES

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ABSTRACT

This study focuses on the immunohistochemical changes in endothelial cells of lung vessels in experimental diabetes. The increased expression of VEGFA-1 and ICAM1 markers indicates endothelial cell damage, new vessel formation, and activation of the neoangiogenesis process. These markers play a crucial role in assessing damage and inflammation processes, as well as understanding pathological vascularization in diabetes. The findings demonstrate the role of VEGFA-1 and ICAM1 markers in the development of diabetic angiopathy and vascular pathology.

Key words: experimental diabetes, immunohistochemistry, endothelial cells, VEGFA-1, ICAM1, neovascularization, pathological vascularization, diabetic angiopathy, inflammation, vascular pathology.

INTRODUCTION

Experimental diabetes - endocrine of the system widespread from diseases is , it is substances for exchange serious impact showing veins and in fabrics various different to pathologies take arrival can In diabetes the most many damage visible from members one is the lung, this on the ground veins in the structure pathological changes observed, diabetic angiosclerosis and neoangiogenesis like complicated situations development possible [4].

Experimental in diabetes lung veins of endothelium immunohistochemical changes study, veins in the system violations mechanisms understanding and for diabetes due vein diseases treatment for new therapeutic methods work on the way out important to the point have [3,5]. Time is time with appearance will be inflammation markers, for example, ICAM-1 and VEGFA-1, vascular diseases in

pathogenesis solution doer role plays, so for them to study diabetic angiopathy diagnostics and in treatment important to the point owner [1,2].

So so, experimental in diabetes lung in the veins immunohistochemical changes to study clinical in terms of It is important because this vein of diseases first signs determination and them effective treatment opportunities create, patients life to the quality impact to show and diabetic angiopathy with related dangers to reduce help to give possible.

Research **purpose**: experimental in diabetes lung veins of endothelium immunohistochemical changes to determine.

Materials and methods: The studies were conducted on male, outbred white laboratory rats with a body weight of 170±185 g. The animals were kept in standard vivarium conditions and provided with natural food and free water consumption. In the experimental diabetes model, alloxan (Lachema, Czechoslovakia) was administered intraperitoneally to the animals at a dose of 130 mg/kg body weight, thereby inducing diabetes. The animals were fasted for 24 hours, thereby ensuring the development of diabetes. To determine the onset of diabetes, the amount of glucose in the blood was measured using a Contour Plus glucometer.

Vascular endothelium:

1. Immunohistochemistry – to detect ICAM -1, VEGFA -1 and other specific proteins.

2. Histological research - methods of preparing sections of lung tissue and staining them (hematoxylin-eosin, silver staining).

3. Morphometric analysis - quantitative assessment of changes in the structure of vessels and endothelial cells.

The ANOVA method was used for statistical processing of data, to analyze differences between groups at different times.

Results: Experimental diabetes induces metabolic injury in pulmonary vessels. A "chemical burn " damages endothelial cells, leading to inflammation and metabolic changes. Studies have shown that markers such as ICAM1, VEGFA-1, CD20, CD3, CD31, and CD34 are important in assessing injury, inflammation, precancerous conditions, neovascularization, and precapillary processes.

The VGFER-1 marker is crucial in assessing vascular endothelial changes and neoangiogenesis in diabetes. It also helps to identify tissue vascularization disorders, injury, and thrombosis.

VEGFR-1 is found in many organs and tissues, including the ovary, placenta, kidney, fetal liver, brain, blood serum, and synovial fluid. This protein is produced by a variety of cells, including macrophages, fibroblasts, lymphocytes, osteoblasts, endothelial cells, and platelets. VEGF is a major angiogenic factor involved in the

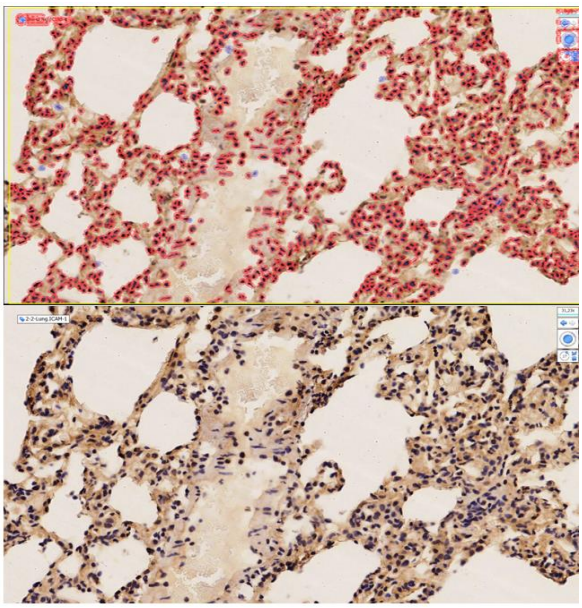
formation of new blood vessels, and its family includes VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, and PLGF.

CD3 and CD20 markers bind to receptors on T and B lymphocytes, allowing us to identify the type of lymphocytes involved in the inflammatory process. Positive expression of T lymphocytes in the vascular perimeter and subendothelial layers helps to assess chronic damage and inflammation in diabetes. These markers also indicate in which layer of the vessel inflammation is occurring and can reflect changes that occur when a secondary infection joins.

The membrane protein CD34 is expressed on cells of many tissues and is involved in cell-cell adhesion in the early stages of hematopoiesis. It is an immunohistochemical marker of vascular endothelium and is studied to assess the level of angiogenesis. Studying the processes of angiogenesis in benign and malignant tumors is important for assessing tumor progression and developing antiangiogenic therapies.

CD31 (PECAM-1) is involved in leukocyte transendothelial migration, angiogenesis, and integrin activation. It also plays a role as a cellular mechanosensor, mediating cell-cell contacts, and is upregulated to maintain stability during mechanical stress.

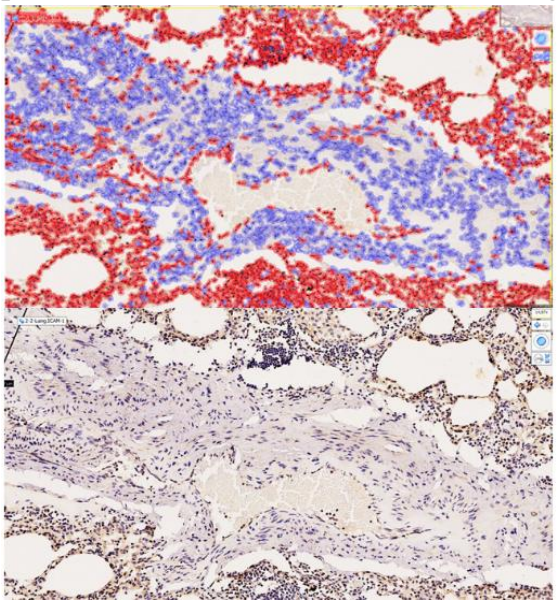
When the vessel dilates, for example, with increased blood flow, the protein is stretched, which leads to phosphorylation of tyrosines by tyrosine kinase and activates a signaling cascade. The cell responds morphologically to the change in blood flow. In immunohistochemical studies, the CD31 marker is a morphological marker indicating damage to endothelial cells.



Number of cells detected	1417
Negative expression	119
Positive expression	1298
Positive expressed cells %	91.6%
Total area of positively expressed cells	1356 mkm2
Total surface area of the tissue being measured	160311 mkm2

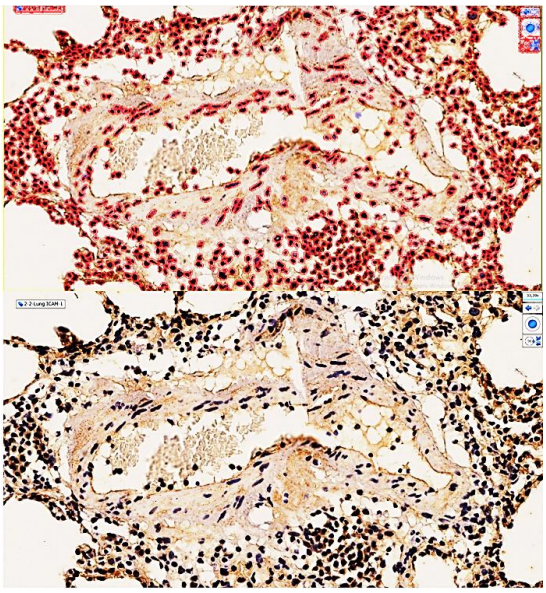
Figure 1. Sample-1. 30-day-old. Low positive expression of ICAM1 marker in lung tissue. Scanned and expression level determined in QuPath-0.4.0.ink. Expressed cells are red. Stain Dab chromogen. Cat. X40.

ICAM1 (Inter-Cellular Adhesion Molecule-1) is a cell adhesion molecule that is expressed in low levels in leukocytes and high levels in endothelial cells. When expressed at high levels, it is found on the membranes of leukocytes and endothelial cells, and sometimes in the cytoplasm. It is activated by cytokines, in particular IL-1 and TNF-α, indicating that the organ is undergoing angiogenesis.



Number of cells detected	4019
Negative expression	1757
Positive expression	2262
Positive expressed cells %	56.28%
Total area of positively expressed cells	3375 mkm2
Total surface area of the tissue being measured	20216 mkm2

Figure 2. Sample-2. 60-day-old. Low positive expression of ICAM1 marker in lung tissue. Scanned and expression level determined in QuPath-0.4.0.ink. Expressed cells are red. Stain Dab chromogen. Cat. X40.



Number of cells detected	1417
Negative expression	119
Positive expression	1298
Positive expressed cells %	91.6%
Total area of positively expressed cells	1356 mkm2
Total surface area of the tissue being measured	160311 mkm2

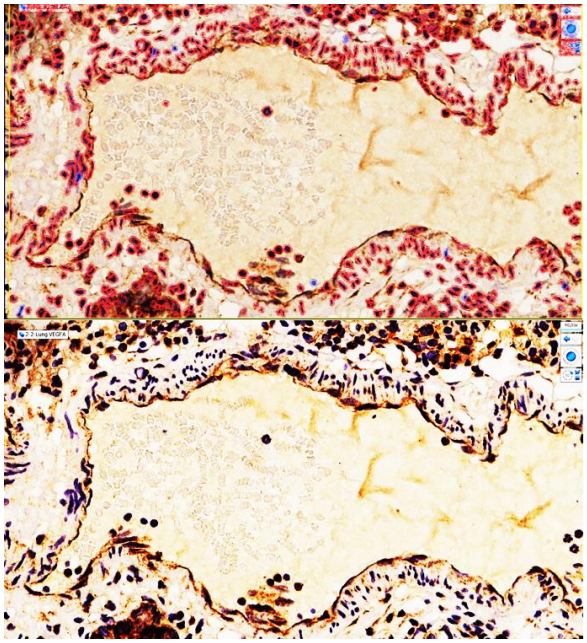
Figure 3. Sample-3. 120-day-old. Low positive expression of ICAM1 marker in lung tissue. Scanned and expression level determined in QuPath-0.4.0.ink. Expressed cells are red. Stain Dab chromogen. Cat. X40.

The study revealed high expression of the ICAM1 marker (91.8%). On the 60th day, experimental diabetes mellitus showed damage to the capillary endothelium, increased adhesion factor, and neoangiogenesis. In hyperglycemia, qualitative reactions associated with a 'chemical burn' in endothelial cells and the formation of new vascular structures were detected.

The study revealed that high expression of the ICAM1 marker was maintained at 30 and 60 days of diabetes, capillary proliferation in the lungs, and diabetic angiosclerosis was exacerbated. At 120 days, high expression of the ICAM1 marker, endothelial cell proliferation, vascular wall transformation, and increased tropocollagen synthesis were observed. As a result, a large amount of obliteration of blood vessels in the lung tissue was observed in 120-day-old rats.

In experimental diabetes mellitus, high expression of the VEGFA-1 marker was observed in damaged capillary and small capillary endothelial cells. This process is a protective mechanism aimed at preventing systemic vascular damage in diabetes, and EGF is an effective regulator of new capillary formation. The effect of VEGFA-1 on endothelial cells is activated through a tyrosine residue, increasing proliferation, migration, and vascular permeability.

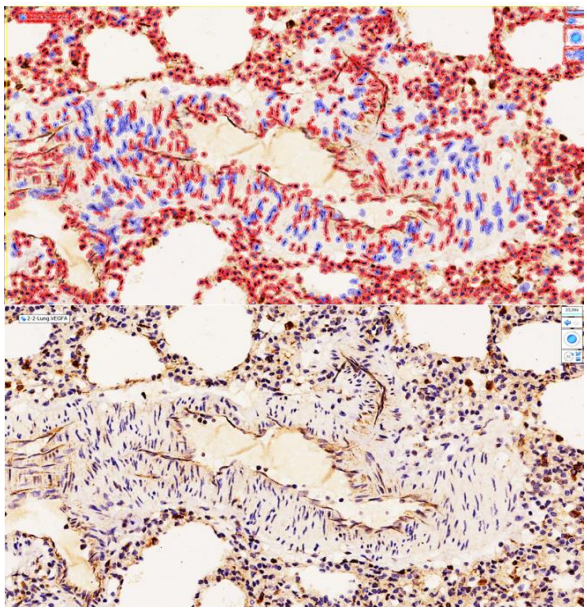
Endothelial cell proliferation and migration play an important role in angiogenesis after hypoxia, able to prevent oxidative stress and influence free radicals and apoptosis processes. They are involved in controlling physiological development and maintaining the structural and functional integrity of neurons.



Number of cells detected	822
Negative expression	229
Positive expression	593
Positive expressed cells %	72.16%
Total area of positively expressed cells	3600 μm ^{2}
Total surface area of the tissue being measured	23521 μm ^{2}

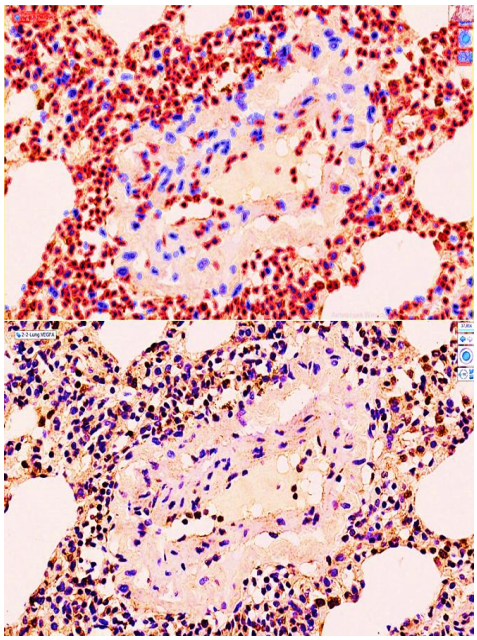
Figure 4. Sample-3. 30-day-old. Low positive expression of VEGFA-1 marker in lung tissue. Scanned and expression level determined in QuPath-0.4.0.ink. Expressed cells are red. Stain Dab chromogen. Cat. 10X40.

In our study, according to the results of staining with the VEGFA-1 marker, high positive expression of the VEGFA-1 marker (72.16%) leads to stimulation of vascular growth factor in diabetes mellitus. This process is associated with a "chemical burn" of all vascular endothelial cells, which increases the production of cytokines and stimulation of endothelial growth factor.



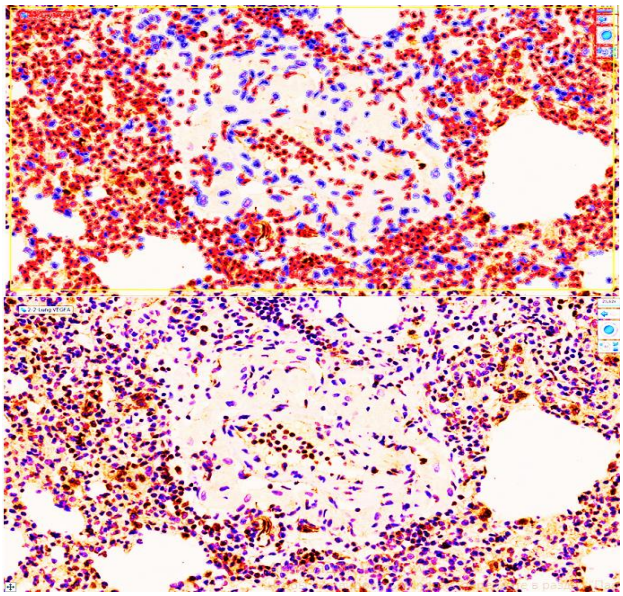
Number of cells detected	1881
Negative expression	343
Positive expression	1538
Positive expressed cells %	81.76%
Total area of positively expressed cells	6400 μm ^{2}
Total surface area of the tissue being measured	41616 μm ^{2}

Figure 5. Sample-3. 60-day-old. Low positive expression of VEGFA-1 marker in lung tissue. Scanned and expression level determined in QuPath-0.4.0.ink. Expressed cells are red. Stain Dab chromogen. Cat. 10X40.



Number of cells detected	1332
Negative expression	236
Positive expression	1076
Positive expressed cells %	82.01%
Total area of positively expressed cells	6400 μm ^{2}
Total surface area of the tissue being measured	41616 μm ^{2}

Figure 6. Sample-3. 90-day-old. Low positive expression of VEGFA-1 marker in lung tissue. Scanned and expression level determined in QuPath-0.4.0.ink. Expressed cells are red. Stain Dab chromogen. Cat. 10X40.



Number of cells detected	2193
Negative expression	488
Positive expression	1705
Positive expressed cells %	77.47%
Total area of positively expressed cells	8100 μm ^{2}
Total surface area of the tissue being measured	765500 μm ^{2}

Figure 7. Sample-3. 120-day-old. High positive expression of VEGFA-1 marker in lung tissue. Scanned and expression level determined in QuPath-0.4.0.ink. Expressed cells are red. Stain Dab chromogen. Cat. 10X40.

High expression of the VEGFA-1 marker in the pulmonary vessels of diabetic rats leads to endothelial cell damage, vacuolar dystrophy, and increased growth factor expression. This process inhibits apoptosis, stimulates angiogenesis and vasculogenesis, and causes the development of pathological vascularization.

Conclusion. Experimental diabetes results in endothelial cell damage and neovascularization in pulmonary vessels. VEGFA-1 and ICAM1 markers play an important role in monitoring endothelial cell damage and neoangiogenesis. VEGFA-1 marker indicates changes in vascular endothelium in diabetes, indicating endothelial cell damage, vacuolar dystrophy, and increased growth factor. This process inhibits apoptosis, stimulates angiogenesis and vasculogenesis, and causes pathological vascularization. ICAM1 marker helps to analyze the processes of inflammation and neovascularization in endothelial cells.

The results of the study allow us to conclude that in diabetes, VEGFA-1 and ICAM1 markers indicate damage to endothelial cells and the formation of new vessels, and their activation is of great importance in the development of diabetic angiopathy and vascularization pathologies.

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