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INVESTIGATION OF THE EXPRESSION OF THE IMMUNOHISTOCHEMICAL MARKER KI-67 IN THE TRACHEA AND MAIN BRONCHI OF RATS: AN EXPERIMENTAL APPROACH

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Summary.

Rheumatoid arthritis represents a chronic systemic inflammatory disorder of connective tissue characterized by progressive damage primarily affecting peripheral (synovial) joints through symmetrical, progressive, erosive-destructive polyarthritis, with some cases demonstrating characteristic extra-articular manifestations.

Objective of the Study. *This investigation aims to evaluate morphological alterations in tracheal and bronchial lymphoid structures in outbred white rats subjected to an experimental rheumatoid arthritis model utilizing complete Freund's adjuvant, with particular focus on Ki-67 proliferation marker expression analysis.*

Materials and Methods. *The experimental cohort comprised 30 outbred white rats aged four months (both sexes) with mean body weight 170.5 ± 9.1 g. All laboratory animals originated from a single vivarium source and were maintained under standardized conditions featuring relative humidity (50-60%), ambient temperature (19-22 °C), and 12-hour light/dark cycles. The principles of bioethics, approved by the Scientific Council of Bukhara State Medical Institute, are preserved and upheld in full compliance. The methods used for statistical data analysis were t-test, χ2-test, and correlation analysis. The study was conducted in accordance with the research plan of the Bukhara State Medical Institute within the framework of the topic «Early detection and diagnosis of pathological factors affecting the health of the population of the Bukhara region in the post-COVID-19 period, as well as the development of new methods of treatment and prevention (2022-2026)».*

Results and Discussion. *Positively stained cells within designated areas were quantified as percentage ratios relative to total cell counts per microscopic field. Expression levels were categorized as follows: low expression (≤20%), moderate expression (20-60%), and high expression (>60%). Tracheal specimen analysis revealed 1,547 total cells, among which 189 cells demonstrated positive Ki-67 proliferation marker expression indicating proliferative activity. The remaining 1,358 cells showed Ki-67 negativity, suggesting predominant cellular quiescence. The overall positive expression rate of 12.21% indicates low proliferative activity in tracheal hyperplastic lymphoid tissue.*

Conclusions. *Heterogeneous Ki-67 proliferation expression patterns in tracheobronchial tissues of experimental rheumatoid arthritis rats may reflect regional vascular perfusion variations across different tracheal and main bronchial segments.*

Keywords: *Expression; Proliferation Marker; Ki-67; Trachea; Main Bronchi; White Outbred Rats; Cell Proliferation; Localization; Inflammatory Processes.*

Introduction

Rheumatoid arthritis (RA) is a chronic systemic inflammatory disease of the connective tissue, characterized by progressive damage primarily to the peripheral (synovial) joints in the form of symmetrical, progressive, erosive-destructive polyarthritis, and in some cases, by characteristic extra-articular manifestations [1-4]. Traditionally, rheumatoid arthritis is regarded as a «joint disease,» although extra-articular manifestations largely determine the disease prognosis [5-7]. Among these, involvement of the respiratory system ranks among the most prevalent and prognostically significant [8-10]. In most guidelines on rheumatoid arthritis and rheumatic diseases in general, pulmonary aspects of the disease receive limited attention. However, despite the diverse manifestations of diffuse connective tissue diseases, pulmonary and pleural changes occur in 50% to 100% of cases, according to various authors [11-13], and are often a direct cause of death. A large-scale population study initiated in 1997 found that bronchial and tracheal involvement of varying severity and clinical presentation occurs in 58% of patients with RA [14-17].

Rheumatoid arthritis (RA) is one of the most common autoimmune diseases, characterized by chronic inflammation and joint damage. However, its systemic manifestations, including effects on the respiratory system, remain insufficiently studied. In particular, the lymphoid structures of the trachea and bronchi play a key role in immune responses and may undergo significant changes during RA-associated inflammatory processes [18-20].

Immunohistochemical analysis of Ki-67 proliferation marker expression allows for the assessment of cellular activity and proliferation in tissues, which is crucial for understanding the pathogenesis of inflammatory diseases [21,22]. In the present investigation, we focus on evaluating morphological alterations within the lymphoid structures of the trachea and bronchi in outbred white rats subjected to an experimental model of RA utilizing complete Freund's adjuvant [23]. The acquired data regarding heterogeneous cellular proliferation contingent upon localization within the trachea and principal bronchus underscore the necessity of investigating these alterations to enhance the understanding of RA pathophysiology and its impact upon respiratory organs.

Objective of the Study

The objective of this study is to evaluate morphological alterations within the lymphoid structures of the trachea and bronchi in outbred white rats subjected to an experimental model of rheumatoid arthritis induced by complete Freund's adjuvant, with particular emphasis on the analysis of Ki-67 proliferation marker expression. The study aims to identify disparities in the level of cellular proliferation dependent upon localization within the trachea and main bronchus, in addition to elucidating the pathophysiological mechanisms associated with

inflammatory processes within the respiratory tract in RA. The findings may contribute to an enhanced comprehension of the relationship between autoimmune diseases and alterations within the respiratory system, which, consequently, could facilitate the development of novel approaches for the diagnosis and management of patients with RA and associated respiratory pathologies.

Materials and Method

The experimental procedures utilized thirty outbred white rats, aged four months, of both sexes, exhibiting an average body mass of 170.5 ± 9.1 g. All laboratory animals were procured from an identical vivarium and maintained under standardized environmental conditions encompassing relative humidity (50-60%), ambient temperature (19-22 °C), and a photoperiod regimen of 12 hours of darkness and 12 hours of light.

To preclude infectious complications, the animals underwent a quarantine period of 21 days, during which their physiological status was monitored, incorporating serial measurements of core body temperature and mass. Body mass accretion was tracked, and no clinical indicators of disease were observed throughout this interval. Core temperature remained within the established normative range (38.5-39.5 °C), and no disturbances in appetite or other external alterations were detected.

The experimental subjects were allocated into two distinct cohorts:

1. Control group

2. Experimental group – animals with experimentally induced rheumatoid arthritis (RA modeling was accomplished utilizing complete Freund's adjuvant).

Upon termination of the experimental protocol, the rats were euthanized, and the trachea in conjunction with the main bronchi were surgically isolated. procured tissues were immersion-fixed in 10% neutral buffered formalin for a duration of 24 hours, followed by sequential dehydration through a graded ethanol series and subsequent embedding in paraffin wax. Histological sections of 5 μm thickness were obtained employing a rotary microtome and mounted upon glass slides.

Immunohistochemical staining procedures were executed utilizing a specific antibody directed against the Ki-67 proliferation marker. A standardized protocol was implemented, incorporating antigen retrieval, incubation with primary antibodies, and subsequent visualization with appropriate secondary antibodies. Control specimens were processed through omission of the primary antibody to preclude nonspecific staining artifacts. The stained histological preparations were examined utilizing a trinocular microscope at magnifications ranging from 200× to 400×. Morphometric analysis was conducted employing QuPath 4.4.0 software. Cells exhibiting positive immunohistochemical staining within the designated area were quantified and expressed as a percentage of the total cellular population within the entire microscopic field of view.

The principles of bioethics, approved by the Scientific Council of Bukhara State Medical Institute, are preserved and upheld in full compliance.

The methods used for statistical data analysis were t-test, $\chi 2$-test, and correlation analysis.

The study was conducted in accordance with the research plan of the Bukhara State Medical Institute within the framework of the topic «Early detection and diagnosis of pathological factors affecting the health of the population of the Bukhara region in the post-COVID-19 period, as well as the development of new methods of treatment and prevention (2022-2026)».

Results and Discussion

Cells demonstrating positive immunohistochemical staining within the selected area were quantified and expressed as a percentage of the total cellular count within the complete field of view. The expression level was categorized according to the following criteria:

- Low expression: ≤20%
- Moderate expression: 20-60%
- High expression: >60%

Results for the Trachea (Experimental Group)

Within the analyzed tracheal specimen, a total of 1,547 cells were identified. Among these, 189 cells exhibited positive expression of the Ki-67 proliferation marker, indicative of their active participation in cellular proliferation processes. The remaining 1,358 cells were negative for Ki-67 immunoreactivity, suggesting a predominance of cells exhibiting low proliferative activity. The aggregate positive expression rate was calculated as 12.21%, indicative of a low level of proliferative activity within the hyperplastic lymphoid tissue of the trachea.

Based upon the immunohistochemical analysis, the subsequent conclusions were formulated:

1. The total cellular population within the analyzed specimen was 1,547.

2. Among these, 189 cells demonstrated positive Ki-67 expression, indicative of their involvement in cellular proliferation.

3. The number of cells exhibiting negative staining was 1,358, confirming the predominance of cells with low proliferative rates within this anatomical region.

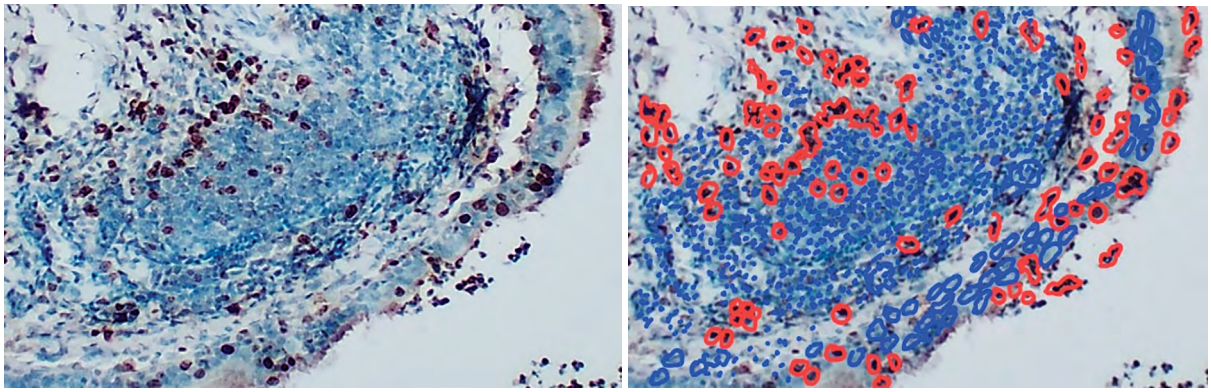
4. The positive Ki-67 expression rate was quantified as 12.21%, suggesting a low level of proliferative activity within the hyperplastic lymphoid tissue of the trachea.

These findings indicate the presence of lymphoid tissue hyperplasia; however, the overall level of cellular proliferation remains comparatively low. This observation suggests that within this experimental model of RA, notwithstanding the activation of lymphoid tissue, the proliferative process fails to attain elevated levels. These results may possess significant implications for further elucidating the pathogenesis of rheumatoid arthritis and the functional role of lymphoid structures within this process.

Results for the Main Bronchus (Experimental Group)

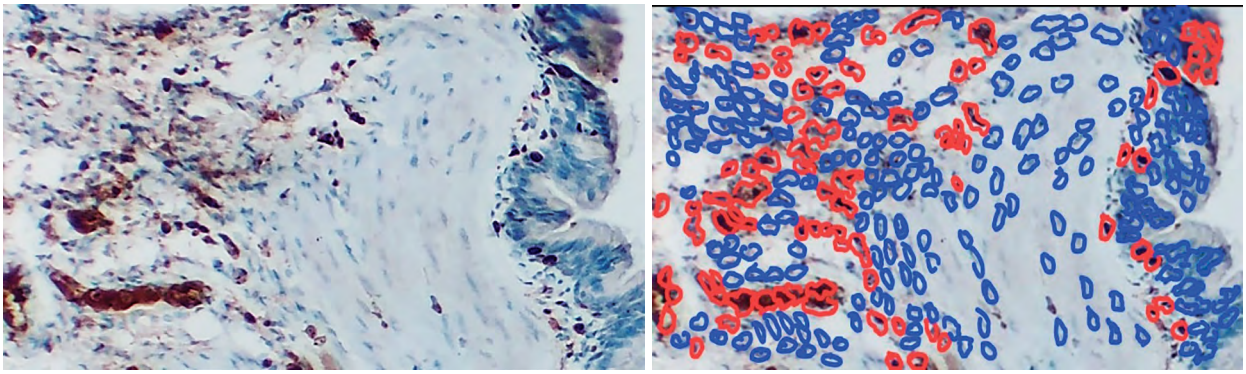
Within the analyzed specimen of the main bronchus, a total of 832 cells were identified. Among these, 83 cells exhibited positive Ki-67 expression, whereas 749 cells were negative. The positive expression rate was calculated as 9.97%, indicative of a low level of proliferative activity within this anatomical region.

These findings suggest that although activation of lymphoid tissue occurs within the respiratory tract in rheumatoid arthritis, the magnitude of cellular proliferation remains relatively subdued, which may reflect differential inflammatory responses contingent upon tissue localization.



Total number of cells detected	832
Number of positive cells	83
Number of negative cells	749
Positive expression	9.97%
Total area	937,513 px^2

Figure 1. Section of hyperplastic lymphoid tissue in the trachea of an outbred white rat from the experimental group. Immunohistochemical staining for Ki-67 marker expression.



Total number of cells detected	832
Number of positive cells	83
Number of negative cells	749
Positive expression	9.97%
Total area	937,513 px^2

Figure 2. Section of Main Bronchus Tissue in an Outbred White Rat from the Experimental Group. Immunohistochemical staining for Ki-67

Immunohistochemical Staining for Ki-67 Marker Expression

Based upon the immunohistochemical analysis of the hyperplastic lymphoid tissue in the trachea of an outbred white rat from the experimental group, the subsequent results were obtained:

• The total number of cells within the analyzed specimen was 832.

• Among these, 83 cells exhibited positive Ki-67 expression, indicative of their involvement in cellular proliferation processes.

• The number of cells exhibiting negative staining was 749, confirming the predominance of cells with low proliferation levels within this region.

• The positive Ki-67 expression rate was quantified as 9.97%, indicative of a low level of proliferative activity within the hyperplastic lymphoid tissue of the trachea.

• The total area of the analyzed specimen was 937,513 px^2.

These findings indicate the presence of lymphoid tissue hyperplasia; however, the overall level of cellular proliferation remains relatively low. A positive Ki-67 expression rate of 9.97% suggests that although lymphoid structures are activated, the cellular proliferation process fails to attain significant levels. These results may be of critical importance for further investigation into the pathogenesis of rheumatoid arthritis and the functional role of lymphoid structures within this process.

Conclusions

The investigation of Ki-67 proliferation marker expression within the tracheal and main bronchial walls of rats in the experimental group revealed disparities in cellular proliferation levels contingent upon anatomical localization.

• Within the trachea (Figure 1): The Ki-67 expression level was quantified as 12.21%, corresponding to a low proliferation rate.

• Within the main bronchus (Figure 2): The Ki-67 expression level was quantified as 9.97%, also indicative of a low proliferation rate.

The results suggest that:

• Within the trachea, heterogeneous cellular proliferation is present. Certain areas exhibit a low

proliferation rate, which may indicate moderate cellular division activity within this region. Other areas demonstrate a moderate proliferation rate, potentially attributable to an inflammatory process or tissue regeneration.

• Within the main bronchus, the cellular proliferation level is inferior to that observed in certain tracheal regions, which may indicate less pronounced cellular division activity within this anatomical area.

• The heterogeneity of Ki-67 proliferation expression within the trachea and main bronchi of rats subjected to experimental rheumatoid arthritis may be associated with disparities in blood supply levels across different regions of the trachea and main bronchi.

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ДОСЛІДЖЕННЯ ЕКСПРЕСІЇ ІМУНОГІСТОХІМІЧНОГО МАРКЕРА KI-67 У ТРАХЕЇ ТА ГОЛОВНИХ БРОНХАХ ЩУРІВ: ЕКСПЕРИМЕНТАЛЬНИЙ ПІДХІД

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Резюме.

Ревматоїдний артрит є хронічним системним запальним захворюванням сполучної тканини, що характеризується прогресуючим ураженням, яке в першу чергу вражає периферичні (синовіальні) суглоби через симетричний, прогресуючий, ерозивно-деструктивний поліартрит, причому в деяких випадках спостерігаються характерні позасуглобові прояви.

Мета дослідження. Це дослідження мало на меті оцінити морфологічні зміни в лімфоїдних структурах трахеї та бронхів у білих щурів, підданих експериментальній моделі ревматоїдного артриту з використанням повного ад'юванта Фрейнда, з особливим акцентом на аналізі експресії маркера проліферації Ki-67.

Матеріали та методи. Експериментальна когорта складалася з 30 нелінійних білих щурів віком чотири місяці (обох статей) із середньою масою тіла 170,5 ± 9,1 г. Усі лабораторні тварини походили з одного віварію та утримувалися в стандартних умовах із відносною вологістю (50-60%), температурою навколишнього середовища (19-22 °C) та 12-годинним циклом світло/темрява.

Принципи біоетики, затверджені Науковою радою Бухарського державного медичного інституту, зберігаються та дотримуються у повній відповідності.

Методи, які використовувалися для статистичної обробки даних, були t-тест, $\chi 2$-тест та кореляційний аналіз. Дослідження було проведене відповідно до плану досліджень Бухарського державного медичного інституту у рамках теми «Раннє виявлення та діагностика патологічних чинників, що впливають на здоров'я населення Бухарського регіону у пост-COVID-19 період, а також розробка нових методів лікування та профілактики (2022-2026)».

Результати та обговорення. Позитивно забарвлені клітини в зазначених ділянках були кількісно оцінені як відсоткові співвідношення відносно загальної кількості клітин на мікроскопічному полі. Рівні експресії були класифіковані наступним чином: низька експресія (≤20%), помірна експресія (20-60%) та висока експресія (>60%). Аналіз зразків трахеї виявив 1547 клітин, серед яких 189 клітин продемонстрували позитивну експресію маркера проліферації Ki-67, що вказує на проліферативну активність. Решта 1358 клітин показали негативність Ki-67, що свідчить про переважну клітинну спокійність. Загальний рівень позитивної експресії 12,21% вказує на низьку проліферативну активність у гіперпластичній лімфоїдній тканині трахеї.

Висновки. Гетерогенні патерни експресії проліферації Ki-67 у трахеобронхіальних тканинах щурів з експериментальним ревматоїдним артритом можуть відображати регіональні варіації судинної перфузії в різних сегментах трахеї та головних бронхів.

Ключові слова: експресія; маркер проліферації; Ki-67; трахея; головні бронхи; білі аутбредні щури; клітинна проліферація; локалізація; запальні процеси.

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