CASE REPORT

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Clinical and histological characterization of an aggressive periodontitis case associated with unusual root canal curvatures

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Abstract

The article presents the histological and clinical characteristics in a severe generalized aggressive periodontitis case associated with multiple root curvatures and the complex therapeutic approach of the severe periodontal destructions. The patient received a complex therapy, including periodontal non-surgical, regenerative and reconstructive approaches, and also endodontic and prosthetic treatments. Recall appointments were fixed at 3-month intervals. One year after the finalization of the active therapy, a hyperplasic, inflamed interdental papilla associated with a recurrent clinical attachment loss was diagnosed at the mesial aspect of the right maxillary second premolar. A biopsy was harvested for histological examination and the recurrent site was treated. The histological study revealed important modifications of the epithelial layer and of the connective tissue of the gingiva. An extremely accentuated pattern of the gingival rete ridges at the epithelial–connective tissue junction, the presence of inflammatory cells infiltrating the epithelial layer and lamina propria and the disorganization of the fascicules of collagen fibers were observed. The inflammatory infiltrate was dominated by plasma and monocytic-like cells as immunohistochemical analyses highlighted. The complex therapeutic approach led to a satisfactory aesthetic and functional outcome. The severe root curvatures may be an unusual trait in this generalized aggressive periodontitis case substantially increasing the amount and the costs of non-periodontal procedures. In this case, the cell make-up of the inflammatory infiltrate and the paucity of collagen in the infiltrated tissue portions are considered to correspond to a fully developed recurrent lesion.

Keywords: aggressive periodontitis, tooth root, abnormalities, therapy.

Introduction

Aggressive periodontitis (AP) comprises a group of rare, often severe, rapidly progressive forms of periodontitis, usually characterized by the early age of clinical manifestations and a tendency to aggregate in families [1], and affects otherwise healthy individuals; the disease is classified in localized and generalized forms [2]. Generalized aggressive periodontitis (GAP) rejoins the most severe forms of periodontal disease [1]; GAP expression involves complex interactions of the subgingival biofilm with the host immunoinflammatory response that subsequently alter periodontal tissue homeostasis [1, 3]. However, microbial factors alone do not explain interindividual differences in the outcome of periodontal disease [4]. Some genetic factors are thought to increase the susceptibility to disease and to periodontal tissue destruction in GAP [5].

The treatment of advanced periodontal destruction in GAP patients represents a significant challenge for the periodontist because it is difficult to manage the biologic phenotype as well as to compensate for the lost tissues. This paper aims to present the histological and immunohistochemical characteristics in a GAP case and the complex therapeutic approach of the severe destruction of the supporting tissues.

Case report

Clinical examination and diagnosis

A 26-year-old woman presented herself to the Department of Periodontology, "Iuliu Haţieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania. The main complaint was fear of tooth loss due to "gum disease" as her general dentist suggested the removal of all her remaining teeth and implant rehabilitation. Other complaints were extreme masticatory pain in the anterior mandibular segment and aesthetic impairment. Her dental history included orthodontic treatment in puberty followed by prosthetic treatment at the age of 19. She did not have any systemic disease and she was a nonsmoker.

The intraoral examination revealed severe aesthetic impairment due to teeth malpositions, unbalanced occlusion, generalized and severe gingival recessions, debris deposits, inadequate prosthetic frontal bridge, and lack of gingival margin alignment (Figure 1a). The oral hygiene was poor as revealed by an O'Leary plaque index of 56%. The full-mouth gingival bleeding index was 78%.

The first periodontal examination revealed the absence of the following teeth: 1.8, 1.6, 2.4, 2.6, 2.8, 3.6, 4.4, 4.5, and 4.6. Extremely severe periodontal destruction and increased mobility were observed in teeth 1.5, 1.2, 2.3,

3.5, 3.1, and 4.1. Four teeth had no periodontal destruction (1.7, 3.8, 3.7, 4.8). The other teeth revealed mild to moderate attachment loss ranging from 1 to 4 mm. The pattern of bone destruction sustained the clinical data. Generalized root curvatures were observed on periapical radiographs. A diagnosis of GAP was made based on the recommendations of literature. The orthodontic diagnosis consisted in dentomaxillary disharmony with spacing successive to dental migrations; crossbite on teeth 1.2, 2.1, and 2.2; posterior left crossbite, and right posterior maxillary teeth overeruption.

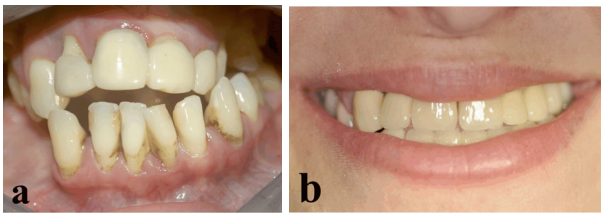

Figure 1 – ***Clinical presentation: (a) Baseline aspect; (b) Unrestrained smile after complex therapy.***

The treatment plan was exposed to the patient and a written informed consent was obtained.

Periodontal treatment

The patient received initial periodontal therapy, consisting in oral hygiene instructions, mechanical debridement associated with adjunctive systemic antibiotherapy, removal of the inadequate prosthetic appliance, and extraction of the teeth with extremely severe periodontal destruction excepting the maxillary left canine. Re-evaluation at six weeks recorded an improvement of the clinical status. Periodontal therapy further included a regenerative surgical treatment and a soft tissue augmentation of the alveolar crest in the frontal region.

Prosthetic rehabilitation

Provisional restorations in both anterior and lateral sectors were provided bimaxillary in the initial phase of the therapy. Six months after the end of the active periodontal treatment, the final metal-ceramic anterior bridges were performed to establish better lateral occlusal contacts and an edge-to-edge incisor relationship and to improve aesthetics.

Endodontic treatment

As the fixed prosthetic restorations implied extensive tooth preparation, endodontic treatment for all the teeth excepting tooth 3.8 was performed. Nickel-titanium manual instruments (Protaper® Universal (Densply Mayllefer, Ballaigues, Switzerland) were used to manage extreme root curvatures in the treated teeth (Figure 2).

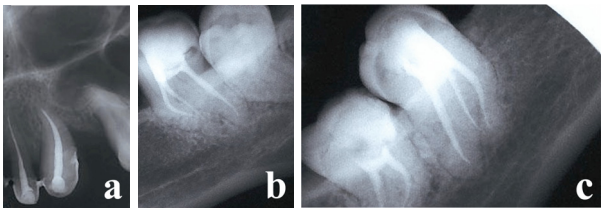

Figure 2 – ***Accentuated root curvatures on periapical radiographs: (a) Left maxillary canine; (b) Left mandibular second and third molars; (c) Right mandibular second and third molars.***

Maintenance

Recall appointments were fixed at 3-month intervals. At the one year recall visit, an extremely hyperplastic interdental papilla at the mesial aspect of the right maxillary second premolar was associated with a 5 mm pocket and 2 mm attachment loss. A biopsy was harvested from the papillae and the area was treated as a recurrence.

Biopsy and histological analysis

Gingival tissue was excised from the area of the hyperplasic papillae with a #15 surgical blade. The orofacial dimension of the sample was around 4 mm. The biopsy specimen was fixed in 10% neutral buffered formalin solution at room temperature for one day. Following fixation, the biopsy was processed following well-established protocols. The specimens were stained in Hematoxylin–Eosin (HE) and in Masson's trichrome. The histological examination was performed by one author (CMM) by light microscopy (Leica DM 750, Germany) and was photographed with Leica ICC 50 HD (Germany) camera connected to the microscope.

Immunohistochemistry

The immunohistochemical analyses evaluated 4 µm-thick sections following the conventional protocol. The primary antibodies used in this study were Monoclonal Mouse Antibodies Anti-Human CD20, CD68, CD138 (DakoCytomation Glostrup, Denmark) and CD3 (Leica BioSystems Newcastle Ltd., United Kingdom) as follows: CD20 (1:100 dilution), code No. M0755; CD68 (1:600 dilution), code No. M0814; CD138 (1:50 dilution), code No. M7228; CD3 (1:50 dilution), code No. NCL-L-CD3-565.

Results

Clinical results

After the specific periodontal therapy, stabilization of the periodontal status was obtained. The patient was extremely motivated and maintained the plaque levels below 25%. The gingival bleeding index decreased to 12% at the 6-week evaluation and no significant changes were noted after one year. Good radiographic outcomes of the endodontic treatments were recorded. Six weeks after the retreatment of the recurrence at the mesial aspect of the right maxillary second premolar normal probing depths were recorded. The fixed prosthetic treatment objectives concerning aesthetics and corrected morphology and occlusal relationships were achieved (Figure 1b).

Histological results

The histological study of the samples revealed important modifications of the epithelial layer and of the connective tissue of the gingiva. An extremely accentuated pattern of the gingival rete ridges characterized the epithelial–connective tissue junction (Figure 3). A normal histological appearance of the basal layer of the gingival epithelium was observed. Dystrophic cells with vacuolization characteristics were present in the spinous layer (Figure 4). Inflammatory cells were observed insight the epithelium (Figure 5a).

Modifications suggesting an inflammatory process were observed in the subepithelial connective tissue. Polymorphonuclear cells, lymphocytes and macrophages infiltrating the lamina propria (Figure 5b) induced the disorganization of the fascicules of the collagen fibers (Figure 5, c and d). Small diameter blood vessels were also observed in the subepithelial connective tissue (Figure 6).

Immunohistochemical analysis of the granulation tissue sections revealed the presence of scarce CD20 positive cells, considered to be B-lymphocytes and of even more rare CD3 positive cells, considered to be T-lymphocytes (Figure 7, a and b).

Many Hematoxylin counterstained positive cells with intense basophilic nucleus characteristic for neutrophils could be observed (Figure 7b).

In the inflammatory infiltrate, many intense CD138-positive cells, namely plasma cells were present. Less intensely colored CD138-positive cells, namely squamous epithelial cells were also present. Rare capillaries could be observed (Figure 7c).

Immunohistochemical staining for CD68 antigen revealed many large cells having irregular and intensely stained cytoplasm in the connective tissue, considered to be macrophages, respectively intraepithelial, considered to be Langerhans cells (Figure 7d).

Discussion

The present paper aimed to characterize a severe case of GAP describing its clinical and histological features and emphasizing the associated root dysmorphological aspects. Besides, the difficulty in managing the devastating effects of the periodontal tissue destruction associated with teeth with severe curvatures was underlined. A complex approach including periodontal, endodontic and prosthetic treatments led to a satisfactory aesthetic and functional outcome.

The periodontal disease often leads to rapid, extensive periodontal tissue destruction, and is often associated with the risk of recurrence. Moreover, the attachment loss may continue, despite the therapeutic efforts. Even modern regenerative strategies have no impact on the genetic local predisposition to periodontal disease, the long-term outcome being uncertain. In the presented case, despite the aggressive maintenance program that enhanced the overall periodontal stability, a recurrent site manifested by clinical attachment loss and hyperplasic inflammation was diagnosed one year after finalizing the active phase of the periodontal therapy. The reinitiation of the treatment in the area of recurrence reestablished the local homeostasis.

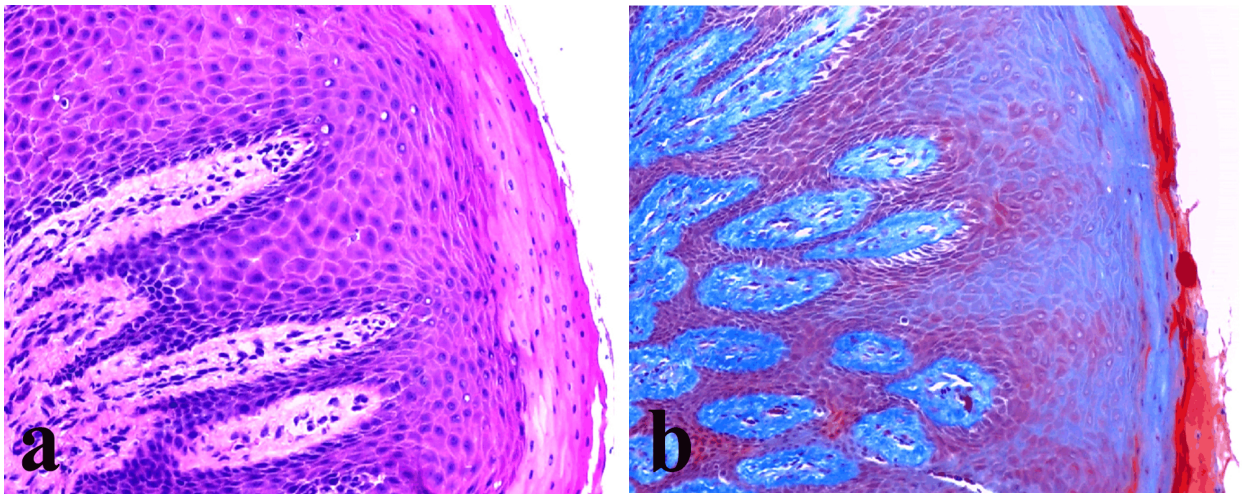

Figure 3 – ***Accentuated rete ridges of the gingiva: (a) HE staining, ×200; (b) Masson's trichrome staining, ×200.***

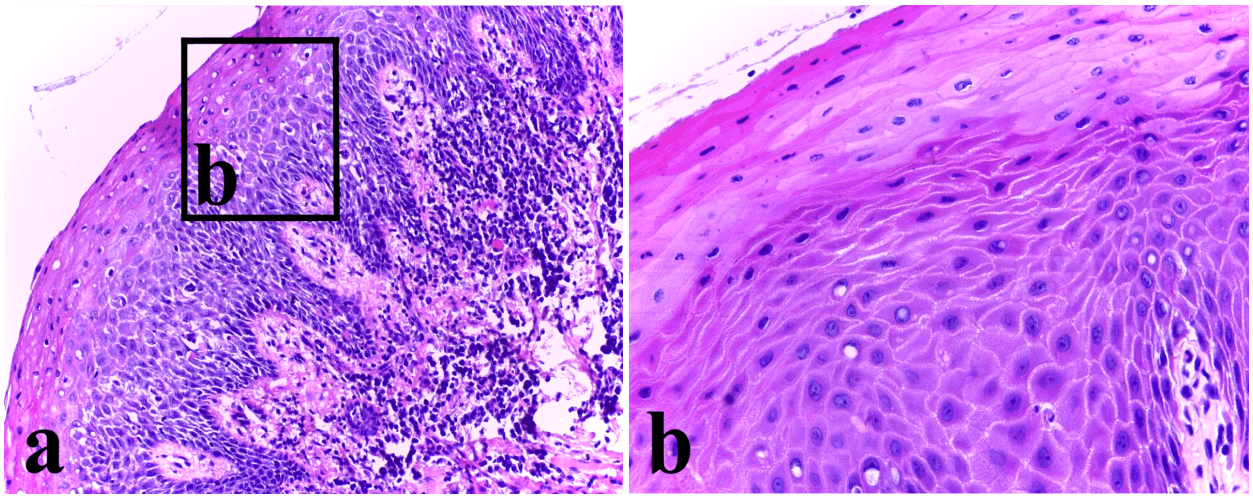

Figure 4 – ***Cells with vacuolization in the spinous layer, HE staining: (a) ×200; (b) ×1000.***

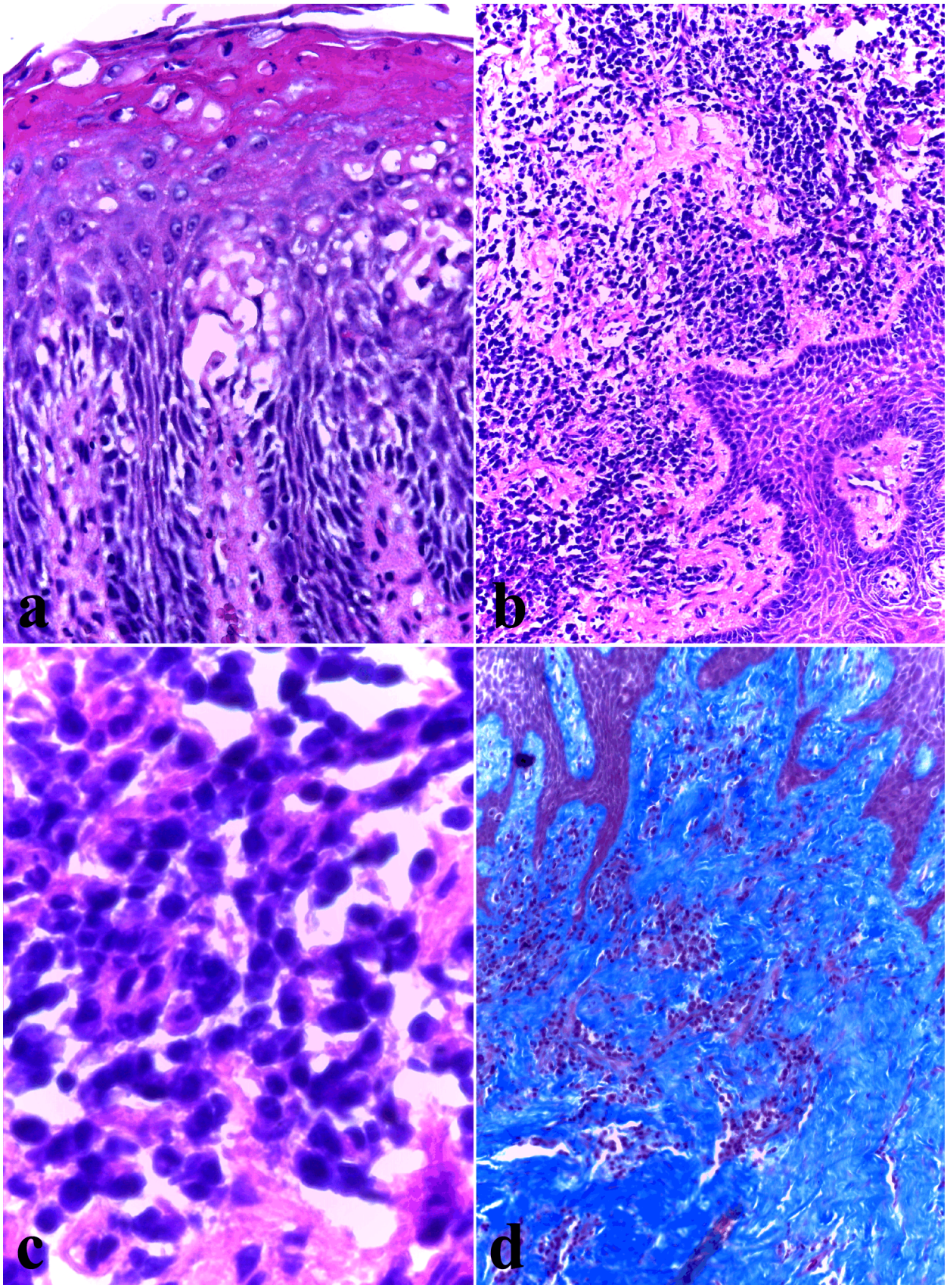

Figure 5 – ***Different inflammatory aspects of the specimens: (a) Inflammatory cells in the gingival epithelium (HE staining, ×400); (b) Inflammatory infiltrate in the gingival lamina propria (HE staining, ×200); (c) Inflammatory infiltrate disorganizing the fascicles of collagen fibers (HE staining, ×1000); (d) Inflammatory infiltrate in the lamina propria in Masson's trichrome staining, ×200.***

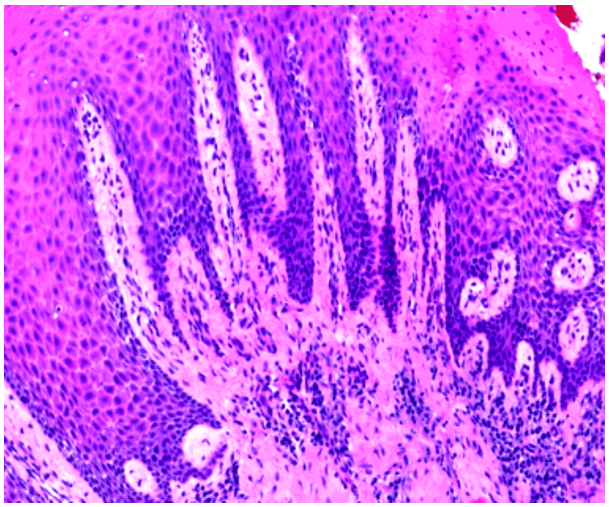

Figure 6 – ***Small diameter blood vessels (HE staining, ×200).***

In the present case, a questionable tooth (bone height loss from ≥50 to <70%) [6] was preserved to be used as prosthetic abutment having in view that the literature highlighted the long-term retention of such teeth in severe AP or chronic periodontitis (CP) cases treated with conservative therapy [7, 8]. Moreover, current data revealed that natural teeth, even those with an initial reduced periodontal attachment level, yielded better long-term results compared with dental implants [9]. However, the tooth retention was significantly dependent on appropriate active periodontal treatment and regular supportive periodontal therapy [10]. Based on the above-mentioned data, the other teeth with reduced periodontium were preserved. Thus, the chosen treatment for the 26-year-old patient seems to be a reasonable therapeutic option.

In the present study, the histological evaluation of the inflamed tissue associated with a recurrent clinical attachment loss showed significant modifications of both epithelial layer and lamina propria. The analysis of the specimens stained in both Hematoxylin and Eosin and Masson's trichrome revealed the presence of elongated rete ridges projected into the gingival connective tissue. Similar findings were described by other authors too [11]. The inflammatory infiltrate observed in the specimens of the present study was characteristic for a chronic inflammatory status, which could be the consequence of an immune dysregulation manifested by a pronounced long-lasting inflammation and weakened self-limitation and resolution of the immune reactions [12, 13]. A decreased density of blood vessels in comparison with normal gingival status was also observed.

The histological analysis observed a large number of lymphocytes and macrophages in the specimens of the present study. Immunohistochemical analyses highlighted many plasma cells and monocytic-like cells that characterized the inflammatory infiltrate of the specimens. In fact, more than 50% of the cells were plasma and monocytic-like cells. The presence of an inflammatory infiltrate of predominantly plasma cells was reported in biopsies from adolescents with localized AP (LAP) [14, 15] and from GAP patients [16]. Plasma cells and macrophages dominated the inflammatory infiltrate of GAP lesions [17]. The presence of plasma cell-dominated infiltrate is considered to correspond to a fully developed lesion of AP [18]. Immunohistochemical analysis used CD138 antibodies to track plasma cells in the present specimens. CD138/syndecan-1 is a cell membrane proteoglycan that functions as a matrix receptor and is expressed on the surface of mature epithelial cells [19] and normal and neoplastic plasma cells. Monoclonal antibodies toward CD138 antigen seem to be plasma cell-specific among hematopoietic elements [20]. CD68 is predominantly an intracellular protein expressed on the lysosomes of tissue macrophages and monocytes, but it is also present to a lesser extent on dendritic cells and peripheral blood granulocytes [21]. In our specimens, CD68-positive cells were present as macrophages in the inflammatory infiltrate of the connective tissue, but also as Langerhans cells in the epithelial layers. The increased number of macrophages observed in the examined specimens may sustain their role of "scavengers" in the elimination of apoptotic cells [22].

The inflammatory infiltrate of the present GAP lesion contained rare B- and T-lymphocytes as revealed by CD20 and CD3 immunohistochemical analyses. CD20 is a common B-cell marker, which is not expressed by haematopoetic stem cells and neither by the earliest B-cell precursors or plasma cells. CD20 molecule is a transmembrane protein thought to function as a calcium channel and to be involved in B-cell activation and proliferation [23]. CD3 is considered the most-specific T-cell marker, usually used to identify T-cells in benign and malign disorders. Other research reported a large number of B- and T-lymphocytes in AP lesions [17, 24].

Our results are in agreement with those of the other studies [14] regarding the paucity of collagen in the infiltrated tissue portions.

In contrast with the current data stating that neutrophils are usually found in very low numbers in the infiltrated connective tissue in both AP and CP [18], the analysis of the present specimens revealed the presence of many polymorphonuclear cells observed both on HE and immunohistochemical stained specimens. It is well known that even with optimal plaque control, neutrophils will still be stimulated to exit the gingival microvasculature, enter the periodontal tissues and, subsequently, migrate toward epithelial high levels of bacteria and neutrophil chemoattractants, which are found in the gingival crevice. The presence of an increased bacterial load or of virulent periodontopathic bacteria could result in the recruitment of vast numbers of polymorphonuclear neutrophil cells into the periodontal pocket [25], which could explain the presented results.

There are some difficulties to compare our histological data with the results of the earlier studies because various disease definitions have been used for the above-mentioned clinical entities.

Many dental anomalies such as dysmorphologies of the roots or supranumerary teeth or roots have been associated with LAP [26–29] and GAP [30, 31]. An unusual combination of dental anomalies (short roots on the maxillary central incisors and premolar talon cusps, dens invaginatus, tubercles of Carabelli, pyramidal root morphology) was reported as being associated with reduced alveolar bone levels in adults [32].

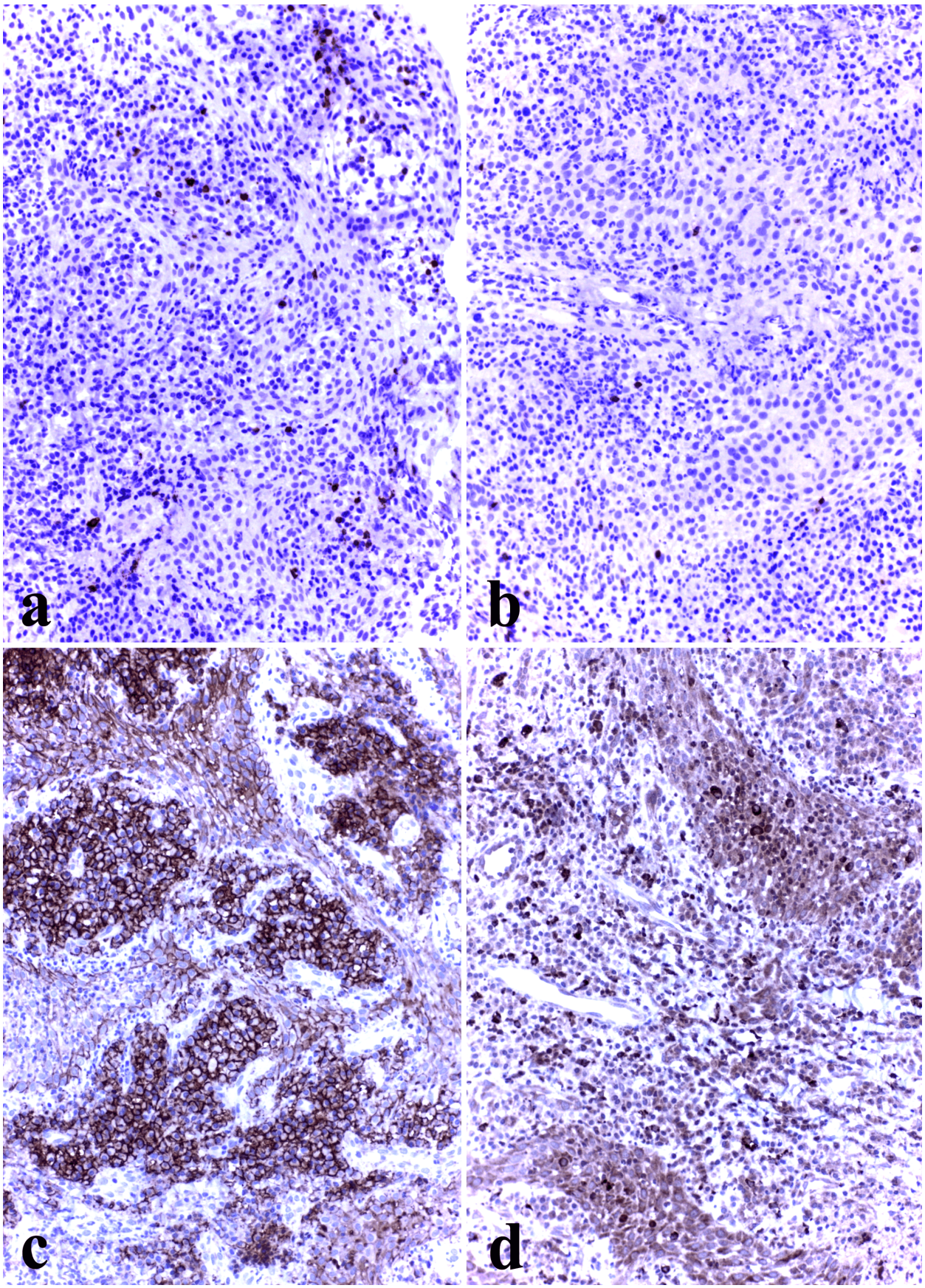

Figure 7 – ***Immunohistochemical analysis of the inflamed tissue: (a) CD20 stained B-lymphocytes, ×200; (b) CD3 stained T-lymphocytes, ×200; (c) CD138 stained plasma and squamous epithelial cells, ×200; (d) CD68 stained macrophages and Langerhans cells, ×200.***

Meng *et al.* (2007) [33] indicated that root form abnormalities can be a susceptibility factor in the development of AP and suggested a root shape classification. The ratio of root abnormalities was found to be 1.76 times higher in AP patients than in normal patients [34]. Anatomical variations in the form of molar roots could contribute to periodontal tissue loss due to plaque retention or unfavorable crown–root ratio resulting in decreased resistance to heavy occlusal forces [35–37]. Root fusion of permanent molars is one of root form abnormalities (type V) [33] that seemed to contribute to periodontal tissue loss in AP patients [31, 38]. Root fusion was the commonest dental anomaly (53%) described in GAP subjects. Curved roots or type III root form anomaly [33] was described in 17% of GAP patients [31].

The presence of supernumerary teeth in AP is rather rare and a tendency for them to aggregate in families has been noticed, suggesting a genetic predisposition [27, 39, 40]. Dens invaginatus is another type of dental anomaly observed in AP [30] with a prevalence of 16% in some populations, value which was significantly higher than that reported for the general population (2.95%) [41].

Thin cementum with irregular external resorption areas was described in localized AP [42]. Some systemic diseases, such as hypophosphatasia, are associated with defective cementum formation that allows a rapid attachment loss [43]; however, in the present case the periodontal destruction did not follow the rapid rhythm described for hypophosphatasia cases.

As in the present case the root curvatures were generalized, the traumatic origin of the bending was excluded. In the reported case, the curvatures of mandibular molars were so accentuated that they were initially considered as root flexions (sharp distortions of root portions, as defined by Fuller & Denehy, 1984) [44], but this characterization was afterwards contradicted by the lack of sharpness of the root bends.

Given the complexity of dental root development, it is not surprising that there are many examples of both genetic and environmental conditions that result in altered root morphology and/or composition [45]. As the genetic basis for various dental anomalies is gradually being revealed [46], it is becoming clearer that genetic predisposition to dental anomalies may be a component of AP in some individuals resulting from specific, possibly related, genetic polymorphisms [30]. The same factors responsible for gene variations that alter host responses and modify the clinical severity of GAP may also interfere in the process of root formation, resulting in abnormal root morphologies, as recorded in the presented case. The association between root abnormalities and some hereditary conditions [47, 48], the description of a genetic predisposition in some dental anomalies [15, 39, 40], the presence of dental/root anomalies in LAP [26–29] and GAP [30, 31] and the fact that GAP has a well-recognized hereditary component could sustain the findings of the present case report. Nevertheless, the extreme root curvatures in all the treated teeth may be an unusual trait in this case of AP. However, genetic and large scale epidemiological studies, designed to investigate the association of AP and dental anomalies are needed.

Conclusions

In this clinical case, the severe root curvatures seemed not to impair the good outcome of the specific therapeutic approach; however, they substantially increased the amount and the costs of the non-periodontal procedures. In the presented case preserving natural teeth seemed to be a reasonable therapeutic option based on the recommendations of the literature. However, tailored oral hygiene and maintenance regimens are mandatory for maintaining low levels of subgingival biofilm and for early identification of recurrences. In this case, the cell make-up of the inflammatory infiltrate and the paucity of collagen in the infiltrated tissue portions are considered to correspond to a fully developed recurrent lesion.

Conflict of interests

The authors declare that they have no conflict of interests.

Acknowledgments

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