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Clinical, imaging and histopathological correlations of gingival overgrowth: a retrospective analysis in northeastern Romanian population

ALEXANDRU NEMŢOI[1,2], MIHAELA MONICA SCUTARIU[3], ANA NEMŢOI[4], LUCIAN EVA[5], GABRIELA FLORENŢA DUMITRESCU[6], PETRU PLĂMĂDEALĂ[7], DAN FERARIU[8], DANISIA HABA[4,9], CLAUDIA FLORIDA COSTEA[10,11]

[1] *Department of Morpho-Functional Sciences I, Faculty of Medicine, "Grigore T. Popa" University of Medicine and Pharmacy, Iaşi, Romania*

[2] *Office of Oral and Maxillofacial Surgery, "Prof. Dr. Nicolae Oblu" Emergency Clinical Hospital, Iaşi, Romania*

[3] *Department of Implantology, Removable Prosthesis, Dental Prosthesis Technology, Faculty of Dental Medicine, "Grigore T. Popa" University of Medicine and Pharmacy, Iaşi, Romania*

[4] *Department of Oral and Maxillofacial Surgery, Faculty of Dental Medicine, "Grigore T. Popa" University of Medicine and Pharmacy, Iaşi, Romania*

[5] *2nd Neurosurgery Clinic, "Prof. Dr. Nicolae Oblu" Emergency Clinical Hospital, Iaşi, Romania*

[6] *Department of Pathology, "Prof. Dr. Nicolae Oblu" Emergency Clinical Hospital, Iaşi, Romania*

[7] *Department of Pathology, "St. Mary" Emergency Children's Hospital, Iaşi, Romania*

[8] *Department of Pathology, Regional Institute of Oncology, Iaşi, Romania*

[9] *Department of Radiology, "Prof. Dr. Nicolae Oblu" Emergency Clinical Hospital, Iaşi, Romania*

[10] *Department of Ophthalmology, Faculty of Medicine, "Grigore T. Popa" University of Medicine and Pharmacy, Iaşi, Romania*

[11] *2nd Ophthalmology Clinic, "Prof. Dr. Nicolae Oblu" Emergency Clinical Hospital, Iaşi, Romania*

Abstract

Background: Gingival overgrowth refers to an increase in the size of the gingival tissue. The etiology varies, and is often a multi-factor issue; what may contribute to gingival enlargement are aspects, such as disease, local and systemic conditions and idiopathic factors. The aim of the present study is to analyze and to correlate the clinical, epidemiological, imaging and histopathological (HP) features of gingival overgrowth in northeastern Romanian population. *Patients, Materials and Methods*: We conducted a clinical, imaging, and pathological study on 98 patients with gingival overgrowth, who underwent a surgical intervention for a gingival biopsy in the Office of Oral and Maxillofacial Surgery, "Prof. Dr. Nicolae Oblu" Emergency Clinical Hospital, Iaşi, Romania, during a 14-month period (January 1, 2018 to February 28, 2019). All patients with localized gingival overgrowth had clinical and imaging investigations done and then were referred to an oral and maxillofacial facility. A surgeon performed the excision of the gingival overgrowth and then sent the surgical specimens to the Laboratory of Pathology for HP examination. *Results*: Local inflammation was found responsible for the gingival overgrowth in most of the cases, with the number of females outnumbering that of the males. A very good correlation was found between clinical and HP diagnosis when epithelial hyperplasia, peripheral giant cell granuloma and pyogenic granuloma were involved and a moderate one when fibrous hyperplasia was involved. *Conclusions*: These findings suggest that the occurrence of gingival overgrowth can have many causes, which highlights the importance of clinical pathology in assisting practitioners with making a better diagnosis.

Keywords: gingival overgrowth, diagnostic imaging, pathology, immunohistochemistry.

Introduction

Many types of lesions share the same site, which is the gingiva [1]. It is commonly affected by non-neoplastic and neoplastic lesions; the latter is usually characterized by a progressive growth, which can be either benign or malignant.

A great number of gingival localized overgrowths are however considered to be reactive lesions rather than non-neoplastic ones [2] and can develop as a result of many underlying both gingival and periodontal diseases. There have been recorded cases of idiopathic hyperplasia (gingival elephantiasis) and of secondary hyperplasia, as well caused by a bacterial infection and associated with other local iatrogenic factors like unadjusted prosthesis or malocclusions. There are also those cases generated in the course of systemic diseases, such as leukemia [3], diabetes, amyloidosis [4], and immunodeficiency disease or as a side effect of specific medications, such as phenytoin, cyclosporine or calcium channel blockers [5]. Also, attention should be given to the new materials used to improve dental implants osseointegration, like strontium ranelate [6], because maxillary bone regeneration could cause an inflammatory response of the maxillary bone and as such an overgrowth of the superjacent gingiva.

Even though almost all gingival overgrowths are

reactive, they demonstrate at the same time tumor-like hyperplasia, which renders very difficult the differential diagnosis with a neoplastic lesion. According to the size of the affected area, gingival overgrowths can be localized, regional or generalized. In the first case, the lesion is painless and has the aspect of a pedunculated or sessile mass of dissimilar colors, ranging from light pink to red. The outside appearance varies from non-ulcerated to ulcerated mass. Lesion dimension extends from a few millimeters to several centimeters [1, 2].

The clinical characteristics of the above-mentioned reactive gingival lesions seem to reflect their various developmental phases. In the early stages they look red, raw, have ulcerated surfaces and bleed spontaneously or on slight touch whereas in the late stages they may be pedunculated, sessile or leaf-shaped growths and look mature, firm, avascular and fibrous [7, 8].

For those gingival overgrowths that cannot be diagnosed on the basis of clinical and radiographic screenings alone, oral tissue biopsy may be required. Biopsy and histological examination represent the golden rule in oral pathology diagnosis and are used to confirm the clinical prognosis [9]. When further information is required for the indicated therapy, oftentimes the ultimate procedure, which can provide tissue for microscopic analysis, is biopsy [2].

As stated above, biopsy is oftentimes the only way to find the cause of a gingival hyperplasia. Furthermore, microscopic analysis is necessary in order to discern neoplasms from non-neoplastic lesions, which range from granulation tissues to avascular masses of collagen [10].

In the medical literature, many studies were aimed at analyzing the occurrence of oral reactive gingival lesions with regard to age, gender and location, but only a few have focused on the histopathological (HP) features of them [11]. Similarly, there are only few Romanian studies on this subject that have considered large number of patients and analyzed their clinical and HP features [12, 13], but there is really no study regarding this issue targeting the northeastern Romanian population.

Therefore, the aim of the present study is to analyze and to correlate the clinical, epidemiological, imaging and HP features of gingival overgrowth in northeastern Romanian population.

Patients, Materials and Methods

We started by selecting 98 patients who needed a medical checkup for the diagnosis and management of a gingival overgrowth, either localized or regional, from a total of 300 that had been referred to the Office of Oral and Maxillofacial Surgery, "Prof. Dr. Nicolae Oblu" Emergency Clinical Hospital, Iași, Romania, during a 14-month period (January 1, 2018 to February 28, 2019). The following exclusion criteria were used: edentulous subjects, generalized gingival enlargement, patients using anticonvulsant drugs, calcium-channel blockers, and immunosuppressants.

For each case, demographic data, such as age and gender, smoking habits and alcohol consumption patterns, also clinical features of the lesions, their location, imaging aspects, and HP diagnosis were recorded.

An oral and maxillofacial surgeon observed and described the clinical characteristics of all the localized gingival overgrowths. After the clinical examination, all the patients with localized gingival enlargements were referred to a panoramic radiograph and a cone-beam computed tomography (CBCT), in order to detect possible lesions in the bone. The radiologist described the images performed by a Panoramic X-ray Machine (Planmeca ProMax® 3D, Planmeca Oy, Helsinki, Finland) and CBCT images that were obtained using an X-ray device (Planmeca ProMax® 3D, Planmeca Oy, Helsinki, Finland) and a spiral technique with 0.2 mm thickness [200 μm voxel size, 200 mm field of view (FOV), 90 kV, 10 mAs, 1 mm pass].

Next, the surgeon performed the excision of the gingival overgrowth and sent the surgical specimens to the Laboratory of Pathology for HP examination.

The tissue samples were labeled, fixed in 4% neutral buffered formalin, dehydrated in a mixture of acetone and xylene, and then embedded in paraffin. Using a microtome, 3 μm thick serial sections were cut and stained with Hematoxylin–Eosin (HE) staining. Representative sections were stained with Szekely staining, in order to identify the fibrous connective tissue presence. Also, other representative sections were used for an immunohistochemical (IHC) two-step staining technique using the EnVision™+ detection system (Dako, Carpinteria, USA).

Briefly, histological slides were dried overnight, at 37°C, then deparaffinized in three washes of xylene and rehydrated in three graded ethanol washes (70%, 80%, 100%). The slides were treated with Dako target retrieval solution sodium citrate, pH 6, 1:10 dilution (Dako, Carpinteria, USA) before antigen retrieval was done by heating them at 95°C, in a steamer, for 30 minutes. Then, the slides were cooled to room temperature (RT) for 30 minutes, and treated with diluted 3% hydrogen peroxide to block endogenous peroxidase activity.

Four primary antibodies [monoclonal mouse anti-human cytokeratin (CK) 19, 1:100 dilution, Dako, Denmark; monoclonal mouse anti-vimentin, 1:100 dilution, Dako, Denmark; monoclonal mouse anti-human cluster of differentiation (CD) 34, class II, 1:50 dilution, Dako, Denmark; monoclonal mouse anti-human CD1a, 1:50 dilution, Dako, Denmark] were applied on the corresponding slides, at RT, for 30 minutes.

After washing them with Tris-buffered saline (TBS), the slides were incubated for 30 minutes, at RT, with Dako Envision™+ Dual Link System (Dako, Carpinteria, USA), followed by a 5-minute incubation with 3,3'-Diaminobenzidine tetrahydrochloride (Dako Liquid DAB+ Substrate Chromogen System, 20 μL:1 mL substrate, Dako, Carpinteria, USA) for color reaction, and then counterstained with Mayer's Hematoxylin (three minutes) to visualize the nuclei. Slides were then immersed in distilled water, dehydrated in graded alcohols (70%, 90%, and 100%), cleared in xylene and mounted in Entellan. The slides were viewed on a Leica DMC 2900 (Germany) light microscope and assessed for the presence of IHC staining.

CK19 immunostaining and vimentin immunostaining,

showing an epithelial origin and a mesenchymal origin of cells, respectively, were considered positive when, in each case, definite brown expression was observed in the cytoplasm of the cells. CD34 immunostaining, expressed on capillary endothelial cells and embryonic fibroblasts, and CD1a immunostaining, useful in differentiating Langerhans cells, were considered positive when a definite brown expression was observed in the membrane of the cells.

Negative controls were obtained through omission of the primary antibody.

All demographic, clinical, imaging, and pathological data collected from each patient were analyzed using descriptive statistics; continuous variables were expressed as mean ± standard deviation (SD). Values were considered significant at $p<0.05$. All statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) software (SPSS® for Windows, version 11, SPSS Inc., Chicago, IL, USA).

The correlation between clinical, imaging and pathological diagnosis was classified as follows: (*1*) expected data 1 (ED1) – provisional clinical diagnosis; (*2*) expected data 2 (ED2) – provisional imaging diagnosis; (*3*) real data (RD) – final histopathology diagnosis; (*4*) concordant data (CD) – correspondence between the expected data and real data. The correlation was calculated as follows: CC (complete concordance) = CD×100/ED, this expressing the percentage in which the clinical or imaging and the histological diagnosis overlapped. This method was adapted from the one previously reported by Patel *et al.* [14].

Results

Our study group involved 98 patients from the northeastern part of Romania (Table 1). There were 56 female and 42 male patients (F:M 1.33), with a mean age at presentation of 49.8 years for women (SD ±12.3) and 52.3 years for men (SD ±11.9).

Table 1 – *Frequency, site and gender distribution of patients with gingival overgrowth*

	Male	Female	Maxillary	Mandible	Total (%)
Fibrous hyperplasia	10	12	9	13	22 (22.44%)
Pyogenic granuloma	18	25	23	20	43 (43.87%)
Peripheral giant cell granuloma	5	13	8	10	18 (18.36%)
Central giant cell granuloma	0	1	0	1	1 (1.02%)
Epithelial hyperplasia	8	5	10	3	13 (13.26%)
Langerhans cell histiocytosis (mimicking gingival overgrowth)	1	0	0	1	1 (1.02%)
Total	42	56	50	48	98

When considering sampling sites, the majority of surgical samples were obtained from the maxillary gingiva (51 patients, 52.04%), followed by the mandibular gingiva (47 patients, 47.95%).

The most frequently observed and biopsied gingival lesions were the exophytic masses, caused by local inflammation.

Within the male group, 55% of the patients were tobacco smokers, 19% were chronic alcohol drinkers and 26% did not use any of these two products. Within the female group, 63% were tobacco smokers, only 7% of them were occasionally alcohol drinkers and 30% were non-smokers and non-alcohol drinkers. 34% of all patients had regular routine dental visits, but 76% had not had a dental checkup for a long period of time.

All patients presented an enlargement of the gingival tissue, which has been located in different regions of the gums, namely marginal, papillary and diffuse. The extension of the gingival overgrowths ranged from limited (gingiva adjacent to a single or two teeth), to regional (gingiva around three or more teeth) or even generalized. In some cases, isolated tumor-like enlargements, sessile or pedunculated, were discovered. Clinical features of the cases presented include lesions which begin as a slight swelling of the papilla or marginal gingiva (Figure 1A; Figure 2, A and B) and which may progressively increase in size and extension until it becomes generalized. Clinically, the enlargements may appear bluish or deep red in color. The gingival overgrowths are often friable and soft; they have a smooth shiny surface and usually bleed easily.

Other types of enlargements, such as firm, pink, non-inflamed mass were clinically presented, which seemed to grow from below the free gingival margin/interdental papilla (Figure 3A). Most often, the lesions were painless. Pain was associated due to secondary trauma *via* brushing, flossing or chewing. A particular type of gingival overgrowth we had to deal with were lesions with significant growth potential, purplish-red in color, and a tendency to bleed and to penetrate interdentally (Figure 4, A and B).

A single case presented a gingival overgrowth on the right part of the mandible between the teeth 4.3–4.6, covering the vestibular and oral surface of the two right premolars, with a soft tissue purple swelling (Figure 5A). On the panoramic and CBCT scan, an osteolytic lesion was found, which had developed on the right side of the body of the mandible and had obliterated the outer cortical (Figure 5, B–E). Another case presented a gingival tissue swelling in the region of the right and left mandibular molars and which was mimicking a gingival overgrowth. The molars had been extracted two months before (Figure 6, A and B). On panoramic and CBCT scan, osteolytic lesions were found (Figure 6, C–G).

The frequencies for each corresponding HP diagnosis are described in Table 1. Out of 98 cases, 43.87% of them were diagnosed as pyogenic granuloma (PG); fibrous hyperplasia accounted for 22.44% of all biopsied gingival lesions; peripheral giant cell granuloma (PGCG) was identified in 18.36% of cases, while epithelial hyperplasia represented only 13.26%. Only two of them (2.04%) presented localized gingival overgrowth as a consequence of a deep intraosseous lesion. One of them was diagnosed with central giant cell granuloma and one with Langerhans cell histiocytosis (LCH).

Correlating the HP aspect of the gingival lesion with the patients' gender, we found out that fibrous hyperplasia, PG and PGCG occurred more frequently in female patients, whereas male patients were more often diagnosed with epithelial hyperplasia (Figure 7).

Regarding the correlation between the HP diagnosis and gingival overgrowth location, we found out that the mandible was the most common site for the development of fibrous hyperplasia (59%) and PGCG (55%), whereas maxilla was the preferred location for epithelial hyperplasia (76%) (Figure 8). Gingival overgrowth in cases with a deep intraosseous lesion developed on both maxillary bones in an equal manner (Figure 2).

The patients who presented a gingival overgrowth due to soft gingival tissues enlargement did not show any change of the radiological investigations (Figures 3B, 4C, 5C and 6C). In the two cases with deep intraosseous lesions, the imaging investigations revealed an osteolytic lesion of the maxillary bones (Figure 5, B–E; Figure 6, C–G).

The HP and IHC analysis of the surgical samples revealed some interesting facts.

The gingival epithelial hyperplasia cases showed an ulcerated gingival surface epithelium that displayed an excessive acanthosis; this resulted in branched epithelial ridges descending deep into the lamina propria, which contained numerous dilated capillaries and inflammatory infiltrate around. IHC staining with anti-CK19 antibody revealed the epithelial ridges that branched and adhered to each other (Figure 1, C and D; Figure 2, D–F).

The gingival fibrous hyperplasia cases displayed an epithelial hyperplasia with parakeratosis and acanthosis, and also long epithelial ridges that looked like "gloves fingers" and descended into the lamina propria (Figure 3D). At the same time, lamina propria has been replaced in almost all of its thickness by a heavy proliferation of fibrous tissue. The fibrous tissue consisted of thick bundles of collagen fibers haphazardly arranged and admixed with numerous dilated new capillaries, presenting inflammatory infiltrate around them (Figure 3E). IHC staining with anti-vimentin antibody revealed reactive proliferation of fibroblasts, the presence of numerous capillaries and lymphocytic inflammation (Figure 3, F and G).

The PGCG cases displayed a non-encapsulated mass consisting of numerous multinucleated giant cells and covered by a hyperplastic gingival epithelium (Figure 4D). The lesion was covered by the gingival squamous cell epithelium, which was ulcerated in its superficial part and presented acanthosis, with thick epithelial ridges descending into the lamina propria. Also, lamina propria was enlarged due to heavy chronic inflammation (Figure 4E). The non-encapsulated mass consisted of a fibrillar connective tissue containing abundant plump mesenchymal cells, ovoidal or spindle-shaped, and many multinucleated giant cells scattered throughout the lesion, along with extravasated red blood cells (Figure 4F).

Gingiva covering the central giant cell granuloma also displayed epithelial hyperplasia due to acanthosis (Figure 5F) and an enlarged lamina propria too, as thick collagen bundles were arranged in a haphazard fashion and admixed with a few dilated new capillaries (more numerous at the papillae level) and few chronic inflammatory cells (Figure 5G). IHC staining with anti-CD34 antibody revealed numerous newly formed capillaries with moderate inflammation, made up of mononucleated and polynucleated cells in the lamina propria (Figure 5H). The deep part of the surgical sample showed a lesion made up of a proliferation of osteoclast-like multinucleated giant cells, with five to 20 nuclei. The stroma also contained plump spindle-shaped mononuclear cells, numerous vascular spaces with erythrocytes inside, and some newly formed bone at the edge of the lesion (Figure 5, I and J).

In the LCH case, gingiva covering the deep lesion showed almost the same morphological changes as those encountered in the LCH case (epithelial hyperplasia with acanthosis and enlarged lamina propria due to bundles of collagen fibers arranged haphazardly). On the other hand, LCH was identified as a proliferation made up of Langerhans cell, presenting irregular nuclei, incisions and fine chromatin, as well as frequent eosinophils. The hallmark of the lesion was CD1a diffuse positivity in Langerhans cells (Figure 6, H–J).

Table 2 presents the correlation between clinical, imaging, and HP data.

Table 2 – ***Correlation between clinical, imaging and histological diagnosis***

	RD	ED1	CC [%]	ED2	CC [%]
Fibrous hyperplasia	22	12	54.54%	–	–
Pyogenic granuloma	43	35	81.39%	–	–
Peripheral giant cell granuloma	18	15	83.33%	3	16.66%
Central giant cell granuloma	1	0	0%	1	100%
Epithelial hyperplasia	13	12	92.3%	2	15.38%
Langerhans cell histiocytosis (mimicking gingival overgrowth)	1	0	0%	1	100%

ED1: Expected data 1 (provisional clinical diagnosis); ED2: Expected data 2 (provisional imaging diagnosis); RD: Real data (final histo-pathology diagnosis); CC: Complete concordance = CD×100/ED; CD: Concordant data (correspondence between the expected data and real data); $p<0.05$.

We found a very good match between clinical aspects and pathological diagnosis in the epithelial hyperplasia cases (92.3%), PGCG cases (83.33%), and PG cases (81.39%), but only a moderate one in the fibrous hyperplasia cases (54.54%). We did not find any match (0%) between clinical aspects and pathological diagnosis in the LCH or central giant cell granuloma cases.

Regarding the correlation between the imaging aspects and pathological diagnosis, we found a strong match (100%) in lesions with bone destruction, such as LCH and central giant cell granuloma and a poor one in epithelial hyperplasia (15.38%) and PGCG (16.66%). We did not consider any correlations for the fibrous hyperplasia and PG, because these two pathologies did not present any changes in the bone structure in the imaging tests.

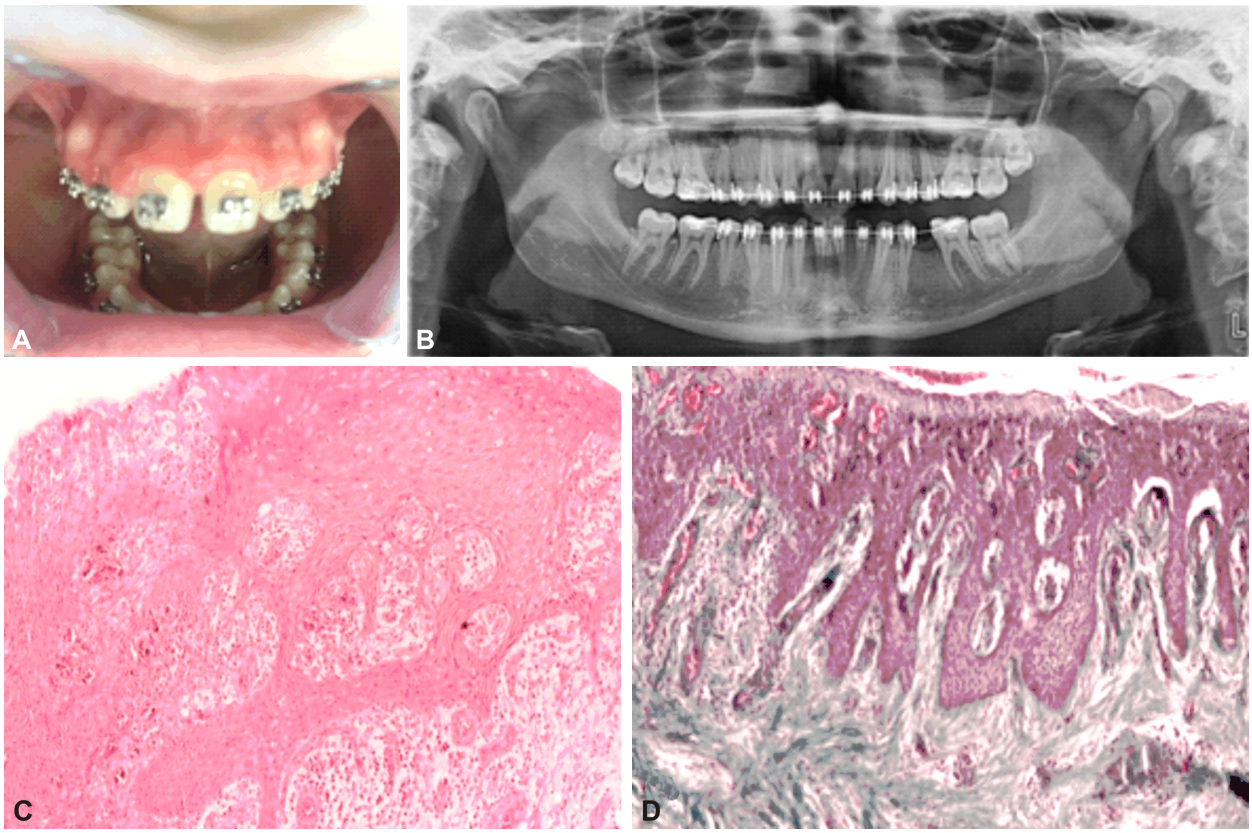

Figure 1 – ***Female patient, 26-year-old, with epithelial hyperplasia in the region of the right maxillary bone: (A) Clinical aspect of gingival tissue enlargement in the region of right maxillary incisors and canine due to the inflammatory reaction in the presence of fixed orthodontic treatment; (B) Imaging aspect without any bone modification in the region; (C) Photomicrograph showed an excessive acanthosis of gingival stratified squamous epithelium, with many branched epithelial ridges descending deep into the lamina propria (HE staining, ×200); (D) Lamina propria was also enlarged due to proliferation of the fibrous connective tissue and of extensive inflammatory cell infiltration (Szekely staining, ×200).***

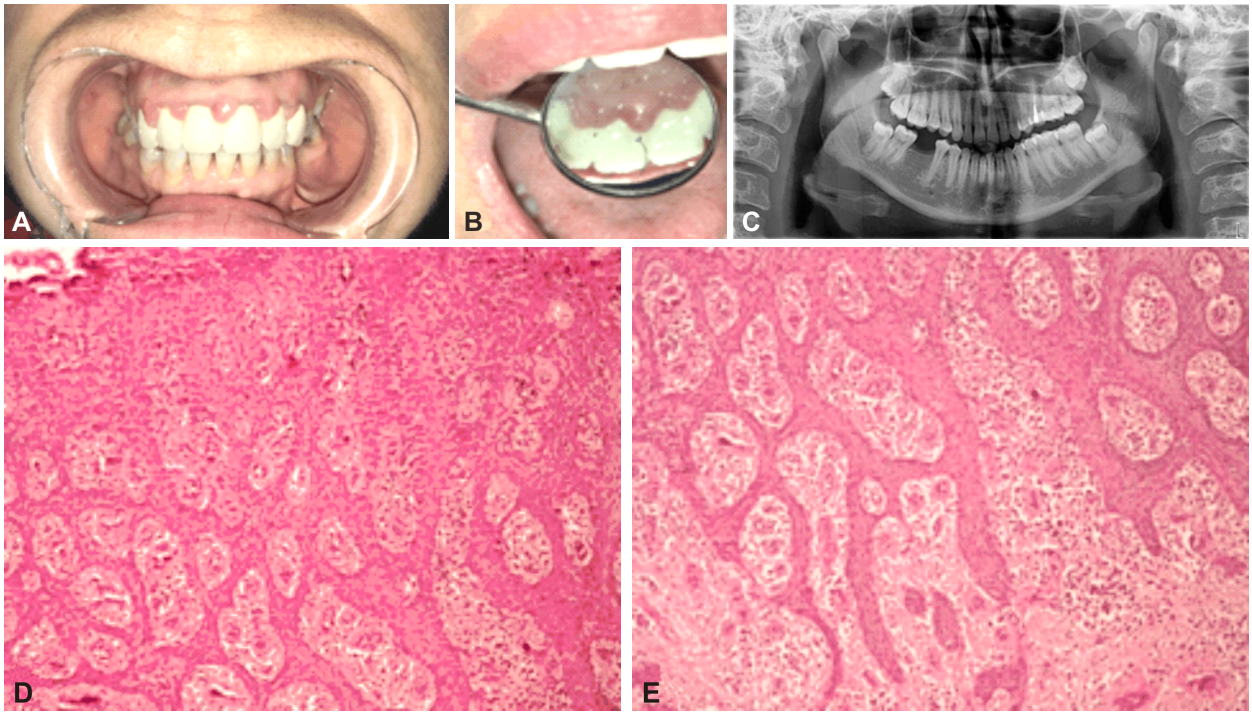

Figure 2 – ***Male patient, 49-year-old, with gingival epithelial hyperplasia in the maxillary region: (A and B) Clinical aspect of gingival tissue enlargement in the region of papilla between maxillary incisors; (C) Imaging aspect without any bone modification in the region; (D and E) Photomicrographs showed ulcerated gingival surface epithelium with excessive acanthosis leading to branched epithelial ridges and extensive inflammatory cell infiltration in the lamina propria (HE staining, ×100) – superficial part of the epithelial hyperplasia (D); deep part of the epithelial hyperplasia (E).***

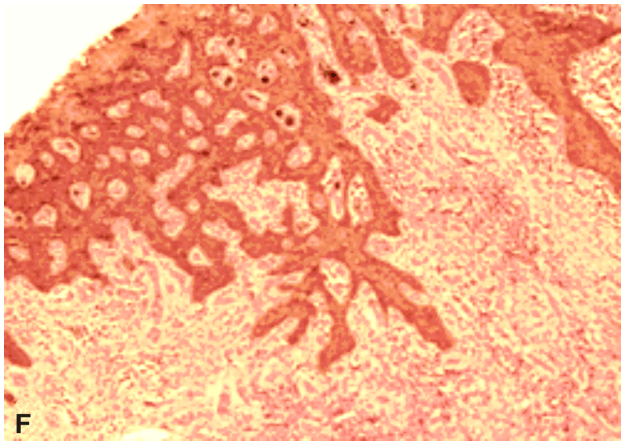

Figure 2 (continued) – ***Male patient, 49-year-old, with gingival epithelial hyperplasia in the maxillary region: (F) Strong immunopositivity for CK19 of the gingival epithelium revealed the heavy branched epithelial ridges that descended deep into the lamina propria (Anti-CK19 antibody immunostaining, ×50). CK19: Cytokeratin 19.***

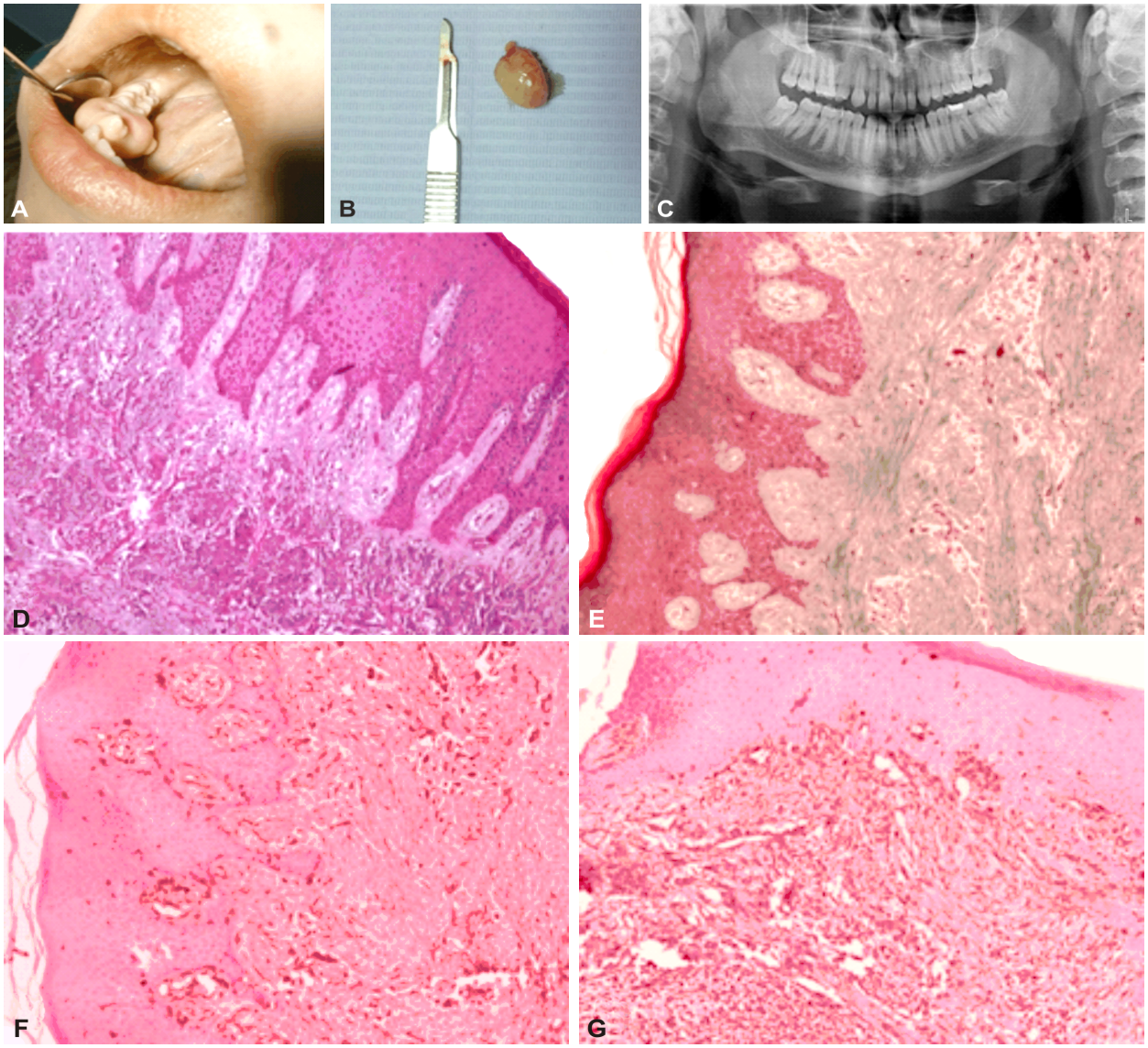

Figure 3 – ***Female patient, 24-year-old, with gingival fibrous hyperplasia in the region of right mandible: (A) Clinical aspect of gingival enlargement in the region of right mandibular premolars; (B) Macroscopic aspect of lesion – a raised sessile mass with a smooth surface, having its external surface of the same color as the surrounding gingiva; (C) Imaging aspect without any bone modification in the region; (D) Gingival epithelial hyperplasia with parakeratosis and acanthosis, with long epithelial ridges having "gloves finger" features and elongated papillae – the hyperplastic epithelium covered a heavy proliferation of fibrous tissue that replaced the lamina propria (HE staining, ×200); (E) The exceedingly dense fibrous connective tissue presented thick collagen bundles arranged in a haphazardly fashion, numerous dilated new capillaries and inflammatory infiltrate around (Szekely staining, ×200); (F and G) Two different areas of gingival hyperplasia revealing strong expression of vimentin immunostaining with different degrees of fibrous hyperplasia (Anti-vimentin antibody immunostaining, ×200) – moderate proliferation of fibroblasts (F); heavy proliferation of fibroblasts and lymphocytic inflammation (G).***

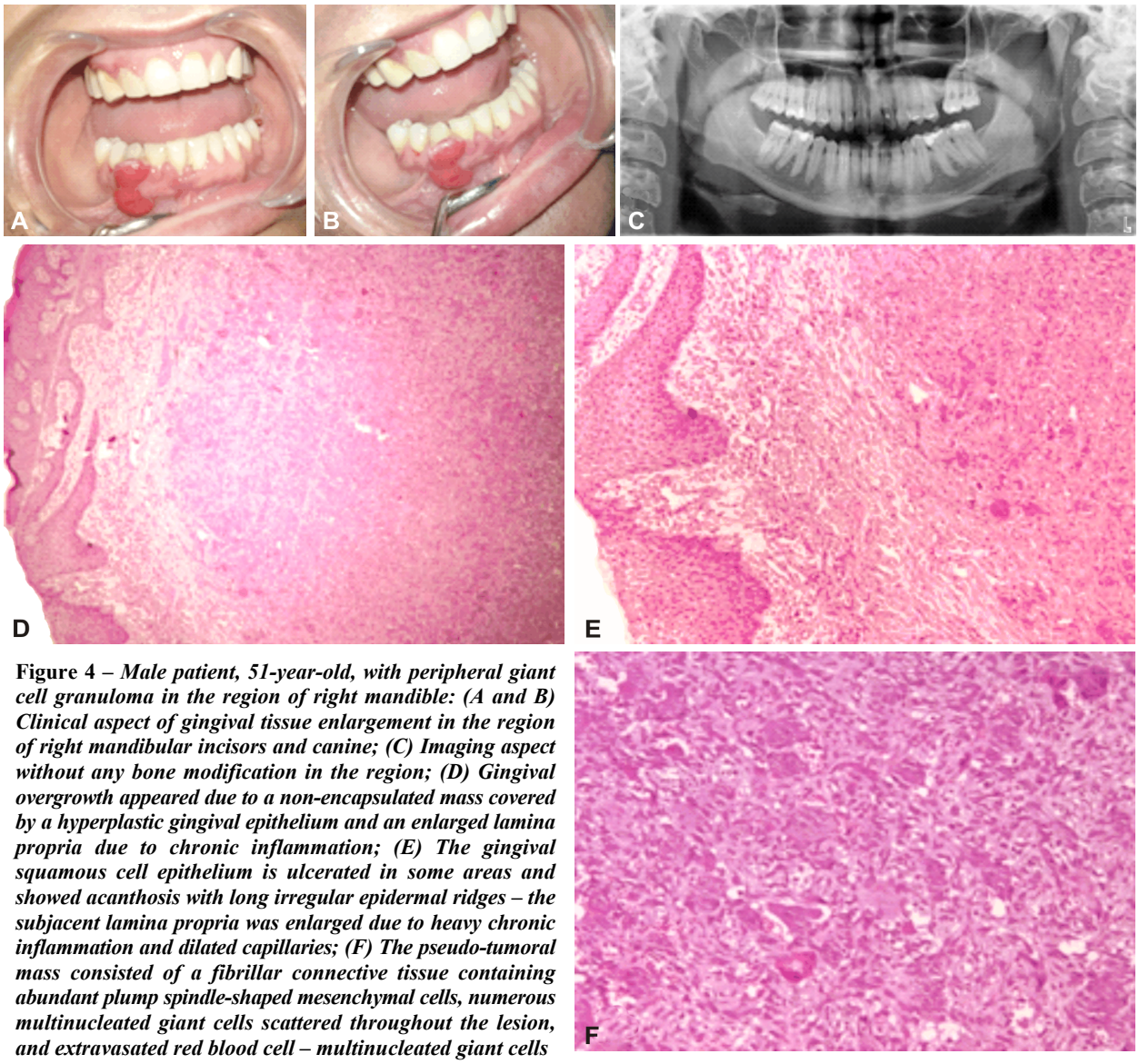

Figure 4 – ***Male patient, 51-year-old, with peripheral giant cell granuloma in the region of right mandible: (A and B) Clinical aspect of gingival tissue enlargement in the region of right mandibular incisors and canine; (C) Imaging aspect without any bone modification in the region; (D) Gingival overgrowth appeared due to a non-encapsulated mass covered by a hyperplastic gingival epithelium and an enlarged lamina propria due to chronic inflammation; (E) The gingival squamous cell epithelium is ulcerated in some areas and showed acanthosis with long irregular epidermal ridges – the subjacent lamina propria was enlarged due to heavy chronic inflammation and dilated capillaries; (F) The pseudo-tumoral mass consisted of a fibrillar connective tissue containing abundant plump spindle-shaped mesenchymal cells, numerous multinucleated giant cells scattered throughout the lesion, and extravasated red blood cell – multinucleated giant cells presented an abundant cytoplasm and five to 20 nuclei. HE staining: (D) ×50; (E) ×100; (F) ×400.***

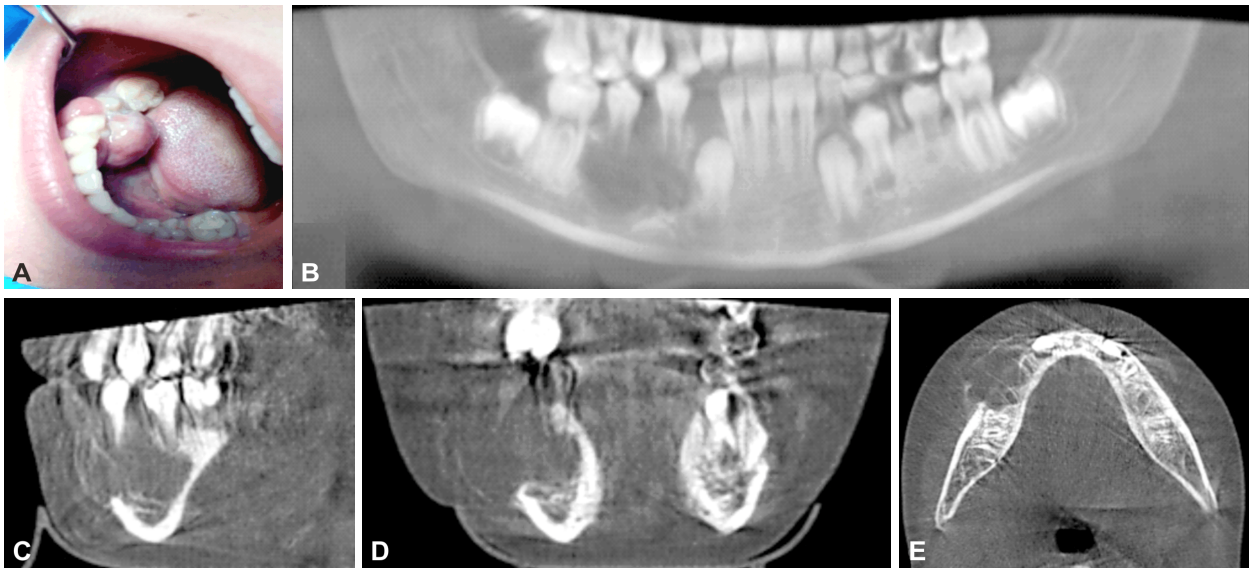

Figure 5 – ***Male patient, 10-year-old, with central giant cell granuloma in the mandible region: (A) Clinical aspect of enlargement gingival tissue in the region of right mandibular premolars; (B–E) Imaging aspects of an osteolytic lesion developed adjacent to dental apexes 4.3–4.6, measuring 23.21/22.63/31.55 mm (vestibulolingual/craniocaudal/transversal), which destroys the outer cortical of the mandible with obvious extension in the soft parts.***

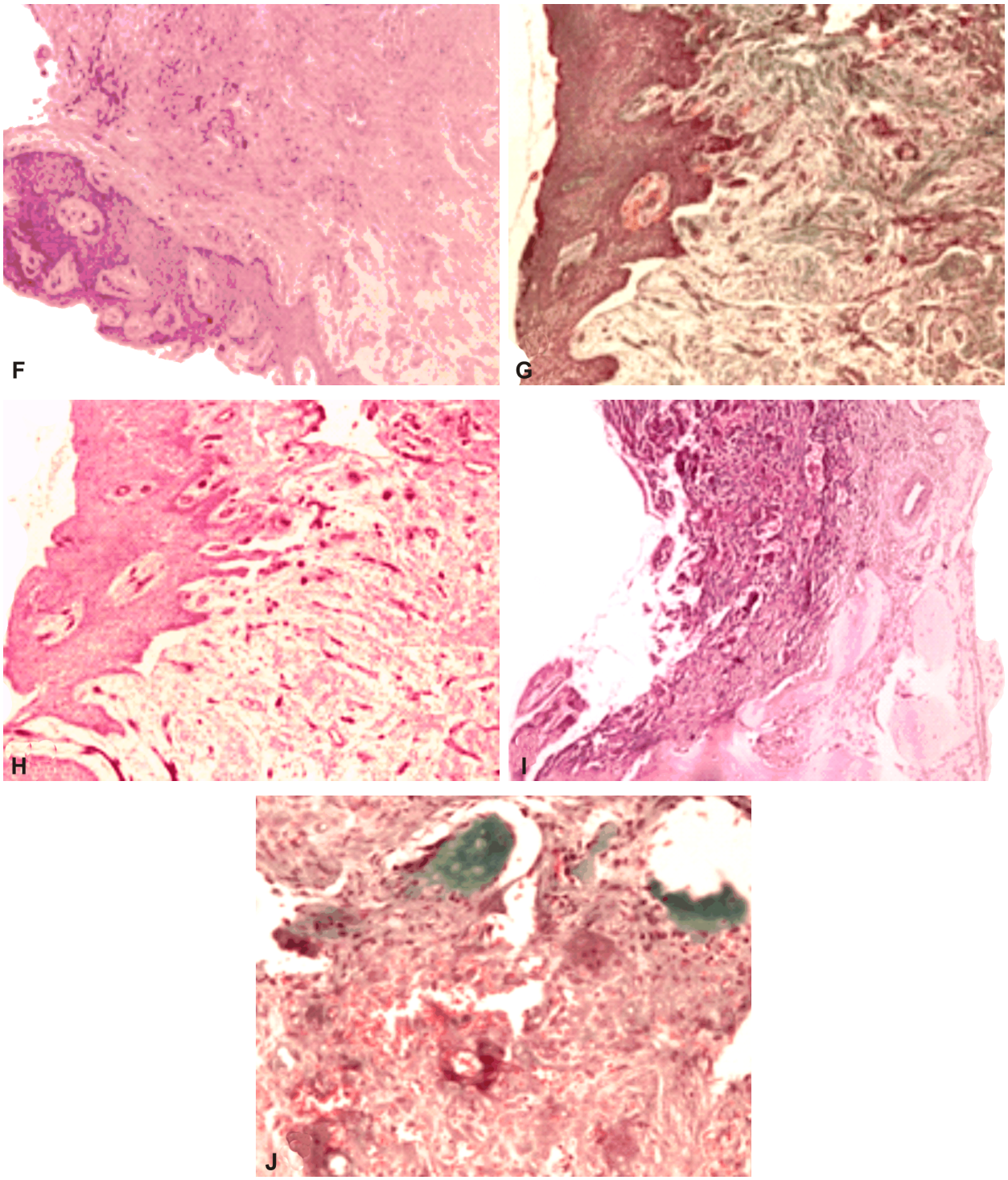

Figure 5 (continued) – ***Male patient, 10-year-old, with central giant cell granuloma in the mandible region: (F) Gingival enlargement in the close proximity of the lesion showed epithelial hyperplasia and lamina propria with moderate inflammation made up of mononucleated and polynucleated cells located around the capillaries; (G) Epithelial hyperplasia and fibrous connective tissue proliferation in the lamina propria, with collagen bundles haphazardly arranged together with numerous newly-formed vessels; (H) Epithelial hyperplasia and numerous newly-formed vessels in the lamina propria; (I) The deep intraosseous lesion was made up of a proliferation of osteoclast-like MGCs dispersed amongst a fibrous stroma; (J) The stroma contained plump spindle-shaped mononucleated cells with smooth oval nuclei, numerous vascular spaces with erythrocytes inside, and some new bone formation at the lesion edge. MGGs contained between five and 20 nuclei. Anti-CD34 antibody immunostaining: (H) ×100. HE staining: (F and I) ×100. Szekely staining: (G) ×100; (J) ×400. MGCs: Multinucleated giant cells.***

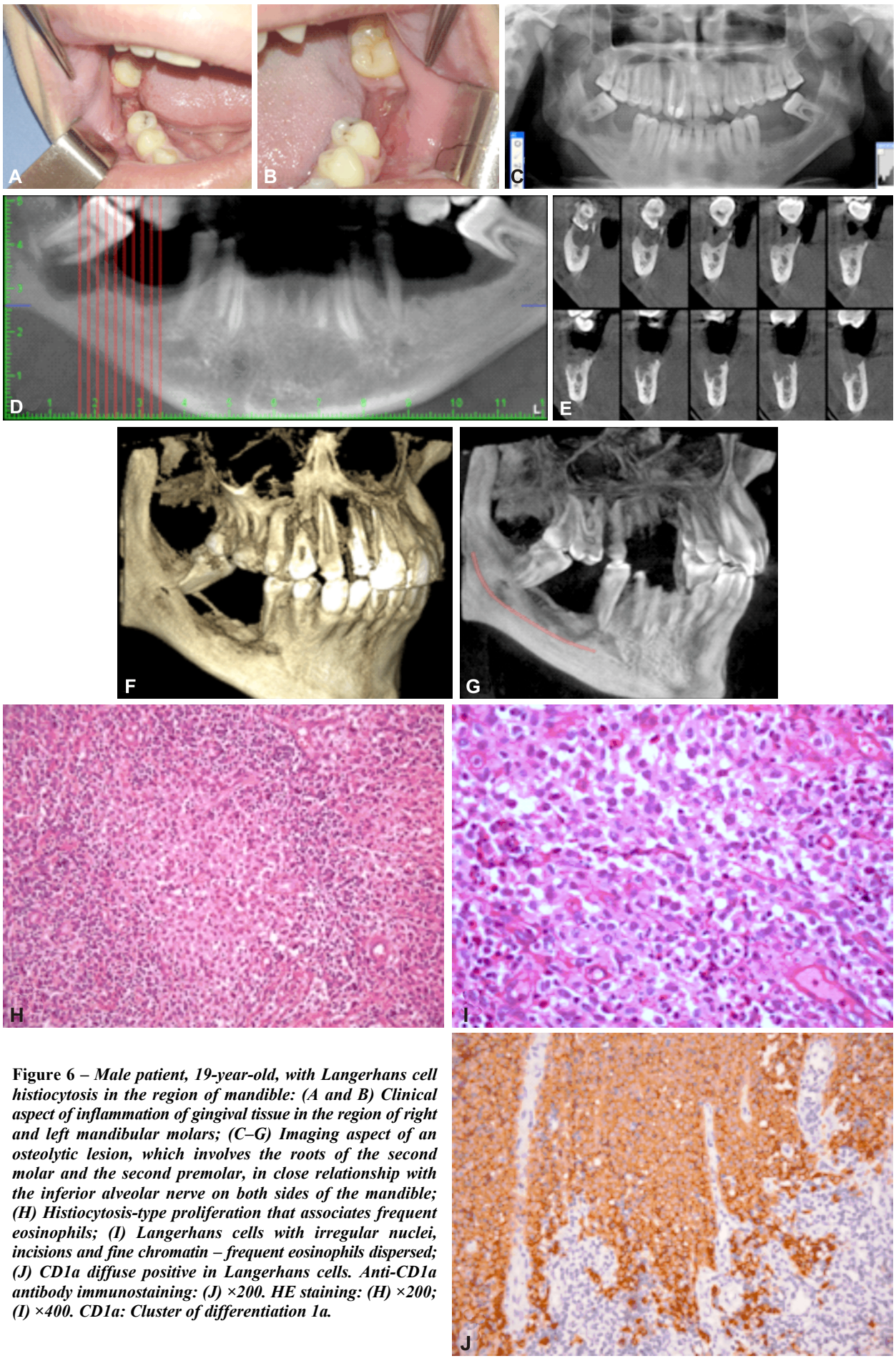

Figure 6 – ***Male patient, 19-year-old, with Langerhans cell histiocytosis in the region of mandible: (A and B) Clinical aspect of inflammation of gingival tissue in the region of right and left mandibular molars; (C–G) Imaging aspect of an osteolytic lesion, which involves the roots of the second molar and the second premolar, in close relationship with the inferior alveolar nerve on both sides of the mandible; (H) Histiocytosis-type proliferation that associates frequent eosinophils; (I) Langerhans cells with irregular nuclei, incisions and fine chromatin – frequent eosinophils dispersed; (J) CD1a diffuse positive in Langerhans cells. Anti-CD1a antibody immunostaining: (J) ×200. HE staining: (H) ×200; (I) ×400. CD1a: Cluster of differentiation 1a.***

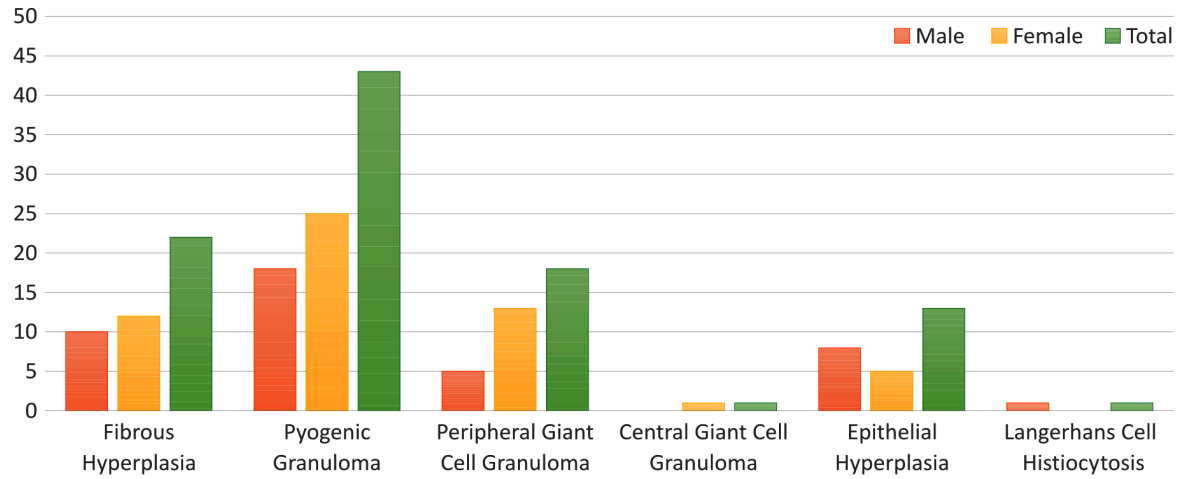

Figure 7 – ***Distribution of patients with gingival overgrowth by gender.***

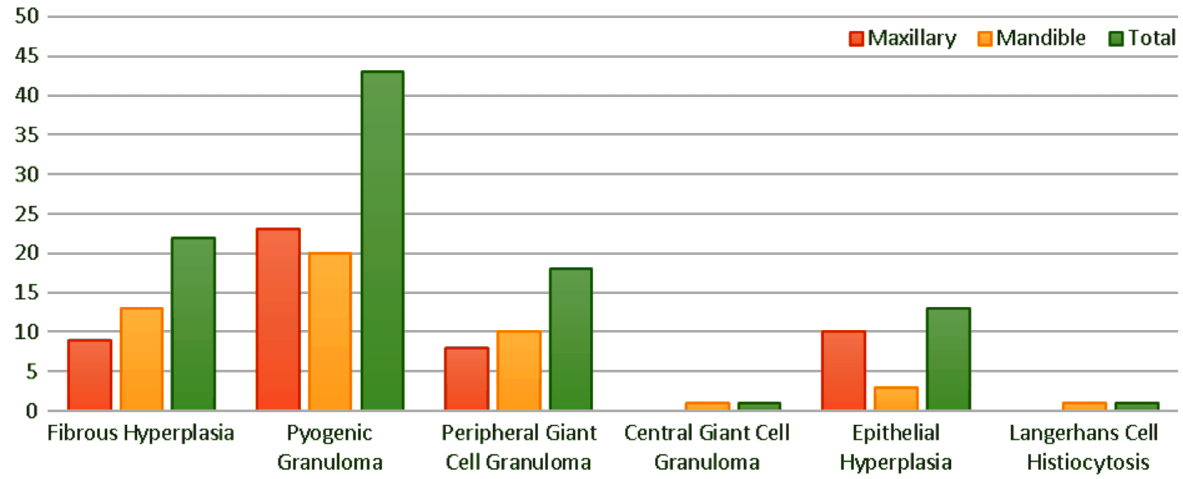

Figure 8 – ***Distribution of patients with gingival overgrowth by localization.***

Discussions

To the best of our knowledge, this study represents the first report on the frequency, location, clinical, radiological, and HP features of gingival overgrowth within the northeastern Romanian population. The data we produced in our survey share many similarities with those previously reported in other regions of Romania or other countries [15, 16], but we also recorded some essential differences that can be attributed to geographic or ethnic factors; yet further studies of larger sample size are needed.

Literature reported that reactive hyperplastic lesions of the gingiva have a high female predominance irrespective of continent or region and the patients' age can vary from the 1st to the 7th decade of life [17, 18]. We also found a slight predominance of female patients, even though our patients were adults in their 5th–6th decades of life.

From a histological point of view, the researchers reported the following entities among their cases of gingival overgrowths: (*1*) PG (pregnancy tumor included); (*2*) peripheral ossifying fibroma (POF) (also referred to as ossifying fibroid epulis; (*3*) focal fibrous hyperplasia (FFH), or fibrous epulis; and (*4*) PGCG.

In literature, the most common lesion was reported to be fibrous hyperplasia (diagnosed in more than 50% of patients) [19], or PG a in some other studies [20], where it made more than 40% of all cases. The HP findings varied from one country to another. However, we did not find any POF. In our survey, the fibrous hyperplasia was the most common form of reactive gingival hyperplasia. Similar to all other studies, PGCG was the form with the lowest occurrence.

Our HP analysis also revealed that an overgrowth of the gingiva consisted of the reactive hyperplasia of both gingival elements: the epithelium and its lamina propria, but in various degrees of development, according to the cause underlying it. However, when an epithelial hyperplasia predominated, the gingival epithelium expressed many epithelial ridges that branched and adhered to each other and went deeply in the lamina propria. At the same time, the lamina propria presented a moderate fibrous hyperplasia. On the other hand, when the fibrous gingival hyperplasia predominated, we also found an epithelial hyperplasia of the surface epithelium, showing parakeratosis and acanthosis. We also found epithelial and fibrous hyperplasia of the gingiva in all cases, even in those diagnosed with deep intraosseous tumor. This can be explained by the fact that gingival tissue is likely to be a very plastic one that promptly reacts to any irritating stimulus by hyperplasia of both elements that constitute its histological structure; the mechanism is correlated with the immune system as numerous inflammatory cells were identified in the lamina propria in all cases.

One of the aims of our study was to establish a correlation between clinical, imaging and pathological findings in gingival overgrowth as some other authors reported a high concordance rate (82.5%) between the clinical and HP diagnosis [17]. In our study, we found a stronger correlation rate (more than 90%) in the epithelial hyperplasia cases and more than 80% in the PG and fibrous hyperplasia cases. Also, PG was the most frequent among gingival overgrowth, and fibrous reactive hyperplasia was the most common pathological diagnosis.

Furthermore, we did not find any correlation between the radiological and pathological tests in the cases with reactive gingival hyperplasia developing in the absence of a deep subjacent lesion; still, we identified a strong correlation between radiological and subsequent pathological diagnosis in the cases with deep intraosseous lesions. It is worth mentioning that in the latter situation there were no correlations whatsoever between clinical and pathological diagnosis.

We only identified one case of LCH, which is an extremely rare dendritic cell disorder with variable clinical evolution and currently described as an inflammatory myeloid neoplasia [21]. LCH occurs less commonly in adults compared to children, with an incidence of 1–2 adult individuals per million and an age ranging between 29 and 38 years old [22]. In the present study, we discovered incidentally one case, who was a 19-year-old young adult. Manifestation of the disease in the oral cavity can be the first and sometimes the only sign of LCH, often resulting in periodontal involvement [23–26]. Periodontal manifestations were similar to those observable in severe periodontal diseases, more exactly the presence of deep periodontal pockets, furcation involvement, recession, gingival bleeding and mobility [27]. In our study, the patient was admitted for proliferative inflammatory condition of the gingiva, which developed after tooth extractions. This clinical aspect, coupled with the rarity of this disease, resulted in an initial incorrect diagnosis. In fact, dentists and periodontists might lack familiarity with the exceptionally rare oral manifestations of LCH. Impaired soft tissue healing after extraction can be suggestive of LCH; however, multidisciplinary approaches are warranted. In everyday clinical practice, dentists and periodontists should be aware that rare systemic diseases, such as LCH, might lead to manifestations in the oral cavity as the first clinical sign [28]. Patients would benefit from a correct multidisciplinary approach for the identification and clinical management of this rare entity.

The accurate diagnosis and treatment of gingival overgrowths should be a crucial aspect of general dental practice. Valuable diagnostic information is often provided by the appearance of the lesion itself. That is why many experienced clinicians use visual inspection and palpation [9] in order to get an accurate provisional diagnosis.

Reactive gingival hyperplasia includes a group of lesions that are commonly the result of either injury or chronic irritation [29, 30]. From a histological point of view, chronic trauma leads to granulation tissue formation, which comprises new capillaries running in an edematous matrix; this matrix is made up of both fibroblasts and mononuclear and neutrophilic inflammatory cells. Later on, this new tissue begins to display fibroblasts differentiation and vessels maturation and to manifest as an overgrowth, which is called reactive hyperplasia. Under the circumstances, even though at first sight they can appear as tumor-like lesions, the gingival overgrowths are not neoplastic; rather they suggest the existence of a chronic process in which an intensified restoration occurs [29].

The favored treatment of choice in gingival overgrowth is surgical excision, when removal of local irritants occurs in order to prevent recurrence. If the lesions fail to resolve, they should be surgically excised. A follow-up checkup is needed as it normally shows a tendency to recur [30–32].

We found out in our study that gingival overgrowth can be triggered by a lot of different diseases, which stresses the importance of pathological examination and differential diagnosis for practitioners. This gives grounds for more prospective studies that can better explain the real incidence of various related gingival diseases.

Conclusions

The data gathered by the present study are similar to those reported in the specialized literature, but there are also some differences, especially regarding the HP features. Furthermore, it was difficult to compare studies carried out in different countries around the world, because of differences in people's attitude towards oral health, on the one hand, and the accessibility of the various population groups to biopsy services, on the other. We believe that similar studies should be conducted in other oral facilities in Romania in order to further investigate not only the epidemiology of gingival overgrowth, but also the numerous different HP facets of this lesion because the fact should not be overlooked that a gingival overgrowth can sometimes hide a deep intraosseous tumor. Moreover, the data presented in this study can be used as a (good practices) guide for additional multicenter studies in our country.

Conflict of interests

The authors declare that they have no conflict of interests.

References

[1] Shamim T, Varghese VI, Shameena PM, Sudha S. A retrospective analysis of gingival biopsied lesions in South Indian population: 2001–2006. Med Oral Patol Oral Cir Bucal, 2008, 13(7):E414–E418.
[2] Gandolfo S, Castellani R, Pentenero M. Proliferative verrucous leukoplakia: a potentially malignant disorder involving periodontal sites. J Periodontol, 2009, 80(2):274–281.
[3] Chowdhri K, Tandon S, Lamba AK, Faraz F. Leukemic gingival enlargement: a case report and review of literature. J Oral Maxillofac Pathol, 2018, 22(Suppl 1):S77–S81.
[4] Costache II, Costea CF, Danciu M, Costan VV, Aursulesei V, Dumitrescu GF, Turliuc MD, Sava A. Amyloidosis – a rare cause of refractory heart failure in a young female. Rom J Morphol Embryol, 2017, 58(1):201–206.
[5] Mironiuc-Cureu M, Dumitriu AS, Gheorghiu IM, Stoian IM. Gingival overgrowth as secondary effect of calcium channel blockers administration. A case report. J Med Life, 2014, 7(2): 241–245.
[6] Nemtoi A, Danila V, Dragan E, Pasca S, Nemtoi A, Constantin M, Sava A, Haba D. The effects of insulin and strontium ranelate on guided bone regeneration in diabetic rats. Rev Chim (Bucharest), 2017, 68(4):693–697.
[7] Bobic AG, Crăiţoiu Ş, Pascu RM, Croitoru IC, Matei M, Obădan F, Crăiţoiu MM. Experimental animal model in a histological study of drug-induced gingival overgrowth. Rom J Morphol Embryol, 2016, 57(3):1003–1010.

[8] Arduino PG, Farci V, D'Aiuto F, Carcieri P, Carbone M, Tanteri C, Gardino N, Gandolfo S, Carrozzo M, Broccoletti R. Periodontal status in oral mucous membrane pemphigoid: initial results of a case-control study. Oral Dis, 2011, 17(1): 90–94.
[9] Ababneh KT. Biopsied gingival lesions in northern Jordanians: a retrospective analysis over 10 years. Int J Periodontics Restorative Dent, 2006, 26(4):387–393.
[10] Stablein MJ, Silverglade LB. Comparative analysis of biopsy specimens from gingiva and alveolar mucosa. J Periodontol, 1985, 56(11):671–676.
[11] Macleod RI, Soames JV. Epulides: a clinicopathological study of a series of 200 consecutive lesions. Br Dent J, 1987, 163(2):51–53.
[12] Drăghici EC, Crăiţoiu Ş, Mercuţ V, Scrieciu M, Popescu SM, Diaconu OA, Oprea B, Pascu RM, Crăiţoiu MM. Local cause of gingival overgrowth. Clinical and histological study. Rom J Morphol Embryol, 2016, 57(2):427–435.
[13] Pisoschi CG, Stănciulescu CE, Andrei AM, Berbecaru-Iovan A, Munteanu C, Popescu F, Baniţă IM. Role of transforming growth factor *β*–connective tissue growth factor pathway in dihydropyridine calcium channel blockers-induced gingival overgrowth. Rom J Morphol Embryol, 2014, 55(2):285–290.
[14] Patel KJ, De Silva HL, Tong DC, Love RM. Concordance between clinical and histopathologic diagnoses of oral mucosal lesions. J Oral Maxillofac Surg, 2011, 69(1):125–133.
[15] Carbone M, Broccoletti R, Gambino A, Carrozzo M, Tanteri C, Calogiuri PL, Conrotto D, Gandolfo S, Pentenero M, Arduino PG. Clinical and histological features of gingival lesions: a 17-year retrospective analysis in a northern Italian population. Med Oral Patol Oral Cir Bucal, 2012, 17(4):e555–e561.
[16] Gambino A, Carbone M, Broccoletti R, Carcieri P, Conrotto D, Carrozzo M, Arduino PG. A report on the clinical-pathological correlations of 788 gingival lesion. Med Oral Patol Oral Cir Bucal, 2017, 22(6):e686–e693.
[17] Dutra KL, Longo L, Grando LJ, Rivero ERC. Incidence of reactive hyperplastic lesions in the oral cavity: a 10 year retrospective study in Santa Catarina, Brazil. Braz J Otorhinolaryngol, 2019, 85(4):399–407.
[18] Sangle VA, Pooja VK, Holani A, Shah N, Chaudhary M, Khanapure S. Reactive hyperplastic lesions of the oral cavity: a retrospective survey study and literature review. Indian J Dent Res, 2018, 29(1):61–66.
[19] Vidyanath S, Shameena PM, Johns DA, Shivashankar VY, Sudha S, Varma S. Reactive hyperplasic lesions of the oral cavity: a survey of 295 cases at a Tertiary Health Institution in Kerala. J Oral Maxillofac Pathol, 2015, 19(3):330–334.
[20] Kashyap B, Reddy PS, Nalini P. Reactive lesions of oral cavity: a survey of 100 cases in Eluru, West Godavari district. Contemp Clin Dent, 2012, 3(3):294–297.
[21] Badalian-Very G, Vergilio JA, Degar BA, MacConaill LE, Brandner B, Calicchio ML, Kuo FC, Ligon AH, Stevenson KE, Kehoe SM, Garraway LA, Hahn WC, Meyerson M, Fleming MD, Rollins BJ. Recurrent *BRAF* mutations in Langerhans cell histiocytosis. Blood, 2010, 116(11):1919–1923.
[22] Berres ML, Merad M, Allen CE. Progress in understanding the pathogenesis of Langerhans cell histiocytosis: back to histiocytosis X? Br J Haematol, 2015, 169(1):3–13.
[23] Milián MA, Bagán JV, Jiménez Y, Pérez A, Scully C, Antoniades D. Langerhans' cell histiocytosis restricted to the oral mucosa. Oral Surg Oral Med Oral Pathol Oral Radiol Endod, 2001, 91(1):76–79.
[24] Cantu MA, Lupo PJ, Bilgi M, Hicks MJ, Allen CE, McClain KL. Optimal therapy for adults with Langerhans cell histiocytosis bone lesions. PLoS One, 2012, 7(8):43257.
[25] Stockschlaeder M, Sucker C. Adult Langerhans cell histiocytosis. Eur J Haematol, 2006, 76(5):363–368.
[26] Chiong C, Jayachandra S, Eslick GD, Al-Khawaja D, Casikar V. A rare case of Langerhans cell histiocytosis of the skull in an adult: a systematic review. Rare Tumors, 2013, 5(3):e38.
[27] Vassallo R, Ryu JH, Schroeder DR, Decker PA, Limper AH. Clinical outcomes of pulmonary Langerhans'-cell histiocytosis in adults. N Engl J Med, 2002, 346(7):484–490.
[28] Merglová V, Hrušák D, Boudová L, Mukenšnabl P, Valentová E, Hostička L. Langerhans cell histiocytosis in childhood – review, symptoms in the oral cavity, differential diagnosis and report of two cases. J Craniomaxillofac Surg, 2014, 42(2):93–100.
[29] Scrieciu M, Mercuţ V, Mercuţ R, Amărăscu MO, Popescu SM, Predescu AM, Baniţă IM. Immunohistochemical aspects of apoptosis in gingival mucosa with papilloma and condyloma acuminata. Rom J Morphol Embryol, 2015, 56(2):425–431.
[30] Shah M, Rathod CV, Shah V. Peripheral giant cell fibroma: a rare type of gingival overgrowth. J Indian Soc Periodontol, 2012, 16(2):275–277.
[31] Scutariu MM, Salamastrakis I, Stan CI, Nedelcu AH, Gavril LC, Costea CF, Dumitrescu AM, Sava A, Şapte E. Histopathological consequences of hyperzincemia on rat teeth. Experimental study. Rom J Morphol Embryol, 2016, 57(3):1057–1061.
[32] Manjunatha BS, Sutariya R, Nagamahita V, Dholia B, Shah V. Analysis of gingival biopsies in the Gujarati population: a retrospective study. J Cancer Res Ther, 2014, 10(4):1088–1092.

Corresponding authors

Monica Mihaela Scutariu, Associate Professor, DMD, PhD, Department of Implantology, Removable Prosthesis, Dental Prosthesis Technology, Faculty of Dental Medicine, "Grigore T. Popa" University of Medicine and Pharmacy, 16 Universităţii Street, 700115 Iaşi, Romania; Phone +40744–845 263, e-mail: monascutaru@yahoo.com

Ana Nemţoi, Assistant, DMD, PhD, Department of Oral and Maxillofacial Surgery, Faculty of Dental Medicine, "Grigore T. Popa" University of Medicine and Pharmacy, 16 Universităţii Street, 700115 Iaşi, Romania; Phone +40740–647 692, e-mail: ana_bamboi@yahoo.com

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