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*E.A. Kharibova, V.I. Ten*Bukhara State Medical Institute named after Abu Ali ibn Sino
(Bukhara, Uzbekistan)STUDY OF PROLIFERATIVE ACTIVITY
OF PAROTID SALIVARY GLAND
CELLS IN OUTBRED WHITE RATS
WITH EXPERIMENTALLY INDUCED
RHEUMATOID ARTHRITIS: THE ROLE
OF KI-67**Summary.**

Extra-articular manifestations are a significant focus in rheumatoid arthritis research. Determination of the proliferation marker Ki-67 expression level is an effective method for evaluating quantitative and qualitative changes in digestive organs in rheumatoid arthritis.

Objective. *The aim of this study is to assess morphological changes in the glandular apparatus of the parotid salivary gland in outbred white rats subjected to a model of rheumatoid arthritis induced by Complete Freund's Adjuvant, using the expression level of the proliferation marker Ki-67 as an indicator of the inflammatory process severity.*

Materials and Methods: *The experimental group comprised 30 four-month-old outbred white rats of both sexes, with a mean body weight of 170.5 ± 9.1 g. All laboratory animals were sourced from a single vivarium and maintained under standard conditions: 50-60% relative humidity, 19-22 °C ambient temperature, and a 12-hour light/dark cycle. Rheumatoid arthritis was induced using Complete Freund's Adjuvant. All studies were approved by the Ethics Committee of the Bukhara State Medical Institute and adhered to bioethical standards, including justification for animal use; compliance with the 3Rs principles; use of humane methods; confirmation of researcher competence. Specimens were analyzed using a trinocular microscope at 200-400× magnification. Morphometric analysis was conducted with QuPath 4.4.0 software. The percentage of Ki-67-positive cells was calculated relative to the total cell count in the field of view. Morphology of Salivary Glands in an Experimental Model of Rheumatoid Arthritis. Code: 14.00.02 Morphology. Implementation Years: 2024-2026. The study was conducted as part of the research plan of the Bukhara State Medical Institute.*

Results and Discussion. *In all examined samples, Ki-67 expression levels ranged from 32.04% to 38.19%, indicating elevated cellular proliferation. High Ki-67 expression in the parotid gland in modeled rheumatoid arthritis does not allow definitive classification of the tumor as aggressive or non-aggressive but may reflect cell cycle activity and proliferation typical of inflammatory processes such as rheumatoid arthritis.*

Conclusions: *The findings confirm proliferative activity in the parotid gland of outbred white rats with experimentally induced rheumatoid arthritis.*

Keywords: *Parotid Salivary Gland; Outbred White Rats; Proliferative Activity; Ki-67 Marker; Immunohistochemistry.*

Introduction

Rheumatoid arthritis (RA) is a chronic systemic autoimmune inflammatory disease. Previous studies have established that RA primarily induces changes in connective tissue, resulting in progressive damage to predominantly peripheral (synovial) joints, manifesting as symmetrical, progressive, erosive-destructive polyarthritis. However, recent evidence suggests that RA is also associated with characteristic extra-articular manifestations affecting various organs and systems, which may even precede musculoskeletal changes [1-3]. Consequently, classifying RA solely as a joint disease is inaccurate.

Numerous studies indicate that internal organ involvement contributes more substantially to disability and mortality in RA than musculoskeletal damage [4,5]. Among the affected systems, the digestive system is frequently involved and significantly influences disease prognosis and severity [6-8].

In most textbooks and guidelines on rheumatoid arthritis or rheumatic diseases, gastrointestinal manifestations receive limited attention. Despite the diverse manifestations of connective tissue diseases, detailed descriptions of digestive system involvement are scarce [10-12].

It is well established that metabolic processes are largely regulated by digestive organs, and disruptions in these processes can result in irreversible health consequences. Large population-based studies confirm that digestive system involvement, with varying degrees and clinical presentations, occurs in a significant proportion of RA patients [13-15].

Rheumatoid arthritis is a prevalent autoimmune diseases characterized by chronic inflammation. However, its systemic manifestations, including digestive organ involvement, remain understudied. Specifically, lymphoid structures within the digestive system play a critical role in the immune response and may undergo alterations during RA-associated inflammatory processes [16, 17].

Immunohistochemical analysis of the Ki-67 proliferation marker allows for assessment of cellular activity and tissue proliferation, which is important for understanding the pathogenesis of inflammatory diseases. The level of Ki-67 expression can serve as an indicator of cellular activity and thus reflect the state of immune response in the digestive organs [18, 19].

This study evaluates morphological changes in the lymphoid structures of the parotid salivary gland in outbred white rats with RA induced by Complete Freund's Adjuvant. The findings demonstrate a high degree of inflammatory activity in the parotid gland, with an uneven pattern of cell proliferation depending on localization within the glandular apparatus, underscoring the importance of investigating these changes to elucidate the pathogenesis and dissemination of RA, and its impact on digestive organs.

Research Objective

This study aims to assess morphological changes in the glandular apparatus of the parotid salivary gland in outbred white rats subjected to experimental rheumatoid arthritis

induced by Complete Freund's Adjuvant. This evaluation is based on analyzing the expression of the proliferation marker Ki-67, which facilitates a comprehensive assessment of the extent of inflammatory changes in the parotid salivary gland under induced rheumatoid arthritis.

The study investigates the intensity of cellular proliferation in the parotid gland caused by rheumatoid arthritis and examines the development of destructive changes in the salivary glands as part of the digestive system. These findings may enhance understanding of the relationship between autoimmune diseases and pathological changes in the digestive organs. In turn, this may inform the development of novel diagnostic and therapeutic approaches for patients with RA and associated gastrointestinal disorders.

Materials and Methods

The experiment included 30 four-month-old outbred white rats of both sexes, with a mean body weight of 170.5 ± 9.1 g. All animals were sourced from a single vivarium and maintained under standard laboratory conditions: 50-60% relative humidity, 19-22 °C temperature, and a 12-hour light/dark cycle.

To prevent infectious diseases, all animals were subjected to a 21-day quarantine, with their health monitored throughout. Body temperature and weight were measured repeatedly. No signs of illness were observed during this period; body temperature remained within the normal range (38.5-39.5 °C), and no evidence of appetite loss or other external abnormalities was noted.

The animals were allocated into two groups:

Group 1 (Control): Healthy animals without induced arthritis.

Group 2 (Experimental): Animals with rheumatoid arthritis induced by Complete Freund's Adjuvant.

At the conclusion of the experiment, the animals were euthanized, and the parotid salivary glands were excised. The glands were fixed in 10% neutral buffered formalin for 24 hours, followed by dehydration in graded alcohols and embedding in paraffin. Tissue sections 5 μm thickness were prepared using a rotary microtome and mounted on glass slides.

Immunohistochemical staining was conducted using a specific antibody targeting the Ki-67 proliferation marker. The standard protocol involved antigen retrieval, incubation with primary antibodies, and subsequent visualization with appropriate secondary antibodies. Negative controls were processed without primary antibodies to rule out nonspecific staining.

Stained sections were analyzed under a trinocular microscope at 200-400× magnification. Morphometric analysis was performed using QuPath 4.4.0 software. The proportion of Ki-67 positive cells in the selected area was calculated as a percentage of the total number of cells within the field of view.

Conclusion

Analysis of Ki-67 proliferation marker expression in the parotid salivary gland of outbred white rats with experimentally induced rheumatoid arthritis demonstrated variations in cellular proliferation levels on localization:

In Figure 1, the Ki-67 expression level was **38.19%**, indicating **high proliferation**.

In Figure 2, the Ki-67 expression was **32.1%**, also corresponding to **high proliferation**.

Across all examined areas, the Ki-67 expression ranged from **32.1% to 38.19%**, reflecting consistently high cellular proliferation.

Immunohistochemical Evaluation of Ki-67 Expression in Parotid Salivary Gland Tissues of Outbred White Rats with Experimentally Induced Rheumatoid Arthritis

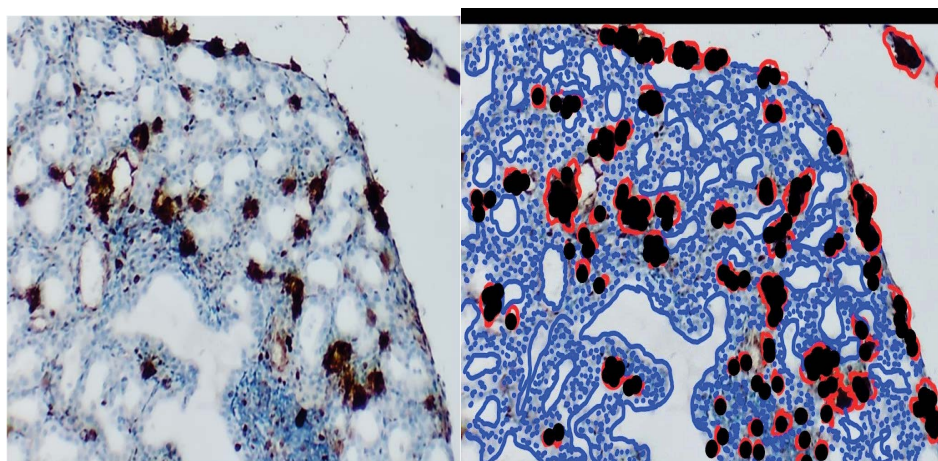


Figure 1. Microscopic view of the parotid salivary gland of an outbred white rat from the experimental group following immunohistochemical analysis to evaluate Ki-67 marker expression level

Total number of cells detected	1,233
Number of positive cells	471
Number of negative cells	762
Positive expression	38.19%
Total area	1,157,413 px ²

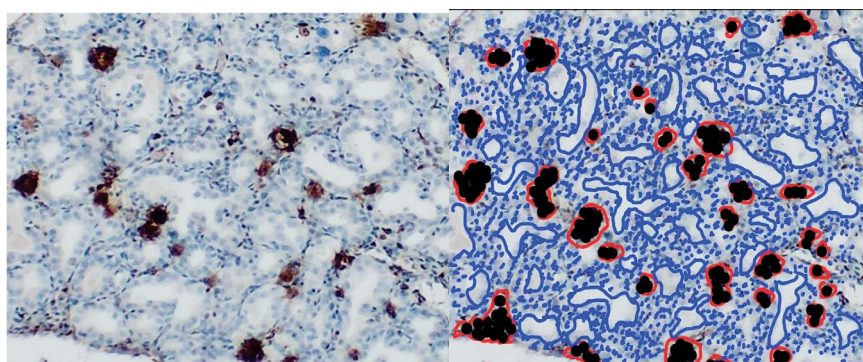


Figure 2. Microscopic view of the parotid salivary gland of an outbred white rat from the experimental group following immunohistochemical analysis to assess Ki-67 marker expression levels

Total number of cells detected	1,231
Number of positive cells	395
Number of negative cells	836
Positive expression	32.1%
Total area	917,743 px ²

Interpretation

High Ki-67 expression in the glandular apparatus of the parotid salivary gland in the presence of induced rheumatoid arthritis does not permit definitive classification of a lesion as aggressive or non-aggressive. However, it may reflect increased cell cycle activity and proliferation,

which are hallmarks of various inflammatory processes, including rheumatoid arthritis.

The obtained data proliferative activity in the parotid salivary gland of outbred white rats subjected to a model of rheumatoid arthritis induced by Complete Freund's Adjuvant.

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ДОСЛІДЖЕННЯ ПРОЛІФЕРАТИВНОЇ АКТИВНОСТІ КЛІТИН ПРИВУШНИХ СЛИННИХ ЗАЛОЗ У БЕЗПОРОДНИХ БІЛИХ ЩУРІВ З ЕКСПЕРИМЕНТАЛЬНО ІНДУКОВАНИМ РЕВМАТОЇДНИМ АРТРИТОМ: РОЛЬ K1-67

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Резюме.

Позасуглобові прояви є значним напрямком досліджень ревматоїдного артриту. Визначення рівня експресії маркера проліферації K1-67 є ефективним методом оцінки кількісних та якісних змін у органах травлення при ревматоїдному артриті.

Мета. Метою дослідження є оцінка морфологічних змін у залозистому апараті привушної слинної залози у безпородних білих щурів, які зазнали впливу моделі ревматоїдного артриту, індукованого повним ад'ювантом Фрейнда, використовуючи рівень експресії маркера проліферації K1-67 як показник тяжкості запального процесу.

Матеріали та методи. Експериментальна група складалася з 30 чотиримісячних безпородних білих щурів обох статей із середньою масою тіла $170,5 \pm 9,1$ г. Усіх лабораторних тварин було отримано з одного виварію та утримувалося за стандартних умов: відносна вологість 50-60%, температура навколишнього середовища 19-22 °C та 12-годинний цикл світло/темрява. Ревматоїдний артрит індукували за допомогою повного ад'юванту Фрейнда. Усі дослідження були схвалені Етичним комітетом Бухарського державного медичного інституту та відповідали біоетичним стандартам, включаючи обґрунтування використання тварин; дотримання принципів 3R; використання гуманних методів; підтвердження компетентності дослідника. Зразки аналізували за допомогою тринокулярного мікроскопа при збільшенні 200-400×. Морфометричний аналіз проводили за допомогою програмного забезпечення QuPath 4.4.0. Відсоток K1-67-позитивних клітин розраховували відносно загальної кількості клітин у полі зору. Морфологія слинних залоз в експериментальній моделі ревматоїдного артриту. Код: 14.00.02 Морфологія. роки реалізації: 2024-2026. Дослідження проводилося в рамках дослідницького плану Бухарського державного медичного інституту.

Результати та обговорення. У всіх досліджених зразках рівні експресії K1-67 коливалися від 32,04% до 38,19%, що свідчить про підвищену клітинну проліферацію. Висока експресія K1-67 у привушній залозі при змодельованому ревматоїдному артриті не дозволяє остаточно класифікувати пухлину як агресивну чи неагресивну, але може відображати активність клітинного циклу та проліферацію, типову для запальних процесів, таких як ревматоїдний артрит.

Висновки: Отримані дані підтверджують проліферативну активність у привушній залозі безпородних білих щурів з експериментально індукованим ревматоїдним артритом.

Ключові слова: привушна слинна залоза; безпородні білі щури; проліферативна активність; маркер K1-67; імуногістохімія.

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