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# DYNAMICS OF DAILY BIORHYTHMIC ADHESIVE ACTIVITY OF ORAL MICROBIOTA IN PATIENTS WITH SECONDARY ADENTIA

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***Анотація.*** *Адгезивна активність мікроорганізмів є одним з критеріїв здатності до прикріплення до будь-яких поверхонь, накопичення мікробної маси та утворення біоплівок, які є причиною періімплантних хвороб. Метою дослідження було визначення динаміки денної біоритмічної адгезивної активності провідних збудників періімплантних ускладень у пацієнтів із вторинною адентією на етапі підготовки до операції дентальної імплантації. Зразки для мікробіологічного дослідження забирали в ділянці ясен у 35 пацієнтів перед операцією дентальної імплантації протягом дня з 9.00 до 18.00 з інтервалами в 3 години. Вивчення циркадної динаміки адгезивної здатності обраних мікроорганізмів дозволило зафіксувати мінімальний рівень її активності о 9.00, максимальний – о 12.00 та о 15.00, після чого відбувалося її зниження до 18.00. Отримані дані доцільно враховувати при проведенні операції дентальної імплантації.*

***Ключові слова:*** *ротова порожнина, мікробіота, біоритми, адгезивна активність, дефекти зубних рядів, дентальна імплантація.*

***Abstract.*** *The adhesive activity of microorganisms is one of the criteria for the ability to attach to any surfaces, the accumulation of microbial mass and the formation of biofilms, which are the cause of peri-implant diseases. The aim of the study was to study the dynamics of the daily biorhythmic adhesive activity of the leading pathogens of peri-implant complications in patients with secondary adentia at the stage of preparation for dental implantation surgery. The samples for microbiological investigation were taken from the gum area of 35 patients before dental implantation during the day from 9.00 to 18.00 with 3-hour intervals. The study of the circadian dynamics of the adhesive ability of the selected microorganisms allowed us to determine the minimum level of its activity at 9.00, the maximum one at 12.00 and 15.00, after which it decreased to 18.00. The obtained findings should be taken into account when performing dental implantation surgery.*

***Key words:*** *oral cavity, microbiota, biorhythms, adhesive activity, dentition defects, dental implantation.*

**Connection of the publication with planned research works.**

The study was conducted within the framework of the planned scientific research work “Optimization of the methods for diagnosis and treatment of main dental diseases”, state registration number 0119U002899.

**Introduction.**

Today it is known that most bacteria do not exist in a monoform, but are in a kind of community in the form of a biofilm [1, 2, 3, 4]. Biofilms are not only the ideal form of existence of any microbiota, but also the main etiological factor of many diseases of the oral cavity, including those associated with dental implantation (DI) [5, 6]. Therefore, understanding the mechanisms of biofilm formation, especially its initial stage, is an important issue for preventing the realization of the aggressive potential of the oral microbiota, including when planning DI.

The first stage of biofilm formation is the stage of microorganisms adhesion, i.e. the ability to directly attach to any surfaces and accumulate microbial mass. It is the adhesive activity of the microbiota that is the basis and starting point for the formation of biofilms and resistance to adverse environmental factors and, as a result, development of the inflammatory process, purulent-inflammatory complications, and rejection of implants [7, 8]. Therefore, the study of the adhesive activity of microorganisms is an important aspect of understanding the development of the pathological process in the peri-implant zone and a possible tool for its prevention.

**The aim of the study.**

To study the dynamics of the daily biorhythmic adhesive activity of the leading causative agents of peri-implant complications in patients with secondary adentia at the stage of preparation for dental implantation surgery.

**Object and research methods.**

The study involved 35 patients aged 35-57 years with small dentition defects (DD). The selected patients had gender equivalence – 18 women and 17 men, did not suffer from diseases of the oral mucosa and periodontal tissues, had a healthy oral cavity, and did not smoke. The selected group of patients had not taken antibiotics or used antimicrobial drugs locally for the previous six months. All patients were at the stages of examination and preparation for dental implantation surgery for the rehabilitation of small dentition defects (DD). The cause of tooth loss in all patients was caries complications. The selected patients underwent professional teeth cleaning and adjustment of their individual oral hygiene a week before collecting the samples for microbiological investigation.

The samples for microbiological investigation were taken from the gingival area of the small DD zone with maximum adherence to the rules of asepsis and traditional requirements for eating and oral hygiene from 9:00 to 18:00 every three hours. The material was trans-

ported as soon as possible after its collection. A previous work on the study of the oral microbiota in patients with secondary adentia allowed us to identify almost all of its resident representatives in the biofilms [9]. In this study, the degree of adhesive activity was determined in isolates that formed biofilms, and according to current data is associated with the development of peri-implant infections: gram-positive – *Streptococcus mutans (S. mutans), Staphylococcus aureus (S. aureus), Peptostreptococcus spp.*; gram-negative *Escherichia coli (E.coli), Fusobacterium nucleatum (F.nucleatum) Porphyromonas gingivalis (P. gingivalis), Prevotella intermedia (P.intermedia)* and fungi *Candida albicans (C. albicans)* [10].

The adhesive activity of the isolates determined according to the generally accepted method of V.I. Brilis (1986) [11, 12]. The result was evaluated as follows: with an adhesion index ≤ 1.75 – microorganisms were considered non-adhesive; from 1.76 to 2.5 – as low-adhesive; from 2.51 to 4.0 – as medium-adhesive and more than 4.0 – as highly adhesive.

The investigation was carried out in accordance with the current regulatory requirements of Ukraine regarding the principles of medical ethics, as well as based on the provisions of the Declaration of Helsinki (1986), the Convention of the Council of Europe "Convention for the Protection of Human Rights and Dignity of the Human Being with regard to the Application of Biology and Medicine" (1997), "Ethical Principles of Medical Research Involving Human Subjects" (WMA, 2000), "General Declaration on Bioethics and Human Rights" (UNESCO, 2005). At the beginning of the study, all patients gave informed written consent to participate in the investigation and use the obtained data in a scientific publication.

Statistical processing of the obtained results was carried out using well-known methods of variation statistics: the average values (M) and the standard error of the arithmetic mean (±m) were determined. The significance of the obtained values was checked using the Student's t criterion.

**Research results and their discussion.**

When analyzing the results of the dynamics of daily biological rhythms of adhesive activity of resident representatives of microorganisms that are capable of forming biofilms in the oral cavity in patients with secondary adentia, certain features were established **(fig. 1 and fig. 2)**.

Thus, the dynamics of adhesive activity of gram-positive microorganisms in patients with small DD was of the same type (**fig. 1**). Namely: at 9.00, the mean adhesion indices in *S. mutans, S. aureus and Peptostreptococcus spp*. were at the lowest level and were equal to 2.1±0.14, 2.5±0.15 and 2.6±0.14, respectively. By 12.00, the most dramatic increase in adhesion indices of selected microorganisms occurred, which reached 7.3±0.16, 6.9±0.18 and 8.3±0.18, respectively (p<0.001 compared to the indices at 9.00).

The results of calculation of the adhesive activity of gram-positive microorganisms at 15.00 showed clear trends to decrease this indicator to 5.4±0.14, 5.1±0.13 and 6.9±0.22, respectively (p<0.001 compared to the indicators at 9.00 and 12.00 hours). By 18.00, the trends towards a decrease in the adhesive ability of *S. mutans, S. aureus* and *Peptostreptococcus spp.* were preserved, which allowed us to record the average adhesiveness index at 3.1±0.21, 3.4±0.18 and 5.8±0.17, respectively (p<0.001 compared to the indicators at 9.00, 12.00, and 15.00). It should be emphasized that the decrease in the adhesive activity of selected gram-positive representatives of the resident oral microbiota at 18.00 still did not reach the level of its activity at 9.00 and was 1.5, 1.4, 2.2 times greater than that, respectively (p<0.001).

As for the dynamics of the adhesion ability of fungi of the genus *Candida*, by analogy with the gram-positive microbiota, the lowest indicators in *C. albicans* were at 9.00 (3.3±0.26) **(fig. 1)**. Further, at 12.00, the maximum value of the adhesive activity index was recorded (9.7±0.39, p<0.001 compared to the previous indicator), after which there was a gradual decrease in adhesive activity to 8.1±0.34 at 15.00 (p<0.001 compared to the indicator at 9.00 and 12.00) and to 5.7±0.31 and 18.00 (p<0.001 compared to the indicators at 9.00, 12.00 and 15.00). The fact of the absence of a return of the adhesive ability indicator to its values at 9.00 (1.7 times more, p<0.001) draws attention.

Regarding the adhesive properties of gram-negative representatives of biofilm isolates in patients with small DD, the information is shown in **fig. 2**. The presented data demonstrate trends similar to those of gram-positive microbiota. Thus, at 9.00, the adhesive activity indicators for *E. coli, F. nucleatum, P. gingivalis, P. intermedia* were the lowest. At 12.00, the highest adhesion indicators of these microorganisms were recorded, after which their gradual decrease occurred from 15.00 to 18.00. It is noteworthy that only the adhesion ability of *E. coli* at 18.00 (3.4±0.19) almost returned to the level at 9.00 (3.3±0.18), p>0.05. As for the adhesive ability of the remaining gram-negative microbiota, namely *F. nucleatum, P. gingivalis, P. intermedia,* at 18.00 it exceeded

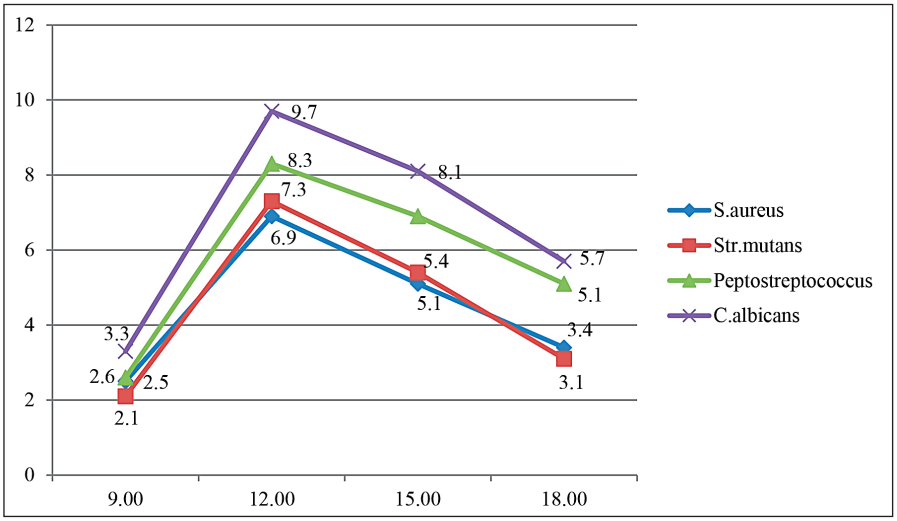


**Figure 1 – Dynamics of daily biorhythmic adhesive activity of gram-positive microbiota (*S. mutans, S. aureus, Peptostreptococcus spp.)* and *C. albicans* in patients with secondary adentia depending on the time of day.**

the initial data at 9.00 by 1.7, 2.0 and 1.7 times (p<0.001).

Thus, the investigation of the dynamics of daily biorhythmic adhesive activity of clinical isolates of gram-positive and gram-negative microbiota revealed the minimum ability to the primary stage of biofilm formation – adhesion, at 9.00, the maximum – at 12.00 and 15.00. Reduction in adhesive activity was recorded at 18.00, but in 7 out of 8 microbiota species it did not reach the 9.00 level and was statistically greater than the morning one (p<0.001). The identified synchronization of the changes in adhesive activity of selected representatives of the oral microbiota confirms the fact of synergism of microbial life in biofilms and the unidirectionality of survival and protection strategies.

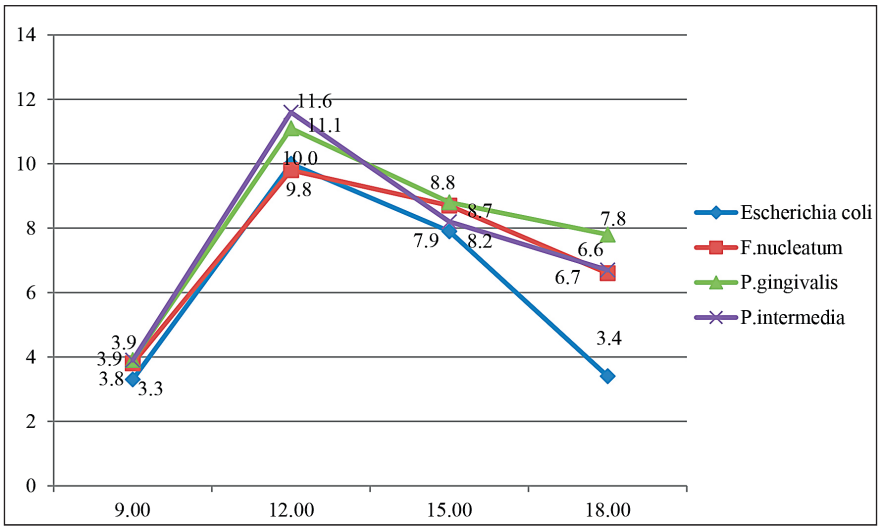


**Figure 2 – Dynamics of daily biorhythmic adhesive activity of gram-negative microbiota (*E.coli, F.nucleatum, P. gingivalis, P.intermedia*) in patients with secondary adentia depending on the time of day.**

The obtained findings should be taken into account when planning surgical interventions, for example, performing DI surgery.

At present it is known that biorhythmic fluctuations in the production of pathogenic factors of the microbiota can affect the metabolism and immunity of the host and, in turn, are directly related to its circadian rhythms [13, 14]. Therefore, a deeper understanding of this influence is interesting and promising not only from a theoretical point of view, but also from a practical one – for the individualization of preventive and treatment measures, including when conducting DI.

## Conclusions.

According to the results of investigating the dynamics of daily biorhythms of adhesive activity of the leading pathogens of peri-implant complications in patients with secondary adentia, a high adhesive potential at 12.00 and 15.00 and a reduced ability to form biofilms at 9.00 and 18.00 were established, which should be taken into account when planning dental implantation surgery.

## Prospects for further research.

The authors plan to investigate circadian changes in the oral microbiome at the stages of dental implantation surgery.

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