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STUDY OF A MARKER OF MICROBIAL CONTAMINATION – UREASE ACTIVITY IN THE ORAL FLUID OF PATIENTS FOLLOWING SURGICAL INTERVENTION FOR TUMOUR REMOVAL AND CHEMOTHERAPY AGAINST THE BACKDROP OF A THERAPEUTIC AND PREVENTIVE COMPLEX

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The study was devoted to the evaluation of the effect of a therapeutic complex of drugs on the marker of microbial contamination in the oral cavity – urease activity in the oral fluid of patients after surgical removal of tumors and chemotherapy. Thirty-five adults (25–55 years) were enrolled: a healthy control cohort, a comparison group receiving only standard oncologic care, and a main group receiving standard care plus the therapeutic and preventive complex. Non-stimulated whole saliva was collected at baseline and at 1, 3, 6, and 12 months post-surgery. Urease activity was quantified. Results were analysed with Student's t-test (p<0.01). The obtained findings indicate that adjunctive administration of the proposed therapeutic-prophylactic complex effectively reduces bacterial contamination of the oral cavity in patients with head and neck cancer after surgery and chemotherapy, thereby supporting the protective potential of this regimen in the correction of therapy-associated oral dysbiosis.

Key words: head and neck tumours, urease, oral fluid, therapeutic and preventive complex.

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ДОСЛІДЖЕННЯ МАРКЕРА МІКРОБНОЇ КОНТАМІНАЦІЇЇ – АКТИВНОСТІ УРЕАЗИ У РОТОВІЙ РІДИНІ ПАЦІЄНТІВ ПІСЛЯ ОПЕРАТИВНОГО ВТРУЧАННЯ З ВИДАЛЕННЯ ПУХЛИН І ХІМІОТЕРАПІЇ НА ТЛІ ЛІКУВАЛЬНО-ПРОФІЛАКТИЧНОГО КОМПЛЕКСУ

Дослідження було присвячене оцінці впливу терапевтичного комплексу препаратів на показник мікробного забруднення порожнини рота – активність уреази в ротовій рідині пацієнтів після хірургічного видалення пухлин та хіміотерапії. До дослідження було залучено 35 дорослих осіб (віком 25–55 років): контрольну групу здорових осіб, групу порівняння, яка отримувала лише стандартну онкологічну допомогу, та основну групу, яка отримувала стандартну допомогу разом із терапевтичним і профілактичним комплексом. Нестимульовану цільну слину збирали на початку дослідження та через 1, 3, 6 і 12 місяців після операції. Визначали активність уреази. Результати аналізували за допомогою t-критерію Стьюдента (p<0,01). Отримані дані свідчать про те, що додаткове застосування запропонованого терапевтично-профілактичного комплексу ефективно знижує бактеріальну контамінацію ротової порожнини у пацієнтів з раком голови та шиї після хірургічного втручання та хіміотерапії, що підтверджує захисний потенціал цього режиму в корекції асоційованої з терапією дисбіозу ротової порожнини.

Ключові слова: пухлини голови та шиї, уреаза, ротова рідина, лікувально-профілактичний комплекс.

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The oral mucosa normally hosts one of the body's largest and most diverse microbial communities [9], in close interplay with local innate defenses. Saliva contains numerous antimicrobial factors (e.g. secretory IgA, histatins, lactoferrin, peroxidases and lysozyme) that help maintain a symbiotic balance between commensals and the epithelium [6]. Tissue-specific immune mechanisms continuously surveil this barrier [4], preserving homeostasis unless disrupted by insults. In cancer patients, however, multiple factors perturb this balance. Surgical resection of head and neck tumors and subsequent chemotherapy inflict mucosal injury and pharmacologic immunosuppression that deplete normal commensals while enabling overgrowth of opportunistic bacteria [7, 8]. For example, chemotherapy-induced mucositis is associated with a shift in the oral microbiome – health-associated taxa such as Streptococcus and Actinomyces decline, while Gram-negative urease-producing species (Fusobacterium nucleatum, Prevotella spp.) become enriched [3]. This dysbiotic environment, together with reduced salivary flow and depressed levels of antimicrobial salivary proteins (e.g. lactoperoxidase, cystatins) after cancer therapy, compromises the oral immune barrier [8, 11]. Indeed, recent proteomic analyses have shown significant reductions in salivary defense proteins following head and neck cancer treatment, suggesting weakened resistance to infection [11].

Given this heightened vulnerability, multimodal prophylactic strategies are being explored to bolster oral immunity and limit microbial contamination. Numerous trials now report that adjunctive probiotics (e.g. Streptococcus salivarius K12, Lactobacillus and Bifidobacterium strains) can significantly reduce the incidence and severity of chemotherapy- or radiotherapy-induced oral mucositis [5, 10]. Similarly, natural antioxidants and anti-inflammatory agents – for instance, the polyphenol curcumin – have shown promise in protecting mucosal tissues by scavenging reactive oxygen species and modulating inflammatory signaling. These observations suggest that combining antimicrobial, immunostimulatory and antioxidant therapies may synergistically preserve mucosal integrity and restrain dysbiosis during cancer treatment.

The purpose of the study was to evaluate the effect of a therapeutic complex of drugs on the marker of microbial contamination in the oral cavity – urease activity in the oral fluid of patients after surgical removal of tumors and chemotherapy.

Materials and methods. Biochemical studies of oral fluid were conducted in 35 patients aged 25–55 years. The study cohort comprised 25 patients with histologically verified malignant tumours of the head and neck who had undergone tumour resection surgery and were scheduled for adjuvant chemotherapy; the control cohort comprised 10 somatically and dentally healthy volunteers whose systemic medical examination and oral status were within normal limits. Individuals who did not meet these inclusion criteria or declined informed consent were excluded. No participants were withdrawn, replaced, or lost to follow-up after enrolment. Biochemical studies were carried out in the «Laboratory of biochemistry and vivarium» of the SE "The Institute of stomatology and maxilla-facial surgery National academy of medical sciences of Ukraine" (SE "ISMFS NAMS"). The study was carried out from 6 February 2023 to 19 February 2024.

Patients with head and neck cancer underwent surgery to remove tumors and were prescribed chemotherapy. The patients observed were divided into two groups as follows:

– Comparison group – after surgery, patients were prescribed treatment in accordance with the "Standards for the Diagnosis and Treatment of Cancer Patients", n=10;

– Main group – after surgery, patients were prescribed a therapeutic and prophylactic complex in addition to the basic standard treatment for cancer patients, n=15.

Both cohorts received guideline-based oncologic care (tumour resection followed by adjuvant chemotherapy in accordance with Order No. 247/2016), while the main cohort additionally underwent a staged therapeutic-prophylactic complex designed to modulate gut/oral microbiota, enhance osteogenesis, and limit oxidative-inflammatory damage. Pre-operative phase (days –14 to 0): Orthomol Pro 6 (INN: Lactobacillus spp. + Bifidobacterium spp. multistrain probiotic; Orthomol GmbH, Germany) – one capsule once daily after meals for 14 days. Post-operative phase: Orthomol Osteo® granules (INN: cholecalciferol 20 µg with calcium, vitamin K1, magnesium and collagen-supporting micronutrients; Orthomol GmbH, Germany) – one sachet dissolved in 150–200 mL water, taken once daily after meals for 30 days; Quertin chewable tablets (INN: quercetin 60 mg; InterChem S.A., Ukraine) – one tablet three times daily 30 min before meals for 60 days; Lizomucoid dental elixir (INN: lysozyme hydrochloride 1 mg mL^{-1} with herbal antiseptics; SPA "Odeska Biotekhnolohiya", Ukraine) – 1 teaspoon diluted in 60 mL of water, rinse for 1 min after meals twice daily for 30 days; Maripolymiel® phytogel (INN: seawater trace-element concentrate 2 % + peppermint hydro-alcoholic extract 5 % with sodium benzoate, carboxymethylcellulose and menthol; SPA "Odeska Biotekhnolohiya", Ukraine) – thin-layer gingival applications (one pump) three to four times daily after meals for 10 days. The entire regimen was re-initiated six months post-surgery to consolidate clinical benefits; no dose modifications or patient withdrawals occurred.

Patients were treated in accordance with the Standards for Diagnosis and Treatment of Cancer Patients, in particular the clinical protocol for providing medical care to patients with oral and oropharyngeal cancer – Order of the Ministry of Health of Ukraine No. 247 of March 28, 2016. "On Amendments to Order No. 554 of the Ministry of Health of Ukraine dated September 17, 2007, "On Approval of Protocols for the Provision of Medical Care in the Specialty of Oncology" as well as the protocols for the provision of medical care to patients with malignant neoplasms developed by the National Cancer Institute in 2011.

All treatment, preventive and diagnostic measures were carried out only after the patients signed a voluntary informed consent in accordance with the principles of bioethics set forth in the Declaration of Helsinki "for Ethical Principles for Medical Research Involving Human Subjects" and "Universal Declaration on Bioethics and Human Rights (UNESCO)". All participants were adults, cognitively competent, and not otherwise classified as a vulnerable population under Good Clinical Practice. Studies recommended by the Commission on Bioethical Expertise (conclusion of the bioethics commission of the SE "ISMFS NAMS", protocol No. 1011 of 04/14/2022).

Oral fluid was collected in the morning, on an empty stomach, by spitting into sterile centrifuge tubes (without prior cleaning or rinsing of the oral cavity) for 5–10 minutes. Before performing biochemical analysis, the oral fluid was thawed at room temperature, centrifuged at 2,500 rpm for 20 minutes at a temperature of +4°C (bench centrifuge RS-6, MedTech, Ukraine), and the supernatant was collected for biochemical analysis. Biochemical studies of urease activity – a marker of microbial contamination of the oral cavity – were carried out in the oral fluid of patients [1]. Determination of urease activity was performed by a colorimetric method based on the ability of urease to hydrolyse urea with the formation of ammonia. The ammonia formed in the reaction interacts with Nessler's reagent, producing a yellow colour, the intensity of which is directly proportional to urease activity in the studied sample. For the assay, 0.4 mL of 0.1 M urea solution and 0.2 mL of oral fluid were mixed and incubated for 1 hour at 37°C. After incubation, 4.4 mL of distilled water and 1.0 mL of Nessler's reagent were added. In parallel, control samples for each specimen were prepared without incubation. All samples were centrifuged for 20 min at 2500 rpm, and the optical density was measured spectrophotometrically at 440 nm against the reagent blank. Urease activity was calculated from the difference between the optical density of the test and control samples and expressed in µkat/L of oral fluid [1].

Data processing was carried out with STATISTICA 6.1. Prior to parametric testing, the Shapiro-Wilk normality test was applied to each continuous variable; none showed significant deviation from a Gaussian distribution ($p>0.05$). Therefore, inter-group comparisons were performed with the two-tailed Student's t-test. When pair-wise contrasts were required (Control × Comparison, Control × Intervention, Comparison × Intervention), the family-wise type-I error rate was controlled with the Bonferroni adjustment. Between-group differences were deemed statistically significant at $p<0.003$ [2].

Results of the study and their discussion. In our studies, we investigated a marker of microbial contamination – urease activity in the saliva of patients with head and neck cancer; the results of these measurements are summarised in Table 1. This indicator was selected because it reflects the metabolic activity of urease-producing opportunistic microflora in the oral cavity under conditions of impaired mucosal homeostasis. Its determination at different time points enabled tracing the direction and persistence of biochemical changes during the postoperative and post-chemotherapy periods.

Table 1

The effect of therapeutic and preventive measures on urease activity in the saliva of patients with head and neck cancer over the course of observation, mU/ml (M±m)

Terms / Groups	Terms of the study				
	Initial state	After 1 month	After 3 months	After 6 months	After 1 year
Reference values for the norm, n=10	0.058±0.003				
Comparison, n=10	0.365±0.028 $p<0.001$	0.326±0.025 $p<0.001$ $p_1>0.3$	0.285±0.020 $p<0.001$ $p_1<0.001$	0.250±0.017 $p<0.001$ $p_1<0.002$	0.270±0.021 $p<0.001$ $p_1<0.01$
Main, n=15	0.380±0.030 $p<0.001$ $p_2>0.8$	0.294±0.024 $p<0.001$ $p_1<0.05$ $p_2>0.4$	0.210±0.017 $p<0.001$ $p_1<0.02$ $p_2<0.01$	0.100±0.010 $p<0.001$ $p_1<0.001$ $p_2<0.001$	0.079±0.005 $p<0.002$ $p_1<0.001$ $p_2<0.001$

Note. p – significance of differences from the norm; p_1 – significance of differences from the initial state. p_2 – significance of differences from the indices in groups.

It is known that there is a direct correlation between the oral microbiome and local immunity. Furthermore, data are available on the composition of the microbiota in malignant tumours of the oral cavity.

Urease is an enzyme that is not produced by somatic cells or probiotic bacteria, but is secreted only by opportunistic and pathogenic microflora [4]. The data in the table indicate that, at the initial stage of observation in cases of oncological pathology, there is an increase in microbial colonisation of the oral cavity and a shift in the balance of the oral microflora towards pathogenic and opportunistic pathogens. At the same time, urease activity was increased by more than sixfold relative to normal values, which is an indicator of increased contamination of the oral cavity with opportunistic microbiota and creates conditions for the development of oral dysbiosis.

Treatment administered according to the standard protocol for cancer patients in the control group resulted in a non-significant reduction in urease activity of 10.7 % ($p_1>0.3$) during the postoperative period, one month after the start of treatment. Subsequently, a statistically significant reduction in the marker under investigation was recorded – by 21.9 % after 3 months ($p_1<0.001$), by 31.5 % after 6 months ($p_1<0.002$), and by 26.0 % ($p<0.01$) after 12 months, relative to baseline data.

More significant positive changes were noted in patients' oral fluid following the removal of malignant head and neck tumours after chemotherapy, against the background of the additional prescription of a therapeutic and prophylactic regimen (twice a year) alongside the main standard therapy. Thus, after just one month, the activity of the studied parameter decreased significantly by a factor of 1.3 ($p<0.05$), after 3 months by a factor of 1.8 ($p<0.02$), after 6 months by a factor of 3.8 ($p<0.001$), and at the final stage of the study after 12 months by a factor of 4.8 ($p<0.001$), almost reaching normal levels.

Thus, the reduction in urease activity in the oral fluid of patients with head and neck tumours that we observed, against the background of the additional use of the therapeutic and prophylactic regimen, indicates a reduction in bacterial contamination of the oral cavity due to the enhanced protective properties of the proposed treatment regimen.

The obtained results indicate that patients with head and neck cancer after tumour resection and chemotherapy develop pronounced microbial contamination of the oral cavity, as evidenced by a more than sixfold increase in urease activity in oral fluid relative to reference values. Such changes are consistent with current concepts of the oral mucosa as a highly specialised barrier system, the stability of which depends on the coordinated interaction between local immune factors, salivary protective components, and the resident microbiota [9,10]. When this equilibrium is disrupted by oncologic treatment, conditions arise for opportunistic microbial overgrowth and persistence of dysbiotic alterations. Our findings agree with the data of Hong et al. [8], who demonstrated that chemotherapy-induced oral mucositis is associated with detrimental bacterial dysbiosis and a shift in the microbial community toward taxa linked to inflammatory and destructive changes. In this context, the marked elevation of urease activity observed in our patients can be interpreted as a biochemical manifestation of such dysbiotic restructuring, since urease is produced by opportunistic and pathogenic microorganisms rather than by host cells or probiotic flora. This interpretation is supported by the study of Dahlén et al. [4], who confirmed the diagnostic value of urease activity assessment for evaluating the presence and metabolic activity of urease-producing oral bacteria.

The gradual but incomplete decrease in urease activity in the comparison group suggests that standard oncologic management alone does not fully restore the microbial balance of the oral cavity during long-term follow-up. At the same time, the significantly greater reduction of this marker in the main group, especially at 6 and 12 months, indicates that the proposed therapeutic-prophylactic complex exerted a substantial corrective influence on the oral ecosystem. These results are in line with the conclusions of Yang et al. [11], whose meta-analysis demonstrated that probiotic-based interventions may reduce treatment-related oral complications by modulating the microbiota and supporting mucosal homeostasis. In our study, the inclusion of a multistrain probiotic, together with local and systemic agents possessing antimicrobial, anti-inflammatory, and protective properties, was associated with an almost complete normalisation of urease activity by the end of follow-up. This favourable trend may also be considered in the broader context of data reported by Golusińska-Kardach et al. [7], who emphasised that head and neck cancer is closely linked with qualitative and quantitative alterations of the oral microbiome, and that correction of these disturbances may have both supportive and preventive clinical significance. Therefore, our findings substantiate the view that adjuvant therapeutic-prophylactic strategies aimed at microbiota stabilisation and enhancement of oral barrier resistance can improve the biochemical indicators of oral health in this category of patients.

Limitations. A limitation of this study is that the evaluation was conducted within a single-centre clinical design and focused on a single defined follow-up model after surgery and chemotherapy, which may limit the extrapolation of the results to other treatment settings. In addition, the observation period, although sufficient to assess one-year dynamics, does not allow conclusions to be drawn regarding the longer-term stability of the obtained effects.

Conclusions

1. Patients with malignant tumours of the head and neck after surgical treatment and chemotherapy demonstrated a marked increase in urease activity in oral fluid, exceeding reference values by more than sixfold, which indicates pronounced microbial contamination of the oral cavity and the development of dysbiotic alterations.

2. Standard treatment according to current oncologic protocols was associated with only moderate improvement in the studied parameter during follow-up, since urease activity in the comparison group remained significantly elevated relative to normal values throughout the observation period.

3. Additional administration of the proposed therapeutic-prophylactic complex led to a significantly more pronounced reduction in urease activity than standard treatment alone: by 1.3 times after 1 month, by 1.8 times after 3 months, by 3.8 times after 6 months, and by 4.8 times after 12 months compared with baseline, with near-normalisation of the indicator by the end of follow-up.

4. The obtained results substantiate the antimicrobial and protective efficacy of the proposed therapeutic-prophylactic complex and support its further use and investigation as an adjunctive strategy for reducing oral bacterial contamination and preventing dysbiosis-related complications in patients with head and neck cancer undergoing combined treatment.

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BRIDGING THE GAP IN EMERGENCY CARE THROUGH THE IMPACT OF TELEMEDICINE ON PATIENT OUTCOMES IN REMOTE REGIONS

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A total of 1115 patients who received emergency medical services between 2015 and 2020 were included in the study. These patients were divided into two groups: the traditional group (527 patients who received standard emergency medical services from 2015 to 2018 and the telemedicine group (588 patients who received additional remote consultative support via telemedicine from 2018 to 2020. Descriptive statistics, logistic regression, ROC analysis, and independent-samples tests were employed to identify key predictors of critical conditions and to assess the effectiveness of telemedicine in patient management. According to the results, 496 patients were identified as having a high likelihood of needing telemedicine. The results of the study demonstrated that telemedicine significantly enhances the management of critical emergencies in rural areas, leading to improved patient outcomes. These findings underscore the potential for telemedicine to transform healthcare, particularly in resource-limited settings.
Key words: telemedicine, emergency response, traffic accidents, acute coronary syndrome, remote areas, high-risk patients.

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ПОДОЛАННЯ РОЗРИВУ У НАДАННІ НЕВІДКЛАДНОЇ МЕДИЧНОЇ ДОПОМОГИ ЗА РАХУНОК ВПЛИВУ ТЕЛЕМЕДИЦИНИ НА РЕЗУЛЬТАТИ ЛІКУВАННЯ ПАЦІЄНТІВ У ВІДДАЛЕНИХ РЕГІОНАХ

До дослідження було включено 1115 пацієнтів, які отримали невідкладну медичну допомогу в період з 2015 по 2020 рік. Пацієнтів було розділено на дві групи: традиційна група (527 пацієнтів, які отримували стандартну екстрену медичну допомогу у 2015–2018 рр.) та група телемедицини (588 пацієнтів, які, окрім стандартної допомоги, отримували дистанційну консультативну підтримку за допомогою телемедицини у 2018–2020 рр.). Для виявлення ключових предикторів критичних станів та оцінки ефективності телемедицини у веденні пацієнтів застосовувалися методи описової статистики, логістична регресія, ROC-аналіз та критерії порівняння незалежних вибірок. Згідно з результатами, у 496 пацієнтів було виявлено високу ймовірність необхідності телемедичної підтримки. Результати дослідження продемонстрували, що телемедицина істотно покращує ведення критичних невідкладних станів у сільських районах, що призводить до поліпшення клінічних результатів. Отримані дані підкреслюють потенціал телемедицини у трансформації системи екстреної допомоги, особливо в умовах обмежених ресурсів.
Ключові слова: телемедицина, невідкладна допомога, дорожньо-транспортні пригоди, гострий коронарний синдром, віддалені регіони, пацієнти групи високого ризику.

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Despite advances in healthcare services, the provision of quality emergency medical services (EMS) in rural areas remains an unresolved issue. Currently, nearly 5 million people worldwide lack the necessary infrastructure for providing emergency care, leading to delayed hospitalization and a need for an additional 143 million surgical interventions annually to prevent disability and other complications [5, 10, 15].

A modern form of EMS has been worldwide provided through telemedicine (TM). TM supports the provision of more specialized care by enabling