The main factor for full-quality existence of a human organism is health. The concept of «health» for any person is defined as a state of complete spiritual, physical and social well-being. The physical component of «health» is represented by a complex morphofunctional relationship and interaction of organs and systems of the human body as a whole [17].

Over the course of life, changes are continuously happening on the molecular level, which ensure growth and development of the body, rate of regenerative processes, and, after their completion, prepare the body for physiological aging. These processes occur as a result of normal cell division and the replication mechanism and are described as shortening of telomere length [7].

Telomeres are specialized DNA-protein structures located at the ends of eukaryotic cell chromosomes. It is important to note that the state of telomeres determines not only the life span of one cell, but also the state of the human body as a whole.

However, a change in the length of telomeres is characteristic not only for physiological aging, but is also a marker of the adverse effects of bad habits, somatic pathology, stress, and other factors on the human body. Chronic systemic low-rate inflammation and oxidative stress adversely impact telomere length and advance premature aging. Therefore, telomeres are currently the key markers of biological aging [5].

Modern studies have proved that changes in telomere length are associated with prevalence and progression of metabolic diseases [13]. One of the somatic diseases, which is based on metabolic disorders, is non-alcoholic fatty liver disease (NAFLD) [16]. Pathogenetic manifestations of NAFLD are obesity, insulin resistance and type 2 diabetes mellitus, hypertriglycerideremia, and hypertension. In NAFLD against the background of insulin resistance and obesity, fat accumulates in hepatocytes, and when such accumulation exceeds 5% of the
liver weight, it initiates non-alcoholic steatosis, and then, under the influence of oxidative stress and cytokines, turns into the most severe form — non-alcoholic steatohepatitis and fibrosis [1, 2].

For now, interdependence between severity of fibrosis and telomere length in patients with NAFLD, especially those complicated by obesity and diabetes mellitus, has been confirmed [12]. These disorders are considered to be the consequence of increased oxidative stress and inflammation in the body, which cause telomere damage and their length decrease. Changes in the length of leukocyte telomeres in patients with cardiovascular disease (CVD) are known. According to the authors, the risk of mortality from CVD in patients with shorter telomeres in blood leukocytes is increased by 3 times [20].

A number of scientific studies prove relation between NAFLD (its basic components) and periodontal diseases. Chronic inflammation is the pathogenetic link that makes connection between these two diseases [11, 14]. Formation of such «vicious circles» in modern medicine is defined by the term «comorbidity» and reasons for requires active participation in such patient’s curation by the specialists from various medical specialties, including a dentist.

Today, it is of interest to study changes in telomere length in patients with chronic periodontitis (CP). T. Takahashi et al. studied the telomere length of gingival fibroblasts in patients with and without aggressive periodontitis, but no significant differences were found [19]. However, S. Masi et al. having conducted a similar study, determined that CP patients have shorter gingival fibroblast telomeres [15].

To date, the scientific problem is the lack of reliable data on the difference in the length of telomeres in periodontal cells in patients with NAFLD and those without metabolic diseases.

Objective: to study the changes in periodontal status in patients with non-alcoholic fatty liver disease.

Materials and methods

Ethical part. Ethical approval for each phase of the study was obtained from the Committee of Government Institution «L. T. Mala Therapy National Institute of the National Academy of Medical Sciences of Ukraine». All study participants signed an informed consent prior to participation with the option to stop participating in the study on their own initiative.

Clinical part. To achieve this goal, 76 patients with a verified diagnosis of non-alcoholic fatty liver disease (41 men and 35 women) were selected, the average age in the group was 47.94 ± 1.16 years. They comprised the main group. The diagnosis was established based on international criteria. Anthropometric parameters were measured in all patients taking into account the anamnesis, clinical and biochemical blood tests were performed to determine cholesterol (of various densities), triglycerides, total protein, albumin, liver tests, and carbohydrate metabolism. Ultrasound of the liver, as well as its elastography, steatography and steatometry were performed. The control group consisted of 14 somatically healthy patients, who were representative by sex and age. Dental examination included determination of clinical status. Particular interest was paid to the periodontium with definition of hygienic and periodontal indices. Also, all patients were interviewed by a dentist to identify the nature of nutrition and knowledge of hygienic oral care.

All patients underwent 2 scrapings from the surface of the attached gums with disposable applicators for PCR studies. Patients were warned about prohibition of eating and smoking 30 minutes before the procedure.

Determination of relative telomere length. Standard procedures were used to extract DNA from the epithelium of the attached gingiva and the extracted DNA was stored at –80 °C until assayed, to prevent sample degradation [4, 9]. DNA concentration was quantified fluorometrically on a fluorometer Qubit 3.0 (Life Technologies, USA) with a Qubit dsDNA HS Assay kit (Thermo Fisher Scientific).

Telomere length was measured using by quantitative PCR [6]. Amplification was performed on the CFX96Touch detection system (Bio-Rad Laboratories, USA) with an initial incubation step at 95 °C for 15 min, followed by 30 cycles of 95 °C for 15 s and 58°C for 1 min; two cycles of 15 s at 94 °C and 15 s at 49 °C; 32 cycles of 15 s at 94 °C, 10 s at 62 °C, 15 s at 74°C with signal acquisition, 10 s at 84 °C, and 15 s at 88 °C with signal acquisition. The telomere length was calculated using the ΔΔCt method with the CFX manager software (Bio-Rad Laboratories) and measured in relative units (RU).

Statistical part. Statistical calculations were performed using SPSS program and were carried out by the parametric method. The results are given as M ± m (M — arithmetic mean, m — error of the arithmetic mean). Comparison of quantitative indicators was carried out using Student’s t-test. The linear relationship between indicators was assessed using the Pearson and Spearman correlation coefficient. Comparison of percentages (parts) was performed using z-test.

Results

When analyzing the questionnaires filled out by patients, it turned out that in the diet of 67 patients (out of 76) with NAFLD and 5 patients from the healthy group (out of 14) carbohydrate food
prevails, most of the respondents prefer fast food restaurants. It is of interest that, the patients suffering from NAFLD prefer having sweet carbonated drinks with their food. A total of 37 patients with NAFLD practice oral hygiene routinely mornings and evenings, while the rest performed it irregularly, and some even forget to brush their teeth. All results of the patient survey are given in Table 1.

The average body mass index in the group with NAFLD was 33.6 ± 1.05 kg/m², while in the healthy group this figure was at the level of 23.03 ± 0.62 kg/m². The main blood biochemical parameters are given in Table 2.

Periodontal diseases in patients with NAFLD had an inflammatory nature of different clinical and morphological forms (Figure) and were represented mainly by chronic generalized periodontitis, while chronic generalized catarrhal gingivitis prevailed in the group of somatically healthy patients.

All changes in periodontal status were confirmed by dental indices (Table 3).

Telomere length of periodontal cells gingival epitheliocytes was significantly shorter in patients with a history of NAFLD (0.9 ± 0.0 and 1.2 ± 0.1 RU, p < 0.05 for main and control group respectively).

In order to define interrelation between the length of telomeres of periodontal cells not only with gum pathology, but also with somatic pathology, search was conducted for linear relationship with available indicators. It can be confidently stated that there is negative average-strength relationship between telomere length and chronic generalized periodontitis (r = –0.590; p = 0.002), NAFLD (r = –0.506; p = 0.045), BMI (r = –0.306; p = 0.031), AST (r = –0.286; p = 0.017). Besides, there was negative correlation of steatosis and fibrosis with duration of NAFLD disease (0.040).

Discussion

As is known, chronic inflammation is base of the pathogenesis of the overwhelming number of diseases. This process is a zone of constant secretion of

Table 1. Patient survey results, %

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Control group</th>
<th>Main group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smoking</td>
<td>14.28</td>
<td>43.42</td>
</tr>
<tr>
<td>Regular consumption of carbohydrates</td>
<td>35.71</td>
<td>88.15*</td>
</tr>
<tr>
<td>Drinking sweet carbonated drinks</td>
<td>42.85</td>
<td>78.94</td>
</tr>
<tr>
<td>Regular two-time brushing of teeth</td>
<td>100</td>
<td>48.68*</td>
</tr>
</tbody>
</table>

Note: * The difference from the control group is statistically significant (p < 0.01).

Table 2. Blood biochemical parameters

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Control group</th>
<th>Main group</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI, kg/m²</td>
<td>23.03 ± 0.62</td>
<td>33.60 ± 1.05*</td>
</tr>
<tr>
<td>AST, U/L</td>
<td>22.35 ± 0.83</td>
<td>33.84 ± 2.08**</td>
</tr>
<tr>
<td>ALT, U/L</td>
<td>17.57 ± 0.81</td>
<td>43.29 ± 3.08*</td>
</tr>
<tr>
<td>ALP, U/L</td>
<td>1313.45 ± 50.67</td>
<td>1474.60 ± 86.78*</td>
</tr>
<tr>
<td>VLDL cholesterol, mmol/L</td>
<td>0.51 ± 0.09</td>
<td>0.80 ± 0.09</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/L</td>
<td>1.32 ± 0.10</td>
<td>1.26 ± 0.08</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.68 ± 0.22</td>
<td>3.30 ± 0.17*</td>
</tr>
<tr>
<td>Glycemia, mmol/L</td>
<td>4.80 ± 0.09</td>
<td>5.84 ± 0.20***</td>
</tr>
<tr>
<td>Glycated hemoglobin, %</td>
<td>5.32 ± 0.08</td>
<td>6.19 ± 0.22</td>
</tr>
<tr>
<td>HOMA-IR index</td>
<td>2.46 ± 0.14</td>
<td>7.40 ± 0.81**</td>
</tr>
</tbody>
</table>

Note. BMI: body mass index; AST: aspartate aminotransferase; ALT: alanine aminotransferase; ALP: alkaline phosphatase; VLDL: very low-density lipoproteins; LDL: low-density lipoproteins; HDL: high-density lipoproteins; HOMA-IR: homeostasis model assessment of insulin resistance.

The difference from the control group is statistically significant: * p ≤ 0.001; ** p < 0.01; *** p < 0.015.

Figure. Structure of periodontal changes

Table 3. Hygiene index and periodontal index results

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Control group</th>
<th>Main group</th>
</tr>
</thead>
<tbody>
<tr>
<td>HI OHI-S, points</td>
<td>1.49 ± 0.15</td>
<td>2.16 ± 0.54*</td>
</tr>
<tr>
<td>PMA, %</td>
<td>12.63 ± 1.86</td>
<td>25.43 ± 0.62*</td>
</tr>
<tr>
<td>CPI, points</td>
<td>0.63 ± 0.13</td>
<td>2.35 ± 0.79*</td>
</tr>
<tr>
<td>PBI points</td>
<td>0.35 ± 0.16</td>
<td>1.60 ± 0.93*</td>
</tr>
<tr>
<td>Loss of attachment, mm</td>
<td>0.28 ± 0.28</td>
<td>3.56 ± 0.20**</td>
</tr>
</tbody>
</table>

Note. OHI-S: simplified oral hygiene index; PI: periodontal index; PMA: papillary-marginal-alveolar index; PBI: papilla-bleeding index. * The difference from the control group is statistically significant (p = 0.001).
pro-inflammatory cytokines, proteolytic enzymes, formation of reactive oxygen intermediates, which will contribute to further tissue destruction [18].

Chronic generalized periodontitis (CGP) and NAFLD are both based on chronic inflammation and have comorbid relationship [8]. In our study, we determined prevalence of CGP in patients of the main group compared to somatically healthy ones, which is confirmed by objective index indicators of periodontal tissues examination. The formed «vicious circle» between CGP and NAFLD does not have possibility of self-interruption and reversal and, therefore, requires active participation from both the dentist and therapist, and the patient.

In CGP treatment, the main task is to stop chronic inflammation and eliminate the focus of chronic sepsis in the oral cavity [3]. With positive dynamics of the treatment process, CGP enters the stage of stabilization, followed by remission. Inactivation of the destruction stage in periodontal tissues is replaced by the proliferative process aimed at restoring damaged tissues, which requires availability of cells with sufficient telomere length. We determined that the length of telomeres of gingival epithelial cells in patients with CGP on the background of NAFLD was significantly shorter compared to those of the control group. Chronic inflammation and oxidative stress are factors in premature cell aging and, accordingly, approaching the Hayflick limit [10].

NAFLD undoubtedly affects the length of adipocyte telomeres and such prematurely aged cells are the source of hypersecretion of pro-inflammatory cytokines and can indirectly accelerate the aging process in the cells of the periodontal complex, especially complement and enhance the negative effect of existing chronic inflammation in form of CGP. Scientific confirmation of influence that chronic inflammation and metabolic lesions makes on acceleration of cellular aging was negative correlation between the telomere length of epithelial cells, on one hand, and CGP and BMI, on the other hand. The sooner CGP treatment is initiated, the more proliferative possibilities will be preserved in the cells of the periodontal complex, and premature cellular aging will be stopped.

In the future, we consider it possible to determine the length of telomeres of gingival epithelial cells as the marker for predicting duration of the remission period in CGP treatment against the background of NAFLD.

Conclusions

The patients with an underlying somatic disease like NAFLD have chronic inflammation in the oral cavity in form of CGP while somatically healthy patients are characterized by chronic generalised catarrhal gingivitis, due to non-compliance with proper oral hygiene.

Chronic inflammation and oxidative stress in periodontal tissues in NAFLD are the factors contributing to premature cellular aging, which is confirmed by significant difference in the length of gingival epithelial cells telomeres between patients of both groups.

Shortening of the length of telomeres of gingival epitheliocytes is in negative correlation with progression of chronic inflammation in periodontal tissues, which is typical for CGP. Besides, high BMI was also negatively correlated with the length of telomeres, which may further worsen the course of CGP and contribute to premature cellular aging in the periodontal complex.

It is possible that Reaching the Hayflick limit will indicate depletion of proliferative opportunities in the periodontium, decrease in the quality of remission after a course of therapy, which means that the risks of chroniosepsis will remain in the oral cavity with negative reverse effect on the course of NAFLD.

Conflicts of interest: none.

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Authorship contributions: conception and design, critical revision of the article — D. V. E.; acquisition of data — V. Y. H., T. M. B., analysis and interpretation of data, drafting the article — D. V. E., V. Y. H., T. M. B.

References

1. Фадєєнко ІД, Гріднєв ОЄ, Кушнір ІЕ. Ендотоксинемія


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**Маркер старіння епітеліальних клітин ясен у пацієнтів з неалкогольною жировою хворобою печінки**

Вкорочення довжини теломер пародонта може бути не лише чинником фізіологічного старіння, а й маркером несприятливого впливу неалкогольної жирової хвороби печінки (НАЖХП).

**Мета**

— вивчити зміни пародонтального статусу у пацієнтів з НАЖХП.

**Матеріал та методи.** У дослідження залучено 76 пацієнтів з НАЖХП та 14 соматично здорових осіб. Проведено анкетування пацієнтів, стоматологічне обстеження з визначенням індексів, qPCR-дослідження зішкрібок з поверхні прикріплених ясен за допомогою одноразових аплікаторів.

**Результати.** Проведено анкетування пацієнтів, стоматологічне обстеження з визначенням індексів, qPCR-дослідження зішкрібок з поверхні прикріплених ясен за допомогою одноразових аплікаторів.

**Висновки.** Анкетування пацієнтів, стоматологічне обстеження з визначенням індексів, qPCR-дослідження зішкрібок з поверхні прикріплених ясен за допомогою одноразових аплікаторів.

**Ключові слова:** епітеліальна клітина, довжина теломер, пародонтит.

**Контактна інформація**

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