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## **Editors office address:**

Osh International Medical University  
723500, 428 Alymbek Datka Str., Osh, Kyrgyz Republic  
E-mail: [journal@humanbiomed.com](mailto:journal@humanbiomed.com)  
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# **АДАМДЫН БИОЛОГИЯСЫ ЖАНА КЛИНИКАЛЫК МЕДИЦИНА ЖУРНАЛЫ**

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*This issue is dedicated to Rakhmanbek Toichuev, a scholar, surgeon, and innovator whose research and clinical achievements had significantly influenced the development of medicine and integrative approaches within the healthcare system of the Kyrgyz Republic. The material included a contemporary perspective by a young researcher on the scientific legacy of R. Toichuev, emphasising the scale of his contribution – from the training of generations of specialists and the development of new medical techniques to the formation of scientific schools and the implementation of research findings into clinical practice. The publication was intended to serve as a token of recognition of an outstanding specialist and as a source of inspiration for young researchers who continued the development of Kyrgyz scientific traditions.*

**Zhanibek Muratov**

Editor-in-Chief,

Journal of Human Biology and Clinical Medicine

## **The greatness of a person reflected in their deeds: The role of Rakhmanbek Toichuev in the integration of paediatric surgery, paediatrics, medical ecology, public health of the Kyrgyz Republic, and the development of fundamental science (on the occasion of the 80<sup>th</sup> anniversary of his birth)**

**Akmaral Argynbaeva\***

PhD in Biological Sciences, Associate Professor  
Osh State University

723500, 331 Alymbek Datka Str., Osh, Kyrgyz Republic  
<https://orcid.org/0000-0003-3835-2282>

**Kyialbek Sakibaev**

PhD in Medical Sciences, Associate Professor  
Osh International Medical University

723500, 428 Alymbek Datka Str., Osh, Kyrgyz Republic  
<https://orcid.org/0000-0001-7303-1252>

**Jildyz Toichueva**

Senior Lecturer

Osh State University

723500, 331 Alymbek Datka Str., Osh, Kyrgyz Republic  
<https://orcid.org/0000-0001-5544-5021>

Rakhmanbek Toichuev was born on 29 November 1945 in the village of Burgondu (now Nokén District) of the Jalal-Abad Region of the Kyrgyz Republic. In 1964, he completed Secondary School No. 2 named after Karl Marx in the Lenin District (now Nokén District) of the Jalal-Abad Region. In 1971, he graduated from the Kyrgyz State Medical Institute in Frunze with a degree in paediatrics. After graduation, R. Toichuev began his medical

practice in 1971 at the Osh Regional Children's Hospital, where he served as an intern doctor – the first in the Kyrgyz SSR to specialise in paediatric surgery. For twenty-three years (1972-1995), his professional activity remained closely connected with this institution, where he progressed from senior resident (1972-1976) to head of the paediatric surgery department (1976-1977 and 1980-1995). During this period, he also fulfilled the

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\*Corresponding author



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duties of chief freelance paediatric surgeon of the Osh Region, providing specialised surgical care to children and contributing to the establishment of the paediatric surgical service in the south of the Kyrgyz Republic.

For the performance of complex surgeries and the saving of children's lives in 1979-1980, a documentary film entitled *Pain* was made about R. Toichuev by the director Tynchtyk Razzakov and the cinematographer Evgenii Baryshnikov, which was considered rare in the Soviet period. In the years 1985-1989, he undertook postgraduate training at the Institute of Paediatrics of the Academy of Medical Sciences of the USSR in Moscow, specialising in paediatric surgery. On 8 January 1991, he defended his PhD dissertation "The biocenosis of the gastrointestinal tract of pregnant women and purulent-septic diseases of newborns and infants" in two specialties: Paediatric Surgery (14.00.35) and Paediatrics (14.00.09), under the supervision of the renowned paediatric surgeon Professor L.M. Roshal (later President of the Research Institute of Emergency Paediatric Surgery and Traumatology and Hero of Labour of Russia). The clinical practice of Rakhmanbek Toichuev was characterised not only by its scale but also by the introduction of original, highly effective surgical and paediatric techniques aimed at substantially improving treatment outcomes in children.

During the period of active surgical practice, more than 5,000 surgical procedures were performed by him. The scope of R. Toichuev's clinical work was exceptionally broad and encompassed virtually all areas of paediatric surgery, including neurotrauma and microsurgical interventions for combined injuries such as urethral rupture with immediate urethral reconstruction, as well as trauma and orthopaedics, urology, oncology, dentistry, and paediatric gynaecology, including the creation of a urinary bladder and an artificial vagina using local tissue. Numerous surgical techniques were developed and implemented by him, including those for congenital malformations. Among the most significant contributions were innovative approaches to the management of purulent-inflammatory and traumatic conditions.

During his internship in 1971-1972, R. Toichuev developed a method of prolonged thoracocentesis that allowed a drain to be maintained at a single point without suppuration of the thoracocentesis site for up to 45 days. In 1972, when managing staphylococcal pyopneumothorax, he introduced a "traction" drainage technique using running water and a compressor, which ensured effective evacuation of purulent-necrotic material. In cases of lung abscesses and pericarditis, a puncture method for placing a micro-irrigator was developed and applied by him, including through Larrey's point in purulent pericarditis. The use of these technologies made it possible to reduce mortality in purulent-destructive diseases of the lungs and pericardium to 1.08%. Particular importance was attributed to the introduction, in 1975, of an osteoperforation

method for acute haematogenous osteomyelitis in children, which reduced the average duration of treatment more than twofold and significantly decreased mortality rates. In April 1976, he introduced a bronchial blockade technique for bronchial fistulas, which markedly reduced the need for thoracotomy and substantially lowered mortality in this group of patients. In 1977, he was awarded the highest qualification category in paediatric surgery for his achievements.

On 24 November 1994, he was appointed as the organising director, and on 5 October 1995 he was elected Director of the Institute of Medical Problems (IMP) of the Southern Branch of the National Academy of Sciences of the Kyrgyz Republic (NAS KR). The institute had been established by Order No. 22/11 of 28 November 1994, based on the resolutions of the Presidium of the NAS KR dated 29 December 1993 (No. 3, "On the Establishment of New Institutes") and the decisions of the Bureau of the Southern Regional Branch of the NAS KR dated 23 September 1991 (No. 5/6) and 28 November (No. 6/1).

While serving as Director from 1995 until September 2017, the Institute of Medical Problems developed, under his leadership, a full production cycle for surgical suture materials made from the serosa of small and large cattle (catgut) and from silkworm cocoons (silk). The authors of this development were R. Toichuev, E. Tostokov, and N. Toichuev, all holding the degree of PhD in Medical Sciences. These technologies were introduced into industrial production, and he became the only scientist who succeeded in transferring a scientific development into full-scale manufacturing. According to R. Toichuev, "As a scientific institute, we had to develop the entire production cycle of catgut and silk from scratch". A patent and a full set of documents required for establishing production and further implementation of the innovative products, carried out on a tender basis, were obtained. In addition, under his supervision, a technology for producing bifidumbacterin and colibacterin from local strains was developed.

Since February 2018, R. Toichuev had served as a leading research fellow at the Institute of Mountain Physiology and Medicine in the Department of Medical Problems, and from April 2022 he was transferred to the position of leading research fellow and, on a voluntary basis, appointed Deputy Director of the Research Institute of Biomedical Problems. R. Toichuev had worked in the healthcare system for more than fifty-five years. During this time, he trained more than 170 specialists in paediatric surgery, who subsequently practised in the Federal Republic of Germany, Greece, Israel, and the countries of the Commonwealth of Independent States. Under his supervision, three individuals obtained the degree of PhD in Medical Sciences, and during his tenure as head of the Institute of Medical Problems, ten doctoral dissertations and more than fifty PhD dissertations in various fields were defended. He published scientific studies and their findings in six hundred

scholarly works, including more than four hundred articles abroad, among them in the United States of America, France, the Czech Republic, the Republic of Korea, Ireland, Canada, Spain, Switzerland, Singapore, Turkey, Romania, Belarus, Georgia, Moldova and other countries. He developed advanced integrative methods for research and student training, and authored one monograph and seven methodological guidelines.

R. Toichuev was the author of thirty inventions, three of which had been developed upon the request of the International Science and Technology Centre. He received one hundred and thirty authorship certificates and acknowledgements for more than one hundred and twenty rationalisation proposals that had been introduced into production and applied in practical medicine in the Kyrgyz Republic and the countries of the Commonwealth of Independent States. He developed more than seventy methods for the prevention and treatment of various diseases using therapeutic agents derived from local raw materials and biopreparations obtained from local strains. He also created methods for the treatment of typhoid fever and typhoid carrier states, as well as techniques for eliminating chlorinated organic pesticides from the gastrointestinal tract, which were applied in the treatment of children and their mothers.

In addition, R. Toichuev proposed to the scientific community the concept of a theoretical medico-biological science, formulated the laws of renewal and equilibrium of the relative relativity of the avatar and the informational creator of the energy "Zhanai", and further elaborated the principles of triplicity. He was included in a group of scientists under the World Health Organization, consisting of forty-three leading specialists worldwide who worked on issues of primary health care for patients with coronavirus infection. He also delivered scientific reports in more than forty countries on topics related to medicine, ecology, biology, biosafety, biosecurity, and economics. In 2020, through the General Assembly of the International Pollutants Elimination Network (IPEN) under the United Nations, R. Toichuev was included in a global working group of three thousand leading scientists, prominent politicians, and major business representatives who addressed issues of climate change, environmental pollution, cyber- and biosecurity, bio-protection, and the prevention of future biological threats.

R. Toichuev served as a member of the editorial board of the scientific journal *Paediatric Surgery* of the Russian Federation and actively participated in the work of leading professional communities and international organisations. He was a member of the Association of Paediatric Surgeons of Russia and the Commonwealth of Independent States, as well as a member of the international organisations ISES–ISEE, the General Assembly of IPEN, and the Association of Infectious Disease Specialists of the Kyrgyz Republic. He also acted

as a representative of the International HCH and Pesticides Association. In addition, he was part of the Executive Committee of the Association for Biosafety of Central Asia and the Caucasus and was a member of the Association for Bioethics of Eastern Europe, the Caucasus, and Central Asia.

As of 2025, R. Toichuev engaged in the training and education of young researchers, specialists, postgraduate students, applicants, and students, applying his own integrative methods developed at the intersection of chemistry, physics, biology, mathematics, geometry, informatics, the social sciences, and cognitive science. These methods contributed to the development of perceptiveness, logical thinking, and creative potential among learners. For his international contribution and outstanding achievements in medicine, as well as for his active expert work, R. Toichuev was awarded a number of honorary distinctions: in 2002, the honorary title of Distinguished Worker of Science of the National Academy of Sciences of the Kyrgyz Republic; in 2004, the honorary title of Distinguished Worker of Healthcare of the Kyrgyz Republic; in 2013, the academic title of Professor of the Russian Academy of Natural Sciences (Professor's Diploma of the Academy of Natural Sciences, Moscow); and in 2024, the badge "Excellence in Science".



Visit of the institute by a Canadian delegation (2009)



R. Toichuev together with the internationally renowned paediatric surgeons of the Russian Federation, L. Roshal – President of the Research Institute of Emergency Paediatric Surgery and Traumatology, Professor and Doctor of Medical Sciences, Academician – and V. Shafranov, Professor of the Department of Paediatric Surgery of the N. Pirogov Research Institute and Doctor of Medical Sciences

## **Determinants and prevalence of urinary tract infections among international medical students in Osh, Kyrgyz Republic**

**Veronika Tursunova\***

Teacher

Osh International Medical University  
723500, 428 Alymbek Datka Str., Osh, Kyrgyz Republic  
<https://orcid.org/0000-0002-3042-2664>

**Zhanibek Muratov**

Doctor of Medical Sciences, Professor  
Osh International Medical University  
723500, 428 Alymbek Datka Str., Osh, Kyrgyz Republic  
<https://orcid.org/0000-0003-1366-2567>

**Nazgul Karimova**

PhD in Medical Sciences, Associate Professor  
Osh State University  
723500, 331 Alymbek Datka Str., Osh, Kyrgyz Republic  
<https://orcid.org/0000-0002-4018-0873>

**Vidhya Gnanam**

Student  
Osh State University  
723500, 331 Alymbek Datka Str., Osh, Kyrgyz Republic  
<https://orcid.org/0009-0009-9786-5177>

**Mohammad Saiff**

Student  
Osh International Medical University  
723500, 428 Alymbek Datka Str., Osh, Kyrgyz Republic  
<https://orcid.org/0009-0007-4735-2854>

**Abstract.** The present study was aimed at identifying the factors and prevalence of urinary tract infections (UTIs) among Indian students studying at Osh State University and Osh International Medical University in the Kyrgyz Republic, as well as at assessing their patterns of seeking medical care. In March 2025, a cross-sectional study was conducted using an anonymous online questionnaire completed by 178 medical students with a mean age of 20.9 years, enrolled across five years of study at two medical universities. Data were collected using structured questionnaires that included demographic characteristics, the presence of symptoms, daily water intake, satisfaction with the hygiene of toilet facilities, habits of delayed urination and self-reported history of a UTI diagnosis. Approximately 20.79% of participants reported having previously been diagnosed with a UTI. Students who were dissatisfied with the hygienic conditions of toilet facilities significantly more often reported having an infection. The most common symptoms were burning sensation and pain during urination (33.15%), frequent urges to urinate (31.46%) and passing small amounts of urine (31.46%). Many students managed their symptoms on their own: 54.49% ignored them, and 38.20% resorted to self-medication. Only 2.81% sought medical care in hospitals of the Kyrgyz Republic. The findings showed that, despite the fact that most students were aware of UTI symptoms, their preventive and treatment measures remained unsatisfactory. Poor hygiene of sanitary facilities markedly increased the risk of infection, whereas self-medication and ignoring symptoms could have led to complications. The obtained results were considered potentially useful for the development of targeted prevention

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\*Corresponding author



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programmes, the improvement of sanitary and hygienic conditions and the enhancement of the effectiveness of measures for early detection and prevention of urinary tract infections in organised populations

**Keywords:** risk factors; sanitary hygiene; urinary retention; self-medication; medical students

## INTRODUCTION

Urinary tract infections (UTIs) were among the most common bacterial conditions, affecting people of all age groups and exerting a substantial impact on quality of life and the functioning of the urinary system. Their development was determined by a combination of microbiological, behavioural and environmental factors, and the prevalence of the condition remained high both in countries with well-developed healthcare systems and in developing regions. In the context of changing environmental conditions, lifestyle factors and differences in sanitary and hygienic conditions, the investigation of the causes of UTIs, approaches to their prevention and patterns of seeking medical care became particularly relevant, representing a key direction for improving the effectiveness of public health measures [1].

UTI was a common bacterial infection that could occur in any individual; however, women were more susceptible. The study by V.F. Johny *et al.* [2] demonstrated the prevalence of recurrent urinary tract infections and key risk factors among women of reproductive age working in a medical college in Kerala. The authors showed that the high recurrence rate was associated with behavioural and sanitary-hygienic characteristics, which underlined the need for preventive measures in organised groups. The findings of S.D. Advani *et al.* [3] indicated that 40-50% of women and about 5% of men experienced a UTI at least once in their lifetime. In the studies by S. Acharya *et al.* [4] and A. Patjas *et al.* [5], it was shown that women encountered UTIs more frequently, with *Escherichia coli* being the most common causative agent, and that international students were additionally at risk because of unfamiliar bacterial strains and unusual local sanitary and hygienic conditions. In the study by F. Tabasum *et al.* [6], it was demonstrated that around 66% of students who had lived abroad for less than two years were not aware of the basic symptoms and signs of UTIs. This gap in knowledge made students particularly vulnerable during the first years of residence in another country. Even as they adapted, they could continue to remain at risk.

In accordance with the findings of Z.A. Abdirasulova *et al.* [7], differences in sanitary and hygienic conditions, access to safe drinking water and behavioural habits were able substantially to influence the prevalence of UTIs, as evidenced by the higher frequency of recurrent UTIs in India compared with the Kyrgyz Republic. In the study by C. Birimoglu Okuyan *et al.* [8], it was reported that 15.7% of students regularly experienced UTIs, and about 30% had various voiding disorders; their impact on quality of life depended

substantially on the individual characteristics of students and the severity of urological symptoms. Studies [9-11] showed that 20-65% of female medical students had experienced UTI symptoms at least once. The higher frequency of UTIs in this group was explained by various risk factors, including clinical practice, long hours of study, specific toilet habits and the type of toilets used. Earlier, N. Kundu *et al.* [12] showed that 63% of students did not perceive UTI symptoms as a serious health problem. Such an attitude posed a serious danger, as many began to regard mild or recurrent symptoms as 'normal' and postponed seeking medical care. This increased the risk of the infection spreading from the lower to the upper urinary tract, which could lead to more severe chronic conditions. The situation was aggravated by the fact that many students in the Kyrgyz Republic preferred not to consult local doctors when UTI symptoms appeared, opting instead for self-medication or remote consultations. Such behaviour led to delays in correct diagnosis and hindered timely and effective treatment [13].

Against the background of the rapid increase in the number of international students choosing medical education in the Kyrgyz Republic, the state implemented special measures to support their adaptation and to ensure safe sanitary and hygienic learning conditions, which made the study of risk factors and the prevalence of UTIs in this group particularly relevant [14]. However, there were very few data specifically devoted to UTIs among international medical students in the Kyrgyz Republic. Thus, an important scientific and practical gap existed: the extent to which adaptation, living and hygiene conditions and behavioural habits of international students in the Kyrgyz Republic influenced the frequency and characteristics of UTIs had been insufficiently studied. The present study was intended to address this gap by assessing the factors and prevalence of UTIs among Indian students studying at medical universities in the Kyrgyz Republic and by identifying characteristic patterns of seeking medical care.

The aim of the study was to determine the prevalence of UTIs and the associated risk factors among international medical students, and to assess the sanitary and hygienic conditions of their living environment and their behavioural patterns when symptoms occurred.

## MATERIALS AND METHODS

**Study population.** A cross-sectional study was conducted using an anonymous Google Forms questionnaire, in which 178 international medical students from years 1-5 in the city of Osh, Kyrgyz Republic, participated

(140 students from the International Medical Faculty of Osh State University and 38 students from Osh International Medical University). The study was carried out during March 2025 using a voluntary sampling method open to all international students of medical universities in Osh. Information about participation was disseminated through official student groups in messengers and WhatsApp channels. Participation was entirely voluntary and involved no material or academic incentives. Inclusion criteria were: international medical student (years 1-5), age 17-30 years, ability to complete the questionnaire independently in English and provision of consent to participate. Exclusion criteria were: pregnancy, presence of chronic kidney disease, indwelling urethral catheter, current use of systemic antibiotics and diagnosed neurological conditions affecting urination.

The study complied with the principles of the Declaration of Helsinki [15]. Approval was obtained from the local ethics committee of Osh State University (No. 17 dated 03.02.2025). Additional clarifications were provided that a cross-sectional design had been selected to assess the prevalence of UTI symptoms and risk factors at a specific point in time, which corresponded to the research aim. Informed consent was obtained by asking participants to tick a dedicated field before starting the questionnaire. Anonymity and confidentiality of data were fully ensured. Data were stored in a password-protected file accessible only to the research team. The results were presented in aggregated form to guarantee confidentiality and privacy of all participants. A cross-sectional design was chosen because it allowed rapid collection of data from a large group of participants at a single time point.

**Data collection.** Data were collected using a detailed structured questionnaire. The questionnaire was developed on the basis of an analysis of publications in PubMed and included elements of validated instruments (ICIQ-SF, UTI-SIQ-8) adapted for students.

Before the main data collection, a pilot test of the questionnaire was carried out among 10 international students. Based on the pilot results, the wording of several questions was refined for greater clarity, and additional explanations were added to some items.

The instrument comprised several key sections:

1. Demographic and anthropometric data: age, sex, year of study, height (cm) and body weight (kg);

2. Exposure and risk factors: self-reported daily water intake (litres), satisfaction with the level of hygiene of toilet facilities and the habit of delaying urination for a long time;

3. Symptomatology: presence of complaints such as burning during urination, frequent urges, cloudy or foul-smelling urine, as well as participants' awareness that these signs indicated a UTI;

4. Behaviour in seeking medical care: actions taken when symptoms appeared (ignoring, self-medication, consulting a doctor in India or in the Kyrgyz Republic) and history of a diagnosed UTI.

The questionnaire included items on both self-reported symptoms and the presence of a UTI diagnosis confirmed by a physician, including indication of a urine test performed (general urinalysis and/or urine culture). In this way, subjective symptoms and objectively diagnosed episodes of infection were distinguished.

**Statistical analysis.** Descriptive, comparative and statistical analyses were performed using STATA SE 12. Descriptive statistics included calculation of means, standard deviations (SD) and absolute and relative frequencies for the characteristics of the study population. Categorical variables were compared using the  $\chi^2$  (chi-square) test, and numerical variables were compared using the t-test.

## RESULTS AND DISCUSSION

Table 1 presents the results of the initial analysis of the sample, reflecting the socio-demographic characteristics of the study participants.

**Table 1.** Socio-demographic characteristics of the study population

| Variable                                       | N (%)       | Mean $\pm$ SD   | p-value* |
|------------------------------------------------|-------------|-----------------|----------|
| Age (years)                                    |             | 20.9 $\pm$ 2.8  | 0.45     |
| 17–19                                          | 43 (24.16)  |                 |          |
| 20–24                                          | 108 (60.67) |                 |          |
| $\geq 25$                                      | 27 (15.17)  |                 |          |
| Sex                                            |             |                 | 0.0001   |
| Female                                         | 98 (55.06)  |                 |          |
| Male                                           | 80 (44.94)  |                 |          |
| Height (cm)                                    |             | 167.5 $\pm$ 9.5 | 0.08     |
| Weight (kg)                                    |             | 64.1 $\pm$ 11.2 | 0.05     |
| Year of study                                  |             |                 |          |
| 1 <sup>st</sup> –2 <sup>nd</sup> year (junior) | 71 (39.89)  |                 |          |
| 3 <sup>rd</sup> –5 <sup>th</sup> year (senior) | 107 (60.11) |                 |          |

**Note:** \* $p \leq 0.05$  – the association between variables was considered statistically significant

**Source:** developed by the authors

Data from Table 1 indicated that most of the 178 participants were young adults, with a mean age of 20.9 years, and approximately 60.67% were in the 20-24-year age group. More than half (55.06%) were women, which was important, as the risk of UTIs was considerably higher in females. The mean height of participants was

167.5 cm, and the mean body weight was 64.1 kg. Most students were in years 3-5 of their studies, which implied a longer stay in the local environment and, consequently, a higher likelihood of exposure to risk factors. Table 2 presents the association between selected risk factors and the diagnosis of UTI among the study participants.

**Table 2.** Analysis of behaviour and environmental factors

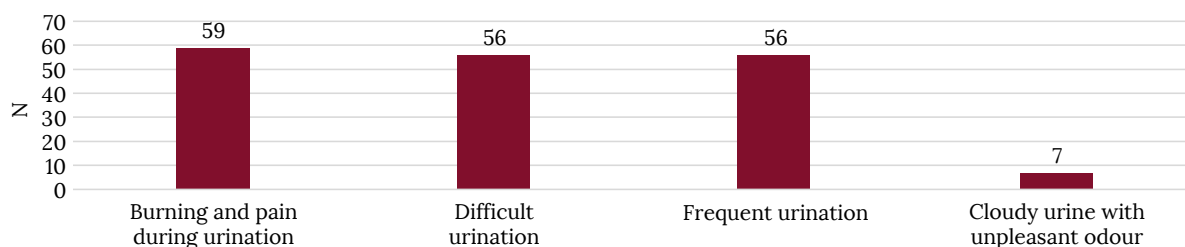
| Risk factor                                      | With UTI diagnosis N (%) | Without UTI diagnosis N (%) | Total | p-value* |
|--------------------------------------------------|--------------------------|-----------------------------|-------|----------|
| Satisfaction with toilet hygiene (not satisfied) | 16 (36.4)                | 28 (63.6)                   | 44    | 0.001    |
| Water intake (litre)                             | 6 (13.0)                 | 40 (87.0)                   | 46    | 0.29     |
| Habit of delaying urination (yes)                | 7 (5.1)                  | 132 (94.9)                  | 139   | 0.14     |

**Note:** \* $p \leq 0.05$  – the association between variables was considered statistically significant

**Source:** developed by the authors

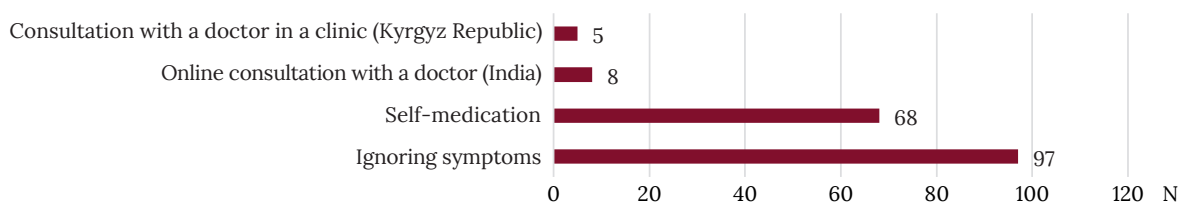
According to Table 2, among students who reported dissatisfaction with the hygienic condition of toilet facilities, 36.4% had been diagnosed with a UTI, whereas 63.6% had no diagnosis. This association was statistically significant ( $p = 0.001$ ), suggesting that dissatisfaction with sanitary conditions was an important risk factor for the development of UTIs. With regard to daily water consumption, 13.0% of students who reported drinking two litres of water or less per day had been diagnosed with a UTI, compared with 87.0% in whom infection had not been detected. This association was not statistically significant ( $p = 0.29$ ), indicating that low water intake was not a major determinant in this sample. As for the habit of delaying urination, only 5.1% of students who reported such a habit had a UTI diagnosis, whereas 94.9% did not. This relationship was likewise

not statistically significant ( $p = 0.14$ ), suggesting that delayed urination did not exert a substantial influence on the incidence of UTIs in this group. Among students who did not delay urination, the frequency of UTI diagnosis was slightly higher, which was possibly related to the fact that individuals with recurrent UTIs were compelled to void more frequently (Fig. 1). Among the participants, burning during urination was reported by 59 students (33.15%), and 56 (31.46%) indicated frequent urges to urinate. This was followed by passing urine in small amounts, which was also reported by 56 students (31.46%), whereas 7 (3.93%) noted cloudy or foul-smelling urine. These findings reflected the distribution of the most common urological symptoms among the surveyed students. Figure 2 presents data on how participants responded to health problems or the onset of symptoms.



**Figure 1.** Frequency of UTI symptoms

**Source:** developed by the authors



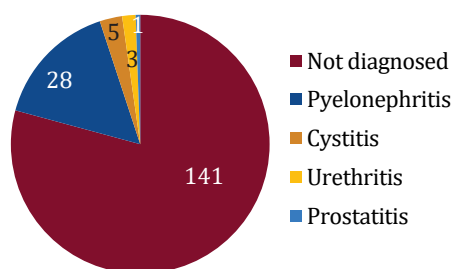
**Figure 2.** Prevalence of health-seeking behaviours in response to UTI symptoms

**Source:** developed by the authors

Ignoring symptoms – 97 (54.49%): more than half of respondents preferred not to pay attention to their symptoms, which indicated a rather high level of neglect in seeking medical care. Self-medication – 68

(38.20%): participants preferred to treat themselves without professional advice, which might have reflected such issues as the availability and cost of medical services or the level of trust in the healthcare system.

Online consultation (India) – 8 (4.49%): this proportion of participants resorted to online consultations, which suggested a growing recognition of telemedicine, as well as greater trust in healthcare professionals in their home country than in local doctors. Hospital visit (Kyrgyz Republic) – 5 (2.81%): this percentage of respondents preferred to attend hospital, which showed that a smaller proportion of students still favoured traditional face-to-face medical care. Data on the prevalence of conditions that had previously been diagnosed and treated were presented in Figure 3.



**Figure 3.** Prevalence of UTIs among participants

Source: developed by the authors

In Figure 3, pyelonephritis, recorded in 28 participants (15.73%), was the predominant diagnosis and occurred markedly more often than other conditions. The presence of urethritis in 3 students (1.69%), prostatitis in 1 (0.56%) and cystitis in 5 (2.81%) indicated a combination of infections of both the upper and lower urinary tract. At the same time, most cases remained undiagnosed and therefore unrecognised, with 141 participants (79.21%) falling into this category. Most of the study participants were young adults aged 20-24 years, with a predominance of women, which in itself increased the likelihood of developing a UTI. Studying abroad was considered an additional risk factor due to stress, environmental change and altered dietary habits, as has been supported by previous research [1].

According to the study by S.B. Alwindy [16], the overall incidence of UTIs was 63.9%, with approximately 68.2% of cases occurring in women, and the most vulnerable group being those aged 20-22 years. In the current study, UTIs were identified in only 20.79% of participants, which was substantially lower than the figures reported by S.B. Alwindy. In the work of M. Mititelu *et al.* [17], it was shown that unhealthy diet, excess body weight, sedentary lifestyle and a tendency towards self-medication significantly increased the frequency and recurrence of UTIs, whereas adherence to a healthy diet and adequate hydration was associated with rare or absent infections. These findings were consistent with the present study: among participants who reported adequate water intake and rational voiding habits, the frequency of diagnosed UTIs was lower, whereas dissatisfaction with sanitary conditions and ignoring symptoms were associated with an increased risk of infection.

In the study by S. Youssef *et al.* [18], 40.2% of female students had significant bacteriuria, with about 33% having previously experienced UTIs; the most frequent complaints in their medical history were urgency (31%) and flank pain (26.6%). The authors also suggested that the UTI rate might be higher in India; however, Indian students in Osh also experienced a high burden of disease, in line with the present findings: among international students in Osh there was a high frequency of UTI symptoms, including burning during urination (33.15%), frequent urges (31.46%) and passing small amounts of urine (31.46%), while a considerable proportion of participants ignored symptoms or resorted to self-medication. This confirmed that the main risk factors were behavioural and environmental rather than unique to this particular sample. Among university students in Nigeria, the prevalence of UTIs was relatively low (4.5%), which, according to J.D. Florence *et al.* [19], was attributed to a relatively high level of knowledge and awareness regarding UTI prevention, whereas among international students in Osh in the present study, the frequency of UTI symptoms was considerably higher.

In study [20] it was noted that the risk factors for UTIs included living conditions in student dormitories, non-compliance with sanitary and hygienic standards and behavioural characteristics of students. These data were consistent with the findings of the present study: dissatisfaction with the hygienic condition of toilet facilities was associated with a higher frequency of diagnosed UTIs, whereas the habit of delaying urination and low water intake did not have a statistically significant effect. The study by O. Olabimtan *et al.* [21] showed that among junior students the prevalence of UTIs reached 48%, with recurrent episodes occurring significantly more often in female students, against a background of low awareness (especially among male students) and very low rates of seeking medical care, which was in line with the findings of the current study. One of the common risks was lack of knowledge of appropriate hygiene practices. Ignoring symptoms and self-medication were also reported in the study by S. Muchukota *et al.* [22]. Many students sought advice on the Internet before visiting a doctor, which led to remote consultations or self-medication instead of obtaining an accurate diagnosis. As 66% of new students were unaware of UTI symptoms, there was an obvious need for structured educational programmes. These conclusions were consistent with the results of similar studies among students after the implementation of educational interventions.

Taking into account the identified risk factors, students' behavioural patterns and the findings of other studies, it appeared necessary to develop a set of practical measures aimed at reducing the prevalence of UTIs among international medical students. The implementation of such measures could substantially

improve sanitary and hygienic living conditions, raise the level of health literacy and reduce the frequency of self-medication, which, according to the present data, remained one of the most common patterns of behaviour when symptoms occurred. In this regard, it was considered appropriate to distinguish the following directions for preventive and organisational work:

- improvement of sanitary and hygienic living conditions – ensuring regular cleaning and disinfection of toilets and showers in student dormitories, as well as monitoring the availability of sufficient sanitary and hygiene supplies;

- increasing students' awareness – organisation of educational seminars on the signs, symptoms and risk factors of UTIs, as well as on appropriate voiding habits and personal hygiene;

- encouraging timely health-seeking – establishment of accessible in-person and online consultation services for international students and provision of information on the risks of self-medication and possible complications associated with delayed medical contact;

- preventive measures – promotion of adequate water intake and regular urination, and implementation of preventive medical check-up programmes for the early detection of UTIs;

- monitoring and further research – conducting periodic surveys and analyses of risk factors among international students, as well as investigating the influence of seasonality, diet and stress on UTI incidence.

Thus, the study findings showed that the prevalence of UTIs among international medical students remained a significant problem directly related to sanitary and hygienic living conditions and behavioural characteristics. The most frequent symptoms were burning during urination, increased urinary frequency and passing small amounts of urine; however, the rate of seeking medical care remained extremely low, which contributed to the widespread practice of self-medication. At the same time, low water intake and the habit of delaying urination did not demonstrate a statistically significant effect, whereas dissatisfaction with the hygiene of toilet facilities showed a clear association with the presence of a diagnosed UTI. The data obtained highlighted the need for a comprehensive approach that would combine improvement of living conditions, educational interventions and the development of accessible medical services for international students.

## CONCLUSIONS

In the present study among international students of medical universities in the city of Osh, UTIs were found to represent a noticeably common problem: 20.79% of participants reported a previously diagnosed infection. The most pronounced risk factor was dissatisfaction with the hygienic condition of toilet facilities, with 36.4% of students who expressed dissatisfaction with sanitary conditions having a diagnosed UTI ( $p = 0.001$ ). At the same time, such factors as the habit of delaying urination (5.1% had a diagnosis,  $p = 0.14$ ) and low water intake (13.0%,  $p = 0.29$ ) did not demonstrate a statistically significant effect on the prevalence of UTIs.

UTI symptomatology among students was manifested mainly as burning during urination (33.15%), increased urinary frequency (31.46%) and passing small amounts of urine (31.46%), indicating a high prevalence of typical urological complaints. Analysis of behavioural responses showed that more than half of respondents ignored their symptoms (54.49%), and 38.20% resorted to self-medication, which reflected insufficient awareness and low health-seeking activity. Seeking professional medical care remained extremely rare: only 2.81% of students visited hospitals in the Kyrgyz Republic when symptoms occurred.

The findings highlighted the need to develop targeted preventive programmes aimed at improving sanitary and hygienic conditions in dormitories, increasing students' awareness of UTI risk factors and early symptoms, and fostering a culture of timely consultation with healthcare professionals. Implementation of such measures could substantially reduce UTI morbidity among international students and improve their overall health status. Prospects for further research included expanding the sample by involving students from other regions and universities of the Kyrgyz Republic and conducting longitudinal studies to assess the dynamics of UTIs over time.

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## CONFLICT OF INTEREST

None.

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## Кыргыз Республикасынын Ош шаарындагы чет өлкөлүк медицина студенттеринин арасында заара чыгаруучу жолдордун инфекцияларынын факторлору жана таралышы

**Вероника Турсунова**

Окутуучу

Ош эл аралык медициналык университети  
723500, Алымбек Датка көч., 428, Ош ш., Кыргыз Республикасы  
<https://orcid.org/0000-0002-3042-2664>

**Жанибек Муратов**

Медицина илимдеринин доктору, профессор

723500, Алымбек Датка көч., 428, Ош ш., Кыргыз Республикасы  
<https://orcid.org/0000-0003-1366-2567>

**Назгул Каримова**

Медицина илимдеринин кандидаты, доцент

Ош мамлекеттик университети  
723500, Алымбек Датка көч., 331, Ош ш., Кыргыз Республикасы  
<https://orcid.org/0000-0002-4018-0873>

**Видхья Гнанам**

Студент

Ош мамлекеттик университети  
723500, Алымбек Датка көч., 331, Ош ш., Кыргыз Республикасы  
<https://orcid.org/0009-0009-9786-5177>

**Мохаммад Саиф**

Студент

Ош эл аралык медициналык университети  
723500, Алымбек Датка көч., 428, Ош ш., Кыргыз Республикасы  
<https://orcid.org/0009-0007-4735-2854>

**Аннотация.** Бул изилдөөнүн максаты – Кыргыз Республикасындагы Ош мамлекеттик университети жана Ош эл аралык медициналык университеттеринде окуган индиялык студенттер арасында заара чыгаруучу жолдордун инфекцияларынын (ЗЧЖИ) таралышын жана аны аныктоочу факторлорду изилдөө, ошондой эле алардын медициналык жардамга кайрылуу жүрүм-турумун баалоо. 2025-жылдын март айында 178 студент (орточо жашы 20,9 жаш) катышкан кесилиштик (cross-sectional) изилдөө жүргүзүлдү. Сурамжылоо эки медициналык университеттин беш окуу жылындагы студенттер арасында онлайн түрүндө анонимдүү анкета аркылуу жүргүзүлдү. Анкетада демографиялык маалыматтар, белгилер (симптомдор), күнүмдүк суу ичүү көлөмү, даараткананын тазалыгына канааттануу деңгээли, заараны кармоо адаты жана өз алдынча билдирилген ЗЧЖИ диагнозу тууралуу суроолор камтылган. Катышуучулардын 20,79 % мурда заара чыгаруучу жолдордун инфекциясына чалдыкканын билдиришкен. Даараткананын тазалыгына нааразы болгон студенттерде инфекцияга чалдыгуу ыктымалдыгы кыйла жогору экени аныкталды. Эң кеңири таралган белгилер – заара кылуу учурунда ачышуусу жана ооруусу (33,15 %), заара кылууга тез-тез муктаж болуу (31,46 %) жана зааранын аз өлчөмдө бөлүнүшү (31,46 %) болду. Көптөгөн студенттер белгилер менен өз алдынча күрөшүшкөн: 54,49 % аларды этибарга алышкан эмес, ал эми 38,20 % өз алдынча дарыланууга аракет кылышкан. Болгону 2,81 % гана Кыргызстандагы ооруканаларга кайрылышкан. Изилдөөнүн жыйынтыгында көпчүлүк студенттер ЗЧЖИ белгилерин билгени менен, аларды алдын алуу жана туура дарылануу иш-чаралары жетишсиз экени аныкталды. Даараткананын начар гигиеналык абалы инфекциянын пайда болуу коркунучун жогорулатат, ал эми өз алдынча дарылануу жана белгилерди көз жаздымда калтыруу терс натыйжаларга алып келиши мүмкүн. Гигиеналык шарттарды жакшыртуу, жетиштүү суу ичүү жана өз убагында дарыгерге кайрылуу боюнча маалымдуулукту арттыруу, ошондой эле өз алдынча дарыланууну азайтуу студенттер арасында инфекциялардын деңгээлин олуттуу түрдө төмөндөтө алат. Алынган жыйынтыктар максаттуу алдын алуу программаларын иштеп чыгуу, санитардык-гигиеналык шарттарды жакшыртуу жана студенттер жамаатынын ичинде заара чыгаруу жолдорунун инфекцияларын эрте аныктоо жана алдын алуу боюнча чаралардын натыйжалуулугун жогорулатуу үчүн колдонулушу мүмкүн.

**Негизги сөздөр:** коркунуч факторлору; санитардык жайлардын гигиенасы; заараны кармап туруу; өз алдынча дарылануу; медициналык окуу жайлардын студенттери

## **Факторы и распространённость инфекций мочевых путей среди иностранных студентов-медиков в городе Ош, Кыргызская Республика**

**Вероника Турсунова**

Преподаватель

Ошский международный медицинский университет  
723500, ул. Алымбек Датка, 428, г. Ош, Кыргызская Республика  
<https://orcid.org/0000-0002-3042-2664>

**Жанибек Муратов**

Доктор медицинских наук, профессор

Ошский международный медицинский университет  
723500, ул. Алымбек Датка, 428, г. Ош, Кыргызская Республика  
<https://orcid.org/0000-0003-1366-2567>

**Назгул Каримова**

Кандидат медицинских наук, доцент

Ошский государственный университет  
723500, ул. Алымбек Датка, 331, г. Ош, Кыргызская Республика  
<https://orcid.org/0000-0002-4018-0873>

**Видхья Гнанам**

Студент

Ошский государственный университет  
723500, ул. Алымбек Датка, 331, г. Ош, Кыргызская Республика  
<https://orcid.org/0009-0009-9786-5177>

**Мохаммад Саиф**

Студент

Ошский международный медицинский университет  
723500, ул. Алымбек Датка, 428, г. Ош, Кыргызская Республика  
<https://orcid.org/0009-0007-4735-2854>

**Аннотация.** Данное исследование было направлено на выявление факторов и распространённости инфекций мочевых путей (ИМП) среди индийских студентов, обучающихся в Ошском государственном университете и Ошском международном медицинском университете в Кыргызской Республике, а также на оценку их особенностей обращения за медицинской помощью. В марте 2025 года было проведено поперечное (кросс-секционное) исследование с использованием анонимной онлайн-анкеты, которую заполнили 178 студентов-медиков в возрасте в среднем 20,9 лет, обучающихся на пяти курсах двух медицинских вузов. Данные собирались с помощью структурированных опросников, включающих демографические сведения, наличие симптомов, объём потребляемой воды в день, удовлетворённость гигиеной туалетных помещений, привычки задержки мочеиспускания и самооценку диагноза ИМП. Около 20,79 % участников сообщили, что им ранее был поставлен диагноз ИМП. Студенты, неудовлетворённые гигиеническим состоянием туалетов, значительно чаще сообщали о наличии инфекции. Наиболее распространёнными симптомами были жжение и боль при мочеиспускании (33,15 %), частые позывы к мочеиспусканию (31,46 %) и выделение небольшого количества мочи (31,46 %). Многие студенты справлялись с симптомами самостоятельно: 54,49 % игнорировали их, а 38,20 % прибегали к самолечению. Лишь 2,81 % обращались за медицинской помощью в больницы Кыргызской Республики. Результаты исследования показали, что, несмотря на осведомлённость большинства студентов о симптомах ИМП, их профилактические и лечебные меры остаются неудовлетворительными. Низкий уровень гигиены санитарных помещений значительно повышает риск инфицирования, а самолечение и игнорирование симптомов могут привести к осложнениям. Полученные результаты могут быть использованы для разработки целевых профилактических программ, совершенствования санитарно-гигиенических условий и повышения эффективности мероприятий по раннему выявлению и профилактике инфекций мочевых путей в организованных коллективах

**Ключевые слова:** факторы риска; гигиена санитарных помещений; задержка мочеиспускания; самолечение; студенты медицинских вузов

## **Association of desmin expression with tumour size in colorectal carcinomas**

**Andrey Filin\***

PhD in Medical Sciences, Associate Professor  
Federal State Budgetary Educational Institution of Higher Education "Voronezh State Medical University named  
after N.N. Burdenko"  
394036, 10 Studencheskaya Str., Voronezh, Russian Federation  
<https://orcid.org/0000-0003-1670-3694>

**Elena Pripitnevich**

Medical Resident  
Federal State Budgetary Educational Institution of Higher Education "Voronezh State Medical University named  
after N.N. Burdenko"  
394036, 10 Studencheskaya Str., Voronezh, Russian Federation  
<https://orcid.org/0000-0002-7763-9932>

**Viktoria Shishkina**

PhD in Medical Sciences, Associate Professor  
Federal State Budgetary Educational Institution of Higher Education "Voronezh State Medical University named  
after N.N. Burdenko"  
394036, 10 Studencheskaya Str., Voronezh, Russian Federation  
<https://orcid.org/0000-0001-9185-4578>

**Abstract.** Desmin, which was expressed by muscle cells, myofibroblasts and certain pathological cells, remained poorly investigated in the context of the morphogenesis of colorectal cancer – the third most common oncological disease in the world. The aim of the study was to examine the characteristics of desmin expression in colorectal carcinomas and to compare the intensity of this expression with tumour nodule size. During the study, 24 samples of malignant large-intestinal tumours were subjected to histological examination and were stained using the immunohistochemical method with mouse monoclonal antibodies against desmin. Desmin expression was identified in all analysed samples. Desmin was expressed both within tumour structures and in the surrounding stromal tissue of the organ. Quantitative heterogeneity of desmin expression was revealed, ranging from extensive to scarce across different samples. When the volumetric density of desmin was compared with tumour nodule area, it was found that the overall amount of expressed desmin was statistically significantly higher in samples with large tumour nodules. In addition, a tendency was observed towards an increase in the amount of desmin localised within tumour structures with increasing tumour area. The study also included a comparison of the presence of desmin in the tumour with the size of the tumour conglomerate. It was established that desmin was present within the tumour considerably more often in large tumour nodules than in samples with small tumours. The findings obtained in the course of the study indicated a potential possibility of using desmin to assess the risk of tumour progression, as well as its potential use as a prognostic marker

**Keywords:** tumour volume; intermediate filament protein; stroma; immunohistochemical method; tumour nodule

### **INTRODUCTION**

Colorectal cancer remained one of the most widespread and socially significant forms of malignant neoplasms, occupying leading positions in both morbidity and mortality. Despite substantial progress in diagnosis

and treatment, the biological mechanisms determining tumour morphogenesis and progression continued to be actively investigated. Particular interest was directed towards molecular and morphological markers ca-

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\*Corresponding author



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pable of reflecting the features of tumour growth and its invasive potential. One such marker was desmin, an intermediate filament protein that was expressed in muscle cells and in a number of tumour structures, for which new patterns of expression continued to be identified in epithelial neoplasms, including colorectal cancer.

According to F. Bray *et al.* [1], colorectal cancer was the third most common malignant neoplasm, surpassed only by lung cancer and breast cancer, with 1,926,118 new cases registered in 2022, accounting for 9.6% of all forms. In addition, mortality from colorectal cancer was among the highest: in 2022 this disease caused the deaths of 903,859 individuals, representing 9.3% of all cancer-related deaths, which placed it in second position among all malignant neoplasms. Colorectal cancer occurred with nearly equal frequency in men and women. The mean age at diagnosis was 68 years for men and 72 years for women.

Colorectal cancer was most frequently diagnosed at an age above 50 years. Opinions regarding trends towards increasing or decreasing incidence of large-intestinal cancer remained somewhat contradictory. According to E. Morgan *et al.* [2], by 2040 the number of newly diagnosed cases of colorectal cancer would reach 3.2 million, and the number of deaths would rise to 1.6 million. At the same time, E.M. Stoffel & C.C. Murphy [3] reported an overall trend towards a decline in the incidence of large-intestinal cancer in the general population, while emphasising another issue: colorectal cancer had become more common among younger individuals. According to their data, the incidence of colorectal cancer in the general population had decreased from 1990 to the present, yet the number of registered cases in young patients had almost doubled, a trend particularly evident in the 40-49-year age group. This trend was further confirmed by the study of L.-F. Hu *et al.* [4], which reported an annual increase of 2% in new colorectal cancer cases in patients younger than 50 years. The same study highlighted specific clinical features in this age group, such as a predominance of poorly differentiated tumours, left-sided localisation of the process and more frequent diagnosis at advanced stages. The issue of rising incidence of colorectal cancer was also addressed in the study of S.G. Patel *et al.* [5], which noted an annual increase in large-intestinal cancer among younger patients and drew attention to the potential causes underlying this trend. The annual rise in colorectal cancer incidence among young individuals indicated the relevance of this problem and the necessity of its detailed investigation.

In the investigation of colorectal cancer, considerable attention was directed towards various clinical and morphological indicators, such as tumour nodule size. In malignant neoplasms of different localisations, tumour nodule size was most often regarded

as a poor prognostic factor. Tumour size frequently correlated with the character and rate of its growth. Large tumours generally demonstrated more aggressive behaviour and a greater potential for invasion and metastasis. Opinions regarding the prognostic role of tumour nodule size in colorectal cancer remained contradictory. According to the study by Y. Meng *et al.* [6], no statistically significant correlation was identified between tumour nodule size and patient survival. For small tumours (less than 3.5 cm) and large tumours (greater than 5 cm), patient prognosis was equally poor, whereas tumours of intermediate size (3.5-5 cm) were associated with better survival outcomes. However, these findings were contradicted by the study of S. Saha *et al.* [7], which demonstrated a correlation between tumour size and patient survival in colorectal cancer. When tumour nodules exceeded 6 cm, patient survival was significantly worse than in cases with smaller tumours.

Desmin was an intermediate filament protein localised in sarcomeres near the Z-line. Since its discovery, various functions had been attributed to desmin, ranging from the regulation of myofibrillogenesis, the establishment of mitochondrial positioning within the cell and participation in their functioning, to determining the resistance of muscle cells to stretching [8]. Desmin attached the Z-disc to the cytoplasmic portion of the muscle-cell membrane, which ensured the preservation of structural and mechanical integrity of the muscle cell during contraction. Desmin was expressed both directly by muscle cells and by tumour cells that had acquired muscle-cell properties [9].

Desmin expression was most frequently examined in tumours arising from muscle tissue; however, in recent years the number of studies addressing the role of desmin in the morphogenesis of tumours of epithelial origin had increased. These were most commonly tumours of the gastrointestinal tract. Although the number of studies on this topic had grown annually, the role of desmin in the morphogenesis of colorectal cancer remained far from fully understood. Most often, desmin expression in colorectal carcinomas had been compared with such clinical and morphological indicators as tumour differentiation grade, disease stage and depth of invasion. In the study by G. Arentz *et al.* [10], desmin was found to be expressed more extensively at advanced stages of the disease than at the first stage. In the study of M. Yoshida *et al.* [11], the authors concluded that desmin was effective for assessing the degree of tumour invasion in colorectal cancer. All the above indicated the relevance of the topic and the necessity of investigating the role of desmin in the morphogenesis of colorectal cancer.

The aim of the study was to investigate the morphological patterns of desmin expression in colorectal cancer and to relate these patterns to a significant morphological indicator, namely tumour nodule size.

## MATERIALS AND METHODS

A histological examination was carried out on material from 24 patients with diagnosed colorectal cancer. Material selection was performed at the Voronezh Regional Pathoanatomical Bureau (Russia, Voronezh). Further processing and analysis of the material were conducted at the Research Institute of Experimental Biology and Medicine (Russia, Voronezh), as well as at the Departments of Pathological Anatomy and Histology of N.N. Burdenko Voronezh State Medical University (Russia, Voronezh). The study was performed in accordance with the ethical standards of the Declaration of Helsinki [12]. The study protocol was approved by the local ethics committee of N.N. Burdenko Voronezh State Medical University (Protocol No. 6 of 14.11.2022). All participants were provided with written information about the aims and procedures of the study, and voluntary informed consent was obtained from each participant prior to inclusion. Material collection was conducted during 2023-2025. The analysed samples were represented by segments of the large intestine removed during surgical interventions. For this study, only tumours localised in the sigmoid colon were selected. Exclusively tumours not exposed to neoadjuvant treatment were used, in order to exclude the potential influence of treatment-induced pathomorphosis on the results. According to histological type, all samples belonged to adenocarcinomas of various differentiation grades: 12 well-differentiated (Grade 1), 9 moderately differentiated (Grade 2) and 3 poorly differentiated (Grade 3) adenocarcinomas. To assess tumour size, its area on the gross specimen was measured. Each sample was photographed in a single plane together with a reference scale ruler. The tumour nodule area in square centimetres (cm<sup>2</sup>) was calculated by a planimetric method using Adobe Photoshop 2024, based on the known scale. To detect desmin, the immunohistochemical method was applied to paraffin sections using monoclonal antibodies against desmin. Histological material was fixed in 10% neutral buffered formalin immediately after sampling. Fixation was performed at room temperature for 3 days, after which standard tissue processing with paraffin embedding was carried out [13]. For histological examination of desmin (the cytoskeletal protein) expression, thin 2-µm paraffin sections were prepared and subjected to standard processing. Immunohistochemical detection was performed manually according to a standard protocol [14]. Samples of skeletal muscle tissue served as the positive control, whereas lymph-node sections served as the negative control. According to the protocol, primary monoclonal rabbit antibodies Anti-Desmin antibody [Y66] (catalogue number #ab32362, dilution 1:2000, manufacturer: Abcam, United Kingdom) were applied and incubated for 12 hours, after which secondary Goat anti-rabbit IgG H&L (HRP), conjugated with horseradish peroxidase (catalogue number #ab205718, dilution 1:2000,

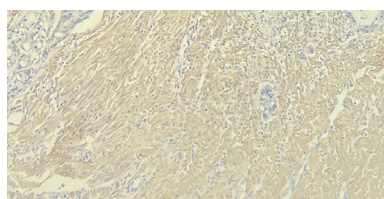
manufacturer: Abcam, United Kingdom), were applied. Nuclear contrast was achieved by counterstaining with Mayer's haematoxylin. Desmin expression intensity and its predominant localisation were assessed: in the stroma or in close proximity to tumour structures (hereafter referred to as in the tumour or in tumour structures). To quantify desmin expression, its volumetric density (Ss) was calculated in each sample by determining the ratio of the area occupied by desmin in a given region to the total section area. In addition, the mere presence or absence of desmin expression directly within tumour structures was evaluated. For each sample, ten random fields of view were selected. Images were captured using a Carl Zeiss Primo Star microscope (Germany), 40×/0.45 objective. Volumetric density was calculated using Adobe Photoshop 2024. Counting was performed by two independent observers. For statistical analysis of the obtained results, Microsoft Excel 2024 was used. The normality of distribution of quantitative data was assessed by graphical analysis (Q-Q plots) and the Shapiro-Wilk test. In all cases, the significance threshold for deviation from normality was set at  $p < 0.05$ . Non-parametric statistical methods were applied for data analysis. Differences between two independent groups for quantitative variables were evaluated using the Mann-Whitney U-test. Pearson's  $\chi^2$  test was used to assess associations between qualitative variables.

## RESULTS

A total of 24 samples of malignant large-intestinal neoplasms were examined. Desmin expression was identified in all analysed samples. The localisation of desmin varied: in 9 samples, desmin expression was observed exclusively in the surrounding stroma of the organ, and no expression was detected within the tumour itself. In 8 samples, by contrast, desmin was present exclusively in tumour structures, with no expression observed at the tumour periphery. In 7 samples, desmin was present both within the tumour and in the stroma. It should be noted that the amount of desmin also varied: in some cases, desmin occupied a large proportion of the section area and was well distinguishable, whereas in others it was barely visible. In samples where desmin was present exclusively in the stroma, its expression was almost always extensive (Fig. 1). However, samples with scant stromal desmin expression were also present, although they constituted a minority. In samples with desmin expression only in tumour structures, its amount varied considerably: in some cases, expression was extensive and occupied a significant part of the field of view, whereas in others desmin appeared as isolated deposits and was barely noticeable (Fig. 2). In addition, a pattern of stromal desmin expression was often observed in which the stroma appeared to "encircle" the tumour structures. It should be noted that desmin expression in areas of mucosa unaffected by tumour structures, which were present in the analysed samples, had its

own characteristics: desmin was located exclusively within the muscularis mucosae. Its amount was extremely small and appeared as a thin band. No desmin

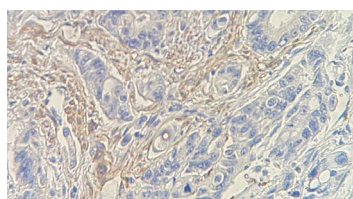
expression was observed in the crypt regions of normal mucosa. The described pattern was identical in all samples that contained areas of normal mucosa (Fig. 3).



**Figure 1.** Extensive desmin expression in the tumour stroma

**Note:** immunohistochemical staining using monoclonal antibodies against desmin; magnification  $\times 100$

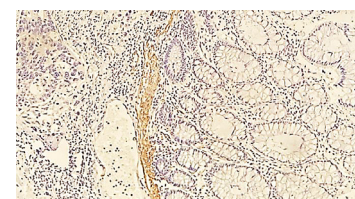
**Source:** authors' image



**Figure 2.** Weak desmin expression in tumour structures

**Note:** immunohistochemical staining using monoclonal antibodies against desmin; magnification  $\times 400$

**Source:** authors' image



**Figure 3.** Desmin expression in the muscularis mucosae unaffected by the tumour

**Note:** Immunohistochemical staining using monoclonal antibodies against desmin; magnification  $\times 100$

**Source:** authors' image

The relationship between desmin expression and tumour nodule size was then examined. The area of the tumour nodules ranged from 3.8 to 70.0 square centimetres. The mean nodule area in the sample was 30.4 square centimetres. For further analysis, the material was divided into four groups: the first group included samples with a tumour nodule area of less than 15 square centimetres; the second group included cases with areas ranging from 15 to 30 square centimetres; the third group comprised tumour nodules with areas from 30 to 45 square centimetres; and the fourth group included material with tumour nodule areas of 45 square centimetres or more. Each group consisted of 7, 6, 6 and 5 samples, respectively. For quantitative assessment of desmin expression, the mean volumetric density of desmin was calculated by determining the ratio between the area occupied by desmin in a given region and the total section area. Volumetric density was calculated both for all desmin present in the sample, localised in the stroma and in tumour structures (hereafter referred to as total volumetric density), and separately for desmin located exclusively within tumour structures (hereafter referred to as volumetric density of desmin in tumour structures).

At the first stage, the relationship between desmin expressed directly within tumour structures and tumour nodule area (S) was examined. The volumetric

density of desmin in tumour structures in the group with a nodule area of less than 15 square centimetres was 1.29%; in the group with an area of 15-30 square centimetres it was 3.04%; in the group with an area of 30-45 square centimetres it was 4.13%; and in the group with an area greater than 45 square centimetres it was 5.23%. These data indicated a tendency towards an increase in the amount of desmin within the tumour itself as tumour nodule size increased; however, the findings did not reach statistical significance ( $p > 0.05$ , Mann-Whitney test).

At the second stage, the relationship between the mean total amount of desmin (both in the tumour itself and in the stroma) and tumour nodule area was investigated. The total volumetric density of desmin in the group with a tumour nodule area of less than 15 square centimetres was 4.38%; in the group with an area of 15-30 square centimetres it was 8.08%; in the group with an area of 30-45 square centimetres it was 11.92%; and in the group with a tumour nodule area greater than 45 square centimetres it was 13.51%. These values indicated a tendency towards an increase in the total desmin content in the sample as tumour nodule size increased. Statistical analysis of the obtained results demonstrated a high level of significance ( $p < 0.05$ , Mann-Whitney test). For greater clarity, the above data are presented in Table 1.

**Table 1.** Comparison of desmin volumetric density with tumour nodule area

| Tumour nodule area (S)                               | $S < 15 \text{ cm}^2$ | $15 \leq S < 30 \text{ cm}^2$ | $30 \leq S < 45 \text{ cm}^2$ | $S \geq 45 \text{ cm}^2$ |
|------------------------------------------------------|-----------------------|-------------------------------|-------------------------------|--------------------------|
| Total volumetric density of desmin, %                | 4.38                  | 8.08                          | 11.92                         | 13.51                    |
| Volumetric density of desmin in tumour structures, % | 1.29                  | 3.04                          | 4.13                          | 5.23                     |

**Source:** calculated by the authors

In addition, the number of cases in which desmin was present within tumour structures and cases in which no tumour desmin expression was observed was compared with tumour nodule area. In the group with a tumour nodule area of less than 15 square centimetres,

there were 2 cases with desmin present in the tumour and 5 cases without such presence. In the group with a tumour nodule area greater than 45 square centimetres, there were 4 cases with desmin present in the tumour and 1 case without it. These data indicated a

tendency towards an increase in the number of samples in which desmin was present within the tumour as tumour nodule area increased; however, statistical analysis showed that the findings were not significant ( $p = 0.079$ , chi-squared test) (Table 2). This outcome

might have been due to the small number of samples in the groups, particularly in the second group. It was therefore necessary to repeat the described calculations with larger sample sizes in all groups to obtain a more complete and reliable picture.

**Table 2.** Statistical analysis of the comparison between the presence of desmin expression within tumour structures and tumour nodule area

| Four-field table analysis                                | $S < 15 \text{ cm}^2$ | $S \geq 45 \text{ cm}^2$  |
|----------------------------------------------------------|-----------------------|---------------------------|
| Number of cases with desmin present in tumour structures | 2                     | 4                         |
| Number of cases with desmin absent in tumour structures  | 5                     | 1                         |
| Chi-squared test                                         | value: 3.086          | significance level: 0.079 |

**Source:** developed by the authors

The data indicating an increase in the total amount of desmin with increasing tumour nodule size were statistically significant. At the same time, there was a tendency towards an increase both in the amount of desmin located directly within tumour structures and in the number of cases in which desmin was present in the tumour as tumour nodule size increased; however, these findings did not reach statistical significance. It should be taken into account that the calculations were performed on relatively small sample groups; therefore, to clarify the results, it will be necessary in future to increase the number of samples in all groups examined and to repeat the described calculations.

## DISCUSSION

The increase in the intensity of desmin expression in colorectal carcinoma samples with increasing tumour nodule size could theoretically indicate a greater potential of such tumours for further progression, invasion and metastasis. At present, the scientific literature contains a limited number of studies specifically examining the role of desmin expression in the pathogenesis and progression of colorectal cancer. In the review by G. Gomes *et al.* [15], an analysis of publication activity related to the term “desmin” was carried out. The authors noted that over recent decades the number of studies devoted to investigating its functions and its role in the pathogenesis of different diseases remained limited. It was also emphasised that a substantial proportion of publications on this topic belonged to a narrow group of authors, indicating insufficient involvement of the wider research community and highlighting the relevance of expanding investigations in this field. The overwhelming majority of existing publications concentrated on more general aspects, such as correlations between desmin levels and the invasive and metastatic potential of tumours of various localisations, as well as on its value as an immunohistochemical marker for identifying invasion into different structures. In the study by Y. Ma *et al.* [16], a systematic comparative analysis of desmin expression patterns at consecutive stages of colorectal oncogenesis was conducted: from normal colonic mucosa through the adenoma stage

to primary adenocarcinoma and, finally, to its hepatic metastases. The results demonstrated a clear gradient: the level of expression of this marker consistently increased and reached its maximum in liver metastases. This upward trend correlated with clinical outcome: high desmin expression was statistically significantly associated with reduced overall patient survival. All these findings indirectly suggested a high progression potential of tumours with pronounced desmin expression, which could correlate with the results obtained in the present study. In the research by J. Shin *et al.* [17], the diagnostic and prognostic value of desmin as a marker of venous invasion was assessed. Through the use of dual immunohistochemical staining (CD31/Desmin), the authors demonstrated that morphologically verified venous invasion served as an independent factor associated with a statistically significant reduction in survival among patients with pancreaticobiliary malignancies. The problem of objectively verifying early invasion in colorectal cancer which directly influences therapeutic decision-making was further developed in the study by M. Yoshida *et al.* [11]. That work systematically examined and confirmed the clinico-morphological value of desmin as an immunohistochemical marker for assessing the condition of the muscularis mucosae. The use of this method ensured reproducible differentiation between pseudoinvasion and true invasion and allowed accurate quantitative measurement of carcinoma penetration into the submucosa, which was of primary importance for correct staging and prognostication. Thus, an analysis of contemporary studies revealed the formation of a stable paradigm in which the assessment of desmin expression was regarded as a promising method for predicting tumour progression. The empirical data obtained in the present study were consistent with this emerging trend, mutually complementing and strengthening the evidence base for the clinical application of this marker. The work of Q. Yan *et al.* [18] highlighted the importance of distinguishing macroscopic tumour size as a separate, significant prognostic category. Their analysis convincingly demonstrated that a critical diameter of 5 cm served as a clear marker of increased biological

aggressiveness, directly correlating with poorer prognosis. This observation allowed tumour diameter to be viewed not merely as a descriptive characteristic but as an independent predictor deserving consideration in risk stratification. Within the framework of analysing factors determining tumour-cell dissemination, H. Feng *et al.* [19] empirically confirmed a significant positive correlation between the macroscopic size of the primary lesion and its metastatic capacity. This finding allowed tumour size to be considered not only as a staging parameter but also as an indirect indicator of its invasive and disseminative potential. Of particular interest were the results of the study by T. Kato *et al.* [20], which examined the relationship between characteristics of the primary tumour and the risk of developing metachronous colorectal cancer. The authors established that primary tumour size served as a strong independent predictor of this risk. Notably, the prognostic sensitivity of this parameter for metachronous cancer was more than twice its significance in the context of synchronous tumours, underscoring the fundamentally different prognostic roles of tumour size in these two clinical scenarios. In the work of W. Dai *et al.* [21], the relationship between the macroscopic characteristic of tumour size and long-term oncological outcomes in colorectal cancer was systematically investigated. The data convincingly showed that the volume of the primary lesion constituted a significant factor integrating biological properties such as duration of growth and invasive potential, which ultimately manifested in reduced survival among patients with larger neoplasms. Based on this, close examination of tumour progression characteristics particularly the analysis of expression patterns of specific proteins was of great importance. The data presented in the present study indicated the presence of a direct relationship between the amount of expressed desmin and tumour size, suggesting its potential prognostic significance. However, further research is required to draw definitive conclusions regarding the clinical value of desmin as a marker, including detailed comparison of the presence and intensity of its expression with long-term survival in patient cohorts.

Based on the results of the present study, it could be concluded that desmin was a promising biomarker involved in key aspects of colorectal cancer progression. The established positive correlation between its expression level and the size of the primary tumour was consistent with the emerging scientific paradigm in which desmin was associated with invasive growth, metastasis and an unfavourable prognosis. However, this field remained insufficiently developed, as evidenced by the scarcity of large targeted studies. This defined the main directions for further research. A promising avenue involved conducting large-scale prospective studies aimed at formalising the clinico-morphological significance of desmin. The key objectives would include:

- 1) standardising methods for quantitative assessment of desmin expression (immunohistochemical scoring, digital image analysis);
- 2) determining relevant diagnostic threshold values enabling risk stratification;
- 3) validating the independent prognostic value of desmin in multivariate models incorporating traditional parameters (stage, size, grade). The implementation of these measures could make it possible to move from a fundamental understanding of the role of desmin to its practical use as a diagnostic and prognostic marker.

## CONCLUSIONS

Desmin expression was identified in all 24 analysed samples. Desmin was expressed both in the stroma distant from tumour structures and within the tumour itself. In the stroma, desmin was more frequently expressed extensively and appeared as layers or thick bands. In the vicinity of the tumour, the amount of desmin varied: it was visualised as isolated deposits, thin bands, or occupying a substantial portion of the section. In areas of mucosa unaffected by tumour structures, desmin in all examined cases was located exclusively within the muscularis mucosae, where it appeared as a thin band. The conducted study made it possible to characterise in detail the patterns of desmin expression in colorectal cancer tissue and to establish their relationship with primary tumour size. A key finding of the work was the identification of a statistically significant ( $p < 0.05$ ) positive correlation between total desmin volumetric density and tumour nodule area. The total volumetric density of desmin in the group with a tumour nodule area of less than 15 square centimetres was 4.38%; in the group with an area of 15-30 square centimetres it was 8.08%; in the group with an area of 30-45 square centimetres it was 11.92%; and in the group with an area greater than 45 square centimetres it was 13.51%. These findings indicated that as tumour mass increased, a systemic rise in desmin expression occurred, potentially reflecting activation of stromal processes and mesenchymal transformation associated with disease progression. An evident, though not statistically significant ( $p > 0.05$ ), tendency was also observed towards an increase both in the volumetric density of desmin within tumour structures and in the frequency of its presence in the tumour in groups with larger neoplasms. Thus, the obtained results were consistent with the hypothesis that desmin was involved in tumour progression processes in colorectal cancer and supported its potential role as a marker associated with biological aggressiveness. To confirm these findings definitively particularly regarding desmin expression within tumour cells and to establish accurate prognostic threshold values, further studies involving larger and more representative patient cohorts are required.

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**CONFLICT OF INTEREST**

None.

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## Колоректалдык карциномалардагы десмин экспрессиясынын шишик көлөмү менен байланышы

**Андрей Филин**

Медицина илимдеринин кандидаты, доцент

Федералдык мамлекеттик бюджеттик жогорку билим берүү мекемеси  
«Н.Н. Бурденко атындагы Воронеж мамлекеттик медициналык университети»  
394036, Студенческая көч., 10, Воронеж ш., Россия Федерациясы  
<https://orcid.org/0000-0003-1670-3694>

**Елена Припутневич**

Ординатор

Федералдык мамлекеттик бюджеттик жогорку билим берүү мекемеси  
«Н.Н. Бурденко атындагы Воронеж мамлекеттик медициналык университети»  
394036, Студенческая көч., 10, Воронеж ш., Россия Федерациясы  
<https://orcid.org/0000-0002-7763-9932>

**Виктория Шишкина**

Медицина илимдеринин кандидаты, доцент

Федералдык мамлекеттик бюджеттик жогорку билим берүү мекемеси  
«Н.Н. Бурденко атындагы Воронеж мамлекеттик медициналык университети»  
394036, Студенческая көч., 10, Воронеж ш., Россия Федерациясы  
<https://orcid.org/0000-0001-9185-4578>

**Аннотация.** Булчуң клеткалары, миофибробласттар жана айрым патологиялык клеткалар тарабынан экспрессиялануучу десмин дүйнөдө кездешүү жыштыгы боюнча үчүнчү орунда турган колоректалдык рактын морфогенези контекстинде караганда азыркыга чейин жетишсиз изилденген. Изилдөөнүн максаты колоректалдык карциномаларда десминдин экспрессиясынын өзгөчөлүктөрүн аныктоо жана экспрессиянын деңгээлин шишик түйүнүнүн көлөмү менен салыштыруу болуп саналат. Изилдөөнүн жүрүшүндө жоон ичегиден алынган 24 даана залалдуу шишик үлгүсү гистологиялык жактан изилденип, иммуногистохимиялык ыкмасы аркылуу десминге каршы моноклоналдык чычкан антителолору колдонулду. Десминдин экспрессиясы бардык изилденген үлгүлөрдөн аныкталды. Десмин шишик түзүмдөрүндө да, органды курчап турган стромада да экспрессияланды. Ар кандай үлгүлөрдө десмин экспрессиясынын сандык гетерогендүүлүгү белгиленип, ал жогорку (массивдүү) деңгээлден төмөнкү деңгээлге чейин өзгөрүп турганы аныкталды. Десминдин көлөмдүк тыгыздыгын шишик түйүнүнүн аянты менен салыштырганда, жалпы экспрессияланган десминдин көлөмү ири шишик түйүндөрү бар үлгүлөрдө статистикалык жактан ишенимдүү түрдө көбүрөөк экени белгиленди. Мындан тышкары, шишик аянты чоңойгон сайын шишик түзүмдөрүндө локализацияланган десминдин көлөмү өсүү тенденциясы байкалды. Ошондой эле шишикте десминдин болуш-болбош фактысы шишик конгломератынын өлчөмү менен салыштырылды. Натыйжада ири шишик түйүндөрүндө десминдин шишик ичинде кездешүү жыштыгы кичине өлчөмдөгү шишиктерге салыштырмалуу кыйла жогору экени аныкталды. Алынган жыйынтыктар десминди шишик прогрессиясынын тобокелдигин баалоодо жана потенциалдуу прогностикалык маркер катары колдонуу мүмкүндүгүн көрсөтөт.

**Негизги сөздөр:** шишиктин көлөмү; аралык филаменттердин белугу; строма; иммуногистохимиялык ыкма; шишик түйүнү

## **Связь экспрессии десмина с размером опухоли в колоректальных карциномах**

**Андрей Филин**

Кандидат медицинских наук, доцент

Федеральное государственное бюджетное образовательное учреждение высшего образования

«Воронежский государственный медицинский университет имени Н.Н. Бурденко»

394036, ул. Студенческая, 10, г. Воронеж, Российская Федерация

<https://orcid.org/0000-0003-1670-3694>

**Елена Припутневич**

Ординатор

Федеральное государственное бюджетное образовательное учреждение высшего образования

«Воронежский государственный медицинский университет имени Н.Н. Бурденко»

394036, ул. Студенческая, 10, г. Воронеж, Российская Федерация

<https://orcid.org/0000-0002-7763-9932>

**Виктория Шишкина**

Кандидат медицинских наук, доцент

Федеральное государственное бюджетное образовательное учреждение высшего образования

«Воронежский государственный медицинский университет имени Н.Н. Бурденко»

394036, ул. Студенческая, 10, г. Воронеж, Российская Федерация

<https://orcid.org/0000-0001-9185-4578>

**Аннотация.** Десмин, экспрессируемый мышечными клетками, миофибробластами и некоторыми патологическими клетками, остаётся слабо изученным в контексте морфогенеза колоректального рака – третьего по распространённости онкологического заболевания в мире. Целью работы явилось изучение особенностей экспрессии десмина в колоректальных карциномах, а также сопоставление выраженности экспрессии с размером опухолевого узла. В ходе работы гистологическому исследованию были подвергнуты 24 образца злокачественных опухолей толстого кишечника, окрашенных иммуногистохимическим методом с применением моноклональных мышечных антител к десмину. Экспрессия десмина была выявлена во всех исследуемых образцах. Десмин экспрессировался как в опухолевых структурах, так и в окружающей строме органа. Выявлена количественная неоднородность экспрессии десмина, варьирующая от массивной до скудной в различных образцах. При сопоставлении объемной плотности десмина с площадью опухолевого узла было выявлено, что общее количество экспрессированного десмина было статистически значимо большим в образцах с крупными опухолевыми узлами. Кроме того, была обнаружена тенденция к увеличению количества десмина, локализованного в опухолевых структурах с возрастанием площади опухоли. В ходе работы также проводилось сопоставление факта наличия десмина в опухоли с величиной опухолевого конгломерата. Выяснилось, что в крупных опухолевых узлах десмин присутствовал в опухоли значительно чаще, чем в образцах с небольшими по размеру опухолями. Полученные в ходе исследования результаты говорят о потенциальной возможности использования десмина для оценки риска прогрессии опухоли, а также потенциального его использования как прогностического маркера

**Ключевые слова:** объем опухоли; белок промежуточных филаментов; строма; иммуногистохимический метод; опухолевый узел

## **Morphological justification of the effectiveness of osteopathic treatment of temporomandibular joint dysfunction**

**Rahat Abirova\***

PhD in Medical Sciences, Associate Professor  
I.K. Akhunbaev Kyrgyz State Medical Academy  
720020, 92 Akhunbaev Str., Bishkek, Kyrgyz Republic  
<https://orcid.org/0000-0001-5204-5583>

**Abdyrakman Eshiev**

Doctor of Medical Sciences, Professor  
Research Institute of Medical and Biological Problems, Southern Branch  
of the National Academy of Sciences of the Kyrgyz Republic  
723500, 130-A Uzgenskaya Str., Osh, Kyrgyz Republic  
<https://orcid.org/0000-0003-2617-8360>

**Daniyar Eshiev**

Doctor of Medical Sciences, Director  
Dental-Osh Clinic  
723500, 9 Kurmanjan Datka Str., Osh, Kyrgyz Republic  
<https://orcid.org/0009-0001-6658-0752>

**Abstract.** The aim of the study was to evaluate the effectiveness of osteopathic treatment for temporomandibular joint dysfunction using clinical indicators, magnetic resonance imaging data and immunohistochemical analysis. The study included 60 patients suffering from pain syndrome, limited mandibular mobility, joint clicking and impaired occlusal relationships. Treatment was carried out at the Dental-Osh dental clinic and involved the application of osteopathic techniques, including craniosacral, myofascial, structural and visceral methods, aimed at restoring biomechanics and normalising muscle tone. Treatment effectiveness was assessed using magnetic resonance imaging and the Temporomandibular Joint Symptom Intensity Scale (TMJ SIS), which reflected pain intensity and the range of mandibular movements. The results demonstrated pronounced morphological changes, including restoration of the physiological position of the articular disc, normalisation of the joint space and reduction of inflammation in soft tissues. A significant decrease in pain intensity and improvement in mandibular mobility were also observed. Immunohistochemical analysis revealed a statistically significant reduction in the expression of pro-inflammatory markers: tumour necrosis factor alpha (TNF- $\alpha$ ) by 41.6%, vascular endothelial growth factor A (VEGF-A) by 27.9%, and matrix metalloproteinase-13 (MMP-13) by 35.8%. At the same time, increased expression of cartilage regeneration factors was noted: collagen type II alpha 1 chain (COL2A1) by 43.9% and transforming growth factor beta 1 (TGF- $\beta$ 1) by 40%, which confirmed successful structural and functional restoration of temporomandibular joint tissues. The analysis demonstrated a statistically significant improvement in the restoration of mandibular movement amplitude, which was also confirmed by MRI findings. Reduction in pain severity and recovery of joint functional status were recorded both by clinical scales and immunohistochemical data, indicating a positive effect of osteopathic intervention. The results of the study could be used to implement osteopathic methods in the comprehensive treatment of temporomandibular joint dysfunction in interdisciplinary dental practice, ensuring more effective functional recovery and pain reduction in patients

**Keywords:** craniosacral therapy; myofascial techniques; structural techniques; visceral techniques; cartilage tissue regeneration

### **Suggested Citation:**

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\*Corresponding author



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## INTRODUCTION

The temporomandibular joint (TMJ) was described as a complex biomechanical structure that ensured coordinated movements of the mandible and participated in such vital functions as mastication, speech, and facial expression. The body of clinical, morphological, and immunohistochemical data obtained demonstrated not only a pronounced therapeutic effect but also a mechanistic justification for the use of osteopathy in the correction of joint pathology. A tendency towards an increase in the prevalence of TMJ dysfunctions was noted, which was associated with heightened psycho-emotional stress, impaired occlusal balance, as well as postural and myofascial disorders. The pathogenesis of TMJ dysfunction was a polyetiological and multifactorial process involving neuromuscular, vascular, and structural-morphological components. The totality of the collected data indicated positive structural-functional remodelling of TMJ tissues and confirmed the effectiveness of the osteopathic intervention carried out [1].

F. Moscagiuri *et al.* [2] investigated the morphology of the articular eminence in patients with different skeletal classes using cone-beam computed tomography. The authors showed that changes in the inclination of the articular eminence correlated with the type of skeletal growth, which highlighted the biomechanical complexity of the TMJ. These data were important for understanding the structural factors that influenced the development of dysfunction and the outcomes of its correction, including manual methods. S. Kadekuzhi *et al.* [3] conducted a protocol-based systematic review dedicated to the morphological changes of the TMJ in dysfunctions. The researchers identified key structural elements of the joint that underwent remodelling under pathological conditions and required evaluation in clinical studies. The work emphasised the importance of a comprehensive analysis of TMJ morphology, which resonated with the present study that used magnetic resonance imaging (MRI) and morphological criteria to assess the effectiveness of therapy.

S. Sirli Yilmazturk *et al.* [4] examined the morphological and morphometric characteristics of the TMJ in children with different skeletal patterns. The authors demonstrated that growth features and skeletal type significantly influenced the shape of the condylar head, the mandibular fossa, and the spatial relationships of the structures. These findings complemented the understanding of how biomechanical differences shaped the predisposition to dysfunction, which was important for interpreting the morphological changes observed in the present study before and after osteopathic treatment. S. Sritara *et al.* [5] investigated the association between TMJ morphology and individual masticatory pattern types. The authors identified that the structural features of the condylar head and the mandibular fossa correlated with the characteristics of masticatory movements, reflecting adaptive biomechanical changes

of the TMJ. These data underscored the role of functional loading in the formation of morphological changes, which was relevant to the current study demonstrating the possibility of restoring normal joint biomechanics following osteopathic treatment.

According to the study by E. S. Mamedov *et al.* [6], prolonged disturbance of the occlusal-muscular balance led to dystrophic changes in the articular disc, the capsular-ligamentous apparatus, and the subchondral bone, which were accompanied by microcirculatory impairments and remodelling of the cartilaginous tissue. J. Song *et al.* [7] investigated the morphological features of the TMJ in patients with different Angle's occlusal classes using cone-beam computed tomography. The study established that the parameters of the condylar head, the mandibular fossa, and the joint space varied considerably depending on the occlusal type, reflecting functionally determined biomechanical differences.

Y. Wei *et al.* [8] examined TMJ changes in adult patients with skeletal class II and a high mandibular growth angle, focusing on the influence of vertical control on joint morphology. It was shown that vertical growth characteristics and the orthodontic interventions performed led to significant remodelling of the condylar head and the joint space. These findings emphasised the sensitivity of the TMJ to alterations in biomechanical loading, which resonated with the present study demonstrating structural restoration of the joint following osteopathic treatment. I.I. Agadzhanov & Yu.Yu. Yakimova [9] investigated the features of diagnosing and treating TMJ dysfunction, paying particular attention to functional methods of joint assessment. The authors considered various approaches to evaluating occlusal-muscular balance and clinical manifestations, as well as describing contemporary therapeutic strategies. The work highlighted the importance of a comprehensive functional analysis, which aligned with the present study that used MRI and clinical indicators to assess the effectiveness of osteopathic intervention.

Y.A. Milutka & A.E. Fortin [10] considered the possibilities and organisational challenges of diagnosing and treating patients with pain-related TMJ dysfunction. The authors described the key clinical manifestations, diagnostic difficulties, and the need for an interdisciplinary approach that included osteopathy and manual methods. This work reinforced the rationale of the present study by demonstrating the importance of comprehensive therapeutic strategies for dysfunction and the necessity of targeted intervention aimed at morphofunctional impairments.

Thus, there was a clear need for comprehensive studies combining clinical, morphological, and molecular methods to achieve a deeper understanding of the mechanisms of osteopathic action in TMJ dysfunctions and to improve treatment effectiveness. Addressing

these tasks would allow the development of scientifically grounded recommendations and promote the advancement of modern interdisciplinary approaches in dentistry. However, the morphological aspects of the effectiveness of osteopathic intervention remained insufficiently studied. The absence of integrated data on structural and cellular changes within TMJ tissues after osteopathic correction limited the scientific justification of this method and its implementation in clinical practice. The relevance of the present study was determined by the necessity to provide a morphological justification for the effectiveness of osteopathic treatment of TMJ dysfunction based on the analysis of tissue and cellular indicators of joint structure remodelling. The results obtained would allow clarification of the mechanisms underlying osteopathic influences and enhance the effectiveness of comprehensive therapy for patients with TMJ dysfunction.

The aim of the present study was to assess the effectiveness of osteopathic treatment in patients with TMJ dysfunction based on clinical indicators and MRI findings obtained before and after the course of therapy.

## MATERIALS AND METHODS

The study included 60 patients with TMJ dysfunction who underwent treatment at the Dental-Osh dental clinic in the city of Osh between January and December 2024. The inclusion criteria were: age between 18 and 55 years; the presence of TMJ dysfunction symptoms (pain syndrome, restricted mobility, joint clicking) lasting for at least three months; and the absence of severe somatic or neurological disorders. Patients with acute inflammatory processes, TMJ trauma, or contraindications to manual therapy were excluded from the study. Before enrolment, all patients signed informed consent forms. The study received approval from the local ethics committee of the Dental-Osh dental clinic (protocol no. 12 dated 15.12.2023) and adhered to the principles of the Declaration of Helsinki [11].

Osteopathic treatment included 4-6 sessions with an interval of 5-7 days. The duration of each session was 45-60 minutes. Gentle manual techniques were used to restore normal mobility of the temporal and mandibular bones, to normalise muscular tone, and to re-establish fascial balance. Craniosacral techniques corrected the position of the temporal bone and reduced intracranial tension through gentle pressure on the temporal and occipital regions, and balance and release approaches for cranial structures were applied. Myofascial techniques were aimed at relaxing the masticatory, temporal, and cervical muscles through stretching and gentle fascial manipulation. Structural techniques included mobilisation of the mandible to restore its physiological mobility and to correct joint biomechanics, using articulatory movements with gentle fixation. Visceral techniques were directed at relieving tension of the hyoid bone and cervical fascial chains,

which contributed to improved occlusal balance. Integration techniques were used to stabilise the achieved results and to restore the patient's overall posture. All osteopathic interventions were performed by certified specialists with more than five years of experience, according to standard protocols adapted for patients with TMJ dysfunction.

The assessment of effectiveness was carried out on the basis of clinical data and MRI of the TMJ performed before and after completion of the treatment course. MRI examinations were conducted using a Philips Achieva 1.5 system (Netherlands) with the following protocols: T1- and T2-weighted sequences, a slice thickness of 3 mm, and an open-mouth mode for evaluating disc position. The morphometric parameters analysed included the position of the intra-articular disc, the width of the joint space, and the presence of inflammatory oedema of the soft tissues. Clinical assessment included measurement of mandibular movement amplitude and the intensity of pain syndrome using the TMJ SIS (Temporomandibular Joint Symptom Index Score), which comprised the domains of pain intensity, functional limitations, clicking, and crepitus. Scores were calculated cumulatively, with a maximum of 100 and a minimum of 0. Statistical processing of the data was performed using SPSS version 26.0.

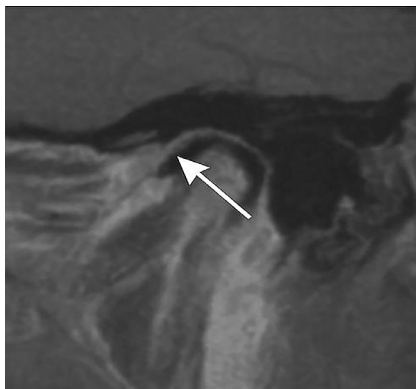
Normality testing was performed using the Shapiro-Wilk criterion. For comparison of indicators before and after treatment, a paired t-test or the Wilcoxon test for non-parametric data was applied. For multiple comparisons, ANOVA with Tukey post hoc testing was used. The level of statistical significance was accepted as  $p < 0.05$ . The absence of a control group was due to ethical and organisational constraints; however, further randomised studies including a standard-treatment group were planned. Immunohistochemical analysis was carried out in five anatomical zones of the TMJ: the synovial membrane, retrodiscal tissue, joint capsule, fibrocartilaginous articular disc, and para-articular muscles. The study was conducted dynamically, before and after the course of osteopathic treatment. Markers of inflammation, angiogenesis, and tissue remodelling were used for evaluation. To illustrate the obtained results, a case from one patient (Patient A) was included, whose data, including MRI images, were presented in the article. Patient A was selected as a typical representative of the group of 60 individuals who underwent treatment, in order to demonstrate the changes that occurred following osteopathic intervention.

## RESULTS

The MRI morphometric data were supported by the clinical dynamics: statistical analysis using the TMJ SIS scale demonstrated a significant reduction in pain intensity in the mandibular region both during typical manifestations ( $p = 0.0007$ ) and during the most pronounced episodes of pain ( $p = 0.032$ ). Painful joint

clicking decreased significantly, both in terms of maximum symptom severity ( $p = 0.0076$ ) and during usual manifestations ( $p = 0.0220$ ). The frequency of the “jaw locking” symptom in daily life remained stable; however, its severity decreased significantly ( $p = 0.0077$ ). Thus, the combination of morphological changes (according to MRI data) and functional improvements (according to TMJ SIS scores) confirmed objective restoration of the anatomical and functional relationships within the TMJ and regression of the muscular–ligamentous imbalance under the influence of osteopathic treatment.

The application of osteopathic techniques exerted a positive influence on reducing the severity of cephalgic manifestations associated with TMJ dysfunction, which indicated the appropriateness of their use as part of an interdisciplinary approach to TMJ therapy. In the sagittal MRI image of the TMJ, the main anatomical structures were demonstrated, with an arrow indicating the intra-articular structure (Fig. 1). Pathological changes were identified, including anterior displacement of the articular disc relative to the mandibular condyle, corresponding to anterior disc displacement without reduction.



**Figure 1.** MRI image of the TMJ before osteopathic treatment (Patient A)

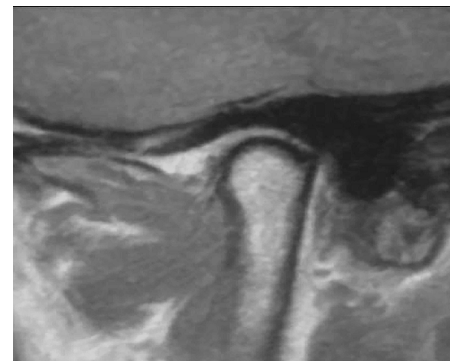
**Note:** displacement of the articular disc and inflammation of the soft tissues were observed in the image

**Source:** authors of the study

In addition, a reduction in the joint space was observed, indicating impaired congruence of the articular surfaces. Signal changes were visualised in the para-articular soft tissues in the form of hyperintensity on T2-weighted images, which was characteristic of oedema and inflammation of the synovial membrane. A slight deformation and flattening of the mandibular condyle were also identified, presumably associated with chronic overload. Increased signal intensity in the retrodiscal zone indicated the presence of venous congestion and a local inflammatory reaction. Thus, the MRI features demonstrated TMJ dysfunction with anterior disc displacement, signs of synovial membrane inflammation, and oedema of the para-articular tissues. The obtained data corresponded to the active phase of

the inflammatory-degenerative process and confirmed the necessity of correcting the muscular-ligamentous imbalance through osteopathic methods.

In the sagittal MRI image presented (Fig. 2), the TMJ demonstrated normal congruence of the articular surfaces with no signs of disc displacement. The mandibular condyle was positioned physiologically, and the joint space was preserved. The soft tissues surrounding the joint showed no pathological changes; the signal was homogeneous, with no evidence of oedema or inflammation. This image reflected the normal anatomy of the TMJ in the sagittal projection.



**Figure 2.** MRI image of the TMJ after osteopathic treatment (Patient A)

**Source:** authors of the study

Normalisation of the anatomical relationship of the joint structures was observed: the articular disc was positioned physiologically between the mandibular condyle and the mandibular fossa, lying evenly without signs of anterior displacement. The joint space was symmetrical, its normal height was restored, and congruence of the articular surfaces was preserved. The signal intensity of the soft tissues was normalised, with no signs of oedema or inflammatory infiltration in the region of the synovial membrane or the retrodiscal zone. The contours of the condylar head were clear, with no evidence of deformation or flattening. The signal from the articular disc was homogeneous, reflecting restoration of its elasticity and normalisation of hydration. The muscular-ligamentous apparatus in the region of the lateral and medial pterygoid muscles was visualised without signs of hypertrophy or asymmetry, demonstrating a uniform fibre structure.

Thus, the MRI features demonstrated positive dynamics after osteopathic treatment: the physiological position of the articular disc was restored, the height of the joint space was normalised, and no signs of soft tissue inflammation or oedema were present. Structural-functional restoration of the TMJ and a reduction in dysfunction signs were established. The data obtained on MRI indicated the restoration of normal TMJ anatomy after osteopathic treatment (Fig. 2), which was also supported by positive changes at the molecular

level. Immunohistochemical analysis showed significant improvements in the anatomical zones of the TMJ,

reflecting reduced inflammation and enhanced tissue regeneration (Table 1).

**Table 1.** Immunohistochemical analysis by anatomical zones of the TMJ

| Anatomical zone                                                | Before treatment                                                                                                                 | After treatment                                                                                                                                  |
|----------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Synovial membrane</b>                                       | High expression of TNF- $\alpha$ , IL-1 $\beta$ , IL-6; NF- $\kappa$ B p65 translocation (40–50% of cells); active inflammation. | Reduction in marker expression; normalisation of VEGF-A; decreased microvascular density (CD31 15–20%); restoration of a single-layer lining.    |
| <b>Retrodiscal tissue (bilaminar zone)</b>                     | Enhanced angiogenesis; increased expression of VEGF-A, CD31, COX-2, iNOS; inflammation.                                          | Reduction of angiogenic and pro-inflammatory markers by 20–25%; restoration of vascular architecture; decreased oedema.                          |
| <b>Joint capsule</b>                                           | Increased expression of MMP-1, MMP-3, MMP-13; reduced TIMP-1 and TIMP-2.                                                         | Normalisation of the MMP/TIMP ratio; moderate increase in TGF- $\beta$ 1; organised orientation of collagen fibres.                              |
| <b>Fibrocartilaginous articular disc</b>                       | Rarefaction of collagen fibres; focal expression of MMP-13; decreased COL2A1.                                                    | Activation of cartilage matrix synthesis; increased COL2A1, aggrecan, COMP, SOX9; increased TGF- $\beta$ 1.                                      |
| <b>Para-articular muscles (masticatory, lateral pterygoid)</b> | Marked expression of TNF- $\alpha$ , IL-6, iNOS; myogenic inflammation.                                                          | Reduction in TNF- $\alpha$ , IL-6, iNOS; decreased COX-2; increased TGF- $\beta$ 1; restoration of metabolic activity and reduced hypertonicity. |

**Note:** TNF- $\alpha$  – tumour necrosis factor- $\alpha$ ; IL-1 $\beta$  – interleukin-1 $\beta$ ; IL-6 – interleukin-6; NF- $\kappa$ B – nuclear factor  $\kappa$ B; VEGF-A – vascular endothelial growth factor A; CD31 – endothelial marker; COX-2 – cyclooxygenase-2; iNOS – inducible nitric oxide synthase; MMP – matrix metalloproteinases; TIMP – tissue inhibitors of metalloproteinases; TGF- $\beta$ 1 – transforming growth factor  $\beta$ 1; COL2A1 – type II collagen; COMP – cartilage oligomeric matrix protein; SOX9 – transcription factor of chondrogenesis

**Source:** developed by the authors

In Table 2, the data reflected changes in the expression of key immunohistochemical markers associated with inflammation, angiogenesis, tissue degradation, and remodelling of the TMJ before and after osteopathic

treatment. The analysis included evaluation of the expression of TNF- $\alpha$ , VEGF-A, MMP-13, COL2A1, and TGF- $\beta$ 1 in various anatomical structures of the joint using semi-quantitative metrics (H-score and IRS).

**Table 2.** Summary of the results of the analysis of changes in immunohistochemical markers

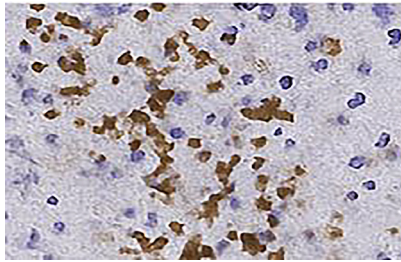
| Marker                                     | Before treatment | After treatment | Change               | p-value   |
|--------------------------------------------|------------------|-----------------|----------------------|-----------|
| <b>TNF-<math>\alpha</math></b>             | 185 $\pm$ 46     | ↓ by 41.6%      | Significant decrease | p < 0.001 |
| <b>VEGF-A</b>                              | 136 $\pm$ 35     | ↓ by 27.9%      | Significant decrease | p = 0.002 |
| <b>MMP-13</b>                              | 162 $\pm$ 42     | ↓ by 35.8%      | Significant decrease | p < 0.001 |
| <b>COL2A1</b>                              | –                | ↑ by 43.9%      | Significant increase | p < 0.001 |
| <b>TGF-<math>\beta</math>1 (IRS score)</b> | 5.0 (3-7)        | 7.0 (5-9)       | ↑ by 40.0%           | p = 0.004 |

**Source:** developed by the authors

Before the start of therapy, elevated values of the pro-inflammatory markers TNF- $\alpha$  (185  $\pm$  46) and MMP-13 (162  $\pm$  42) were observed, as well as moderately pronounced expression of the angiogenic factor VEGF-A (136  $\pm$  35). After completion of the treatment course, a significant reduction in the expression of TNF- $\alpha$  by 41.6% (p < 0.001), VEGF-A by 27.9% (p = 0.002), and MMP-13 by 35.8% (p < 0.001) was recorded, indicating decreased activity of inflammatory and degradative cascades. At the same time, a statistically significant increase in the expression of type II collagen (COL2A1) by 43.9% (p < 0.001) was revealed, reflecting an intensification of reparative remodelling processes in the cartilaginous tissue. In parallel, the level of TGF- $\beta$ 1 in the capsular structures increased from a median of 5.0 (3-7) to 7.0 (5-9) on the IRS scale (+40.0%; p = 0.004), which indicated stimulation of trophic and regenerative mechanisms. Thus, the obtained data demonstrated a pronounced anti-inflammatory and regenerative

effect of the osteopathic intervention, accompanied by normalisation of the expression of key markers of inflammation and tissue restoration in the TMJ. In Figure 3, the microscopic image of the synovial membrane stained for tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ) showed pronounced signs of inflammatory activation of the tissues. At  $\times$ 400 magnification, areas of synoviocyte hyperplasia, thickening of the synovial layer, and infiltration of the underlying stroma by mononuclear cells were clearly visualised. The obtained microscopic picture corresponded to a proliferative-inflammatory type of synovitis characteristic of structural disorders of the TMJ in chronic joint dysfunction. This aligned with the literature, which demonstrated that an increased level of TNF- $\alpha$  was an early and persistent marker of joint inflammation, and its intensity could correlate with clinical symptoms. In Figure 4, the identified morphological features were presented, which were consistent with contemporary research showing that TMJ dysfunction

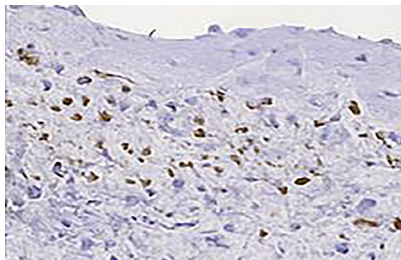
was accompanied by activation of pro-inflammatory cytokines (TNF- $\alpha$ , IL-1 $\beta$ , IL-6) and mediators of extracellular matrix degradation.



**Figure 3.** Synovial membrane (Patient A)

**Note:** microscopic image,  $\times 400$  magnification, obtained using phase-contrast microscopy

**Source:** authors of the study



**Figure 4.** Synovial membrane (Patient A)

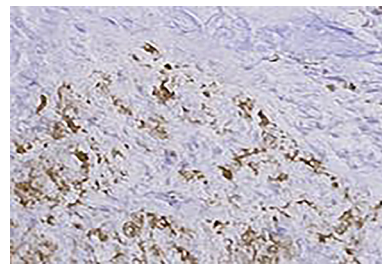
**Note:** microscopic image,  $\times 400$  magnification, obtained using phase-contrast microscopy

**Source:** authors of the study

At  $\times 400$  magnification, the synovial membrane of Patient A demonstrated pronounced inflammatory-reactive changes. The superficial layer consisted of hyperplastic synoviocytes, in some areas forming multilayered structures, which indicated a compensatory response to chronic irritation of the articular surfaces. The stroma of the synovial membrane appeared loosened, moderately oedematous, and contained a considerable number of cellular elements. Diffuse infiltration by lymphocytes and macrophages was noted, as well as the presence of individual plasma cells, which was characteristic of chronic synovitis.

Immunohistochemical analysis of the synovial membrane using the CD31 marker ( $\times 400$ ) (Fig. 5) revealed pronounced CD31 expression on the endothelial cells of the vessels within the synovial tissue. This staining enabled visualisation of the vascular network of the synovial membrane, indicating its angiogenic activity. The  $\times 400$  magnification provided a detailed assessment of the morphology of the vascular structures and the degree of tissue vascularisation. Immunohistochemical analysis of the synovial membrane using the CD31 marker ( $\times 400$ ) revealed pronounced CD31 expression on the endothelial cells of the vessels within the synovial tissue. This staining allowed visualisation

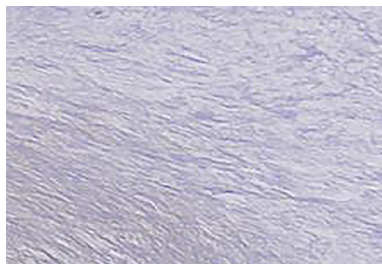
of the vascular network of the synovial membrane, indicating its angiogenic activity. The  $\times 400$  magnification provided a detailed assessment of the morphology of the vascular structures and the degree of tissue vascularisation. The application of osteopathic techniques exerted a positive influence on reducing the severity of inflammatory processes in the TMJ tissues, which was confirmed by the results of the immunohistochemical examination of the joint capsule (Fig. 6).



**Figure 5.** Immunohistochemical examination of the synovial membrane (Patient A)

**Note:** microscopic image obtained using immunohistochemical analysis at  $\times 400$  magnification

**Source:** authors of the study



**Figure 6.** Immunohistochemical examination of the joint capsule (Patient A) using the COX-2 marker

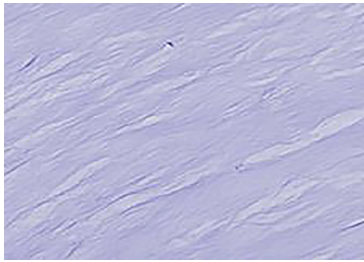
**Note:** COX-2 – cyclooxygenase-2; image obtained using immunohistochemical analysis at  $\times 400$  magnification

**Source:** authors of the study

Immunohistochemical examination of the joint capsule using the COX-2 marker ( $\times 400$ ) revealed pronounced COX-2 expression in the cells of the synovial tissue. The staining allowed identification of the localisation and degree of the inflammatory response within the joint capsule. Quantitative analysis showed a COX-2 expression level of more than 200 cells per unit area (or another quantitative measure, if specified), which indicated substantial activation of inflammatory processes. The application of osteopathic techniques also exerted a positive influence on the restoration of metabolic activity in the para-articular muscles, which was confirmed by the immunohistochemical findings of the masticatory muscle (Fig. 7).

In Figure 7, the masticatory muscle was examined using immunohistochemistry with antibodies to iNOS at  $\times 400$  magnification. The findings showed an absence

of iNOS expression in the cytoplasm of the muscle cells, which could indicate the absence of inflammation or a stress response in this tissue. The results obtained demonstrated a pronounced positive dynamic in the clinical, morphological, and immunohistochemical indicators in patients with TMJ dysfunction after the course of osteopathic treatment. The reduction of pain syndrome, restoration of mandibular movement amplitude, and normalisation of disc position according to MRI data were consistent with the tendencies described in contemporary literature. In the systematic review by V. Villar-Aragón-Berzosa *et al.* [12], it was noted that manual therapy was comparable in clinical effectiveness to occlusal splints and, in some cases, demonstrated more pronounced outcomes in TMJ-related pain. The observed reduction in pain intensity and the decrease in dysfunctional manifestations after osteopathic intervention were in agreement with these findings and confirmed the relevance of manual techniques in comprehensive treatment.



**Figure 7.** Immunohistochemical examination of the masticatory muscle (Patient A) using the iNOS marker

**Note:** image obtained using immunohistochemical analysis at  $\times 400$  magnification

**Source:** authors of the study

According to large-scale meta-analytic research by G. Zieliński *et al.* [13], TMJ dysfunction was a highly prevalent condition, the pathogenesis of which involved a combination of biomechanical, inflammatory, and psychosomatic factors. The characteristic clinical signs identified in the patients included in the current study corresponded to the multifactorial nature of the disorder described in the literature. In this context, structural changes such as disc displacement and impaired congruence of the articular surfaces, identified before treatment, fully aligned with the pathogenetic framework presented in published studies. The results of the meta-analysis by L.F. Valesan *et al.* [14] also highlighted the high frequency of structural-inflammatory alterations in the TMJ, which corresponded to the pre-treatment signs observed in this study, including synovial membrane inflammation, oedema of the para-articular tissues, and deformation of the condylar head.

Additional comparison with the literature confirmed the significance of the identified morphological

and biomolecular changes. In the study by L.H.S. Cevdanes *et al.* [15], osteoarthritic changes in the condylar region of the TMJ were shown to be closely associated with levels of systemic and local biomarkers of inflammation and tissue degradation. The correlation demonstrated by the authors between joint morphology and the expression of inflammatory markers aligned with the observed reduction in TNF- $\alpha$ , VEGF-A, and MMP-13 following osteopathic intervention. Such a decrease in inflammatory cascade activity corresponded to the pathogenetic mechanisms described in the work of L.H.S. Cevdanes *et al.* and indicated the potential for structural remodelling of the TMJ under therapeutic influence. A number of systematic reviews dedicated to manual therapy have confirmed its high effectiveness in functional TMJ disorders. In the review by F.M. Liberato *et al.* [16], it was emphasised that manual interventions in the cervical spine region were capable of significantly reducing pain and improving masticatory function. A similar trend was observed in the present findings: reduced pain intensity and improved mouth-opening amplitude were confirmed by TMJ SIS results. Comparable conclusions were reported by A.N. Jácome *et al.* [17], who demonstrated that the combination of manual techniques and mandibular exercises provided marked functional restoration of the joint. These data further supported that the therapeutic effect of osteopathic techniques could be comparable to the best outcomes of conservative rehabilitation for TMJ dysfunction. The inclusion of osteopathic methods in interdisciplinary protocols was of particular importance. In the work by S. Easterbrook *et al.* [18], the clinical relevance of osteopathic manipulative treatment for TMJ pain symptoms was underscored. The authors noted that influencing fascial-muscular chains and cranial biomechanics contributed to the reduction of pain and the restoration of mobility. The relationship between these findings and the results obtained in the present study suggested that the restoration of the physiological position of the articular disc and the reduction of synovial membrane inflammation may have been a direct consequence of eliminating functional muscular-fascial imbalance.

Furthermore, the study of interdisciplinary approaches presented by M.V. Martyusheva *et al.* [19] highlighted the necessity of integrating diagnostic methods, manual interventions, and correction of occlusal disturbances. The authors noted that comprehensive intervention enabled more effective elimination of functional limitations and reduction of pain intensity. Comparison of these data with the findings of imaging and immunohistochemical analysis showed that the complex restoration of TMJ biomechanics was accompanied by objectively verifiable tissue improvements. Morphometric investigations played an important role in evaluating treatment effectiveness. D.P. De Melo *et al.* [20] demonstrated that changes in the size

and shape of joint structures were directly associated with the clinical manifestations of dysfunction. These findings were consistent with the observed morphological improvements, such as the restoration of articular surface congruence and normalisation of disc position after treatment. Similarly, S. Han *et al.* [21] established a relationship between the incisal path angle, the inclination of the occlusal plane, and morphological variability of the TMJ, which underscored the influence of occlusal-biomechanical factors on the development of dysfunction. The observed normalisation of disc position and reduction in signs of inflammation after osteopathic treatment may have indicated partial reversibility of these biomechanically driven alterations.

Thus, comparison of the results of the present study with contemporary research indicated a high effectiveness of osteopathic treatment for TMJ dysfunction, confirmed by clinical, morphological, and molecular data. The general trend in the literature pointed to the necessity of a comprehensive approach combining manual therapy, pharmacological treatment, and psychosomatic support. A distinctive feature of the current study was the integration of MRI findings with the objective TMJ SIS scale and the analysis of immunohistochemical markers, which enabled a comprehensive evaluation of the therapeutic effect and the mechanisms of osteopathic action.

## CONCLUSIONS

In the present study, a comprehensive morphological and immunohistochemical analysis of TMJ tissues was carried out before and after osteopathic treatment. The microphotographs of the immunohistochemical reactions demonstrated a statistically significant reduction in the level of inflammatory markers: TNF- $\alpha$  expression decreased on average by 41.6%, and NF- $\kappa$ B p65 by 40–50% compared with baseline values ( $p < 0.05$ ). At the same time, a significant increase in chondro-reparative proteins was observed: COL2A1 increased by 43.9%,

aggrecan by 47%, and TGF- $\beta$ 1 by 40% ( $p < 0.05$ ), which indicated stimulation of cartilage tissue regeneration. Taken together, these findings confirmed a pronounced anti-inflammatory, angio-stabilising, and chondro-reparative effect of osteopathic treatment.

The morphological changes, including normalisation of the structure of the synovial membrane and restoration of the vascular pattern, were consistent with the immunohistochemical indicators demonstrating recovery of trophic and functional activity within the TMJ tissues. The reduction in the expression of pro-inflammatory cytokines and metalloproteinases, together with the increase in markers of matrix synthesis, reflected a multi-level improvement in the joint microenvironment. Thus, osteopathic intervention exerted a comprehensive positive influence on the morpho-functional state of the TMJ, confirming its effectiveness as a pathogenetically justified non-pharmacological method for treating joint dysfunction. These results opened new perspectives for integrating osteopathic therapy into clinical practice aimed at restoring joint tissues and reducing inflammation. Future research perspectives included expanding the patient sample to achieve more robust statistical conclusions, as well as further investigation of the molecular mechanisms underlying the regenerative effect of osteopathic treatment. In addition, long-term clinical follow-up was considered advisable to assess the stability of the therapeutic changes and the impact of therapy on functional TMJ indicators.

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None.

## CONFLICT OF INTEREST

None.

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## **Самай жана астыңкы жаак сөөк муунунун жабыркашынын остеопатиялык дарылоо эффективдүүлүгүнүн морфологиялык негиздемеси**

**Рахат Абирова**

Медицина илимдеринин кандидаты, доцент  
И.К. Ахунбаев атындагы Кыргыз мамлекеттик медициналык академиясы  
720020, Ахунбаев көч., 92, Бишкек ш., Кыргыз Республикасы  
<https://orcid.org/0000-0001-5204-5583>

**Абдыракман Ешиев**

Медицина илимдеринин доктору, профессор  
Кыргыз Республикасынын Улуттук илимдер академиясынын Түштүк бөлүмүнүн медициналык-  
биологиялык көйгөйлөрдү илимий изилдөө институтунун  
723500, Өзгөн көч., 130-А, Ош ш., Кыргыз Республикасы  
<https://orcid.org/0000-0003-2617-8360>

**Данияр Ешиев**

Медицина илимдеринин доктору, директор  
«Dental-Osh» стоматологиялык клиникасы  
723500, Курманжан Датка көч., 9, Ош ш., Кыргыз Республикасы  
<https://orcid.org/0009-0001-6658-0752>

**Аннотация.** Изилдөөнүн максаты – самай жана астыңкы жаак сөөк муунунун жабыркашынын остеопатиялык дарылоо натыйжалуулугун клиникалык көрсөткүчтөрдүн, магниттик-резонанстык томографиянын (МРТ) маалыматтарынын жана иммуногистохимиялык анализдин негизинде баалоо болду. Изилдөөгө ооруу синдрому, астыңкы жаактын кыймылынын чектелиши, муундун чыкылдашы жана окклюзиялык катыштардын бузулушу менен мүнөздөлгөн 60 бейтап киргизилди. Дарылоо «Дентал-Ош» стоматологиялык клиникасында остеопатиялык ыкмалардын комплекси аркылуу жүргүзүлдү: краниосакралдык, миофасциалдык, структуралык жана висцералдык техникалар колдонулуп, алар муундун биомеханикасын калыбына келтирүүгө жана булчуң тонусун нормалдаштырууга багытталды. Натыйжалуулук МРТ жана оорунун интенсивдүүлүгүн жана астыңкы жаактын кыймыл амплитудасын чагылдырган TMJ SIS шкаласы аркылуу бааланды. Натыйжалар муундагы айкын морфологиялык өзгөрүүлөрдү көрсөттү: муун дискинин физиологиялык абалы калыбына келди, муун көңдөйү нормалдашты, жумшак ткандардагы сезгенүүнүн белгилери азайды. Ошондой эле ооруу синдрому олуттуу төмөндөп, астыңкы жаактын кыймылдуулугу жакшырды. Иммуногистохимиялык анализ сезгенүү маркерлеринин экспрессиясынын статистикалык жактан ишенимдүү азайганын аныктады: TNF- $\alpha$  – 41,6%га, VEGF-A – 27,9%га, MMP-13 – 35,8%га. Ошол эле учурда кемирчек тканынын регенерациясына байланышкан факторлордун экспрессиясы жогорулаганы белгиленди: COL2A1 – 43,9%га, TGF- $\beta$ 1 – 40%га. Бул самай жана астыңкы жаак сөөк муунунун ткандарынын структуралык жана функционалдык калыбына келүүсүн тастыктайт. Талдоо астыңкы жаактын кыймыл амплитудасы статистикалык жактан маанилүү деңгээлде жакшырганын көрсөттү жана бул МРТ аркылуу да ырасталды. Оорунун күчүнүн азайышы жана муундун функционалдык абалынын калыбына келиши клиникалык шкалалар боюнча да, иммуногистохимиялык көрсөткүчтөр боюнча да тастыкталып, остеопатиялык кийлигишүүнүн оң таасирин билдирет. Изилдөөнүн жыйынтыктары самай жана астыңкы жаак сөөк муунунун дисфункциясын комплекстүү дарылоодо остеопатиялык ыкмаларды стоматологиялык көп тармактуу практикага интеграциялоого негиз болуп, бейтаптарда функцияны натыйжалуу калыбына келтирүүгө жана ооруу синдромун азайтууга көмөктөшө алат.

**Негизги сөздөр:** краниосакралдык терапия; миофасциалдык техникалар; структуралык техникалар; висцералдык техникалар; кемирчек тканынын регенерациясы

## Морфологическое обоснование эффективности остеопатического лечения дисфункции височно-нижнечелюстного сустава

**Рахат Абирова**

Кандидат медицинских наук, доцент  
Киргизская государственная медицинская академия им. И.К. Ахунбаева  
720020, ул. Ахунбаева, 92, г. Бишкек, Кыргызская Республика  
<https://orcid.org/0000-0001-5204-5583>

**Абдыракман Ешиев**

Доктор медицинских наук, профессор  
Научно-исследовательский институт медико-биологических проблем Южного отделения Национальной академии наук Кыргызской Республики  
723500, ул. Узгенская, 130-А, г. Ош, Кыргызская Республика  
<https://orcid.org/0000-0003-2617-8360>

**Данияр Ешиев**

Доктор медицинских наук, директор  
Стоматологическая клиника «Dental-Osh»  
723500, ул. Курманжан Датка, 9, г. Ош, Кыргызская Республика  
<https://orcid.org/0009-0001-6658-0752>

**Аннотация.** Целью исследования было оценить эффективность остеопатического лечения дисфункции височно-нижнечелюстного сустава с использованием клинических показателей, данных магнитно-резонансной томографии и иммуногистохимического анализа. В исследование были включены 60 пациентов, страдающих болевым синдромом, ограничением подвижности нижней челюсти, суставными щелчками и нарушением окклюзионных соотношений. Лечение проводилось в стоматологической клинике «Дентал-Ош», с применением остеопатических техник, включая краниосакральные, миофасциальные, структурные и висцеральные методы, направленных на восстановление биомеханики и нормализацию мышечного тонуса. Эффективность оценивалась с использованием магнитно-резонансной томографии и шкалы TMJ SIS, отражающей интенсивность болевого синдрома и амплитуду движений нижней челюсти. Результаты показали выраженные морфологические изменения, включая восстановление физиологического положения суставного диска, нормализацию суставного пространства и уменьшение воспаления в мягких тканях. Также наблюдалось значительное снижение болевого синдрома и улучшение подвижности нижней челюсти. Иммуногистохимический анализ выявил статистически достоверное уменьшение экспрессии провоспалительных маркеров: TNF- $\alpha$  – на 41,6 %, VEGF-A – на 27,9 %, MMP-13 – на 35,8 %. Одновременно отмечено повышение экспрессии факторов регенерации хрящевой ткани: COL2A1 – на 43,9 %, TGF- $\beta$ 1 – на 40 %, что подтверждает успешное структурное и функциональное восстановление тканей височно-нижнечелюстного сустава. Анализ показал статистически значимое улучшение в восстановлении амплитуды движений нижней челюсти, что также подтвердилось через МРТ-данные. Уменьшение выраженности болевого синдрома и восстановление функционального состояния сустава были зафиксированы как по клиническим шкалам, так и по данным иммуногистохимического анализа, что свидетельствует о положительном влиянии остеопатического вмешательства. Результаты исследования могут быть использованы для внедрения остеопатических методов в комплексное лечение дисфункции височно-нижнечелюстного сустава в междисциплинарной стоматологической практике, что обеспечит более эффективное восстановление функций и уменьшение болевого синдрома у пациентов

**Ключевые слова:** краниосакральная терапия; миофасциальные техники; структурные техники; висцеральные техники; регенерация хрящевой ткани

## **Influence of the eccentric mode of muscle contraction on pain intensity and mobility limitations in individuals with impaired knee joint function**

**Kirill Chikhalin\***

Postgraduate Student

Russian University of Sports "State Central Order of Lenin Institute of Physical Culture"

105122, 4 Sirenevyy Blvd., Moscow, Russian Federation

<https://orcid.org/0009-0006-9968-3201>

**Tatyana Grichanova**

PhD in Biological Sciences, Associate Professor

Russian University of Sports "State Central Order of Lenin Institute of Physical Culture"

105122, 4 Sirenevyy Blvd., Moscow, Russian Federation

<https://orcid.org/0000-0001-7359-4338>

**Abstract.** With the increase in the number of patients presenting with pain and reduced mobility of the knee joint, the search for effective methods of functional restoration at the early stages of osteoarthritis became particularly important. The aim of the study was to identify a possible relationship between pain intensity, movement limitations in the joint and parameters of the eccentric mode of muscle contraction in individuals with impaired knee joint function. Within the study, an experiment was carried out in a medical centre to investigate the eccentric mode of muscle contraction in knee osteoarthritis. During the rehabilitation sessions, emphasis was placed on the eccentric phase of contraction of the quadriceps femoris muscle. Electromyograph readings, pain intensity on the visual analogue scale (VAS) and range of motion in the joint measured by goniometer were recorded before and after the experiment. The mean amplitude of eccentric contraction of the quadriceps femoris muscle more than doubled; for example, in the vastus lateralis muscle it increased from 98.7  $\mu$ V to 298.4  $\mu$ V. After the course of sessions, full knee extension was achieved in six of nine participants, and the level of pain decreased in all subjects, including complete disappearance of pain in two individuals. According to the results of the study, the eccentric mode of muscle contraction showed its effectiveness in reducing pain level, increasing range of motion and enhancing indices of electromyographic activity. The eccentric mode developed muscle strength and neuromuscular control during movement and reduced mechanical load on the articular cartilage. The results of this study demonstrated that exercises in an eccentric mode for the quadriceps femoris muscle at the initial stages of knee osteoarthritis may be effective, acting simultaneously on the cause (muscle weakness) and the consequences (pain, limitation of joint range of motion). The obtained findings may be used in the development of individual rehabilitation programmes for patients with early forms of knee osteoarthritis

**Keywords:** gonarthrosis; electromyography; negative phase; knee pain; range of motion

### **INTRODUCTION**

Functional impairment and the number of complaints of pain and restricted mobility of the knee joint in the general population had been steadily increasing. Various factors could have contributed to this, such as a sedentary lifestyle and obesity, which increased the load on the joints. Pain was the main subjective symptom, which determined everyday activity, work capacity and quality of life. Limitation of joint

mobility was an objective sign of joint dysfunction and directly affected the biomechanics of walking, stair ascent and descent, and other everyday activities. Particular attention was given to the eccentric mode of muscle contraction. Eccentric control was critically important for stabilising the knee joint during the stance phase of gait, especially during stair descent; this mode also absorbed impact loading

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\*Corresponding author



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and protected the articular cartilage from excessive compression [1, 2].

In the study by R. Çiftçi *et al.* [3] it was demonstrated that dysfunction of the quadriceps femoris muscle was not only a consequence, but also a significant factor in the progression of many joint diseases, including osteoarthritis. C.Y. Zeng *et al.* [4] reported that therapeutic exercise as an adjunct method of physiotherapy could prevent degeneration of cartilage tissue, suppress inflammation and prevent the loss of subchondral bone and metaphyseal trabecular bone. There had been increasing evidence that therapeutic exercise could reduce pain, stiffness, joint dysfunction and muscle weakness in patients with knee osteoarthritis. The authors noted that several types of therapeutic exercise for the management of knee osteoarthritis were available, including aerobic exercise, resistance training, neuromuscular exercises, balance training, proprioceptive training, aquatic exercise and traditional exercise programmes.

In the scientific article by S. Hody *et al.* [5], increases in strength and muscle mass as a result of the negative phase of muscle contraction were associated with DOMS (delayed-onset muscle soreness related to micro-damage of muscle fibres). The authors considered that eccentric work allowed meaningful functional improvements to be achieved with a relatively small training volume; therefore, with appropriate dosing and periodisation, DOMS was regarded as the main driver of unique adaptive responses. The same article also highlighted the use of eccentric exercise programmes in individuals with sarcopenia, where such training was found to effectively counteract fatty degeneration of the muscles. The systematic review by K. Čretnik *et al.* [6] showed that eccentric training in older adults led to more pronounced gains in strength and functional mobility compared with traditional methods. The authors emphasised the high mechanical efficiency of eccentric contractions under low metabolic load, which made this mode particularly promising for patients with limited exercise tolerance, including individuals with knee osteoarthritis. These data confirmed the importance of eccentric work in restoring muscle function and controlling movement.

In a randomised controlled trial by E. Hanci *et al.* [7], the effect of a 4-week eccentric training programme for the peroneal muscles in patients with chronic ankle instability was evaluated. The influence on range of motion was not directly assessed in that study, but increases in strength and control of dynamic range of motion were key outcomes. The results showed that training based on eccentric exercises could positively affect neurophysiological parameters in patients with joint instability, which suggested improved functional stability and control throughout the full range of motion. However, it remained unclear how long these neurophysiological adaptations persisted.

For athletes, emphasis on eccentric exercises was reported by O. Younes-Egana *et al.* [8] to improve sports performance through increases in explosive strength and speed-strength qualities. Most studies on this topic had been short-term; long-term effects and the impact on injury prevention had not been sufficiently investigated. There was also an opinion that, for highly trained athletes, focusing on a particular contraction mode provided only a modest advantage. The effect on pain was examined in the study by S. Holden *et al.* [9]. Patients with knee pain were asked to perform a single session of exercises for the quadriceps femoris muscle in isometric and eccentric modes. Pain intensity was assessed using the visual analogue scale (VAS). The results showed that isometric exercises significantly reduced pain, whereas eccentric exercises did not lead to a meaningful change in pain severity.

Despite the importance of each component (pain, range of motion, muscle function), the relationship between subjective pain perception, objective restriction of mobility and neurophysiological parameters of eccentric contraction in individuals with impaired knee function had not been studied in a sufficiently comprehensive manner. The relevance of the present study was determined by the need for a deeper understanding of the complex interrelations between subjective and objective manifestations of dysfunction, the potential for developing new diagnostic criteria and the design of individualised rehabilitation approaches. The aim of the study was to determine the relationship between pain intensity, mobility limitations and parameters of eccentric contraction in individuals with impaired knee joint function. The objectives of the study were as follows: (1) to determine the subjective perception of pain in the participants and the objective limitations in range of motion; (2) to carry out spectral analysis of the data recorded by the electromyograph; and (3) to assess the relationship between objective and subjective indicators and the electromyogram of the quadriceps femoris muscle in an eccentric mode.

## MATERIALS AND METHODS

To address the objectives of the study, the following methods were used: the visual analogue scale (VAS) for quantitative assessment of pain intensity; goniometry for objective measurement of range of motion; and surface electromyography for recording the electrical potential of the muscle under investigation. The VAS was applied to quantify pain intensity, where 0 corresponded to no pain and 10 to the maximum imaginable pain. The values were interpreted as follows: 1-3 points indicated mild pain, 4-6 points moderate pain, and 7-10 points marked pain syndrome. The scale made it possible to objectify the subjective sensations of the patient and to assess their changes over time.

An eight-channel electromyograph of Russian manufacture, KBK BP-08-4, was used in the study.

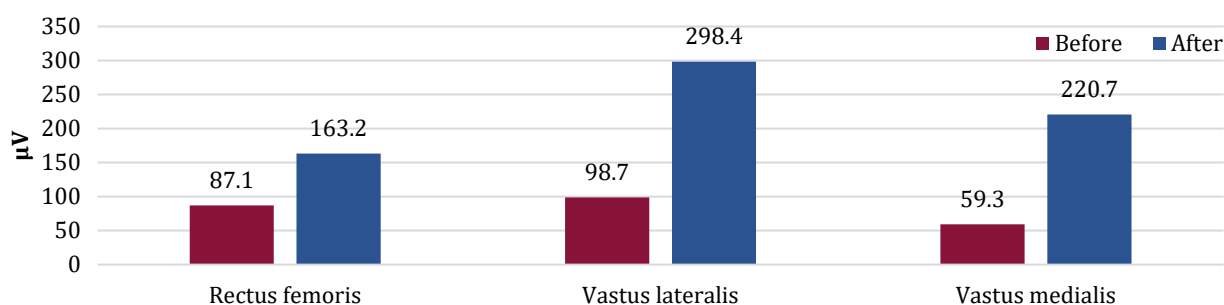
Interpretation of the recorded data was carried out using the EMGAnalyzer software. A root mean square (RMS) filter with a window length of 1000 was applied to the electromyographic signals. Before signal processing, a visual inspection of the raw electromyographic trace was performed to exclude artefacts related to poor electrode contact, electrical interference or excessive skin movement. Application of the RMS filter made it possible to obtain averaged amplitude values reflecting the level of neuromuscular activation during the eccentric phase of movement.

The study was conducted at the Ariadna Medical Centre (Moscow, Russian Federation). The sample consisted of 9 individuals aged 44-60 years, including 7 women and 2 men. The study was carried out in accordance with the ethical principles of the Declaration of Helsinki [10] and was approved by the local ethics committee of the Ariadna Medical Centre. The study protocol No. 2 dated "06" June 2025 was registered. All participants had been informed in advance about the aims, possible risks and conditions of participation and had signed voluntary informed consent. Personal data of the participants were processed confidentially and were used exclusively for scientific purposes. All participants presented with impaired knee joint function, and the diagnosis of gonarthrosis of grade 1-2 had been established by a physician. Inclusion criteria were stable clinical status, absence of arthritis exacerbation and absence of lower limb injuries within the previous 6 months. Exclusion criteria were severe pain syndrome above 7 points on the VAS, as well as the presence of neurological disorders or comorbidities that limited the ability to perform physical exercises.

The participants were instructed to perform knee extension in an eccentric mode for 12 repetitions, 4 sets, with a 2-minute rest interval. The effort was performed with maximal possible force but without pronounced pain (up to 4 points on the VAS). Baseline testing was carried out before the start of the course, prior to 12:00. The eccentric phase was provided by manual resistance from the instructor. As pain intensity decreased during the course of sessions, the manual resistance was gradually increased, while pain was continuously monitored (kept at or below 4 points on the VAS). The use of manual resistance allowed individual dosing of the load and prevented excessive increase in pain response. Before each training session, a 15-minute massage of the anterior thigh and stretching of the quadriceps femoris muscle using the method of post-isometric relaxation (1 set, 5 repetitions) were performed. This preliminary myofascial intervention reduced muscle tone, improved local perfusion and prepared the muscle for eccentric loading. After completion of the treatment course, which included 12 sessions performed every other day, repeated testing was carried out. It was also performed before 12:00 to minimise the influence of circadian variations in pain sensitivity and muscle tone.

## RESULTS AND DISCUSSION

The study yielded data reflecting the dynamics of pain intensity, range of motion and muscle activation level after the course of eccentric training. The graphical materials presented below demonstrated changes in the investigated parameters compared with baseline values. Analysis of the results made it possible to identify stable positive trends in most participants (Fig. 1).



**Figure 1.** Mean amplitude of eccentric contraction of the quadriceps femoris muscle

Source: compiled by the authors

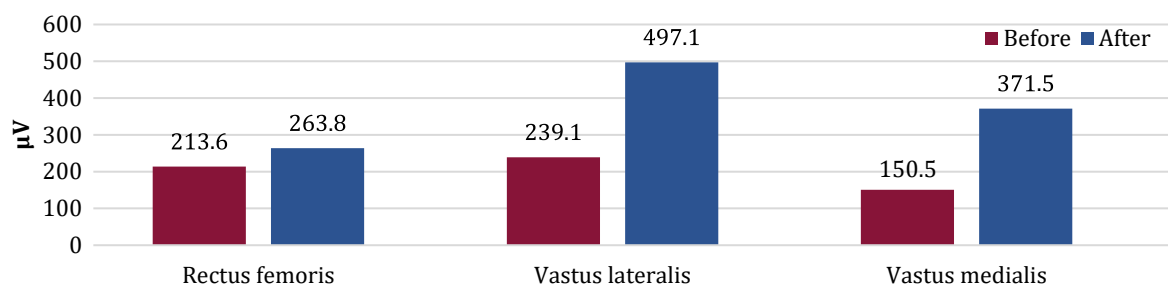
Figure 1 shows the mean electromyographic amplitude of eccentric contraction of the quadriceps femoris muscle: the rectus femoris increased from 87.1  $\mu$ V to 163.2  $\mu$ V, the vastus lateralis from 98.7  $\mu$ V at baseline to 298.4  $\mu$ V at the end of the intervention, and the vastus medialis from 59.3  $\mu$ V before to 220.7  $\mu$ V after the experiment. Analysis of the electromyography (EMG) results for the quadriceps femoris muscle indicated that the increase in mean EMG amplitude for each head had important functional significance. The increase in

rectus femoris amplitude reflected its active contribution to knee extension, which was essential for movements requiring strength and power, such as jumping and sprinting. The rise in activity of the vastus lateralis indicated its role in stabilising the knee joint during dynamic loading, which was critical for injury prevention and for maintaining appropriate movement mechanics. Similarly, the increased EMG amplitude of the vastus medialis pointed to its contribution to knee stabilisation, particularly during eccentric movements such as

downhill walking or landing after a jump. Increased activation of the deeper portions of the quadriceps could have been related to their role in supporting overall knee joint stability and distributing load between the other heads.

The pronounced increase in activity of the vastus medialis and vastus lateralis could be explained by the need to enhance knee joint stabilisation during eccentric exercises. These muscles helped to control knee motion and prevented lateral displacement, which was crucial for reducing the risk of injury. During eccentric exercise, load redistribution between the four heads

of the quadriceps might have occurred, leading to increased activity of the vastus medialis and vastus lateralis. This was important for appropriate force distribution and prevention of overloading of other muscles. Moreover, the rise in activity of these muscles could be regarded as an adaptive response to eccentric loading, allowing the musculature to cope better with mechanical demands and to increase its strength. To assess peak values of muscle activation, the maximal amplitude of eccentric contraction of the quadriceps femoris muscle was additionally analysed, and these results are presented in Figure 2.

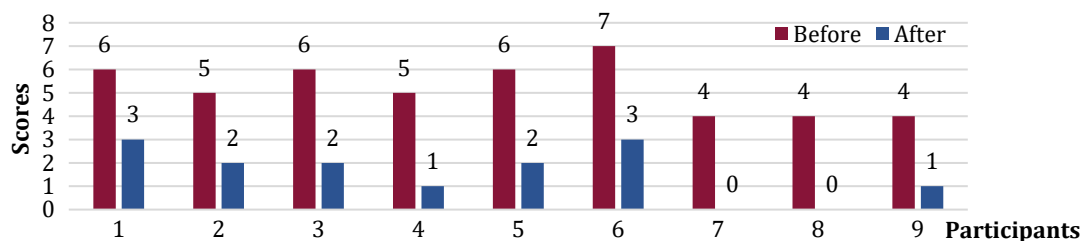


**Figure 2.** Maximal amplitude of eccentric contraction of the quadriceps femoris muscle

Source: compiled by the authors

Analysis of the EMG results for the maximal amplitude of the quadriceps femoris muscle demonstrated differences in the change of mean EMG amplitude for each head. The rectus femoris showed the smallest increase, from 213.6  $\mu$ V to 263.8  $\mu$ V, which indicated stable but relatively modest activity under eccentric conditions. In contrast, the vastus lateralis exhibited a pronounced rise in amplitude, increasing from 239.1  $\mu$ V to 497.1  $\mu$ V. This pointed to its important role in ensuring knee joint stability and adapting to eccentric loading, which could be critical for injury prevention and optimisation of movement mechanics. The vastus medialis also demonstrated a marked increase, with values rising from 150.5  $\mu$ V to 371.5  $\mu$ V. This confirmed its active involvement in controlling and stabilising the knee joint during eccentric exercise.

The difference in activity between the muscle heads could be explained by their specific functions in maintaining knee stability. The vastus lateralis and vastus medialis, as key stabilisers, were activated in response to eccentric loading, enabling more efficient force distribution and reducing the risk of injury. The rectus femoris, although active, did not show the same level of adaptation, which might have indicated a more limited role in this context. Overall, the increased activity of the vastus medialis and vastus lateralis reflected their capacity to adapt to eccentric modes of work, contributing to improved functional characteristics and enhanced overall strength. Figure 3 presents the results for pain intensity according to the VAS, where a reduction in pain syndrome by the end of the experiment was observed in all participants.



**Figure 3.** Results of pain assessment on the visual analogue scale

Source: compiled by the authors

The reduction in pain intensity after the eccentric exercises was associated with improved movement control, a decrease in the inflammatory component

and adaptation of the muscle-tendon structures. The experimental results showed heterogeneity in pain reduction among the participants, which might have

been due to differences in baseline pain levels. The obtained data were consistent with the known rehabilitative effects of eccentric loading in gonarthrosis. Previous studies indicated that eccentric exercises could lead to a considerable decrease in pain and an improvement in the functional status of the joints. In particular, they helped to strengthen the muscles sup-

porting the joint and contributed to enhanced joint stability. To evaluate the overall muscular response to the course of eccentric training, the total amplitude of electrical activity of the quadriceps femoris muscle was additionally analysed, which made it possible to assess the integrated contribution of all heads to knee joint extension (Fig. 4).

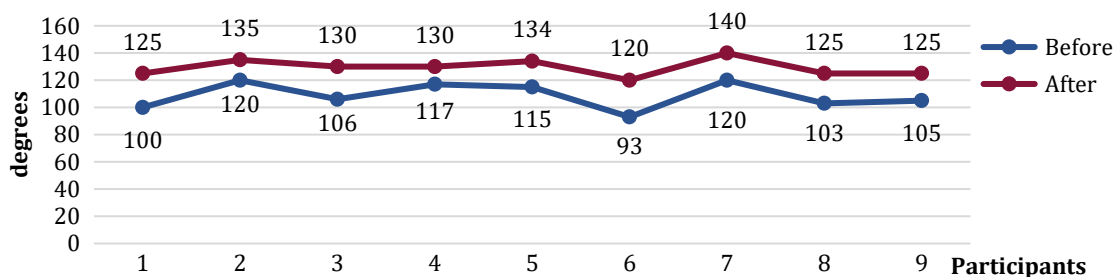


Figure 4. Knee flexion range of motion

Source: compiled by the authors

For a more comprehensive evaluation of the findings from Figure 4, they were compared with normal values of knee flexion range of motion for the corresponding age group. Normal values may vary, but on average, in healthy individuals aged 50-70 years, knee flexion is approximately 130-140 degrees. At the beginning of the course, many participants demonstrated a marked deficit in flexion range of motion, for example 93 degrees in one participant, which indicated pronounced limitation of mobility. After the course of sessions, most participants reached values close to normal, which testified to substantial progress. Improvement in knee flexion could have had a considerable

impact on gait. Due to the increase in flexion range, gait dynamics became smoother and more natural, which could lead to reduced fatigue during prolonged walking. Enhanced mobility also facilitated the performance of such tasks as squatting, stair climbing and other everyday activities, thereby improving quality of life. From a biomechanical perspective, an increased range of motion promoted more free execution of movements in daily life, which was important for preserving functional capacity. Joint and soft-tissue loading was reduced, which decreased the risk of injury. Figure 5 presents the results for knee extension range of motion before and after the experiment.

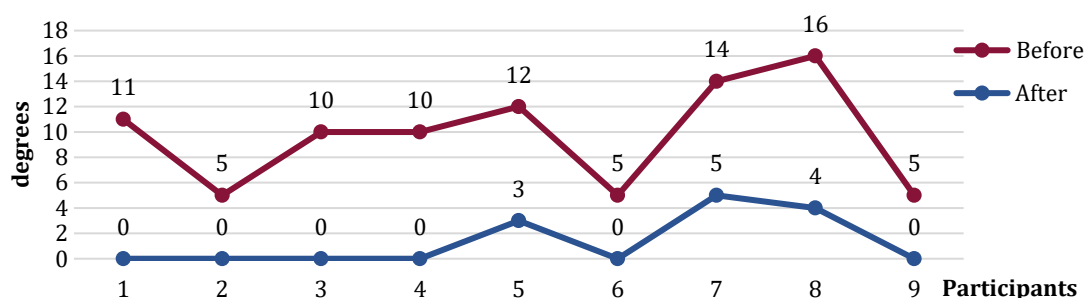


Figure 5. Knee extension range of motion

Source: compiled by the authors

A deficit of knee extension could have led to deterioration of gait biomechanics. Incomplete extension disrupted the normal rhythm and range of motion, resulting in inefficient distribution of load across the joints and muscles. The load on the anterior compartment of the knee joint also increased, which could have caused excessive cartilage wear and accelerated progression of gonarthrosis, as well as an increased risk of osteoarthritis or other degenerative changes in the patellofemoral joint. Reduced extension was likely to be

associated with pain, which limited physical activity and decreased patients' quality of life. As shown in Figure 5, the observed improvement in knee extension directly influenced pain scores on the VAS.

The present study was an initial stage aimed at evaluating the effectiveness of a short-term course of eccentric exercises for the quadriceps femoris muscle in patients with gonarthrosis of grades 1-2 (n=9). Standard tools commonly used in rehabilitation research were applied: subjective pain assessment using the VAS,

objective goniometry and surface electromyography to analyse muscle activation. The obtained results demonstrated positive dynamics for all three parameters: a substantial reduction in pain syndrome, an increase in range of motion (both knee flexion and extension) and an increase in EMG amplitude, particularly in the vastus medialis and vastus lateralis muscles. On the basis of this study, the method could be considered effective, with its effects explained through neuromuscular, morphofunctional and biomechanical components.

The studies by E. Hanci *et al.* [7] on the ankle joint were methodologically similar to the present research. They also employed methods consistent with the contemporary evidence-based rehabilitation approach, which required a combined assessment of subjective (pain, function) and objective (biomechanical, neurophysiological) parameters, including the same triad of VAS, goniometry and EMG. Comparable findings in the interpretation of EMG data were reported by P. Correia *et al.* [11], who examined the potential consequences of insufficient eccentric strength of the hamstring muscles. The results of the present study regarding the greatest increase in activation of the vastus medialis and vastus lateralis, which are key dynamic stabilisers of the knee joint, were in line with the findings of P. Correia *et al.*, who identified an activation deficit of specific heads of the hamstring muscles after injury. This confirmed that the beneficial effect of eccentric work was mediated not only by an increase in strength but also by normalisation of the recruitment pattern of motor units and improvement of joint control.

The present data were consistent with the results of the systematic review by H.Y. Lim & S.H. Wong [12], which showed that eccentric exercises exerted a pronounced analgesic effect and improved functional outcomes in athletes with patellar tendinopathy. Despite differences between populations, the proposed adaptation mechanisms – increased neuromuscular activation, improved movement coordination and redistribution of muscular effort – could have been universal and manifested in various musculoskeletal disorders. According to H.Y. Lim & S.H. Wong, eccentric contractions created conditions for more effective management of load on the affected structures, potentially reducing pain and improving movement control. These conclusions were in agreement with the observed changes in EMG amplitude and pain reduction in response to eccentric training, which supported its promise for both sports and clinical rehabilitation. P.R. Camargo *et al.* [13], in a study on shoulder impingement, used methods that differed from those of the present research. Their work employed ultrasound imaging and multiple orthopaedic tests and was characterised by a larger sample and longer duration (6 weeks with sessions every other day). Ultrasound imaging was able to visualise tendon integrity, degenerative changes, muscle thickness, micro-damage and

other structural alterations, which could be highly valuable in such studies.

The focus on joint pathology (gonarthrosis) and the use of eccentric training to improve joint function were directly related to the studies by D. Stasinopoulos *et al.* [14], who investigated tendinopathies. A common feature with the present research was the attempt to demonstrate that controlled eccentric loading, despite a potential risk of pain exacerbation, ultimately resulted in pain reduction and improvement in objective measures. In a study on the effects of isokinetic eccentric exercise on strength, muscle volume and postural stability, A.D. Kay *et al.* [15] reached similar conclusions that eccentric exercises promoted improvement of thigh muscle function and reported increases in strength and muscle volume of the knee extensors. The main difference from the present study was the evaluation of postural stability, which, according to the authors, was not influenced by exercises performed in the selected mode. It should also be noted that ultrasound imaging was used in that work. In a randomised controlled trial, B.A. Baxter *et al.* [16] indicated that one weekly session of submaximal eccentric resistance training over 12 weeks led to improvements in neuromuscular function comparable to those achieved with the currently recommended twice-weekly training. Given the considerable improvement in neuromuscular function and previously documented low adherence to twice-weekly training recommendations, eccentric training could have played a key role in developing minimal-load strategies to prevent neuromuscular decline. That study differed substantially from the present one, as it included a larger sample ( $n = 36$ ), employed a control group and had a 12-week intervention period. The main similarities with the current research lay in the focus on the effects of eccentric loading and the assessment of thigh muscles.

In the studies by E. Hanci *et al.* [7] and D. Stasinopoulos *et al.* [14], and in the present research, the methodology, results and data interpretations were broadly comparable. Their main difference lay in the fact that the above-mentioned works were large studies designed as randomised controlled trials that included a control group performing alternative exercises or receiving standard therapy, and they involved a larger sample. The present study, as noted earlier, represented an initial stage and conclusions regarding the effectiveness of the proposed protocol were therefore preliminary. A similar object of research was found in the work of J.P. Gordon *et al.* [17] on eccentric muscle contraction and its influence on functional outcomes. Both articles regarded eccentric exercises as a powerful training stimulus. Convergence was observed in the assessment of their key role in joint stabilisation and improvement of movement biomechanics, which in the present study was directly linked to pain reduction. The differences between the studies were fundamental: the

article by J.P. Gordon *et al.* investigated adaptations in healthy trained individuals with an emphasis on gains in strength, power and athletic performance, whereas the present research had a rehabilitative focus, involving a sample with gonarthrosis, and the key dependent variables were pain, range of motion and EMG activity as a marker of neuromuscular control rather than maximal strength. In the study by J.P. Gordon *et al.* the effectiveness of the protocol for performance enhancement was assessed, whereas in the current work its therapeutic effect (reduction in pain and increase in range of motion) was evaluated.

Additional mechanistic aspects of eccentric contractions could be explained in terms of the dependence of muscle force on the preceding history of stretch and shortening. As demonstrated by W. Herzog & T.R. Leonard [18], the force generated by skeletal muscle depended substantially on whether the contraction was preceded by stretch or shortening, which was reflected in the phenomena of residual force enhancement and residual force depression. These effects were particularly pronounced during eccentric contractions, when prior muscle stretch facilitated a subsequent increase in force. In view of these mechanisms, the increase in electromyographic amplitude observed after the course of eccentric exercises might have been related not only to enhanced neuromuscular activation but also to changes in the mechanical properties of the muscle in accordance with these regularities. This supported the view that eccentric loading was capable of eliciting specific adaptive responses that improved the efficiency of muscle work and enhanced movement control in the presence of functional knee joint disorders.

The findings were consistent with current concepts of the role of knee joint morphotype in the development of degenerative changes. In particular, the study by L. Kylymniuk [19] showed that structural abnormalities in the proximal tibia could influence load distribution within the knee joint and promote the progression of degenerative processes. Taking these data into account, the increase in neuromuscular activation and stabilising function of the quadriceps femoris observed after eccentric training could be considered a compensatory mechanism that potentially reduced loading of altered joint structures. This underlined the clinical relevance of eccentric exercises in patients with early manifestations of gonarthrosis. The data obtained from the analysis of electromyographic activity were in line with the results of F. Hanno *et al.* [20], who reported that amplitude and frequency characteristics of the EMG signal are important indicators of the functional state of muscles and allow an objective assessment of the degree of their activation. The authors emphasised that changes in EMG amplitude may reflect both neuromuscular and mechanical adaptive processes induced by the training stimulus. These conclusions were consistent with the observed increase in EMG amplitude of the quadriceps

femoris after eccentric exercises, indicating the development of adaptations aimed at improving movement control and strengthening the stabilising function of the knee joint.

Modern approaches to the management of gonarthrosis have implied a comprehensive intervention aimed at reducing pain, improving joint function and enhancing patients' quality of life. In the review by M.A. Kolesnikov [21] it was emphasised that non-pharmacological strategies, including therapeutic exercise and graded resistance training, were key components of treatment because they strengthened the musculo-ligamentous apparatus and optimised knee joint biomechanics. In this context, the increase in neuromuscular activation and the improvement in range-of-motion characteristics observed after the application of eccentric exercises in the present study could be viewed as consistent with contemporary principles of rehabilitation for gonarthrosis. These results supported the notion that eccentric training could serve as an effective functional intervention aimed at reducing load on damaged joint structures and stabilising joint performance.

Thus, the obtained data demonstrated a marked reduction in pain, an increase in range of motion and a substantial rise in neuromuscular activation of the quadriceps femoris muscle after the course of eccentric exercises. The positive dynamics were observed in all participants, indicating the universality of the identified adaptive changes. Improvements in mean and peak EMG values reflected an enhanced functional capacity of the muscle to control movement and stabilise the knee joint. Taken together, these changes indicated the formation of stable adaptations directed towards optimisation of motor function in the presence of degenerative knee joint alterations.

## CONCLUSIONS

The results of the present study showed that a course of eccentric exercises exerted a pronounced positive effect on the functional state of the quadriceps femoris muscle and on pain intensity in patients with gonarthrosis of grades 1-2. After only 12 training sessions performed every other day, a clear positive trend was observed across all key parameters. According to the visual analogue scale (VAS), a reduction in pain was recorded in all participants. The maximal baseline pain level was 7 points, whereas by the end of the course the values had decreased to the range of mild or moderate pain, which confirmed the clinical effectiveness of eccentric loading in relation to pain symptoms. Electromyographic analysis also demonstrated significant improvements. The mean amplitude of electrical activity of the rectus femoris increased from 87.1  $\mu$ V to 163.2  $\mu$ V, reflecting an enhanced contribution to knee extension. The vastus lateralis showed an even more pronounced change, from 98.7  $\mu$ V to 298.4  $\mu$ V, indicating a strengthening of its stabilising role. The amplitude of the vastus medialis rose

from 59.3  $\mu\text{V}$  to 220.7  $\mu\text{V}$ , which testified to improved movement control and redistribution of load within the knee joint. The increase in EMG activity of all investigated heads confirmed the development of adaptive neuromuscular responses to the eccentric work regime.

In addition, an increase in knee joint range of motion was observed, reflecting improved biomechanics and enhanced functional mobility of the limb. This was of practical importance for patients with osteoarthritis, in whom restricted mobility is a major factor contributing to reduced quality of life. Thus, eccentric exercises demonstrated effectiveness in reducing pain, increasing muscle activation and improving range of motion. The obtained findings supported the feasibility of incorporating eccentric training into rehabilitation programmes for patients with early stages of knee gonarthrosis. Further research is required to examine the long-term effects of eccentric exercises in order to determine whether improvements in pain, strength and range of motion are maintained after completion of the

course and whether maintenance therapy is necessary. An important avenue for future work is the comparison of eccentric exercises with other rehabilitation modalities to assess their relative effectiveness and to define optimal conditions for their use. The development of individualised eccentric training programmes with tailored intensity, frequency and volume for different patient groups also remains a promising direction. Subsequent studies in these areas may help to increase the effectiveness of rehabilitation and improve the quality of life of patients with knee osteoarthritis.

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## CONFLICT OF INTEREST

None.

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## **Эксцентрикалык режимде булчуң жыйрылуусунун тизе муунунун функциясы бузулган адамдарда оорунун интенсивдүүлүгүнө жана кыймылдын чектелишине тийгизген таасири**

**Кирилл Чихалин**

Аспирант

«Ленин ордендүү Мамлекеттик борбордук дене тарбия институту» Россия спорт университети  
105122, Сиреневый б-р, 4, Москва ш., Россия Федерациясы

ORCID

**Татьяна Гричанова**

Биология илимдеринин кандидаты, доцент

«Ленин ордендүү Мамлекеттик борбордук дене тарбия институту» Россия спорт университети  
105122, Сиреневый б-р, 4, Москва ш., Россия Федерациясы

<https://orcid.org/0000-0001-7359-4338>

**Аннотация.** Тизе муунунда оору жана кыймылдын чектелиши менен кайрылган бейтаптардын санынын өсүшү остеоартроздун баштапкы стадияларында функцияны калыбына келтирүүнүн эффективдүү ыкмаларын издөөдөн өзгөчө мааниге ээ кылды. Изилдөөнүн максаты – тизе муунунун функциясы бузулган адамдарда оорунун интенсивдүүлүгү, муундагы кыймылдын чектелиши жана булчуң жыйрылуусунун эксцентрикалык режими боюнча параметрлердин ортосундагы мүмкүн болгон байланышты аныктоо болгон. Изилдөөнүн алкагында медициналык борбордо тизе остеоартрозунда булчуң жыйрылуусунун эксцентрикалык режими изилденип, эксперимент жүргүзүлгөн. Реабилитациялык сессиялар учурунда сан сөөгүнүн төрт баштуу булчуңунун жыйрылуусунун эксцентрикалык фазасына өзгөчө көңүл бурулган. Экспериментке чейин жана андан кийин электромиографтын көрсөткүчтөрү, оорунун интенсивдүүлүгү визуалдык аналогдук шкала (ВАШ) боюнча, ошондой эле муундагы кыймыл амплитудасы гониометрдин жардамы менен катталган. Сан сөөгүнүн төрт баштуу булчуңунун эксцентрикалык жыйрылуусунун орточо амплитудасы эки эседен ашык өскөн; мисалы, латералдык кең булчуңда көрсөткүчтөр 98,7 мкВдан 298,4 мкВга чейин жогорулаган. Сабактар курсунан кийин тогуз катышуучунун алтысында тизе муунун толук жазуу калыбына келтирилип, бардык изилденгендерде оорунун деңгээли азайган, алардын экөөндө оору толугу менен жоголгон. Изилдөөнүн жыйынтыктары боюнча булчуң жыйрылуусунун эксцентрикалык режими оорунун деңгээлин төмөндөтүүдө, кыймыл амплитудасын жогорулатууда жана электромиографиялык активдүүлүктүн көрсөткүчтөрүн күчөтүүдө эффективдүү экени көрсөтүлгөн. Эксцентрикалык режим булчуң күчүн жана кыймыл учурундагы нейромускулалык көзөмөлдү өнүктүрүп, муун кемирчегине түшкөн механикалык жүктү азайтты. Бул изилдөөдө алынган натыйжалар сан сөөгүнүн төрт баштуу булчуңу үчүн эксцентрикалык режимде аткарылган көнүгүүлөр тизе остеоартрозунун баштапкы стадияларында булчуң алсыздыгы сыяктуу себептерге жана оору, муундагы кыймыл амплитудасынын чектелиши сыяктуу кесепеттерге бир убакта таасир этүү аркылуу эффективдүү болушу мүмкүн экенин көрсөттү. Алынган маалыматтар тизе остеоартрозунун эрте формалары бар бейтаптар үчүн индивидуалдык реабилитациялык программаларды иштеп чыгууда колдонулушу мүмкүн.

**Негизги сөздөр:** гонартроз; электромиография; негативдүү фаза; тизе оорусу; кыймыл амплитудасы

## **Влияние эксцентрического режима мышечного сокращения на интенсивность боли и ограничения подвижности у лиц с нарушением функций коленного сустава**

**Кирилл Чихалин**

Аспирант

Российский университет спорта

«Государственный центральный ордена Ленина институт физической культуры»

105122, б-р Сиреневый, 4, г. Москва, Российская Федерация

ORCID

**Татьяна Гричанова**

Кандидат биологических наук, доцент

Российский университет спорта

«Государственный центральный ордена Ленина институт физической культуры»

105122, б-р Сиреневый, 4, г. Москва, Российская Федерация

<https://orcid.org/0000-0001-7359-4338>

**Аннотация.** С ростом числа пациентов с болевым синдромом и ограничением подвижности коленного сустава особое значение приобретает поиск эффективных методов восстановления функции при начальных стадиях остеоартроза. Целью исследования было выявить возможную взаимосвязь интенсивности боли, ограничений движений в суставе и параметров эксцентрического сокращения у лиц с нарушением функций коленного сустава. В рамках проведенного исследования на базе медицинского центра проведен эксперимент по изучению эксцентрического режима мышечного сокращения при остеоартрозе коленного сустава. Во время реабилитационных сессий делался упор на эксцентрическую фазу сокращения четырехглавой мышцы бедра. Оценивались показания миографа, уровень боли по визуально-аналоговой шкале и амплитуда движения в суставе при помощи гониометра до и после эксперимента. Средняя амплитуда эксцентрического сокращения четырехглавой мышцы увеличилась более чем в два раза: например, у латеральной широкой мышцы – с 98,7 мкВ до 298,4 мкВ. После курса занятий у шести из девяти участников было достигнуто полное разгибание в коленном суставе, а уровень болевого синдрома снизился у всех испытуемых, включая полное исчезновение боли у двух человек. По результатам исследования эксцентрический режим мышечного сокращения показал свою эффективность в снижении уровня боли, повышении амплитуды движений, а также увеличения показателей электромиографической активности. Эксцентрический режим развил силу мышц, нейромышечный контроль при движениях, снижает механическую нагрузку на суставной хрящ. Результаты данного исследования продемонстрировали, что упражнения в эксцентрическом режиме для четырехглавой мышцы бедра, при начальных стадиях остеоартроза коленного сустава, могут быть эффективны, воздействуя одновременно на причину (мышечная слабость) и следствие (боль, ограничение амплитуды движений в суставе). Полученные результаты могут быть использованы при разработке индивидуальных реабилитационных программ для пациентов с начальными формами остеоартроза коленного сустава

**Ключевые слова:** гонартроз; электромиография; негативная фаза; боль в коленном суставе; амплитуда движения

## **Comparative analysis of diet, protein and vitamin-mineral status of Indian vegetarian students with different durations of stay in the Kyrgyz Republic and permanent residents of India**

**Ravshanbek Dzhumaev\***

PhD in Medical Sciences, Associate Professor  
Osh International Medical University  
715000, 428 Alymbek Datka Str., Osh, Kyrgyz Republic  
<https://orcid.org/0009-0006-2129-4943>

**Lazokatkhon Dzhumaeva**

PhD in Medical Sciences  
Osh State University  
715000, 331 Alymbek Datka Str., Osh, Kyrgyz Republic  
<https://orcid.org/0000-0002-5465-2528>

**Nasiba Dzhumaeva**

Student  
Osh State University  
715000, 331 Alymbek Datka Str., Osh, Kyrgyz Republic  
<https://orcid.org/0009-0002-1158-6142>

**Abstract.** The aim of the study was to examine the characteristics and composition of the diet, including macro- and micronutrients, of Indian students adhering to a vegetarian lifestyle while residing in the Kyrgyz Republic, taking into account the duration of their stay, and to compare these indicators with a control group residing in India. The study included three groups of 50 participants each: newly arrived students in the Kyrgyz Republic (Group 1), students residing in the Kyrgyz Republic for more than four years (Group 2), and a control group living in India. Dietary assessment was performed using a 30-day food diary method. Based on the collected data, average daily energy intake, consumption of proteins, fats and carbohydrates, as well as dietary diversity, were analysed. Using food composition reference tables, the approximate intake of macronutrients and selected micronutrients in the participants' diets was calculated. The results demonstrated that both Group 1 and Group 2 showed reduced intake of proteins and fats, as well as a lower proportion of legumes and dark green vegetables, compared with the control group from India. Adequate overall caloric intake was achieved mainly through increased carbohydrate consumption, including foods with low nutritional value (snacks, sweets), particularly among newly arrived students. In Group 1, reduced dietary intake of iron, vitamin B<sub>12</sub> and vitamin A was also observed. Students who had resided in the Kyrgyz Republic for a longer period demonstrated a tendency towards dietary improvement; however, diet quality remained inferior to that of the control group. Conclusions drawn from the obtained data indicated a risk of developing protein-energy and vitamin-mineral deficiencies. This highlighted the need to develop adapted dietary patterns with increased diversity of plant-based foods to prevent deficiencies and improve the health status of foreign students during the adaptation period. The study results could be used in the development of adapted diets for foreign vegetarian students aimed at preventing nutritional deficiencies

**Keywords:** diet; dietary intake; protein-energy deficiency; vitamin and mineral deficiency; dietary adaptation

### **Suggested Citation:**

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\*Corresponding author



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## INTRODUCTION

A significant proportion of international students from India, due to cultural and religious traditions, adhered to a vegetarian dietary pattern. Vegetarianism, despite certain positive aspects, was associated with a number of potential risks and specific features that needed to be taken into account when organising adequate nutrition. An unbalanced vegetarian diet was often characterised by a deficiency of several key nutrients, such as proteins, essential amino acids, iron, and vitamins, particularly vitamin B<sub>12</sub>. A lack of these elements could lead to deficiency states, reduced immunity, deterioration of general well-being, and decreased work capacity [1].

In the study by R.M. Dzhumaev *et al.* [2], the impact of a vegetarian diet on the development of protein-energy and vitamin-mineral deficiencies in Indian students studying abroad was examined. It was demonstrated that an unbalanced plant-based diet was accompanied by reduced intake of protein, iron, and several vitamins, which increased the risk of nutritional deficiencies and deterioration of overall health status. At the same time, in India, the majority of the population obtained approximately 60% of protein from cereal crops. However, even with adequate availability of legume products, the requirement for certain individual amino acids might not have been fully met [3].

A number of studies also emphasised that relocation was accompanied by changes in diet and potential health risks. In the article by R. Ysmailova *et al.* [4], the socio-psychological aspects of adaptation of international students in the Kyrgyz Republic were examined. The influence of nutrition and unfamiliar foods on students' health and academic performance was also noted. In the study by I.A. Lesser *et al.* [5], the relationship between acculturation and dietary habits among immigrants from South Asia (including India) who had relocated to Canada was investigated. It was shown that with increasing length of residence in the host country, the diet of the surveyed participants changed: consumption of local vegetables and fruits increased, food preparation methods improved, but at the same time intake of processed foods, ready-to-eat meals, and sugar-sweetened beverages rose. As a result, both positive and negative dietary changes were demonstrated. However, the conclusions were limited to Western settings and did not identify vegetarians as a separate group. In the study by L.S. Panchenko [6], the nutrition of international students studying in Volgograd was analysed. The study showed that many students retained previous dietary habits typical of a tropical climate; however, these diets were insufficient in terms of protein-fat composition and vitamin content, especially during the cold season in a sharply continental climate, which was also characteristic of the Kyrgyz Republic. As a consequence, students experienced difficulties with physiological adaptation. J.V. Varre *et al.* [7] emphasised that after relocation,

migrants' habitual diets changed: among representatives from South Asia, consumption of traditional whole grains, legumes, vegetables, and fibre-rich foods decreased, while the intake of processed foods increased. Dietary changes were influenced by factors such as length of residence, food availability, and socio-economic status. These changes also led to adverse outcomes, including an increased risk of obesity and metabolic diseases, confirming potential health problems during dietary adaptation. T.P. Yurochko *et al.* [8] reported that the diet of international students in Ukraine changed after relocation; dietary composition, types of foods, and frequency of consumption were assessed. Most students confirmed changes in their diet; however, no comparative "before/after" analysis was conducted. At the local level in the Kyrgyz Republic, A. Murzalieva & M. Manivannan [9] identified that international students faced problems of undernutrition and unbalanced diets. However, the study was predominantly descriptive and did not include dietary intake analysis, highlighting the need for more detailed research. In the study by E. Filipchenko *et al.* [10], the adaptation of Indian students in the Kyrgyz Republic was examined. It was demonstrated that adaptation affected physiological health and body condition, and that nutrition, through dietary characteristics, was considered a possible influencing factor. This reinforced the importance of studying diet and eating habits. However, it should be noted that the article did not provide a detailed dietary analysis and assessed only indirect biomarkers, namely the condition of the buccal epithelium and teeth. The study by A. Moldobaeva *et al.* [11] revealed that after relocation, students of medical universities in the southern part of the Kyrgyz Republic experienced dietary changes. Most students showed reduced consumption of fruits, vegetables, and whole-grain products, indicating a potential deficiency of essential vitamins, minerals, and dietary fibre. In addition, a large proportion of students relied on eating outside the home, which created difficulties in controlling food choices. Problems in finding healthy and preferred foods after relocation were also identified. This study highlighted that the nutrition of international students in the Kyrgyz Republic required special attention. Students faced the need to adapt to a new food environment, organise their own meals, and cope with limited access to familiar foods. Moreover, in the studies mentioned above, vegetarianism was not identified as a separate research group. Given that a vegetarian diet with an established system for compensating potential deficiencies (for example, through legumes and spices) was widespread in India, while the cuisine of the Kyrgyz Republic predominantly emphasised meat-based dishes with limited variety of plant foods, it could be assumed that international students from India who

continued to adhere to vegetarian diets under new conditions encountered difficulties in maintaining their previous nutritional balance.

The aim of the present study was to assess the diet of Indian vegetarian students residing in the Kyrgyz Republic, including the dietary content of macronutrients, iron, and vitamins B<sub>12</sub> and A, taking into account the duration of their stay, as well as to compare these indicators with a group continuing to reside in India. The study hypothesis was that the characteristics and quality of the diet of students from India underwent changes during residence in the Kyrgyz Republic, which could lead to reduced intake of key nutrients, primarily protein, iron, and vitamins B<sub>12</sub> and A.

The objectives of the study were as follows:

- to conduct a comparative analysis of the actual dietary intake of three groups: students and employees residing in India (control group), students from India in their first-second year of study in the Kyrgyz Republic, and students from India in their fourth-fifth year of study in the Kyrgyz Republic;

- to assess changes in the intake of key nutrients (protein, vitamins B<sub>12</sub> and A, and iron) depending on the duration of residence in the Kyrgyz Republic;

- to analyse possible causes and factors hindering adherence to a habitual vegetarian diet abroad (food availability, dietary preferences, and eating conditions).

## MATERIALS AND METHODS

Participant recruitment, data collection, and data analysis were conducted from October 2022 to May 2023. Based on data reported in the studies by R.S. Bhatnagar & O.I. Padilla-Zakour [12] and S. Agrawal *et al.* [13] indicating a substantial proportion (>29%) of the vegetarian population in India, fourteen research publications were reviewed using the scientific electronic library eLibrary and the biomedical literature search engine PubMed to examine the impact of an unbalanced vegetarian diet on population health. An analysis of the risks of development and consequences of protein-energy malnutrition, as well as mineral and vitamin deficiencies, was carried out.

Subsequently, an assessment of the diets of three groups of 50 individuals each was conducted using the dietary record method to evaluate actual food intake. Participants who adhered to a vegetarian diet with permitted consumption of eggs and dairy products were selected. The first group consisted of newly arrived students from India in the Kyrgyz Republic, while the second group included students who had been residing in the Kyrgyz Republic for more than 4-5 years. Students of both sexes aged 18-34 years who were studying at Osh State University at the International Medical Faculty were selected. The third group served as a control for the first two groups and consisted of students and employees from various sectors residing in India and consuming a traditional plant-based diet. This group

included individuals living in different states and cities of India corresponding to the regions of origin of students in the first two study groups: the states of Tamil Nadu, Sikkim, Uttar Pradesh, Bihar, West Bengal, Telangana, Puducherry, Karnataka, the city of Delhi, and the Kashmir region. This group comprised students and workers from the educational, banking, and industrial sectors aged 19-42 years. The control group included individuals without severe chronic diseases and not experiencing material deprivation.

All participants provided voluntary informed consent to participate. The collected data were anonymised to ensure confidentiality. Study participants were asked to keep dietary records designed to be completed over a 30-day period. Each day was divided into four sections, in which all foods consumed at breakfast, lunch, dinner, and during snacks were to be recorded. The diaries listed mixed dishes as well as individual foods and beverages consumed during the day. For more accurate data analysis, participants were encouraged to specify the individual ingredients comprising mixed dishes; however, this clarification was not a mandatory requirement. For dishes for which full ingredient composition was not indicated, data from open sources on ingredient content were used. The study was conducted in accordance with the ethical principles of the Declaration of Helsinki [14] and was approved by the Ethics Committee of the International Medical Faculty of Osh State University (Protocol No. 16, 07.10.2022). All participants provided voluntary informed consent, and the collected data were anonymised to ensure confidentiality.

The foods consumed by the study participants were classified into main food groups using the minimum dietary diversity recommendations, which included the following categories: (1) potatoes, (2) beans, peas, and other legumes, (3) nuts and seeds, (4) dairy products, (5) eggs, (6) dark green leafy vegetables, (7) root vegetables, (8) other vegetables, (9) fruits, and (10) cereals and bakery products. Based on the dietary diary data provided, indicators such as average daily energy intake, quantitative protein consumption, and dietary diversity were analysed. Dietary diversity was defined as the sum of food categories consumed from the ten groups proposed in the present study. In addition, a separate supplementary group of foods was identified that was not included in the calculation of minimum dietary diversity. This group comprised sugar-sweetened beverages, sweets, chocolate, cakes, biscuits, chips, snacks, and other products with high sodium and sugar content. In the present study, these products were considered not to meet healthy requirements for macro- and micronutrients. A 10-point scale was used to assess dietary diversity, corresponding to the ten main food group categories, excluding unhealthy products and oils. The collected data on food consumption formed the basis for assessing macronutrients (proteins, fats, and carbohydrates) and three micronutrients (iron, vitamin B<sub>12</sub>,

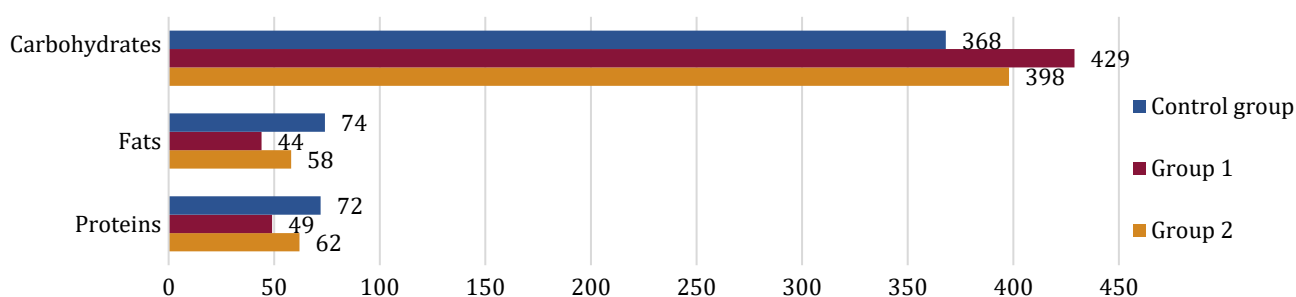
and vitamin A), deficiencies of which vegetarians were most susceptible to. Data on the average nutritional value and micronutrient composition of each food item were obtained from the food composition tables edited by I.M. Skurikhin [15] and the U.S. Department of Agriculture, Agricultural Research Service [16].

To assess the statistical significance of differences between the study groups, a one-way analysis of variance (ANOVA) was performed. This method allowed the full set of observations within each group to be taken into account when calculating the F-statistic and determining whether significant differences existed between groups in terms of protein, fat, carbohydrate, vitamin B<sub>12</sub>, iron, and vitamin A content. The selected significance level was  $\alpha = 0.05$ . The critical F value was 3.06.

No statistically significant differences were identified for mean daily energy intake (F-statistic = 2.25). In contrast, for the remaining indicators (protein intake, proportion of unhealthy foods, iron content, vitamin B<sub>12</sub>, and vitamin A), the differences were statistically significant (F-statistic exceeded the critical F value,  $p < 0.05$ ).

## RESULTS AND DISCUSSION

Within the framework of the study, the mean daily energy intake of the diets of three groups was assessed: the control group (residing in India), newly arrived students from India studying in the Kyrgyz Republic (Group 1), and students from India who had been residing in the Kyrgyz Republic for more than 4-5 years (Group 2) (Fig. 1).



**Figure 1.** Mean daily nutritional value of the diet, g

**Source:** developed by the authors

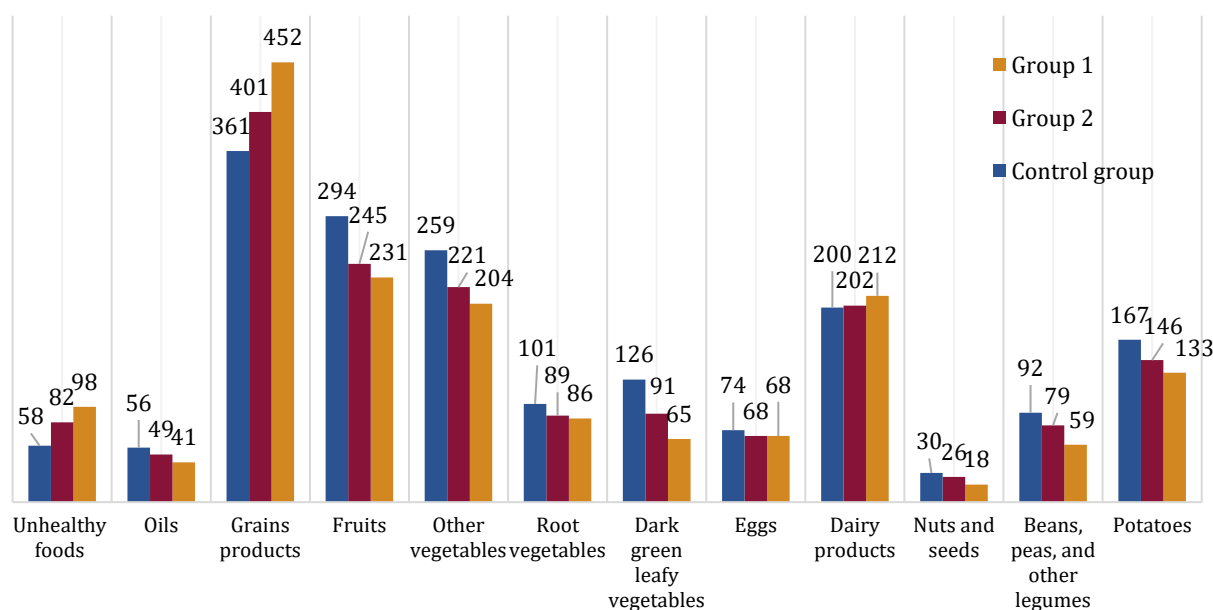
The average daily caloric intake of the control group was 2,426 kcal, in Group 1 – 2,308 kcal, and in Group 2 – 2,362 kcal. Despite a slight decrease in the energy value for students residing in the Kyrgyz Republic, the results of one-way analysis of variance (ANOVA) did not reveal any statistically significant differences in caloric intake between the groups. This indicates that the overall energy provision for the students remained at a comparable level. However, when analyzing average protein consumption, significant differences were observed. In the control group, the average daily protein intake was 72 g, in Group 1 – 49 g (68% of the control group), and in Group 2 – 62 g (86% of the control group). ANOVA showed the statistical significance of these differences: the calculated F-statistic was 103.7. This indicates a significant reduction in protein content in the diet of students who arrived in the Kyrgyz Republic, especially during the early stages of the adaptation period. Thus, despite maintaining overall caloric intake, there was a substantial change in the macronutrient balance, with a decrease in protein and an increase in carbohydrates, which may indicate a risk of protein deficiency among students, particularly during the initial stages of living in a new country. Next, the mass consumption of products from different categories was studied (Fig. 2). The products were divided into 12 categories: potatoes, legumes, nuts and seeds, dairy products, eggs, dark green leafy vegetables, root

vegetables, other vegetables, fruits, grains and bakery products, oils, and unhealthy foods with high added sugars and sodium.

The analysis showed that the main component of the diet in all groups was cereal products. Their consumption among students residing in the Kyrgyz Republic was significantly higher than in the control group, especially during the initial adaptation period (Group 1), where a 25% increase was observed. In Group 2, this indicator decreased but remained 11% higher than in the control group. At the same time, the consumption of most other product categories (legumes, dark green leafy vegetables, fruits, other vegetables, potatoes, root vegetables) among students in the Kyrgyz Republic was lower compared to the control group. A noticeable decrease in the consumption of legumes and dark green leafy vegetables was particularly evident, which may indicate limited diversity and a lack of certain nutrients in the diet of Group 1. Dairy products were consumed at approximately the same level as in the control group, and the consumption of eggs and oils was slightly reduced. An interesting feature was the higher consumption of “unhealthy” products with high added sugar and sodium content among students in the Kyrgyz Republic, especially among newcomers, which was statistically significant. The results of the one-way analysis of variance showed high statistical significance of the differences (F-statistic = 11.7), indicating an in-

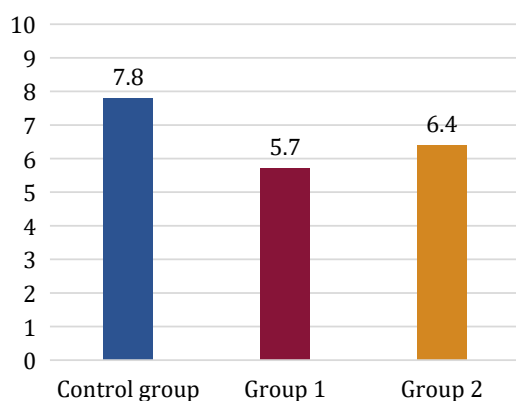
crease in the proportion of unhealthy food in the diet of foreign students, particularly during the early stages of

adaptation. Additionally, the average daily diversity of the students' diet was assessed (Fig. 3).



**Figure 2.** Average daily consumption of products in the specified categories in grams

Source: developed by the authors

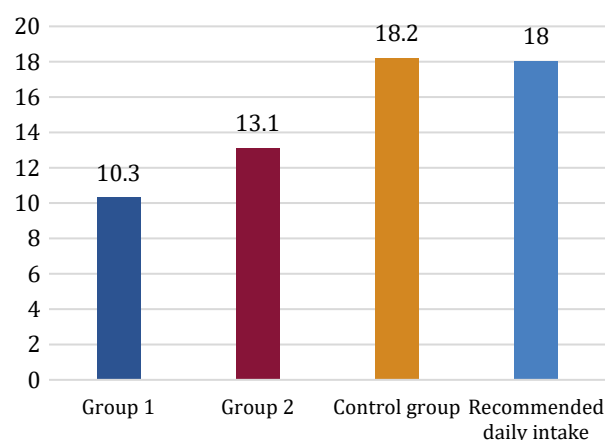


**Figure 3.** Average daily diet diversity

Source: developed by the authors

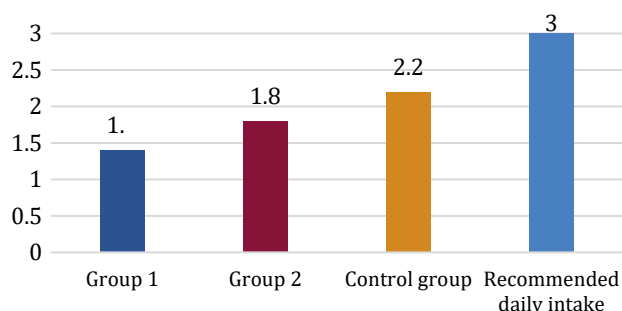
The assessment of the students' diet diversity on a 10-point scale revealed differences between the groups. In Group 1, the students who had just arrived in the Kyrgyz Republic, the average diversity score was 5.7, indicating a limited range of consumed product categories. Students in Group 2 demonstrated a higher level of diversity – 6.4, reflecting the gradual adaptation of their diet and an expansion of the range of consumed products with an increasing length of stay. This may be related to foreign students becoming more familiar with the local food assortment over time, as well as a loss of initial caution towards unfamiliar products and cooking methods. The control group, which continued to live in India, maintained the highest level of diversity at 7.8, which corresponds

to a traditional diet with a broad range of food categories. Thus, long-term stay in the Kyrgyz Republic contributes to a more balanced and diverse diet, but the diet still requires adjustments, especially concerning the consumption of legumes and dark green vegetables. Using food composition and micronutrient reference books, the approximate content of the main micronutrients, which are predominantly found in animal products, was calculated. The consumption of these nutrients is limited for lacto-ovo-vegetarians due to the absence of meat in their diet. Figures 4-6 present the average daily consumption values of iron, vitamin B<sub>12</sub>, and vitamin A.



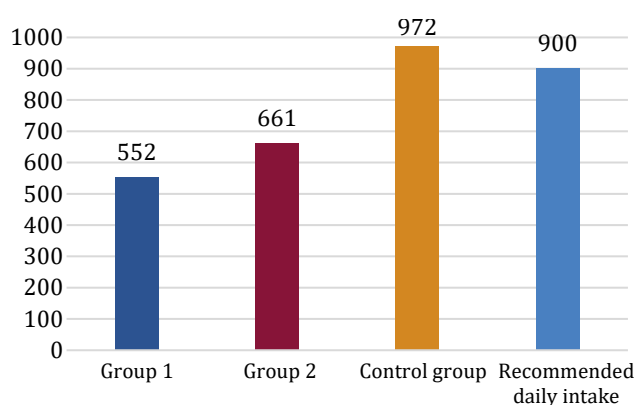
**Figure 4.** Daily iron consumption, mg

Source: developed by the authors



**Figure 5.** Daily vitamin B<sub>12</sub> consumption, µg

Source: developed by the authors



**Figure 6.** Daily vitamin A consumption, mcg

Source: developed by the authors

The recommended daily intake of iron was taken as the average value between the consumption recommendations for women and men, which is 18 mg. The diet of the control group fully meets the body's iron needs with 18.2 mg. Meanwhile, in Group 1, the average daily iron intake was only 10.3 mg, which is significantly lower than the recommended intake and indicates a risk of iron deficiency. In Group 2, iron consumption was higher (13.1 mg), but still insufficient to fully meet the body's needs. The analysis of vitamin B<sub>12</sub> consumption showed that none of the groups reached the recommended daily intake of 3 µg. The highest intake was observed in the control group – 2.2 µg, which is about 73% of the recommended value. Students in Groups 1 and 2 received only 46% (1.4 µg) and 60% (1.8 µg) of the recommended intake, respectively, which indicates a possible risk of vitamin B12 deficiency. The minimum recommended intake of vitamin A is 900 µg. The average vitamin A content in the diet of the control group exceeded this value by 72 µg, which is still below the upper limit for vitamin A consumption. However, in Groups 1 and 2, there is a deficiency of vitamin A: the average consumption is 552 µg and 661 µg, respectively, which is below the recommended value and may indicate a risk of hypovitaminosis. The data obtained demonstrate that students from India living in the Kyrgyz Republic, especially in the early years of their stay, experience significant changes in dietary habits and

food intake compared to the control group remaining in India. Despite comparable total caloric intake, students living in the Kyrgyz Republic show a decrease in protein, vitamin (B<sub>12</sub>, A), and essential micronutrient intake, such as iron.

The main reason for the change in the quality of the diet is related to the limited availability of legumes, which are typical in Indian cuisine, as well as a limited range of vegetables and fruits, particularly in the autumn and winter periods. In the Kyrgyz Republic, replacing familiar plant-based proteins is difficult due to the smaller selection of legumes, which can be explained by differences in food traditions – eat is the primary source of protein in the local diet, which reduces the demand for and availability of legume products. Furthermore, seasonal fluctuations in the availability of fresh vegetables and fruits exacerbate deficiencies in vitamins and minerals. An additional factor contributing to changes in the diet is adaptation to a new lifestyle. Studying, practical training, stress, and other everyday circumstances influence eating habits and food choices. It is noticeable that students who have been living in the Kyrgyz Republic for more than 4-5 years have a more balanced diet, indicating gradual adaptation to the new conditions. However, despite the improvements, the diet of all students living in the Kyrgyz Republic still requires adjustments and rationalisation.

The study of the eating habits of the investigated groups of vegetarian students from India revealed deficiencies and potential risks associated with the lack of macro- and micronutrients, as well as imbalances in energy provision. To gain a deeper understanding of the dietary characteristics of vegetarians, it is advisable to compare the results of this study with the findings of D.O. Gorbachev *et al.* [17], which examined the actual content of macro- and micronutrients in the diet and biochemical status of vegetarians in Samara. The study focused on vegetarians without specifying their status as immigrants, while the current study focused on the diet of foreign students. Nevertheless, comparing the two studies allowed us to observe which nutrients were actually deficient according to the biochemical markers in the blood of vegetarians. The protein consumption analysis showed that vegetarian students from India had insufficient intake (49 g/day and 62 g/day), which is below the control group value (72 g/day). These results are consistent with the findings of D.O. Gorbachev *et al.*, where vegetarian consumption was 57-58 g/day. As for micronutrients, the deficiency of vitamin B<sub>12</sub> and iron was observed in all groups, with the lowest levels in Group 1 (B<sub>12</sub> – 1.4 µg/day, iron – 10.3 mg/day). A similar situation was observed in Samara, where most vegetarians had lower levels of vitamin B<sub>12</sub> and iron in their blood. The concentration of retinol in plasma was close to the lower limit of the norm, which correlates with the results of the current study, as a clear decrease in retinol in the blood is only observed after its reserves

in the liver are depleted. Thus, the comparison of the data shows that regardless of geographical and social context (Samara vs immigrant students), the diet of vegetarians can lead to deficiencies in protein and key vitamins, particularly B<sub>12</sub>, iron, and vitamin A. In the study by D.S. Yasakov *et al.* [18], the impact of a vegetarian diet on nutrient status, health, and deficiency risks was analysed. This article identified typical deficiencies common among vegetarians – iron, vitamins A, B<sub>12</sub>, and others – which confirms the relevance of the micronutrients selected for investigation in the current study. The paper states that lacto- and lacto-ovo-vegetarian diets can fully provide adequate protein intake, which aligns with the fact that the protein consumption in the control group was indeed normal (72 g/day). Additionally, the study by D.S. Yasakov *et al.* highlighted different types of vegetarianism and identified other micronutrients (zinc, vitamins D, B<sub>2</sub>), which vegetarians are particularly deficient in but were not addressed in the current study.

In the study by L.D. D'Aloisio *et al.* [19], the adaptation of the diet of migrants and its impact on changes in gut microbiota due to lifestyle changes was examined. The authors showed that among Indians maintaining a traditional diet, the bacteria *Prevotella* spp., which are associated with the breakdown of complex carbohydrates, dominated. In contrast, immigrants demonstrated a transitional flora: a decrease in *Prevotella* spp. and an increase in bacteria typical of Western populations, which correlated with an increase in the consumption of processed foods, unhealthy foods, and a reduction in dietary fibre intake, as observed in the current study when evaluating changes in the consumption of different product categories among the groups. Such data underscore that changes in diet can lead not only to biochemical changes but also shifts in the microbiome, which can alter nutrient absorption, metabolism, and mineral and vitamin status.

The review by G. Holmboe-Ottesen & M. Wandel [20] provided a deeper understanding of the mechanisms underlying the observed changes in the diet of Indian students after migration. The review showed that after the migration of South Asians to Europe, eating habits almost always changed, including a decrease in the consumption of traditional sources of dietary fibre (legumes, vegetables, whole grains) and a reduction in the content of vitamins and minerals in the diet. However, the study did not specifically examine vegetarians. Nevertheless, the results of the current study logically align with the findings of the review: the transition to a new food and social environment leads to a reduction in the consumption of familiar foods that are key sources of micronutrients in India (legumes, various vegetables, spices). At the same time, migrants faced limited access to familiar products, which is reflected in the decrease in the variety of food categories consumed by students arriving in the Kyrgyz Republic. This review

confirms that the observed changes in diet are part of a larger process of dietary restructuring when changing geographical and socio-cultural environments.

In the study by A.S. Vorontsova *et al.* [21], the change in folate levels among Indian students in Arkhangelsk was examined depending on the duration of their studies. Notably, the serum folic acid levels of first-year students were higher than those of sixth-year students (10 ng/ml vs. 4 ng/ml), which was associated with a decrease in vegetable consumption, as well as an increase in smoking and alcohol consumption. In contrast, the current study observed a gradual improvement in the diet as the length of stay increased. This comparison highlights that the consumption of different nutrients may either increase or decrease as the duration of stay in the new country lengthens. It also underscores that deficiencies are formed not only under the influence of diet but also due to other lifestyle factors.

The study found that Indian students living in the Kyrgyz Republic experience significant changes in their diet compared to the control group from India. Despite maintaining overall caloric intake, students in their early years of stay showed a marked decrease in the consumption of protein, iron, vitamins B<sub>12</sub> and A, as well as a reduction in dietary diversity and an increase in the proportion of refined foods and those with lower nutritional density. For students who had been living in the Kyrgyz Republic for more than 4-5 years, partial compensation for these changes was observed; however, their diet still lagged behind the control group in terms of quality. These results confirm the hypothesis that adaptation to the new food environment in the Kyrgyz Republic leads to a reduction in the consumption of key nutrients and a shift in the dietary structure of Indian vegetarian students. The data obtained underscore the need for targeted measures to optimise the diet of foreign students during their adaptation period.

## CONCLUSIONS

The research hypothesis regarding the reduction in the consumption of protein, iron, vitamins B<sub>12</sub> and A among Indian students living in the Kyrgyz Republic was confirmed by quantitative data. It was found that the average daily protein consumption in the control group was 72 g, whereas in Group 1 it decreased to 49 g, and in Group 2 to 62 g. A similar trend was observed for micronutrients: iron consumption decreased from 18.2 mg in the control group to 10.3 mg and 13.1 mg in Groups 1 and 2, respectively; vitamin B<sub>12</sub> – from 2.2 µg to 1.4 µg and 1.8 µg; vitamin A – from 972 µg to 552 µg and 661 µg. Dietary diversity was also significantly lower among students who had recently arrived in the Kyrgyz Republic (5.7 points compared to 7.8 in the control group), while students with over 4-5 years of residence showed partial recovery to 6.4 points.

The structure of food groups demonstrated a decrease in the consumption of legumes and dark green

leafy vegetables in both groups of students residing in the Kyrgyz Republic, as well as a statistically significant increase in the proportion of foods with low nutritional density. Despite comparable caloric intake (2,308 and 2,362 kcal compared to 2,426 kcal in the control group), a marked change in the macronutrient balance was observed, with relatively higher carbohydrate consumption and reduced intake of protein and fats. However, students who had been living in the country for more than 4-5 years showed improvement in several indicators, although their diet still did not reach the control group values.

The prospects for further research include several directions. A more detailed analysis is required of the factors determining the reduction in dietary diversity among Indian migrants during the early stages of their stay in the Kyrgyz Republic. Special attention should be given to issues such as the availability of plant-based products specific to tropical regions, local limitations in the food assortment, and the related barriers in forming a balanced diet. It would also be reasonable to delve deeper into studying the dynamics of dietary adaptation among vegetarians in the Kyrgyz Republic in the long term. It is important to assess whether the recovery of dietary habits, followed prior to migration, is taking place or if a stable adaptation to the local food assortment is forming. Such an approach will allow for

a clearer understanding of the dietary mechanisms of vegetarians in the context of long-term residence in a different food environment. Another promising direction is the expansion of the range of evaluated indicators affecting the nutrient status of students. Specifically, it would be prudent to include an analysis of other micronutrients, such as vitamin D, zinc, folates, etc. Furthermore, it is essential to assess the factors influencing their levels, such as behavioural, psychological, and other aspects that potentially affect adherence to and the quality of a vegetarian diet. Additionally, a significant potential lies in including the analysis of biochemical markers for the body's macro- and micro-nutrient status. This would allow for a comparison of the data on the composition of the actual diet with objective physiological indicators, thus providing a more accurate evaluation of the potential changes in diet and their impact on health status.

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## CONFLICT OF INTEREST

None.

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## Кыргыз Республикасында ар кандай мөөнөттө жашап жаткан индиялык вегетариан студенттердин жана Индияда туруктуу жашаган тургундардын азыктануу статусунун салыштырма анализи

**Равшанбек Джумаев**

Медицина илимдеринин кандидаты, доцент  
Ош Эл аралык медициналык университети  
723500, Алымбек Датка көч., 428, Ош ш., Кыргыз Республикасы  
<https://orcid.org/0009-0006-2129-4943>

**Лазокатхан Джумаева**

Медицина илимдеринин кандидаты  
Ош мамлекеттик университети  
723500, Алымбек Датка көч., 331, Ош ш., Кыргыз Республикасы  
<https://orcid.org/0000-0002-5465-2528>

**Насиба Джумаева**

Студент  
Ош мамлекеттик университети  
723500, Алымбек Датка көч., 331, Ош ш., Кыргыз Республикасы  
<https://orcid.org/0009-0002-1158-6142>

**Аннотация.** Изилдөөнүн максаты – Кыргыз Республикасы жашаган жана вегетариандык тамактануу режимин сактаган индиялык студенттердин рационунун мүнөзүн жана курамын, анын ичинде макро- жана микронутриенттерди, алардын өлкөдө жашаган мөөнөтүн эске алуу менен баалоо, ошондой эле бул көрсөткүчтөрдү Индияда жашаган контролдук топ менен салыштыруу болуп саналат. Изилдөөгө 50 адамдан турган үч топ катышты: Кыргызстанга жаңы келген студенттер (1-топ), өлкөдө 4 жылдан ашык жашаган студенттер (2-топ) жана Индияда жашаган контролдук топ. Тамактанууну баалоо үчүн 30 күндүк азык-түлүк күндөлүгүн жүргүзүү ыкмасы колдонулду. Алынган маалыматтардын негизинде суткалык калориялык нарк, белок, май, углеводдордун керектелиши жана рациондун ар түрдүүлүгү анализденди. Тамак-аштын химиялык курамы боюнча маалымдамалардын жардамы менен рациондогу макро- жана айрым микронутриенттердин болжолдуу көлөмү эсептелди. Натыйжалар көрсөткөндөй, 1 жана 2-топтордо Индиядагы контролдук топко салыштырмалуу белок жана май керектөөсүнүн төмөндөшү, буурчак өсүмдүктөрү менен коюу жашыл жалбырактуу жашылчалардын үлүшүнүн азайышы байкалган. Жалпы калориялуулук негизинен углеводдорду, анын ичинде азыктык баалуулугу төмөн продуктуларды (закускалар, таттуулар) көбүрөөк керектөөнүн эсебинен камсыздалган, айрыкча жаңы келген студенттер арасында. Ошондой эле 1-топто темирдин,  $B_{12}$  витамининин жана А витамининин керектөөсү төмөндөдү. Кыргызстанда узак жашаган студенттер рационду жакшыртуу тенденциясын көрсөткөнү менен, анын сапаты дагы эле контролдук топко жетпейт. Алынган маалыматтар белок-энергетикалык жана витамин-минералдык жетишсиздик коркунучун көрсөтөт. Бул чет өлкөлүк студенттердин адаптация шарттарында ден соолугун жакшыртуу жана дефициттердин алдын алуу үчүн өсүмдүккө негизделген тамак-аштын ар түрдүүлүгүн көбөйткөн адаптацияланган рациондорду иштеп чыгуунун зарылдыгын айгинелейт. Изилдөөнүн жыйынтыктары чет өлкөлүк вегетариан студенттер үчүн нутритивдик дефициттердин алдын алууга багытталган адаптацияланган тамактануу программаларын түзүүдө практикалык колдонулушу мүмкүн.

**Негизги сөздөр:** диета; тамактануу рациону; белок-энергетикалык жетишсиздик; витамин жана минералдык дефицит; тамактанууга адаптация

## **Сравнительный анализ пищевого статуса индийских студентов-вегетарианцев с разным сроком пребывания в Кыргызской Республике и постоянных жителей Индии**

**Равшанбек Джумаев**

Кандидат медицинских наук, доцент  
Ошский международный медицинский университет  
723500, ул. Алымбек Датка, 428, г. Ош, Кыргызская Республика  
<https://orcid.org/0009-0006-2129-4943>

**Лазокатхан Джумаева**

Кандидат медицинских наук  
Ошский государственный университет  
723500, ул. Алымбек Датка, 331, г. Ош, Кыргызская Республика  
<https://orcid.org/0000-0002-5465-2528>

**Насиба Джумаева**

Студент  
Ошский государственный университет  
723500, ул. Алымбек Датка, 331, г. Ош, Кыргызская Республика  
<https://orcid.org/0009-0002-1158-6142>

**Аннотация.** Целью исследования было изучение характера и состава рациона, включая макро- и микронутриенты, индийских студентов, проживающих в Кыргызской Республике и ведущих вегетарианский образ жизни, с учётом длительности их пребывания, а также сравнение этих показателей с контрольной группой, проживающей в Индии. В исследовании приняли участие три группы по 50 человек: новоприбывшие в Кыргызскую Республику студенты (1 группа), студенты, проживающие в Кыргызской Республике более 4 лет (2 группа), и контрольная группа, проживающая в Индии. Для оценки питания использовался метод ведения пищевого дневника в течение 30 дней. На основе полученных данных анализировались среднесуточная калорийность, потребление белков, жиров, углеводов, а также диетическое разнообразие. С использованием справочников по химическому составу продуктов питания было рассчитано примерное содержание макро- и некоторых микронутриентов в рационе исследуемых. Результаты показали, что в 1 и 2 группах наблюдалось снижение потребления белков и жиров, а также уменьшение доли бобовых и темно-зеленых овощей по сравнению с контрольной группой из Индии. Удовлетворительная общая калорийность достигалась в основном за счёт увеличения потребления углеводов, в том числе продуктов с низкой питательной ценностью (снеки, сладости), особенно среди недавно прибывших студентов. Также у 1 группы снижено содержание железа, витамина B<sub>12</sub>, витамина А в рационе. Студенты, прожившие в Кыргызской Республике длительное время, демонстрировали тенденцию к улучшению рациона, однако его качество всё ещё уступает контрольной группе. Выводы на основе полученных данных указывают на риск развития белково-энергетической и витаминно-минеральной недостаточности. Это свидетельствует о необходимости разработки адаптированных рационов с увеличением разнообразия растительных продуктов для предотвращения дефицитов и улучшения состояния здоровья иностранных студентов в условиях адаптации. Результаты исследования могут быть использованы при разработке адаптированных рационов питания для иностранных студентов-вегетарианцев в целях профилактики нутритивных дефицитов.

**Ключевые слова:** диета; рацион питания; белково-энергетическая недостаточность; дефицит витаминов и минералов; адаптация питания

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## **Managing Editors**

Akmaral Argynbaeva  
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## **Publishing Address:**

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# **АДАМДЫН БИОЛОГИЯСЫ ЖАНА КЛИНИКАЛЫК МЕДИЦИНА ЖУРНАЛЫ**

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Кыялбек Сакибаев

## **Редакциянын дареги:**

Ош эл аралык медициналык университети  
723500, Алышбек Датка көч., 428, Ош ш., Кыргыз Республикасы  
E-mail: [journal@humanbiomed.com](mailto:journal@humanbiomed.com)  
<https://humanbiomed.com/ky>

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## **Ответственные редакторы:**

Акмарал Аргынбаева  
Кыялбек Сакибаев

## **Адрес редакции:**

Ошский международный медицинский университет  
723500, ул. Алымбек Датка, 428, г. Ош, Кыргызская Республика  
E-mail: [journal@humanbiomed.com](mailto:journal@humanbiomed.com)  
<https://humanbiomed.com/ru>