Saliva pH and Flow Rate in Patients with Periodontal Disease and Associated Cardiovascular Disease

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Background:
Periodontal disease, a frequent oral health problem, is connected with cardiovascular morbidity and mortality. This study aimed to assess the unstimulated saliva flow rate and saliva pH as markers of the severity of periodontal disease in patients with cardiovascular disease.

Material/Methods:
A cohort of 155 patients (78 men and 77 women, aged 30-92 years) was included, and a structured questionnaire obtained information about their health status, oral healthcare behaviors, and eating habits. An oral examination was performed to assess periodontal status and presence of dental calculus. The unstimulated whole salivary flow rate and salivary pH were measured. An oral hygienization was performed, and 3 months later, salivary flow rate and pH were reevaluated.

Results:
A severe form of periodontal disease was found in 22.4% of patients. Disease severity was strongly correlated with low pH values (6.25 in stage IV periodontal disease), lower salivary flow rate (0.28 mL/min), smoking, poor oral hygiene habits and obesity, with no significant differences by sex. We observed a significant increase of pH (up to 6.30±0.17) in patients with severe periodontal disease (P=0.001) and salivary flow rate values (0.29±0.07 mL/min; P=0.014) 3 months after oral hygienization. There was a strong association between the severity of periodontal disease and presence of cardiovascular disease (P=0.001).

Conclusions:
Our study suggests that the decrease of salivary flow rate and pH level might be associated with the severity of periodontal disease.

Keywords:
Cardiovascular Diseases • Fluxum • Periodontal Diseases • Saliva

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Background

Periodontal disease, a complex inflammatory disease initiated by bacteria of the oral biofilm [1], is one of the most frequent oral health problems and is possibly involved in the evolution of other chronic systemic diseases [2] by contributing to systemic inflammation [3]. The worldwide prevalence of periodontal disease is reported to be between 20% to 50% [4], with 5% to 11% of adults in Europe reported to have a severe form of periodontitis [5]. Studies performed in several areas in Romania have also shown that periodontal disease is a frequent health problem in 41% to 65% of the adult population [6-8], suggesting the need for further efforts in implementing oral healthcare programs to better control this pathology. The severity of periodontal disease can impact the general health of a population, as multiple studies have reported an association between periodontal inflammation and the increased risk of cardiovascular disease [9,10].

Although the exact mechanisms linking periodontal disease and cardiovascular disease are yet undiscovered, research has shown that periodontal disease is associated with cardiovascular mortality [11]. According to several studies, oral bacterial can enter the systemic circulation [12], especially in patients with gingival inflammation and multiple dental interventions [13]. At the same time, the presence of bacteria has been shown to influence the appearance and progression of atheromatosis in animals [14] and humans [15], with studies showing invasion of the endothelium in vitro [16].

Recent studies on oral biofilm in patients with periodontal disease found that bacteria (Porphyromonas gingivalis) directly mediate vascular damage in vivo by degrading endothelial adhesion, thus increasing vascular permeability and modulating leucocyte recruitment at the endothelium surface [17]. The local inflammation enhances cytokine and other inflammatory markers [18], which promotes the further destruction of dental tissue. Different inflammatory markers have been identified [19] as being associated with an increased cardiovascular risk in patients with periodontal disease [20].

The persistent inflammation caused by bacterial infection is an independent predictor of acute cardiovascular events [21]. In addition, improved oral hygiene has been shown to reduce cardiovascular events in patients [22]. Salivary secretion has an important role in maintaining the health of the oral cavity [23] by adjusting the pH level and thereby intervening in the regulation of teeth mineralization and gum health [24]. The saliva flow rate and saliva composition depend on various factors such as age, sex, body mass index (BMI) [25], medication use [26], and oral hygiene. Any modification in saliva properties can lead to oral health problems, including caries, dental calculus, gingivitis, and periodontal disease [27].

Gingival crevicular fluid is not easy to collect in a typical dental practice [28], but rather requires a skilled health provider using a correct technique [29]. Saliva is available in a large quantity, is easy to collect, is suitable for repetitive examinations, has been proven to be a suitable noninvasive diagnostic tool, and is suitable for mass-screening programs [30]. In addition, biomarkers have been identified in saliva that originate from both biofilm bacteria and the host.

Studies have shown that exposing the gingiva to saliva with a neutral or more alkaline pH level improves gingival healing in patients with periodontitis, while a lower pH level might have a superficial necrotizing effect [31]. Furthermore, bacteria multiplication, such as by Porphyromonas gingivalis, which has been shown to be implicated in the pathogenesis of periodontal disease, is influenced by pH level [32]. Also, bacteria present in the oral biofilm induce local inflammation and determine the increase of inflammatory markers [33]. Poor oral hygiene leads to gum problems [34], favoring bacteria multiplication and inducing modifications in saliva properties; therefore, there are several reasons to use saliva as a diagnostic and follow-up tool in periodontal disease [35].

Changes in saliva properties influence the progression of periodontal disease, which could increase cardiovascular risk. The purpose of this study was to investigate the correlation between saliva pH and flow rate and the severity of periodontal disease in patients with cardiovascular disease and to determine if saliva pH and flow rate variation could be used as potential markers of disease severity. Our objectives were to (1) evaluate the periodontal status and oral healthcare habits of patients with periodontal and cardiovascular disease; (2) identify factors that could influence the saliva pH and saliva flow rate in the selected group of patients; and (3) assess the modification of salivary pH and salivary flow rate after dental hygienization.

Material and Methods

We performed a cohort study between June 2018 and December 2019, including 155 patients selected from the Emergency Clinical County Hospital Sibiu, Sibiu County. Approval for the project was obtained from the Ethics Committee of the Emergency County Clinical Hospital Sibiu, Sibiu County, Romania (10936/25.05.2018). The study was conducted in accordance with the principles of the Declaration of Helsinki research guidelines as well as with Romanian legislation applying to biomedical research and data protection. All participants provided informed, written consent.

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Study Participants

The inclusion criteria for the study group were as follows: patients with cardiovascular disease, including arterial hypertension, valvular disease, myocardial infarction, stable coronary disease, chronic heart failure, and peripheral artery disease. A control group consisted of patients without cardiovascular disease. Patients in both groups agreed to undergo an oral health examination and oral healthcare services.

The exclusion criteria were as follows: hemodynamically unstable patients, situations that could influence systemic inflammatory response (such as recent surgical interventions, including dental surgery in the last 6 months), signs of acute infections or patient under treatment for acute/chronic infections, use of anti-inflammatory medications, other chronic diseases (such as diabetes, kidney disease, pulmonary diseases, bronchial asthma, autoimmune, and autoinflammatory diseases under treatment), malignancy, cognitive and psychiatric disorders, lactation or pregnancy, oral lesions other than dental calculus, caries, and generalized periodontal disease. The final patient sample selection is shown in Figure 1.

Patients’ medical information was obtained from their medical records. A physical examination assessed anthropometric measures including weight, height, and BMI. An initial group of 315 patients agreed to participate in the study. Of those, 107 patients were excluded for not meeting the inclusion and exclusion criteria. From the 208 remaining patients, 32 refused oral intervention. Of the remaining 176 patients, 21 did not come to the 3-month follow-up and were also excluded. Of the total of 155 patients finally included in the study, 112 had cardiovascular disease and 33 were in general health (Figure 1).

Subsequently, the 155 patients completed a structured questionnaire that collected information on their oral hygiene habits (frequency of teeth brushing per day, use of mouth rinse, dental floss, frequency of visits to the dentist, dental calculus removal procedures), alimentation habits, and physical exercise.

Oral Health Examination and Dental Scaling

Oral examinations of all patients were conducted by the same experienced dentist to minimize evaluation bias. Periodontal status was evaluated according to the 2018 periodontal disease assessment recommendations [36]. Periodontal disease severity was assessed using a plane examination mirror (Carl Martin, Germany) and dental probe (1-mm periodontal probe, Fima Instruments). The dentist recorded incidences of bleeding on probing, the pocket depth of all teeth, and clinical attachment loss using 6 index teeth (all first molars, or second molars as substitutes, the upper right central incisor, and lower left central incisor). Third molars, root remnants, and dental implants were excluded from all measurements. Clinical attachment loss was determined with the periodontal probe, and measurements were recorded on 4 sites per tooth, from the cemento-enamel junction to the base of the gingival sulcus. Pocket depth was measured from the gingival margin to the base of the gingival sulcus. Bleeding on probing was noted when it was present during or immediately after introduction of the probe in the gingival sulcus and on performing a smooth lateral move along the pocket wall on 5 sites per tooth (buccal and oral). No radiological assessments were performed owing to technical difficulties.

The oral health index-simplified (OHI-S) index [37] was calculated using 6 index teeth (1.1, 1.6, 2.6, 3.1, 3.6, 4.6). In cases of missing teeth, the adjacent teeth were used, and in cases of missing adjacent teeth, the opposite teeth were used. If dental plaque was found during the examination, the dentist proposed and performed oral hygienization for patients who agreed with the procedure. Dental scaling is defined as supra-gingival scaling to remove the plaque and calculus for preventive oral health. A Woodpecker DTE D1 ultrasonic scaler with Satelec probes (GD1, GD2) was used for supra-gingival scaling of all dental surfaces.

Three months later, the OHI-S score, clinical attachment loss, salivary flow rate, and saliva pH level were reevaluated using the same examination methods.

Saliva Analysis

Saliva analysis, performed for all participants in the study, consisted of determining the unstimulated whole salivary flow rate and measuring the salivary pH level before any dental procedures were conducted. The whole saliva collection procedure was explained to the patients to ensure compliance. Unstimulated whole saliva was collected following the drainage technique [38]. Patients were instructed to let saliva drop from their mouths into a graded tube. This was carried out between 8:00 AM and 10:00 AM on the day of the oral examination before dental procedures were performed. For this
morning saliva collection, patients were asked to not smoke, eat, or drink any beverages except water, and to not perform any particular oral hygiene except rinsing the mouth with drinking water to avoid influencing the final results.

The patients were given drinking water and asked to rinse their mouth out well. Five minutes after this oral rinse, patients were comfortably seated and asked to spit whole saliva into a sterile grade collection tube for 10 min. They were asked to refrain from talking and to drop the head down and let the saliva run naturally to the front of the mouth. The patients were also asked to not cough up mucus during saliva collection. All saliva collections were supervised. After recording the saliva flow rate results, the pH of the saliva was measured immediately to prevent any deterioration of the sample.

Salivary pH level was measured using a single-electrode digital pH meter (Mettler Toledo Seven Compaq PH, Autolinc, USA). The electrode was submerged in hydrochloric acid of 0.1 N standard solution the night before measurements. The pH meter was then calibrated using freshly prepared buffers of pH 7 and pH 4. Following this, the electrode was kept dipped in double-distilled water. Prior to dipping the electrode in the sample, it was gently and completely dried each time using fresh sterile tampons. After analyzing the pH, the electrode tip was again washed with a gentle stream of distilled water and then dipped in the double-distilled water.

The dentist performed dental scaling to remove the plaque and calculus and used an airflow and ultrasound scaling technique, which was followed by professional brushing. Subgingival scaling and root planing were not performed. Patients agreeing to oral hygienization were reexamined 3 months after the procedure. The dentist reevaluated the clinical attachment loss and determined the OHI-S score, salivary flow rate, and salivary pH level. The results were compared with the initial measurements.

Definition of Other Variables

Patients were asked about their living conditions (rural/urban area), education level, which was categorized as no education, elementary (≤8 years), or higher education (high school and university), and level of physical activity in the last 12 months (sedentary, 1-3 h activity per week, >3 h per week).

Smoking, coffee, and alcohol consumption was recorded. Patients were classified as smokers if they reported cigarette use for at least 5 years, and alcohol consumption was regarded as at least 3 occasions of drinking alcohol per week. No heavy alcohol drinkers were reported. Oral healthcare habits were measured as follows: frequency of tooth brushing, use of dental floss, use of mouthwash, and number of visits to the dentist per year.

Data Analysis

Statistical analyses were performed using SPSS version 17. For numeric variables, descriptive statistics were performed, and the comparisons between these variables were made with the nonparametric Kruskal-Wallis test for more than 2 independent series and with the Mann-Whitney U test for comparisons between 2 sets of independent values with no Gaussian distribution. For comparisons between 2 paired numerical series, the Wilcoxon signed-rank test was used. The correlations between numerical variables were made by determining the Spearman’s correlation coefficient. Results were considered significant with a value of P<0.05.

Results

Of the total 155 patients (50.3% men and 49.7% women, aged 64.49±14.72 years), 122 patients (78.8%) had been previously diagnosed with cardiovascular diseases and were receiving treatment. Of the 155 patients, 102 (65.8%) had periodontal disease. Table 1 presents data on the patient population included in our study (characteristics, oral hygiene habits, eating habits, physical activity level).

Analysis of individual characteristics (sex, background, smoking, alcohol consumption, coffee consumption, presence of dental caries) did not reveal any significant associations with the presence or the severity of periodontal disease. A statistically significant association was found between periodontal disease and age (P<0.001), obesity (P<0.001), and the presence of cardiovascular disease (P<0.001). The severity of periodontal disease was significantly associated with the number of missing teeth (P<0.001) and poor oral hygiene habits (P=0.002). As expected, bleeding on probing occurred more frequently in severe forms of periodontal disease (P<0.001). Annual dental scaling (P=0.003), regular mouthwash use (P=0.002), and dental flossing (P=0.005) were significantly associated with the absence of periodontal disease. Moreover, dental plaque was found to be a significant risk factor for periodontal disease (chi-squared test, OR 2.01, P<0.05, confidence interval [CI] 1.02-3.93, P=0.046).

Oral Healthcare Outcomes

Concerning oral healthcare, 20% of all patients reported that they had never been to a dentist, and 77.5% of patients with
<table>
<thead>
<tr>
<th>Variable</th>
<th>Without (n=53)</th>
<th>Stage I (n=44)</th>
<th>Stage II (n=35)</th>
<th>Stage III and IV (n=23)</th>
<th>p*&lt;sup&gt;**&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Population characteristics</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>54.26±16.23</td>
<td>67.86±9.75</td>
<td>69.00±12.55</td>
<td>74.30±5.46</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>Age groups</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25-49</td>
<td>19 (90.5%)</td>
<td>1 (4.8%)</td>
<td>1 (4.8%)</td>
<td>0 (0.0%)</td>
<td></td>
</tr>
<tr>
<td>50-70</td>
<td>25 (52.9%)</td>
<td>25 (32.9%)</td>
<td>20 (26.3%)</td>
<td>6 (7.9%)</td>
<td></td>
</tr>
<tr>
<td>Above 70</td>
<td>9 (15.5%)</td>
<td>18 (31.0%)</td>
<td>14 (24.1%)</td>
<td>17 (29.3%)</td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>25.24±3.78</td>
<td>25.49±3.89</td>
<td>27.61±4.23</td>
<td>28.87±3.18</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>31 (39.7%)</td>
<td>21 (26.9%)</td>
<td>18 (23.1%)</td>
<td>8 (10.3%)</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>Women</td>
<td>22 (28.6%)</td>
<td>23 (29.9%)</td>
<td>17 (22.1%)</td>
<td>15 (19.5%)</td>
<td></td>
</tr>
<tr>
<td>Background</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No education</td>
<td>4 (9.5%)</td>
<td>17 (40.5%)</td>
<td>12 (28.6%)</td>
<td>9 (21.4%)</td>
<td></td>
</tr>
<tr>
<td>Elementary</td>
<td>17 (20.0%)</td>
<td>16 (20.0%)</td>
<td>16 (17.8%)</td>
<td>16 (15.8%)</td>
<td></td>
</tr>
<tr>
<td>Higher</td>
<td>34 (64.2%)</td>
<td>10 (18.9%)</td>
<td>7 (13.2%)</td>
<td>2 (3.8%)</td>
<td>&lt;0.250</td>
</tr>
<tr>
<td>Physical exercise level</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sedentary</td>
<td>34 (31.5%)</td>
<td>31 (28.7%)</td>
<td>26 (24.1%)</td>
<td>17 (15.7%)</td>
<td></td>
</tr>
<tr>
<td>1-3 hours/week</td>
<td>18 (40.0%)</td>
<td>13 (28.9%)</td>
<td>8 (17.8%)</td>
<td>6 (13.3%)</td>
<td>0.802*</td>
</tr>
<tr>
<td>Daily</td>
<td>1 (50.0%)</td>
<td>0 (0.0%)</td>
<td>1 (50.0%)</td>
<td>0 (0.0%)</td>
<td></td>
</tr>
<tr>
<td>Alcohol consumption</td>
<td>Yes</td>
<td>12 (41.4%)</td>
<td>6 (20.7%)</td>
<td>7 (24.1%)</td>
<td>0.718&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Smoking</td>
<td>Yes</td>
<td>20 (29.4%)</td>
<td>10 (26.3%)</td>
<td>12 (28.6%)</td>
<td>0.428&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Coffee</td>
<td>Yes</td>
<td>23 (32.9%)</td>
<td>21 (28.9%)</td>
<td>16 (22.9%)</td>
<td>0.975&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cardiovascular disease</td>
<td>Yes</td>
<td>29 (23.8%)</td>
<td>31 (31.1%)</td>
<td>22 (26.2%)</td>
<td>0.001**</td>
</tr>
<tr>
<td>No</td>
<td>24 (72.7%)</td>
<td>6 (18.2%)</td>
<td>3 (9.1%)</td>
<td>0 (0.0%)</td>
<td></td>
</tr>
<tr>
<td>Oral healthcare habits</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Visits to the dentist</td>
<td>Never</td>
<td>7 (21.9%)</td>
<td>11 (34.4%)</td>
<td>7 (21.9%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>When needed</td>
<td>23 (29.9%)</td>
<td>23 (29.9%)</td>
<td>20 (26.2%)</td>
<td>0.196&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>17 (26.5%)</td>
<td>17 (28.7%)</td>
<td>17 (28.7%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1/year</td>
<td>8 (12.5%)</td>
<td>16 (20.0%)</td>
<td>9 (21.4%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2/year</td>
<td>6 (9.5%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3/year</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td></td>
</tr>
<tr>
<td>Scaling</td>
<td>Never</td>
<td>20 (20.2%)</td>
<td>23 (23.2%)</td>
<td>25 (25.3%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1/year</td>
<td>24 (54.1%)</td>
<td>10 (20.8%)</td>
<td>11 (22.9%)</td>
<td>0.003&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>2/year</td>
<td>3 (50.0%)</td>
<td>2 (33.3%)</td>
<td>1 (16.7%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3/year</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td></td>
</tr>
<tr>
<td>Dental brushing</td>
<td>Rarely</td>
<td>1 (33.3%)</td>
<td>0 (0.0%)</td>
<td>1 (33.3%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1/day</td>
<td>15 (24.6%)</td>
<td>21 (34.4%)</td>
<td>15 (24.6%)</td>
<td>0.342&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>2/day</td>
<td>37 (40.7%)</td>
<td>23 (25.3%)</td>
<td>19 (20.9%)</td>
<td></td>
</tr>
<tr>
<td>Mouthwash</td>
<td>Yes</td>
<td>23 (52.3%)</td>
<td>12 (27.3%)</td>
<td>6 (13.6%)</td>
<td>0.002&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>2/day</td>
<td>3 (6.3%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td></td>
</tr>
<tr>
<td>Dental floss</td>
<td>Yes</td>
<td>20 (51.3%)</td>
<td>10 (25.6%)</td>
<td>7 (17.9%)</td>
<td>0.005&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>2/day</td>
<td>2 (5.1%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td></td>
</tr>
<tr>
<td>Oral health examination</td>
<td>Probing depth</td>
<td>1.64±1.16</td>
<td>3.05±1.11</td>
<td>4±1.26</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.87±2.62</td>
<td>4±1.26</td>
<td>3.87±2.62</td>
<td>&lt;0.001**</td>
</tr>
</tbody>
</table>
Periodontal disease had never had dental scaling. Furthermore, 24.5% of patients with periodontal disease were not previously diagnosed by an oral healthcare provider and did not benefit from any specific treatment. The use of mouthwash and dental floss was not a common practice among the patients in the study group, and this was associated with the severity of periodontal disease \((P<0.001)\).

Saliva pH

In all patients, the saliva pH level decreased with the increase of the severity of periodontal disease (Kruskal-Wallis test, \(P<0.001\)). There were significant differences in the pH values between different stages of periodontal disease (Mann-Whitney U test, \(P<0.001\)). By analyzing the parameters used to assess the severity of patients’ periodontal disease, we found a strong association between salivary pH level and the absolute value of clinical attachment loss \((P<0.001, r=-0.56)\). Additionally, as the salivary pH level decreased, the number of missing teeth increased \((P<0.001, r=-0.28)\).

We did not find an association between salivary pH level and age, sex, oral hygiene practices, eating habits, exercise habits, and alcohol consumption \((P>0.05)\), but we found a strong association with smoking \((P=0.028)\) and found significantly low pH levels in patients who were overweight or had obesity.

Correct oral hygienization induced changes in salivary pH levels, which became significantly more alkaline at 3 months after dental scaling (Kruskal-Wallis test, \(P=0.001\)) \((\text{Figure 2})\).

Table 1 continued. Periodontal status and oral healthcare habits in the study group.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Without (n=53)</th>
<th>Stage I (n=44)</th>
<th>Stage II (n=35)</th>
<th>Stage III and IV (n=23)</th>
<th>(P^{*})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bleeding on probing</td>
<td>Yes</td>
<td>18 (17.6%)</td>
<td>35 (34.3%)</td>
<td>31 (30.4%)</td>
<td>18 (17.6%)</td>
</tr>
<tr>
<td>Clinical attachment loss mm</td>
<td></td>
<td>0.11±0.61</td>
<td>1.55±0.76</td>
<td>3.43±0.7</td>
<td>4.13±2.05</td>
</tr>
<tr>
<td>Dental absence Number of absent teeth</td>
<td></td>
<td>3.11±2.33</td>
<td>2.91±2.38</td>
<td>3.77±2.61</td>
<td>14.87±11.29</td>
</tr>
<tr>
<td>OHI-S score</td>
<td>Good (0-0.6)</td>
<td>35 (61.4%)</td>
<td>16 (28.1%)</td>
<td>1 (1.8%)</td>
<td>5 (8.8%)</td>
</tr>
<tr>
<td></td>
<td>Fair (0.7-1.8)</td>
<td>19 (27.5%)</td>
<td>31 (44.9%)</td>
<td>16 (23.2%)</td>
<td>3 (4.3%)</td>
</tr>
<tr>
<td></td>
<td>Poor (1.9-3)</td>
<td>2 (4.1%)</td>
<td>6 (12.2%)</td>
<td>26 (53.1%)</td>
<td>15 (30.6%)</td>
</tr>
</tbody>
</table>

Patients with severe periodontal disease had a decreased salivary flow rate (Kruskal-Wallis test, P<0.001). Analyzing the correlation between the stages of periodontal disease and salivary flow, we found significant differences between the groups with no periodontal disease and those with mild and moderate disease (Mann-Whitney U test, P<0.001). There was no significant difference between the groups with moderate and severe periodontal disease (Mann-Whitney U test, P=0.113). The salivary flow rate was associated with smoking (Mann-Whitney U test, P=0.004) and was decreased in patients who were overweight or had obesity (P=0.017). A lower salivary flow rate was also associated with a higher number of missing teeth (P=0.017) and higher clinical attachment loss (P<0.001, r=-0.52).

Furthermore, the oral hygienization procedure significantly improved the salivary flow rate after 3 months in patients with stage II, III, and IV periodontal disease (Kruskal-Wallis test, P=0.001) (Figure 3).

Patients with severe periodontal disease had a higher OHI-S score at baseline (Kruskal-Wallis test, P<0.001), and, at 3 months after treatment, the differences between OHI-S scores after periodontal treatment were insignificant (Kruskal-Wallis test, P=0.068). Furthermore, patients with severe periodontal disease also had increased clinical attachment loss at baseline and at 3 months after treatment (Kruskal-Wallis test, P<0.001), and there were no significant improvements at 3 months after superficial scaling (Table 2).

Three months after dental scaling, the salivary pH level increase was not significant (Wilcoxon signed-rank test, P=0.515), the salivary flow increase was significant (Wilcoxon signed-rank test, P<0.014), clinical attachment loss decrease was significant (Wilcoxon signed-rank test, P=0.024), and OHI-S score decrease was significant (Wilcoxon signed-rank test, P<0.001).

**Cardiovascular Disease**

There was a strong association between the severity of periodontal disease in patients with cardiovascular disease compared with patients without identified cardiovascular disease (P=0.001). All patients with severe forms of periodontal disease enrolled in the study had associated cardiovascular disease (Figure 4).

In total, 78.7% (122) of patients included in the study had cardiovascular disease. All had hypertension under treatment, and some patients had other associated cardiovascular diseases: 61.67% had stable coronary disease and 45.8% had myocardial infarction. In addition, 60.75% had degenerative valvular disease and 20.8% had arrhythmia. All patients with periodontal disease had a significantly higher prevalence of arrhythmia (P=0.01), peripheral artery disease, and coronary disease (P=0.032). The association between valvular disease and the presence of periodontal disease (regardless of the severity) was nonsignificant (P=0.23). Arrhythmia and myocardial infarction at a younger age (<60 years) was more frequent in patients with a severe form of periodontal disease (6.4% and 2.4%, respectively) (Figure 5).

By conducting logistic regression analysis with cardiovascular disease as a dependent variable, we observed that poor dental hygiene (as evaluated with the OHI-S score) was a significant risk factor for cardiovascular disease. Adapting oral health measures such as regular teeth brushing, flossing, and oral...
Hygienization might have a protective role. Moreover, clinical attachment loss was a risk factor for cardiovascular disease, and an increased saliva flow rate had a protective role (Table 3).

**Discussion**

Periodontal disease and cardiovascular disease are non-comunicable diseases [39,40] with common risk factors including age, sedentarism, and an unhealthy lifestyle [41].

Periodontal disease was present in 65.8% of our patients, a higher rate than in other studies [42], and a more severe form of disease was found in patients over 70 years old. The increasing prevalence of periodontal disease in an aging population has been demonstrated in studies [40,42].

In the present study, 14.8% of all included patients had a severe form of periodontal disease, similar to other reports [43], with no significant difference between men and women, as was found in studies in Europe [44] and Asia [45]. Moreover, 18.9% of patients in our study with cardiovascular disease

<table>
<thead>
<tr>
<th>Table 2. Patient reevaluation 3 months after oral hygienization.</th>
<th>Periodontal disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variables</td>
<td>Without (n=53)</td>
</tr>
<tr>
<td>Salivary pH (initial moment)</td>
<td>6.9±0.21</td>
</tr>
<tr>
<td>Salivary pH (3 months after)</td>
<td>6.99±0.17</td>
</tr>
<tr>
<td>Salivary flow rate (initial moment)</td>
<td>0.39±0.05</td>
</tr>
<tr>
<td>Salivary flow rate (3 months after)</td>
<td>0.39±0.04</td>
</tr>
<tr>
<td>CAL (initial moment)</td>
<td>0.11±0.61</td>
</tr>
<tr>
<td>CAL (3 months after)</td>
<td>0.16±0.8</td>
</tr>
<tr>
<td>OHI-S (initial moment)</td>
<td>0.59±0.50</td>
</tr>
<tr>
<td>OHI-S (3 months after)</td>
<td>0.21±0.30</td>
</tr>
</tbody>
</table>

had a severe form of periodontal disease, and, importantly, a high incidence of periodontal disease among patients with cardiovascular disease has been reported in other studies [42], demonstrating the importance of oral healthcare in this specific group of patients.

Our results showed that patients who are overweight or have obesity and have a tendency toward sedentarism have more severe forms of periodontal disease; BMI values were significantly higher in patients with periodontal disease (28.87±3.18 in patients with severe forms of periodontal disease, $P<0.001$).

Figure 5. Distribution of cardiovascular disease in different stages of periodontal disease. MI – myocardial infarction.

Table 3. Logistic regression (using Enter method) with cardiovascular disease as a dependent variable.

<table>
<thead>
<tr>
<th>Variables in the equation</th>
<th>B</th>
<th>S.E.</th>
<th>Wald</th>
<th>df</th>
<th>Sig.</th>
<th>Exp(B)</th>
<th>95% C.I. for EXP(B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Probing depth (mm)</td>
<td>-0.658</td>
<td>0.681</td>
<td>0.934</td>
<td>1</td>
<td>0.334</td>
<td>0.518</td>
<td>0.136 – 1.968</td>
</tr>
<tr>
<td>Bleeding on probing (yes)</td>
<td>-0.994</td>
<td>1.827</td>
<td>0.037</td>
<td>1</td>
<td>0.845</td>
<td>0.37</td>
<td>0.01 – 13.287</td>
</tr>
<tr>
<td>Clinical attachment loss (mm)</td>
<td>1.765</td>
<td>1.151</td>
<td>2.35</td>
<td>1</td>
<td>0.045</td>
<td>5.84</td>
<td>1.612 – 55.754</td>
</tr>
<tr>
<td>Dental calculus (yes)</td>
<td>3.551</td>
<td>2.417</td>
<td>2.159</td>
<td>1</td>
<td>0.042</td>
<td>34.846</td>
<td>1.306 – 3974.57</td>
</tr>
<tr>
<td>OHI-S</td>
<td>10.489</td>
<td>5.713</td>
<td>3.372</td>
<td>1</td>
<td>0.036</td>
<td>35933.412</td>
<td>2.493 – 2.62E+09</td>
</tr>
<tr>
<td>Saliva pH</td>
<td>-0.429</td>
<td>2.19</td>
<td>0.038</td>
<td>1</td>
<td>0.845</td>
<td>0.651</td>
<td>0.009 – 47.63</td>
</tr>
<tr>
<td>Saliva flow rate (ml/min)</td>
<td>-25.692</td>
<td>16.55</td>
<td>2.41</td>
<td>1</td>
<td>0.041</td>
<td>0.054</td>
<td>0.102 – 0.849</td>
</tr>
<tr>
<td>Dental scaling (yes)</td>
<td>-4.331</td>
<td>2.377</td>
<td>3.32</td>
<td>1</td>
<td>0.038</td>
<td>0.013</td>
<td>0.002 – 0.838</td>
</tr>
<tr>
<td>Dental brushing</td>
<td>-7.192</td>
<td>3.911</td>
<td>3.381</td>
<td>1</td>
<td>0.037</td>
<td>0.011</td>
<td>0.001 – 0.706</td>
</tr>
<tr>
<td>Mouthwash use</td>
<td>0.417</td>
<td>1.908</td>
<td>0.048</td>
<td>1</td>
<td>0.827</td>
<td>1.518</td>
<td>0.036 – 63.905</td>
</tr>
<tr>
<td>Dental floss use</td>
<td>-2.41</td>
<td>1.681</td>
<td>2.057</td>
<td>1</td>
<td>0.047</td>
<td>0.09</td>
<td>0.003 – 0.902</td>
</tr>
<tr>
<td>Constant</td>
<td>4.383</td>
<td>16.455</td>
<td>0.071</td>
<td>1</td>
<td>0.79</td>
<td>80.076</td>
<td></td>
</tr>
</tbody>
</table>
Saliva properties in periodontal and cardiovascular disease

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Lăzureanu P.C. et al:

Obesity has been associated with periodontal lesions, probably because it can enhance local inflammation [46]. Also, obesity is a known cardiovascular risk factor [47]. Smoking also has a negative impact on oral health, not only by inducing oxidative stress [48], but also by modifying saliva properties and composition [49], leading to the further alteration of dental and periodontal structures.

**Oral Hygiene**

Overall, our patients had an alarmingly low oral health hygiene score, with 63.87% of them never having had dental scaling. Other studies have acknowledged the necessity of the participation of a dental hygienist in a periodontal care program [50]. Only 18.2% of our patients with periodontal disease and associated cardiovascular disease reported having regular visits to their oral healthcare provider (once or twice per year) and 6.45% reported having regular scaling, but none of these patients reported receiving specific periodontal treatment prior to our evaluation. A Korean study with a large number of patients [45] showed that constant oral hygiene decreases cardiovascular risk by 14%, and these patients might also have fewer acute cardiovascular events.

Moreover, lack of a proper oral hygiene routine leads to tooth loss, with a median of 5 missing teeth in patients with an OHI-S score >1.9 in the present study. Missing teeth have been associated with high cardiovascular risk, although the mechanism is still unknown [45,51].

Education level also seems to have a strong impact on the progression of periodontal disease, as we observed that patients with a higher education level were more adherent to oral healthcare and had less severe forms of periodontal disease. Access to information and a proper oral health education has a beneficial impact, according to Boillot et al [52].

**Saliva Properties Modification in the Periodontal Group**

In the oral cavity, saliva helps maintain the pH level near neutral (6.78±0.04) [53], but studies have shown that patients with periodontal disease tend to have a more acidic pH [54], which is similar to our findings. The average pH level in our control group of healthy periodontal individuals was 6.982±0.207. Average pH decreased with the increase of the severity of periodontal disease and was highly associated with smoking habits in our study group. Furthermore, the salivary pH level of our patients was not associated with sex, similar to other findings [55], place of residence, and oral hygiene habits, although some studies [56] have shown evidence of differences between men and women. A high proportion of our female patients were smokers (41.5%), with an average of 15.5 packages per year, compared with an average of 15.1 packages per year in men, a factor that could explain the absence of a significant difference in salivary pH between men and women in our study group [57].

**Saliva Flow Rate in the Periodontal Group**

A Japanese study with a large number of participants showed that patients with lower salivary flow rates had an increased risk of developing periodontal disease [58]. In our study, we observed that the unstimulated salivary flow rate decreased with the increase of the severity of periodontal disease, with no significant difference between the moderate and severe stage of the disease; however, the unstimulated salivary flow rate remained lower than in the control group. Other studies also found no significant difference in salivary flow rate in different stages of periodontal disease [59]. We found no significant difference between men and women (P=0.058, Mann-Whitney U test), which was similar to the findings of other studies [60,61]. However, the same high proportion of women in our study were smokers, and smoking is proven to decrease the salivary flow rate [62].

In the present study, the salivary flow rate and salivary pH level showed a significant improvement 3 months after oral hygienization in patients with severe forms of periodontal disease, although they remained lower than in patients with no periodontal disease. Therefore, these factors are potential tools for periodontal disease follow-up.

Furthermore, the severity of periodontal disease and acute cardiovascular events (such as myocardial infarction at an early age) seem to be influenced by severe forms of periodontal disease [63]. In the present study, our group of patients with a severe form of periodontal disease also had more frequent arrhythmias and myocardial infarctions at an early age. Lower pH levels and lower salivary flow rates were reported in patients with cardiovascular disease [64] and are potential aggravating factors of existing periodontal disease and can therefore lead to the progression of systemic inflammation and further influence systemic diseases [65]. Of course, we have to keep in mind that older patients have multiple associated diseases and multiple treatments that could also influence the salivary pH level and salivary flow rate [66], which could further aggravate oral health status.

Our results showed that associated oral health problems such as dental plaque may be additional risk factors for the development and progression of periodontal disease, whereas obesity and smoking are associated with more severe forms of periodontal disease. Further, obesity and smoking are implicated in cardiovascular disease occurrence [67,68]. These are all modifiable risk factors for periodontal disease [3].

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The studies published until now revealed that there is an independent and a significant association between severe periodontal disease and cardiovascular disease [69]. In this context, although a very complex pathology by itself, periodontal disease can be considered a potentially modifiable nontraditional risk factor for cardiovascular disease, which can be considered in cardiovascular pathology [42].

Programs promoting oral health are very important [70] for prevention of diseases related to oral health, and there could be a significant benefit in implementing an educational program addressed to hospitalized patients. Studies show that even Romanian adolescents have lower oral health-related knowledge than other adolescents in Europe [71], and there is an opportunity for improvement through adequate oral health programs [72].

Limitations of the Study

The final sample of patients included in the study was small, with an uneven distribution regarding the presence or absence of cardiovascular disease. Patients were reevaluated 3 months after dental scaling, but long-term surveillance is required to determine how cardiovascular risk might be reduced by improving oral health.

Projection for Future Studies

The saliva pH level in our group of patients with periodontal disease was lower than that reported in other studies, motivating us to further research saliva chemical and microbiological composition on a larger scale in a Romanian population to explain what could influence these values in our country.

Further studies regarding the impact of oral health in patients with chronic diseases in our country are needed, and a regular follow-up program could be developed through the close collaboration with fellow dentists.

Conclusions

A low saliva flow rate and a lower pH level are associated with severe forms of periodontal disease, with significant changes in these measures after performing dental hygiene, which makes them accessible markers in patient follow-up. Arrhythmia and myocardial infarction were common in our group of patients with a severe form of periodontal disease. By improving patients’ oral hygiene and eating habits and increasing referrals to dental healthcare professionals, we could have better results in treating periodontal disease and perhaps also preventing acute cardiovascular events. We consider it necessary to implement specific oral healthcare programs for patients with cardiovascular disease.

Conflicts of Interest

None.
References: