Interrelationship between oral status and histopathological aspects of periodontitis in patients from Craiova, Romania

VERONICA MERCUȚ1, SHLOMO PAUL ZUSMAN2, KENNETH EATON3, MONICA SCRIECIU1, SILVIU-MARIAN SIMION3, RĂZVAN-EUGEN GHIȚĂ1, CRISTIAN ADRIAN RAȚIU6, SILVIU MIREL PITURU3, SANDA MIHAELA POPESCU6

1) Department of Prosthetic Dentistry, Faculty of Dental Medicine, University of Medicine and Pharmacy of Craiova, Romania
2) Division of Dental Health, Ministry of Health, Jerusalem, Israel
3) Visiting Professor, University College London Eastman Dental and King’s College London Dental Institutes, United Kingdom; Honorary Professor, University of Kent, Canterbury, United Kingdom
4) Department of Dentistry, Faculty of Medicine and Pharmacy, University of Oradea, Romania
5) Department II, Faculty of Dental Medicine, “Carol Davila” University of Medicine and Pharmacy, Bucharest, Romania
6) Department of Oral Rehabilitation, Faculty of Dental Medicine, University of Medicine and Pharmacy of Craiova, Romania

Abstract
The aim of the present study was to evaluate the oral status in a group of institutionalized individuals from Craiova, a city in South-Western Romania. Also, another objective of the study was to highlight the clinical, histological and immunohistochemical (IHC) aspects of periodontal disorders in the elderly. The study was performed on 48 institutionalized individuals examined in the Clinic of Dental Prosthetics, Faculty of Dentistry, University of Medicine and Pharmacy of Craiova, Romania, in 2013. The data were collected using medical records and intraoral clinical examination. The data were processed by descriptive statistical analysis. The gingival mucosa samples were harvested from subjects with teeth mobility. The gingival fragments collected were processed through the histological technique of paraffin inclusion. The serial sections were commonly stained with Hematoxylin–Eosin and processed by the IHC technique with anti-CD3, anti-CD45RO and anti-CD20 antibodies. Of the 48 study participants, 20 were males, 28 were females, aged over 56 years old. 66.6% of them had poor oral hygiene, with a mean simplified oral hygiene index (OHI-S) of 1.57, 16 had the generalized periodontitis, three experienced some form of gingival overgrowth at the remaining teeth caused by incorrect fixed prosthesis, and 25 participants presented a form of periodontitis with gingival retraction. Gingival mucosa samples were histologically diagnosed with papillomatosis with discrete parakeratosis and rich chronic inflammatory infiltrate. The IHC reactions for the studied antibodies were different for T- and B-cells, which had different disposition in lamina propria.

Keywords: institutionalized individuals, periodontitis, gingival inflammation, T- and B-cells.

Introduction
In the last decades, we witnessed a major demographic shift, especially in developed countries, represented by the increasing number of the elderly [1]. This is a consequence of the life expectancy increase, due to improved living conditions, the progress of medicine, and also a low birth rate.

In this context, the study on the changes produced in the oral cavity in the elderly is justified.

In 2003, the World Health Organization (WHO) mentioned, in “World Oral Health Report 2003”, that oral health is integral and essential to general health” [1]. The same document shows that oral health means no pain, discomfort, and disease of the craniofacial complex” [3].

Among the elderly population, the presence of oral health pathologies has a major impact on the quality of life, since oral health is also associated with other pathological processes, which also affect the basic nutritional maintenance [4]. For example, untreated or sub-optimally treated dental caries or periodontal infections may lead to malnutrition in elderly people [5].

Moreover, some studies indicated that the elder people who had fewer teeth had a greater risk of malnutrition [6]. More specifically, ageing is associated with problems in oral health such as teeth loss, oral dryness, periodontal diseases, dental caries, painful mucosal disorders and decreased masticatory functioning [7].

A poor oral health status is especially prevalent in the elderly, as it results from cumulative disease progression over time. The negative impact of poor oral conditions on the quality of life was shown in many studies and it represents an important issue [8]. These oral health problems have been shown to be worse in the institutionalized elderly, mainly due to the loss of independence, difficulty in accessing oral care and affordability of oral care [9].
Old age is associated with being edentulous or partially edentulous. Caries, periodontal disease, defective dentures and poor oral and denture hygiene are quite common. In addition, an association between poor oral health and adverse medical outcomes has been well documented [10]. The Report Commissioned by the Platform for Better Oral Health in Europe [11] specifies that public health issues are oral caries, periodontal disease and oral cancer. Between 1998–2001, surveys in Denmark, Germany and UK reported advanced periodontal breakdown in 14% of Danes, 76% of Germans and 31% of British aged between 65–74 years old [12].

The aim of the present study was to evaluate the oral status in a group of patients from Craiova, a city in South-Western Romania. Also, another objective of the study was to highlight the clinical, histopathological and immunohistochemical (IHC) aspects of periodontal disorders in the elderly.

Participants, Materials and Methods

Forty-eight institutionalized persons from Craiova were included in the study. All study participants gave their informed consent and they were examined in the Clinic of Dental Prosthetics, Faculty of Dentistry, University of Medicine and Pharmacy of Craiova.

The study was approved by the Ethical Committee of the University of Medicine and Pharmacy of Craiova. The data were collected using medical records and intraoral clinical examination.

The baseline oral health and hygiene status and oral hygiene procedures of the elderly were evaluated by a clinical examination conducted by two clinicians who were calibrated to ensure consistency before the baseline examinations. They visited the old people’s home and conducted the clinical assessments at baseline. The clinical assessments were performed according to the WHO protocol [13] and baseline decay, missing, filled teeth (DMFT) and DMFT values were established [14]. The oral hygiene was assessed using the simplified oral hygiene index (OHI-S) [15]. The subjects sat facing a window and no dental instruments were used except a dental mirror.

The data were recorded on paper and transferred to electronic spreadsheet by one operator and the accuracy of entry was checked by a second operator. The data included information on age, gender, decayed, missing and filled permanent teeth, denture wearing, oral hygiene index, prevalence of periodontal disease. The data were processed by a descriptive statistically analysis.

The subjects who showed clinical signs of periodontitis were subsequently clinically and radiologically examined. The periodontal parameters recorded were bleeding on probing (BOP), periodontal pocket depth (PPD) and clinical attachment level (CAL). It was set the right treatment strategy for each case. The irrecoverable teeth were extracted. After irrecoverable teeth extractions, the overgrowth gingival fragments were excised in order to favoring wounds healing and to smooth the edges of the wounds.

The gingival fragments collected were fixed in 4% formalin for 48 hours and then processed for paraffin embedding. The serial sections were stained with Hematoxylin–Eosin (HE) and trichromic Goldner–Szekely (GS), and then examined by light microscopy. A set of cluster of differentiation (CD) antigen specific monoclonal/polyclonal antibodies to detect different cell types within the tissues were used. These included anti-CD45RO (clone UCHL1, 1:50 dilution, Dako), anti-CD3 (clone F7.238, 1:25 dilution, Dako), anti-CD20 (clone L26, 1:50 dilution, Dako), anti-CD68 (clone KP1, 1:100 dilution, Dako) and anti-CD34 (clone QBEnd 10, 1:50 dilution, Dako) antibodies.

Results

The study group included 20 (41.66%) males, and 28 (58.34%) females. Most of the subjects, i.e., 33 out of 48 (68.75%), belonged to the +75 age group (Tables 1 and 2). More than half of the study participants, i.e., 66.6% of them had poor oral hygiene, with mean OHI-S index of 1.57, seven (14.58%) of them had total edentulism and 41 (85.42%) had partial edentulism (Table 3). 19.51% of the subjects lost between 1–12 teeth, but 25% of them were females and 75% were males. On the contrary, within the group who lost between 13–32 teeth, the proportion of females and males was inverse: out of 33 (80.48%) study participants, which lost between 13–32 teeth, 66.7% were females and 33.3% were males (Table 4).

| Table 1 – Age group distribution of study participants |
| Age group | No. of subjects | Percent |
| 35–64 | 7 | 14.59% |
| 65–74 | 8 | 16.66% |
| +75 | 33 | 68.75% |
| Total No. | 48 | 100% |

| Table 2 – Gender distribution of study participants |
| Gender | No. of subjects | Percent |
| Males | 20 | 41.66% |
| Females | 28 | 58.34% |
| Total No. | 48 | 100% |

| Table 3 – Distribution of study participants according to edentulism status |
| Edentulism status | No. of subjects | Percent |
| Total edentulous | 7 | 14.58% |
| Partial edentulous | 41 | 85.42% |
| Total No. | 48 | 100% |

| Table 4 – Distribution of study participants according to number of lost teeth |
| No. of lost teeth | Gender of subjects | No. of cases | Percent |
| Males | Females | 1–12 | 6 | 2 | 8 | 16.66% |
| 13–32 | 11 | 20 | 40 | 83.34% |
| Total No. | 17 | 31 | 48 | 100% |

Regarding the type of the used prosthesis, it was found that the majority of participants in the study did not show any prosthesis: 26 (54.17%) of the subjects for maxillary arch and 35 (72.92%) of the participants for mandibular arch (Table 5). Twenty-five participants had a form of localized chronic periodontitis, 13 study participants had generalized chronic periodontitis, three participants experienced localized gingival overgrowth around the remaining teeth caused by incorrect fixed...
prosthesis, and seven participants had total edentulism (Table 6). Periodontal parameters values of bleeding on probing, periodontal pocket depth and clinical attachment level are summarized in Table 7.

**Table 5 – Distribution of study participants according to type used prosthesis**

<table>
<thead>
<tr>
<th>Type of used prosthesis</th>
<th>Maxillary arch</th>
<th></th>
<th>Mandibular arch</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of prostheses</td>
<td>No. of cases</td>
<td>Percent</td>
<td>No. of cases</td>
<td>Percent</td>
</tr>
<tr>
<td>No. of prostheses</td>
<td>26</td>
<td>54.17%</td>
<td>35</td>
<td>72.92%</td>
</tr>
<tr>
<td>One fixed dentures</td>
<td>10</td>
<td>20.84%</td>
<td>6</td>
<td>12.5%</td>
</tr>
<tr>
<td>Two or more fixed dentures</td>
<td>2</td>
<td>4.16%</td>
<td>2</td>
<td>4.16%</td>
</tr>
<tr>
<td>Partial removable dentures</td>
<td>1</td>
<td>2.08%</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Removable and fixed dentures</td>
<td>3</td>
<td>6.25%</td>
<td>4</td>
<td>8.34%</td>
</tr>
<tr>
<td>Complete dentures</td>
<td>6</td>
<td>12.5%</td>
<td>1</td>
<td>2.08%</td>
</tr>
<tr>
<td>Total No.</td>
<td>48</td>
<td>100%</td>
<td>48</td>
<td>100%</td>
</tr>
</tbody>
</table>

**Table 6 – Distribution of study participants according to type of periodontitis**

<table>
<thead>
<tr>
<th>Periodontal disease</th>
<th>No. of subjects</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Localized chronic periodontitis</td>
<td>25</td>
<td>52.08%</td>
</tr>
<tr>
<td>Generalized chronic periodontitis</td>
<td>13</td>
<td>27.08%</td>
</tr>
<tr>
<td>Localized gingival overgrowth</td>
<td>3</td>
<td>6.25%</td>
</tr>
<tr>
<td>Without periodontal disease – total edentulism</td>
<td>7</td>
<td>14.59%</td>
</tr>
<tr>
<td>Total No.</td>
<td>48</td>
<td>100%</td>
</tr>
</tbody>
</table>

**Table 7 – Periodontal parameters values of study participants**

<table>
<thead>
<tr>
<th>Gender</th>
<th>BOP [%] Mean ± SD</th>
<th>PPD [mm] Mean ± SD</th>
<th>CAL [mm] Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>83.47 ± 0.36</td>
<td>3.39 ± 0.62</td>
<td>3.93 ± 1.32</td>
</tr>
<tr>
<td>Females</td>
<td>73.81 ± 0.55</td>
<td>3.19 ± 0.51</td>
<td>3.45 ± 1.73</td>
</tr>
</tbody>
</table>

BOP: Bleeding on probing; PPD: Periodontal probing depth; CAL: Clinical attachment level; SD: Standard deviation.

In cases with chronic localized and generalized periodontitis, the gingiva has presented signs of inflammation associated with gingival recession, bad oral hygiene and, also, subgingival and supragingival microbial plaque and calculus. In cases with gingival overgrowth, gingiva had an aspect of a red, violet, volume-swollen gum, with a smooth surface. The hyperplastic swelling had a hemispherical appearance, both in the gumline and in the gingival papillae. All three cases of gingival overgrowth were associated with poor oral hygiene and teeth mobility (Figure 1, a and b).

The histopathological study on the gingival mucosa fragments, harvested from the subjects with tooth mobility, showed the presence of some areas of hypertrophic epithelium, associated with acanthosis and parakeratosis processes (Figures 2, a and b), and also certain areas of gingival epithelium erosions. In the periodontal conjunctive tissue, in all the investigated subjects, there was highlighted the presence of a chronic inflammatory process, more or less abundant, mainly formed of lymphocytes, plasmocytes, macrophages and, sometimes, granulocytes. The intensity of the inflammatory reaction varied from one patient to another, and even from one area to another in the periodontium of the same patient (Figure 3, a and b), probably according to the quantity of antigens entered in the periodontal conjunctive tissue. There were frequently observed congested blood vessels, including microhemorrhages, because of the vascular wall deterioration. In some subjects, the inflammatory infiltrate was quite abundant, being associated with diffuse necrosis areas of the periodontal conjunctive tissue (Figure 4, a and b).

The IHC study highlighted an intensely positive reaction of the cells in the periodontal inflammatory infiltrate to the CD45RO antibody (Figure 5), while the T-lymphocytes (electively highlighted by the use of anti-CD3 antibody), the B-lymphocytes (electively highlighted by the anti-CD20 marking) and the macrophages (highlighted by the anti-CD68 antibody) had a lower IHC reaction (Figures 6–8). We should mention that most T- and B-lymphocytes were localized perivascularly, which shows their blood origin. Also, we observed that in areas of tissue necrosis, the macrophages were dominated numerically.

The evaluation of blood microvascularization, by using the anti-CD34 antibody, showed the presence of a high number of blood vessels, especially capillaries with heterogeneous lumenus, turgescent endothelium, aspects characteristic to the angiogenesis processes (Figure 9). Most often, the density of blood capillaries was correlated with the intensity of the inflammatory infiltrate of the periodontal conjunctive tissue.

**Figure 1 – (a and b) Patients with chronic periodontitis with gingival overgrowth at the remaining mandibular teeth.**
Figure 2 – (a and b) Gingival mucosa with squamous epithelium with acanthosis and discreet parakeratosis. HE staining: (a) ×100; (b) ×200.

Figure 3 – Gingival mucosa with rich and diffuse chronic inflammatory infiltrate in the lamina propria, with lymphocytes (a) and ectatic blood vessels with passive hyperemia (b). HE staining: (a) ×100. GS trichrome staining: (b) ×100.

Figure 4 – Periodontal area with abundant inflammatory infiltrate, mainly formed of lymphocytes, plasma cells, macrophages and rare granulocytes (a) associated with areas of tissue necrosis (b). HE staining: (a) ×400; (b) ×100.
Discussion

Considering the interconnectedness of oral diseases and general health, we must gain a complete understanding of the physiopathology of oral diseases within the dynamic, complex oral cavity in order to successfully develop potential treatments and accurate patient risk assessments for the prediction of these diseases [16].

In this respect, our study took into account the same aspects with other studies. Studies in the United States, Canada, Great Britain, and Europe have demonstrated that oral health status is deficient in patients and high prevalence rates of caries, poor oral hygiene and denture care, gingival inflammation, dry mouth, bleeding gums, and periodontal disease among nursing home elders [17].

The average age of patients included in this study was much smaller than that shown in other research. A study by Khanagar et al., on 462 institutionalized elderly, showed that the mean age of the dependent elderly...
residents was 76.98±7.33 years. 27.32% of the dependent elderly residents were males and 72.67% of them were females [18]. Regarding gender distribution, the results are consistent with results of other studies, that most of institutionalized persons were female. A study realized within Mount Sinai Visiting Program showed that the mean age of participants was 81.42 (±12.29) years, with 80% being female [19].

Regarding status edentation, most of the study participants (85.42%) were partially edentulous. The study results of Vadavadagi et al., on 394 subjects aged 18 to 35 years, showed that 75% were also partially edentulous even though the average age was much smaller and 46.88% were females [20]. This study indicates similar results with other research regarding significant association between gender and condition edentation [21].

A literature research made by Kossioni, in 2013, on teeth loss, dental decay and periodontal disease in the elderly was performed using available databases and electronic sources. His results revealed that the updated national data are scarce in many parts of the world, particularly in Africa, Asia and South America, and direct comparisons are not always possible due to methodological variations. The available information may indicate that dental disease in older adults worldwide is more prevalent compared to younger age groups, with significant variation between countries and regions. Teeth loss is currently more common in the developed countries, while dental decay and periodontal disease are more widespread globally [22]. In this study, most of the participants had a large number of missing teeth, respectively, between 12 and 32, mostly female.

Regarding the type of prostheses used by study participants, 54.17% of these did not wear any kind of prostheses due to poor conditions of the prosthetic alloy fixed dentures [25].

The results of other study highlighted the association between gingival outgrowing, grade 2 and 3, and fixed dentures, and overflowing fillings [26].

Generally, periodontitis is considered an infection of microbial origin, the pathogenesis of which is mediated through the host immune response [27–29]. It has been suggested that immune response to bacterial aggression plays an important role in periodontal disease [30]. Both T- and B-lymphocytes present in periodontitis tissues sites are considered to play an essential role in the pathogenesis of the disease [31].

Rosenkoetter et al. [32] published, since 1984, the results of a study, which well recognized the central role played by T-cells inmediating the host response against periodontal pathogens in periodontal disease. They regulate polyclonal B-cell activation as well as antigen-specific antibody formation [32]. In this study, we identified T-lymphocytes with CD3 and CD20 intense positive reaction in superficial lamina propria, at which occur exchanges with epithelial layers. Naïve T-cells (CD3) on their own are unable to recognize microbial antigens efficiently. Efficient T-cell response is dependent on the presence of specialized group of cells called the antigen presenting cells (APCs). These APCs recognize pathogen-associated molecular patterns (PAMPS) produced by microorganisms and present them as peptides to the naïve T-cell in a manner that allows them to mount an effective response that clears the bacterial antigen. The exposure of the naïve T-cell to microbial antigens results in the synthesis of new proteins, cellular proliferation, and differentiation of the naïve T-cell into effector and memory T-cells (CD45RO), which is specific for the activating antigen. Majority of T-cells in periodontitis lesions express CD45RO [33, 34]. In gingival samples analyzed in this study were identified lymphocytes with CD45RO positive reaction and diffuse distribution in deep lamina propria. Also, other studies highlighted the increasing frequency of CD45RO+ memory T-lymphocytes in diseased tissues in individuals with various forms of periodontal disease [35].

CD20 immunoexpression in B-cells was weak in deep lamina propria. CD79 represents a protein molecule composed of CD79a and CD79b components expressed almost exclusively on B-cells and B-cell neoplasms. CD79a and CD79b expression precedes immunoglobulin (Ig) heavy-chain gene rearrangement and CD20 expression during B-cell ontogeny and disappears later than CD20 in the late stage of B-cell differentiation into plasma cells [36]. B-cells serve as a well-controlled part of the adaptive host response and act on systems regulated by T-cells [37]. The naïve B-cells with CD20 positive reaction recognize antigens without the help of APCs. For differentiation and class’ switching to occur, it requires direct contact with memory T-cells [38]. Pathogenic bacteria activate B-cells polyclonal in the presence of T-cells into plasma cells [39]. Polyclonal activation of B-cell by pathogenic bacteria results in the development of antibodies with low avidity, which results in inefficient elimination of bacteria, [10] thus, was resulting in persistence of the bacteria, which, in turn, could cause tissue destruction. Jully et al. [40] reported that progressive periodontitis was characterized by a B-cell or plasma cell lesion, which was in agreement with the present study. This may be due to hyperactivity influenced by an imbalance in regulatory T-lymphocytes, which dominated the periodontal lesion [41].

The overall significance of the study consists in highlighting the high treatment needs in elderly patients.
from our country. The study has limitations represented by the reduced number of participants and by periodontal parameters values evaluation by partial mouth assessments [42].

Conclusions

Poor oral status and tooth loss are widespread in this sample of institutionalized elderly population, especially in women. Most of the study participants had significant prosthetic needs. There is a need to develop an oral health program for the elderly institutionalized including prosthetic rehabilitation. Overgrowth gingival mucosa due to poor oral hygiene and over-extended edges of fixed denture was histologically associated with papillomatosis and T- and B-cells rich infiltrate with different disposition in lamina propria, according to the stage of periodontal lesions.

Conflict of interests

The authors declare that they have no conflict of interests.

References


Conflict of interests

The authors declare that they have no conflict of interests.

References


**Corresponding authors**

Monica Scrieciu, Professor, DMD, PhD, Department of Prosthetic Dentistry, Faculty of Dental Medicine, University of Medicine and Pharmacy of Craiova, 2 Petru Rareş Street, 200349 Craiova, Dolj County, Romania; Phone +40723–516 539, e-mail: scrieciu_monica@yahoo.com

Cristian Adrian Raţiu, Associate Professor, DMD, PhD, Department of Dentistry, Faculty of Medicine and Pharmacy, University of Oradea, 1 Universităţii Street, 410087 Oradea, Bihor County, Romania; Phone +40741–077 976, e-mail: ratiu_cristian@yahoo.com

Received: January 25, 2017

Accepted: February 28, 2018