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Drug-induced gingival enlargement

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Abstract

OBJECTIVES: Side effect of medicamentous treatment in hypertension therapy and angina pectoris with calcium channel blockers related to fibrotic gingival enlargement were examined.

METHODS: In our study we deal with clinico-histopathological and microbiological knowledge from this field underpinned by two case reports treated with antihypertensive therapy using calcium channel blockers. In the first case report we were largely concerned with microbiological findings from the area of periodontal pseudopockets diagnosed through a DNA analysis and appropriate antibiotic therapy. In a patient treated with a preparation from amlodipine group we proceeded to a complex treatment involving the change of hypertension therapy, introduction of professional and home oral hygiene and also following surgical and prosthetico-aesthetic rehabilitation.

RESULTS: Case Report 1 who was for a long term medicated with a preparation from the nifedipine group of antihypertensives we detected the presence of periodontal pseudopockets with probing depth of 4 to 7 mm with positive BOP and with marked rigid fibrotic gingival enlargement accompanied with considerable foetor ex ore. In a patient from the Case Report 2 who was for a long term medicated with a preparation from the amlodipine group of antihypertensives with large gingival overgrowth angiogenesis was characterized by cuboidal endothelial cell lining. In the samples under a layer of stratified epithelium there was present dense fibrous connective tissue comprising largely of collagen fiber bundles.

CONCLUSION: Bacterial composition in the patient with a high degree of gingival enlargement and periodontal pseudopockets 4 to 7 mm deep represented a typical spectrum of bacteria occuring in chronic forms of periodontitis. However, we cannot determine, if such distribution of bacteria was primary before the application of nifedipine antihypertensives, or it originated later after the formation of typical anaerobic setting of false periodontal pockets.

CASE REPORT

INTRODUCTION

Drug-induced gingival enlargement is characterized as an increase of the size of the gingiva resulting in alterations of its shape and margin. Enlarged and often inflamed gingival tissue overlaps clinical dental crowns to varied extent and often causes aesthetic concerns to a patient and may induce also psychological disorders and subsequent reduction of speaking and smiling in an attempt to cover up the aesthetic deficit in the frontal area. The first information on drug-induced gingival enlargement originated in 1939, when it was described regarding epilepsy treatment with diphenylhydantoin sodium. The first references on gingival overgrowth in antihypertensive therapy with calcium channel blockers date from 1939 (Fu *et al.* 1998). In 1972 there was invented cyclosporine which is an important immunosuppressant preventing rejection of organ transplants; however, one of its side effects is gingival enlargement (Yng-Tzer *et al.* 2010). Currently, antihypertensive therapy is indicated in a relatively large part of population, while calcium channel blockers are one of several therapeutical groups used in hypertension treatment. The most preferable drugs are nifedipine and amlodipine which have quite different pharmacological properties. Whereas amlodipine has quite long-acting therapeutical effect, action of nifedipine is less short-lasting and in circulation it is present more in a free form and to a larger extent, and thus shows more extensive side effects. In hypertensive patients after kidney transplantation it is necessary to apply medication with cyclosporines and also calcium channel blockers, which results in rapid increasing of prevalence and deteriorating of a clinical picture of gingival enlargement (Fu *et al.* 1998; Yng-Tzer *et al.* 2010). The group of antihypertensives called "dihydropyridins" includes preparations such as nifedipine, amlodipine, felodipine, nicardipine and isradipine. Phenylalkylamines are bound to V binding sites of L-calcium channels and a benzothiazepine group binds to D binding sites and is represented through the active substance verapamil (Seymour *et al.* 2000).

For drug-induced gingival overgrowth there have been described several risk factors which may contribute to increased prevalence and to intensity of clinical manifestations of such complications, including in particular the following: the presence of gingivitis and different localizations and forms of retention of oral biofilms and plaques, genetic factors – HLA-B37 and HLA-DR2 for cyclosporine and simultaneous application of cyclosporines and calcium channel blockers, concomitant medication with cyclosporine and prednisolone together with azathioprine and phenytoin. The origin and course of gingival enlargement are affected also by various properties of drugs, such as their concentration in the serum, saliva and gingival crevicular fluid, the course and dosage of a drug and many other factors (Seymour *et al.* 2000; Ellis *et al.* 1999; Fernandes *et al.* 2010; Ciantar 1996).

CALCIUM CHANNEL BLOCKERS-INDUCED GINGIVAL ENLARGEMENT

Calcium channel blockers prevent calcium from entering into blood vessels wall and cells of the heart. Relieved heart work into distended vessels enables easier squezzing of blood into circulation and blood pressure decreasing. Calcium channel blockers consist of 3 structurally and chemically different therapeutic groups, which bind to an L-type of calcium channels, but each of them binds to particular specific sites.

a. Nifedipine group

Scientific literature is full of published studies of various nature dealing with gingival overgrowth induced by calcium channel blockers. Prevalence of gingival complications is quite high and ranges from 6.3% to 85% (Ellis *et al.* 1999). Some studies report the results on animal models and indicate that application of nifedipine alone without coexistence of other risk factors cannot induce gingival enlargement (Fernandes *et al.* 2010). Although exact pathogenesis of a disease is not clear, the following facts and risk factors affect its incidence and clinical picture:

1. therapy duration and drug dosage (Informational Paper 2004)
2. poor oral hygiene and the presence of gingival inflammation are serious risk factors in pathogenesis of this disease (Sato *et al.* 2005; Santi *et al.* 1998)
3. the presence of dental plaque is a supporting factor of development of gingival enlargement (Ciantar 1996; Deen-Duggins *et al.* 1996)
4. simultaneous medication of drugs from the nifedipine group and cyclosporine A (Flynn *et al.* 2006)
5. clinical picture of gingival enlargement is mostly affected by nifedipine concentration in blood plasma (Thomason *et al.* 1997)
6. IL-1beta is a significant mediator for IL-6 increase and for expression of androgen receptors in nifedipine hyperplasia (Lu *et al.* 2007).

Possible pathogenetic mechanisms of development and histopathologic findings in nifedipine-induced gingival enlargement

Despite the fact that first changes on gingival tissue were described as early as in 1984, a molecular nature of arising of such complication is still not clearly defined (Fu *et al.* 1998). According to several studies and observations the following factors may in particular contribute to disease development:

a. Collagen metabolism. Cultures of gingival fibroblasts from nifedipine-induced gingival enlargement produced more collagen and showed a lower collagenolytic activity compared with healthy fibroblasts of individuals of the same gender and age. These fibroblasts did not produce more fibronectine and their production of glycosaminoglycans was lower.

The results of in vitro experiments point rather at higher collagen deposition than at a hyperproliferative fibroblastic activity, or on higher production of fibronectine and glycosaminoglycans (Tipton *et al.* 1994). Some research has not confirmed assumptions that alteration of collagen metabolism is conditioned by intracellular calcium oscillation (Bullon *et al.* 2007).

b. The role of extracellular matrix. Within particular components of extracellular matrix in health and in nifedipine-induced hyperplasia there was reported significant heterogeneity of affliction of particular components, while these alterations were detected on the following structures in particular: fibronectin was organized in "cloud"-like structures with varied intensity; in structures near the surface of the tooth the increased number of vessels was localized; collagen type I and III showed a diffuse distribution in extracellular matrix and collagen type V and VI displayed completely different patterns of distribution of "crater"-like structure /collagen V/ and "honeycomb"-shaped structure /collagen VI/. Such findings confirm significant differences in structure between healthy and nifedipine-induced extracellular matrix (Romanos *et al.* 1993).

c. Disorder of cell proliferation and apoptosis. Through expression of the proliferative index "FOXO1" and apoptotic index "caspase 3" there was reported that in fibrotic gingival lesions proliferation is stimulated and apoptosis is decreased (Seymour *et al.* 2000; Kantarci *et al.* 2007). Some different markers of apoptosis in patients with nifedipine therapy were reported also in other similar studies (Castro *et al.* 2010).

d. The effect of bFGF on the cell cycle. Basic Fibroblast Growth Factor (bFGF) is a major factor of mitogenesis for fibroblasts, epithelial cells and keratinocytes (Seymour *et al.* 2000; Vojtaššak *et al.* 2006). Cell proliferation depends on gradual progression of series of cell cycles, which are controlled through cyclins and which subsequently stimulate cyclin-dependent kinases (CDKs). Studying gingival fibroblasts harvested from the cells of nifedipine responders showed increased expression of mRNA cyclins A, B1, D1 a CDKs 1, 2, 4, 6 in the presence of bFGF compared to fibroblasts harvested from the cells of nifedipine non-responders. These results indicate that in nifedipine responders increased proliferation can be mediated by growth factors such as bFGF (Seymour *et al.* 2000).

e. The effect of epithelial cell integrins. Integrins are transmembrane glycoproteins that regulate cell adhesion, cell proliferation and tissue fibrosis (Walsh *et al.* 2007). Study of integrins revealed that in patients taking nifedipines and cyclosporines there occurred increased expression of nifedipines and cyclosporines in the epithelial layer what may participate in forming and shaping of elongated rete ridges along with tissue fibrosis (Walsh *et al.* 2007).

f. The synergic effect of nifedipine and cyclosporine A. Nifedipine and cyclosporine A induce significant enlargement and overgrowth of gingival tissue and equally show a relatively strong immunomodulatory effect. Some research suggests that also alteration of an immunological response may cause gingival changes (Pernu *et al.* 2001). With using nifedipine or cyclosporine alone, but particularly with their concomitant application there is increased density of fibroblasts and collagen in a parallel correlation with a degree of gingival enlargement (Spoildorio *et al.* 2002). Concomitant application of nifedipine and cyclosporine A causes gingival overgrowth and increases mitotic activity, specifically in a thickened epithelial layer (Nurmenniemi *et al.* 2001).

In clinical practice a combination of cyclosporine A and nifedipine is applied in patients after kidney transplantation, as cyclosporine A is the most frequently used immunosuppressant. However, cyclosporine A induces hypertension, which is most commonly eliminated by nifedipine, amlodipine or diltiazem (Morisaki *et al.* 2000). In one group of patients (46 subjects), after kidney transplantation, cyclosporine A and nifedipine were administered, where the prevalence of gingival overgrowth accounted for 53% in comparison to a group of patients (89 subjects) taking cyclosporine A and amlodipine, where the prevalence of gingival complications accounted for 72% (James *et al.* 2000). These results are partially inconsistent with the results of studies showing gingival overgrowth in monotherapeutically amlodipine-induced complications as not statistically significant (Ellis *et al.* 1999).

CASE REPORT 1

A 54-year-old woman was diagnosed within accidental treatment with considerably overgrown and bleeding gingival tissue (Figure 1). Gingiva was enlarged, covering the necks of frontal and distal teeth, forming periodontal pseudopockets with the depth ranging from 4 to 7 mm. The tissue itself was of relatively solid, firm consistency corresponding to fibrotic alterations of gingiva in drug-induced hyperplasia of gingival tissue. The patient complained of heavy bleeding when brushing teeth, and thus she had insufficient oral hygiene with the presence of soft dental plaque accompanied with considerable foetor ex ore. However, she herself showed a little interest in her problem and trivialized it. Her social, intelligence and education level was relatively low. In addition, after performing professional oral hygiene she was incapable of maintaining the required care and eventually she discontinued her treatment wilfully.

She even completely ceased visiting the doctor's office after having been offered a possible surgical solution.

Her total medical history revealed a long-time treated presence of hypertension, but we failed to obtain further data from her attending specialist. The patient took a drug nitrendipine from a group of calcium channel blockers in a long term. Nitrendipine relaxes smooth muscle fibres in the vascular walls, and thus lowers resistance of circulating blood and considerably decreases hypertension. Its further beneficial effects include higher excretion of sodium chloride and water through the kidneys.

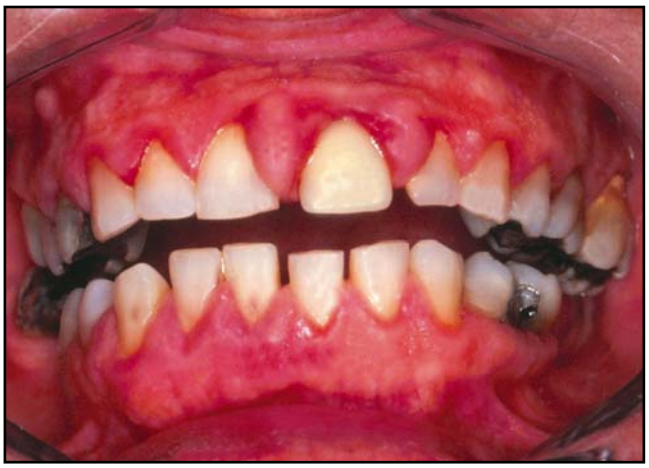

Fig. 1. Case report 1. Nifedipin induced gingival enlargement

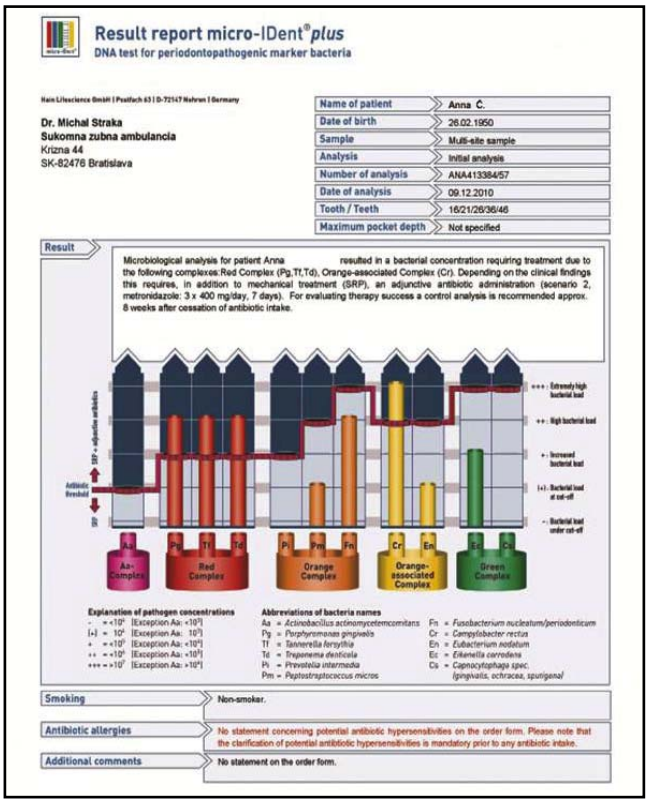

Fig. 2. Case report 1. DNA test for periodontopatic bacteria: Normal levels of A.a., high levels of red complex bacteria (++).

Our attention was focused also on the presence and semiquantitative DNA analysis of classic periodontal pathogens clustered to coloured complexes, whose pathogenicity declines from left to right (Figure 2). The complex *Aggregatibacter actinomycetemcomitans (A.a.)* was not elevated neither in qualitative nor quantitative parameters. The bacteria of the red complex, which is formed by periodontal pathogens *Porphyromonas gingivalis (P.g.), Tannerella forsythia (T.f.)* and *Treponema denticola (T.d.)* were increased above the pathological level. From the bacteria of the orange complex there were found high levels of *Fusobacterium nucleatum (F.s.)*. The bacteria of the yellow complex were in the high percentage /+++ = 1 billion of bacteria/ presented by a bacterium *Campylobacter rectus (C.r.)*. From the least pathogenic species of the green complex there were moderately elevated levels of a species *Eikenela Corrodens (E.c.)*. On the basis of the above microbial findings in our patient it is evident that a bacterial spectrum of periodontal pathogens is very similar to microbial findings in chronic periondotitis, while in our case report we are not able to answer the question whether microbial characteristic findings were primary prior to introducing antihypertensive therapy, or their characteristic composition occurred after the production of anaerobic environment of false periodontal pockets.

Treatment

The patient's treatment was planned to be provided in 4 phases:

1. Depuration phase consisted of introducing professional and home oral hygiene.
2. Systemic antibiotic treatment of periodontal pathogens. In our patient on the basis of the results of periodontal indices /pocket depth up to 7 mm, positive BOP/ we assumed a long-term presence of bacterial pathogens in gingival sulcus and subsequent invasion into the periodontal structures. For these reasons we indicated 400 mg metronidazol 3 times per day for 7 days.
3. Drug substitution in antihypertensive therapy. A cardiologist or specialist for antihypertensive treatment has to change hypertension treatment to another or other groups of drugs for hypertension. Drugs from the group of calcium channel blockers can be altered to antihypertensives from the group of beta blockers, ACE inhibitors and preparations increasing diuresis.
4. Surgical techniques based on gingivectomy of various extent.

Evaluation of treatment after applying first three steps was successful and after 3 months we reported reduction of hyperplastic gingiva, resolution of inflammation and reduction of inflammation at one clinical examination. As already noted, the patient suddenly cut off the contact, so the surgical phase of treatment and thorough clinical examination was not possible to perform and check over.

b. Amlodipine group

The prevalence of amlodipine-related enlarged and overgrown gingiva ranges from 1.7 to 3.3%, accounting for significantly lower percentage in patients undergoing antihypertensive therapy in this group of calcium channel blockers (Jorgensen 1997). Monitoring of the occurrence of abnormal gingival enlargement in three groups of patients using cyclosporines and calcium channel blockers revealed a following distribution of complications: in patients medicated with cyclosporine the prevalence of complications was 51.6%; in a group of patients treated with a combination of cyclosporine A and nifedipine the prevalence of gingival enlargement was 90.3% and in a group of cyclosporine A and amlodipine the prevalence of complications was 58.1% (López-Pintor *et al.* 2009). In this context it is necessary to point out that in the prevention of transplant exclusion /kidneys, liver/ and other post-transplantation complications, and also for treating autoimunne diseases we are often made to eliminate a hypertensive effect of cyclosporines with various types of antihypertensives, most frequently from a group of calcium channel blockers. In this group of patients there is often indicated also the triple combination: cyclosporine A, prednisone and antihypertensive (Jorgensen 1997; López-Pintor *et al.* 2009; Hassell *et al.* 1991).

Amlodipine is categorized in the calcium channel antagonist group, especially in the third generation of dihydropyridine group, and is used for treating hypertension and angina pectoris (Fauci *et al.* 2008). It has been stated that amlodipine has a unique pharmacodynamic profile, which is characterized by slow hepatic degradation, late-peak plasma concentrations and almost complete resorption. Various pharmacological and physicochemical properties of nifedipines and amlodipines are likely to cause different prevalence of gingival complications. Nifedipine as well as amlodipine fall into the group of dihydropyridines and they have similar structure; however, the risk of development of overgrown gingiva is in nifedipine preparations considerably higher, as it has lipophilic properties and is completely dissolved in the cytoplasmic cell membrane and further penetrates into the cytoplasm. On the contrary, amlodipine is from other dihydropyridines markedly polarized with a pKa value of 8.7 (Triveni *et al.* 2009).

The pathogenesis of amlodipine-induced gingival enlargement and gingival complications is uncertain. Some studies suggest the existence of fibroblast subpopulations which respond to amlodipine medication with increased fibrotic reaction. It is evident, that in fibroblasts there is functional variability to various external stimuli (Sharma *et al.* 2012). It shoul be noted that the number of literature data and references regarding a group of amlodipine-induced enlargement is considerably lower than the number regarding nifedipine group due to the fact that a low incidence and prevalence in this group does not indicate high scientific interest and need. Some observations indicate that in a group of 150 cardiac patients taking amlodipine 5.0 mg per day for more than 6 months there did not occur any marked enlargements of gingival tissues (Seymour *et al.* 1994). On the contrary, there is a study describing a case series where after 3 months of oral administration of amlodipine there occurred gingival enlargement in three patients with an unfavourable periodontal status (Srivastava *et al.* 2010). Case reports from Iran suggest possible non-inflammatory mechanism based on defective collagenase activity due to decreased uptake of folic acid resulting in blockade of aldosterone synthesis and consequent feedback increase in adrenocorticotropic hormone (ACTH) levels, with higher levels of keratinocyte growth factor (KGF1). Inflammatory mechanism includes the interaction between increased drug concentrations in crevicular fluid of bacterial biofilms accompanied with hyperproduction of some pro-inflammatory cytokines. The key role is attributed to hyperproduction of TGF-beta1 (Lafzi *et al.* 2006; Odessey *et al.* 2006).

CASE REPORT 2

A 63-year-old male patient, for 14 years treated for hypertension with a preparation Agen (amlodipine) in common daily doses (Figure 3). Clinically present enlargement of gingival tissues of large size. In the upper frontal region overgrown gingiva advanced to the site of incision margins and caused discomfort to the patient during mastication and markedly interfered with his aesthetic appearance. In the lower jaw gingival enlargement and overgrowth were of a lesser extent, however, they overgrew into interdental spaces of an old and long unsatisfactory gold-resin dental bridge. Hygiene habits and oral hygiene itself were absolutely insufficient and largely deteriorated this unfavourable condition. Upon patient's request, mostly from an aesthetic point of view, a therapeutical solution in the upper jaw was preferred.

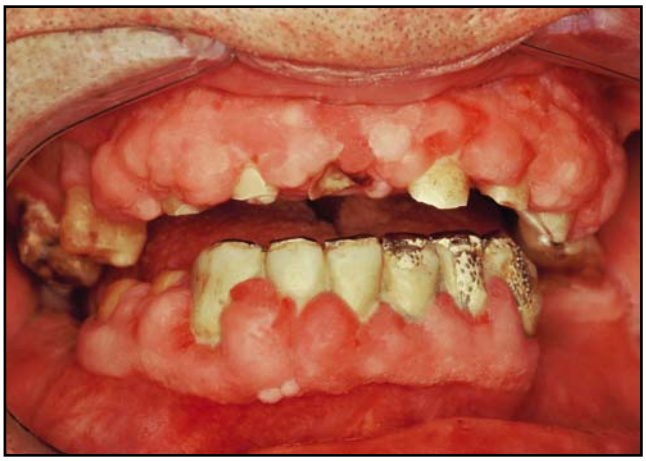

Fig. 3. Case report 2., Amlodipininduced gingival enlargement

The patient's treatment consisted of 3 major points:

1. Change of hypertension treatment was performed on the basis of consultation with an internist, where amlodipine preparation (Agen) was substituted with Rasilez, which is an antihypertensive from the group of inhibitors of a renin-angiotensive system.
2. Performing professional hygiene provided by a dental hygienist and motivation and education towards home hygiene. It should be noted, that individual hygieno-depuration regime was not adequately achieved due to the patient's age.
3. According to a clinical picture of the disease with heavily overgrown gingival tissues a surgical resection of tough fibroticly altered gingiva was considered. We believed that after discontinuation of the main etiologic factor, represented by long-lasting taking of calcium channel blockers, and further normal and healthy neoformation and pathologically unaffected remodelling of collagen and connective tissue, the growth of physiological and normally shaped gingiva would occur. And we ultimately managed to secure it.

The majority of authors present traditional resections of gingiva – gingivectomies as the major therapeutical procedure in patients with drug-induced gingival enlargement. Gingivectomies can be performed either locally, on the site of the greatest growth, or on sites which most traumatize the patient from an aesthetic and psychological point of view, which usually represents the upper frontal part of the dentition. Traditional surgical procedure is presented by gingival resections (gingivectomies) series-performed as "quadrantectomies", in which gradually, on one quadrant of the mouth at a time, gingival resections are performed. However, this procedure shows in long term monitoring fairly common reccurence and a need for corrective interventions. For these reasons only a two-stage surgical procedure is recommended (Sengun *et al.* 2007). A need of reoperation in various time sequences was shown also in other studies (Hart *et al.* 2002). Some studies show a need of reoperation even after 1 or 2 years and also due to other fibrotic gingival enlargement (Hart *et al.* 2002). Gingivectomy as a primary therapeutico-surgical procedure can be performed with using a scalpel, elec-

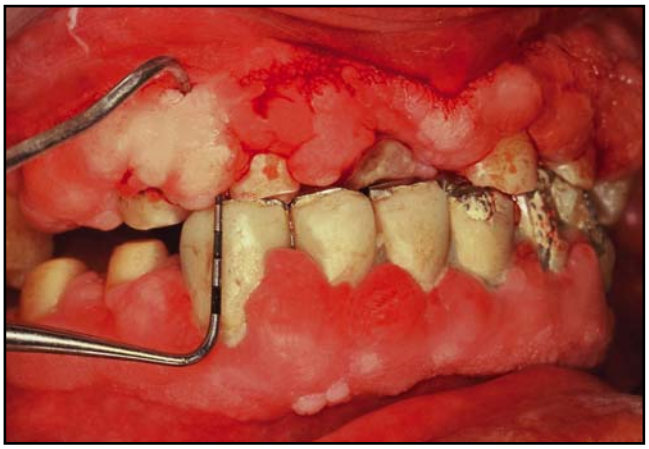

Fig. 4. Case report 2. Probing of false periodontal pockets forming by extremly overgrowth of gingiva

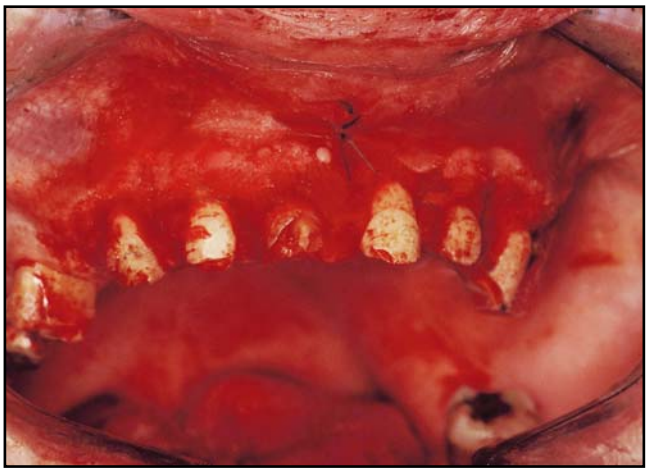

Fig. 5. Case report 1. Perioperative photo during partial gingivectomy

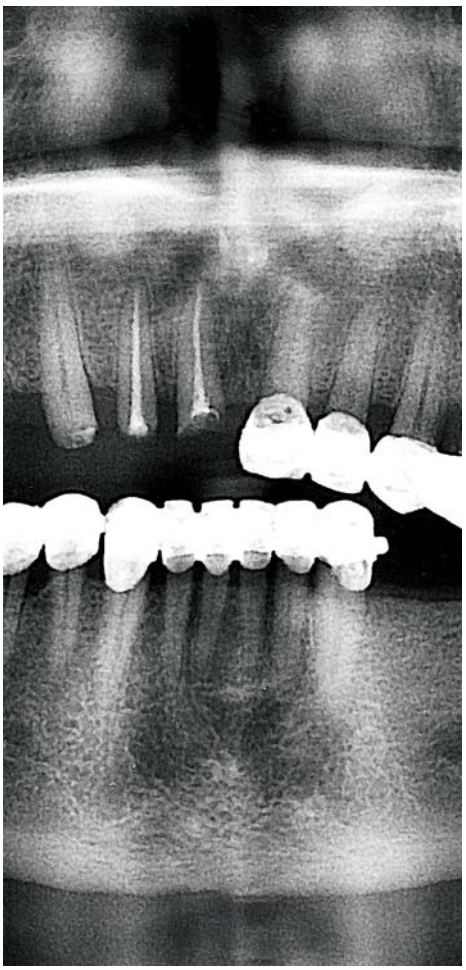

Fig. 6. Case report 2. Results of endodontic treatment 12 and 11, which were reconstructed by metal postcores

trocauter, CO_2 and diode laser. There are often applied modifications of Goldman technique of gingivectomy introduced in 1950 and it represents a quadrant-by-quadrannt approach in which we probe the base of particular pockets (Figure 4), which are then via a probe communicated to the labial aspect of enlarged gingiva, and thus presenting a small bleeding point. Connection of particular bleeding points outlines the primary incision. Primary incision can be completed with different types of vertical incisions, where in some cases mucoperiosteal flaps can be formed, and we can use also various methods of bevelling the primary incision. The exposed firm structures are thoroughly protected by manual and ultrasound decontamination and degranulation. Postsurgical care is followed up with systemic antibiotic use, regular 0.2% chlorhexidine rinse for 14 days and periodontal plastic dressing (Figure 5). With using an electrocauter in the labial region a needle electrode is employed and in the palatal application rather a loop electrode is used (Hart *et al.* 2002). Between successive incisions cooling periods are necessary due to heat generated by an electrode (Hart *et al.* 2002). Currently, in this field and indication methods using CO_2 laser are recommended due to its antimicrobial effect and good post-operative healing (Zhou *et al.* 2007) (Figures 6, 7).

Histological findings

At present there are some histological studies diagnosing amlodipine-induced fibrotic gingival enlargement as fibroepithelial hyperplasia with proliferating hyperkeratinized stratified squamous epithelium. The underlying connective tissue is formed by collagen fibres with a mild chronic inflammatory infiltrate. Severe chronic inflammatory infiltrate with vascular and nerve structures was seen in the deeper layers of connective tissue (Spoildorio *et al.* 2002). Microscopic specimens from another case revealed histological alterations in the form of epithelial acanthosis, hyperplasia of connective tissue, a few inflammatory cells and elongated rete ridges (Lafzi *et al.* 2006). On the contrary, histopathological specimens from other cases stained with hematoxylin and eosin revealed the presence of hyperplastic squamous epithelium and inflammatory cells infiltrate without any dysplastic features (Srivastava *et al.* 2010).

Our histopathological findings resulted from the Case Report 2 and the patient's history, clinical status and treatment are provided in the following part of the paper.

Tissue biopsies from hyperlastic gingiva were also histologically examined. Fragments of the gingiva were fixed in formalin for 24h, embedded in paraffin, and five μm thick sections were stained with hematoxylin and eosin. Histological examination was performed by LEICA DM2500 microscope and images were captured with camera LEICA DFC290HD (Germany).

Histologically the epithelium is hyperplastic (resembling acanthosis, Figure 8) and in the underlyining connective tissue contains lymphoid cell infiltrates (lymphocytes and their activated forms typical for chronic imflammation, Figure 9) and a marked fibrosis is seen (Figure 10). Our findings of microscopic structure of the gingiva correspond with the picture of cyclosporine A-induced gingival hyperplasia described by Nurmenniemi et al. (2001) and Pernu and Knuuttila (2001). The endothelial lining of bloodvessels is atypical – we found cuboidal, and not squamous epithelial cell. This finding correspond with active neoangiogenesis (formation of new blood vessels with higher endothelial cells, Figure 11). Higher level of angiogenesis is also associated with another chronic inflammatory diseases of the epidermis, mucous membranes and tongue, such as lichen planus (Vybohova *et al.* 2014a) or psoriasis (Vybohova *et al.* 2014b).

CONCLUSION

In this literature review underpinned with two case reports of patients taking antihypertensives from the group of calcium channel blockers we have found through a DNA analysis of periodontal pathogens and clinical

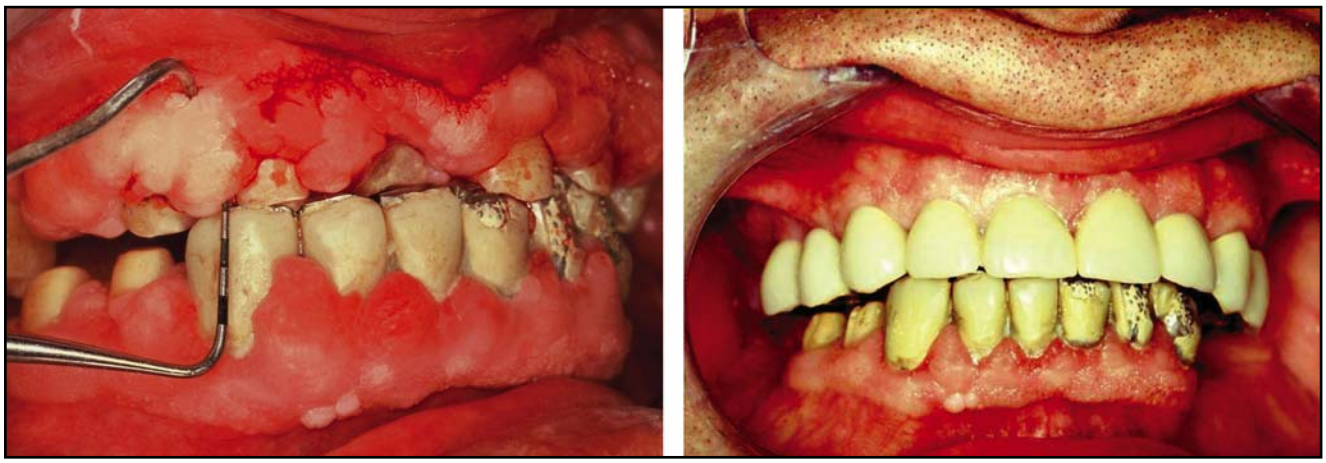

Fig. 7. Case report 2. Finally results of prothetic treatment with fixed bridge.

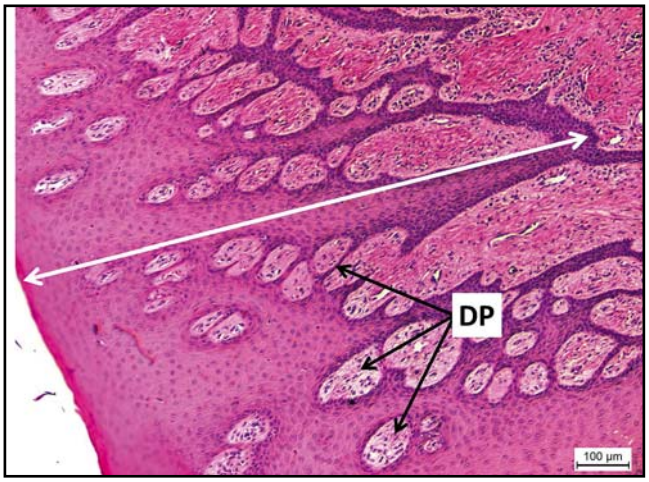

Fig. 8. Case report 2. Detail view on hyperplastic epithelium. Thick stratified squamous epithelium of gingiva (white arrow) and well developed connective tissue dermal papillae (DP) (Hematoxylin & Eosin staining, Orig. Magn. 100x, line in Figure = 100 µm)

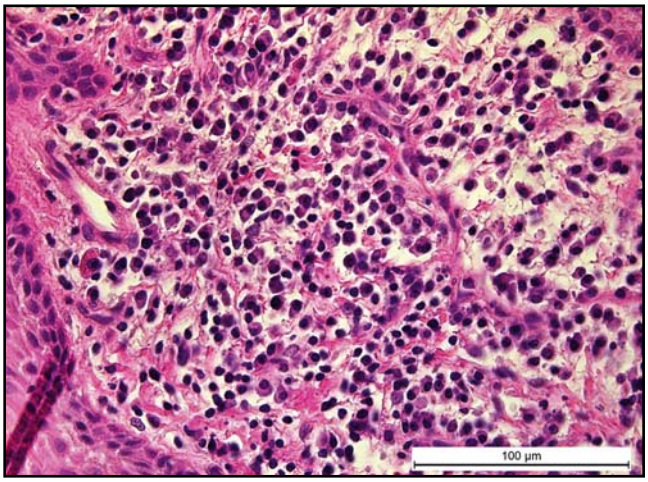

Fig. 9. Case report 2. The connective tissue is infiltrated by lymphocyte and plasma cells – picture of chronic inflammation (Hematoxylin & Eosin staining, Orig. Magn. 400x, line in Figure = 100 µm)

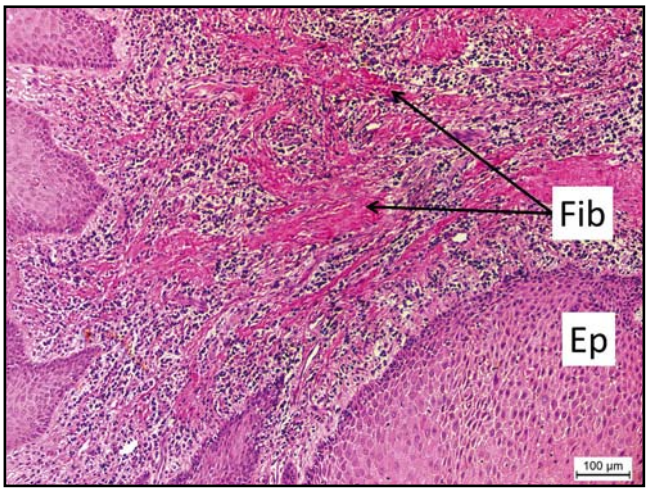

Fig. 10. Case report 2. Bundles of collagen fibres (Fib) of the connective tissue under stratified epithelium (Ep) (Hematoxylin & Eosin staining, Orig. Magn. 100x, line in Figure = 100 µm)

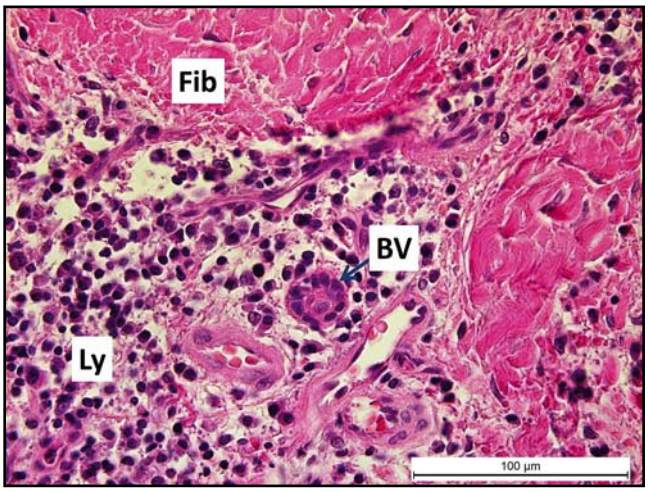

Fig. 11. Case report 2. Detail view on connective tissue under gingival epithelium. The connective tissue is infiltrated by lymphoid cells typical for chronic imflammation (Ly), huge bundles of collagen fibres are visible (Fib) and the endothelial lining of some blood vessels (BV) is cuboidal, what is a posible sign of massive angiogenesis (Hematoxylin & Eosin staining, Orig. Magn. 400x, line in Figure = 100 µm)

histological examination together with a combined conservative and surgical treatmnt the following facts:

1. In a hypertensive patient from the Case Report 1 who was for a long term medicated with a preparation from the nifedipine group of antihypertensives we detected the presence of periodontal pseudopockets with probing depth of 4 to 7 mm with positive BOP and with marked rigid fibrotic gingival enlargement accompanied with considerable foetor ex ore.
2. In the same patient we performed a semiquantitative DNA analysis of classic periodontal pathogens with the following results. A semiquantitative and qualitative bacterial analysis with its proportions corresponded to bacterial periodontal findings in chronic forms of periodontitis:
   a. The main periodontal pathogen of acute periodontitis *Aggregatibacter actinomycetemcomitans* was not elevated regarding the endpoints.
   b. Periodontal bacteria of the so-called red complex (P.g., T.f., T.d) which are characteristic for a group of chronic periodontitis were quantitatively elevated to high concentrations (++). From a group of less pathogenic bacteria of the so-called yellow complex the bacteria *Campylobacter rectus* were elevated to enormously high concentrations (+++). To conclude, a bacterial composition

in the patient with a high degree of gingival enlargement and periodontal pseudopockets 4 to 7 mm deep represented a typical spectrum of bacteria occuring in chronic forms of periodontitis. However, we cannot determine, if such distribution of bacteria was primary before the application of nifedipine antihypertensives, or it originated later after the formation of typical anaerobic setting of false periodontal pockets.

3. In a patient from the Case Report 2 who was for a long term medicated with a preparation from the amlodipine group of antihypertensives with large gingival overgrowth apart from standard therapeutical procedures /change of hypertension therapy, introduction of professional and home oral hygiene/ we used a classic partial gingivectomy. For delineating the surgical incision line a double probing of periodontal pseudopockets was used. The incision line proper was outlined through connecting single bleeding points marked by probing the base of particular pockets. After resolution of postoperative swelling we proceeded in a rapid succession to the follow-up endodontic and fixed prosthetic treatment in the upper jaw.
4. In the aforesaid patient hyperplastic acanthosis-like epithelial tissue was detected in bioptic sections for histological examination. In the underlying connective tissue a chronic inflammatory infiltrate characterized by lymphocytes and their active forms was noticed. Ongoing angiogenesis was characterized by endothelial cell lining with cuboid forms. In the samples under a layer of stratified epithelium there was present dense connective tissue comprising largely of collagen fiber bundles. Future immunohistochemical examination may find answers about the processes of proliferation or apoptosis of different cells of the epithelium and connective tissue of enlarged gingiva (Adamkov *et al.* 2011; Nurmenniemi *et al.* 2001) and elucidate the composition of the chronic inflammantion micro-environment of the gingiva (Pernu & Knuuttila 2001).

In this review study underpinned by two case reports we have dealt with gingival enlargement in hypertensive patients long-term treated with calcium channel blockers. We have covered several aspects of hyperplastic complications: clinical, microbiological and histopathological. The treatment involved the change of a group of antihypertensive drugs, oral-hygienic methods for clearing mouth biotypes, antibiotic therapy against mostly anaerobic oral pathogens completed with classic gingivectomy methods and final prosthetico-aesthetic therapy with good outcomes. Stated therapeutical procedures can be recommended for treating abovementioned complications.

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