The role of inflammation and apoptosis in cyclosporine A – induced gingival overgrowth

Kristina Mitic[1]*, Mirjana Popovska[1], Maja Pandilova[1], Rubens Jovanovic[2], Goce Spasovski[3], Vladimir Nikolov[3]

[1] Dental Clinical Center "St. Pantelejmon"-Clinic for Periodontology, Faculty of Dentistry, Skopje 1000, Macedonia. [2] Institute of Pathology, Faculty of Medicine, 1000 Skopje, Macedonia. [3] University Department of Nephrology, 1000 Skopje, Macedonia

Abstract

Cyclosporin A(*CsA*) - induced gingival overgrowth(GO) is a current problem of tissue-specific mechanism which is still incompletely explained. The apoptotic process has been of particular interest like a new concept in the etiology of this unwanted effect.

The aim of our study was to detect the level of apoptosis, expression bcl-2 and p53, associated with the different dosis of *CsA*. in gingival stroma. A cohort of 84 kidney transplant recipients was divided into four subgroups based on average daily dose of therapeutically applied *CsA* (Neoral®), (100 mg, 125 mg, 150 mg and 175 mg). The control group consisted of 21 patients, clinically diagnosed with periodontitis, who were not subjected to any medicamentous treatment causing gingival overgrowth.

The following indexes were analyzed: plaque index (PI), index of gingival inflammation (GI) according to Loe-Silnes, and gingival overgrowth index (GOI) according to MacGaw et al. The tissue samples were subjected to a semiquantitative analysis to detect apoptotical cells and imunohistochemically stained to detect the expression of the bcl-2 and p53 proteins.

The difference in percentage of apoptotic cells between the group taking 175mg and other subgroups, as well as the control group was statistically significant ($p<0.05$). There was a significant difference in percentage of expression bcl-2 between the 175 mg group compared to the other three subgroups and the control ($p=0.001$). However, a statistically significant positive correlation between the medicament dose, p53, apoptosis, and bcl-2 was registered ($p<0.05$).

Inflammation plays the most important role in the induction of apoptosis and proliferation in gingival tissues.

KEY WORDS: gingival overgrowth, cyclosporin A, gingival inflammation, apoptosis, bcl-2, p53.

INTRODUCTION

Gingival overgrowth (GO) is very often associated with the use of cyclosporine A (*CsA*) [1]. Although some studies indicate several factors as taking part in the pathogenesis of gingival overgrowth (GO), the dosage, the serum and saliva concentration of the medicament, the duration of treatment, oral hygiene and the age of each individual is of course worth emphasizing [2]. However, the genetics of each individual, the susceptibility of the gingiva including also inflammation should not be put a side as well, which can additionally render the diagnosis and prognosis of these conditions more difficult [3, 4, 5]. Some studies report that the fibroblastic gingival substrate is caused by defective collagen synthesis homeostasis and degradation, particularly of type I collagen, resulting in increased accumulation of collagen fibres, extracellular matrix and inflammation cells in gingival fibrotic tissue [6]. Other studies emphasize apoptosis as a controlling mechanism of the GO, as well as cascade of specific biochemical reactions, which go together with increased level of intracellular Ca^{2+}. A common feature of fenitoin, nifedipine and cyclosporine is the fact that they are all Ca^{2+} antagonists. The blockade of one or several steps in the cascade of the *CsA* interactions, may result in apoptotic changes and reduced caspase leading also to an increased level of growth of the gingival tissue [7, 8]. Apoptosis as a physiological death plays a vital role in the cell proliferation and differentiation. In addition, tissue homeostasis is maintained through a balance between the cell proliferation and cell death. The apoptotic cell death is considered as a possible participant in the pathogenesis of many diseases, including cancer, autoimmune illnesses and virus infections alike [9, 10, 11]. The crucial apoptotic regulation is realized through two transmembranic proteins: the deadly p53 gene

* Corresponding author: Kristina Mitic,
Dental Clinical Center "St. Pantelejmon"-Clinic for Periodontology,
Faculty of Dentistry, Skopje 1000, Macedonia
Phone: +38970350909; Fax :
e-mail: 3mkristina@gmail.com

Submitted: 4 May 2012 / Accepted: 10 November 2012

and the survival gene bcl-2. P53 is a protein–transactivator of many genes, and in case of cell DNA damage, p53 is activated, thus initiating the apoptotic process in terminally differentiated cells. The bcl-2 protein is a caspase-9 activation inhibitor, and hence, a cell death inhibitor. The abnormal level of bcl-2 affects tissue homeostasis, its increased expression resulting in cell survival, leading to tumor-genetic conditions [12]. The mechanisms responsible for GO with nephropathies still remain unidentified and the researches concerning the participation of cellular and apoptotic effect have resulted in discrepancies and contradictions. Hence, the consideration that there are still no clear information and knowledge on whether or not GO is a result of one factor or synchronization and a combination of several causes, conditioned by *CsA* therapy dosage, is completely justified. Considering the fact that tissue homeostasis is seriously impaired with *CsA*-induced GO, the aim of our research was to assess the level of apoptosis, bcl-2 and p53 expression in the gingival tissue at different *CsA* doses and compared to findings in healthy individuals with parodontitis.

MATERIAL AND METHODS

Patients

Gingival specimens were collected from eighty four kidney-transplant recipients, diagnosed with GO (*CsA* group) and from 21 healthy controls with periodontitis.All biopsies were obtained under local anesthesia during gingivectomy procedures, according to the guide lines of the University Dental Clinical Centre "St. Pantelejmon", Skopje, Department of periodontology and Ethics Committee. All kidney recipients had severe gingivitis, but no sign of periodontitis. Tissue biopsies from the controls were obtained during tooth extraction and gingivoplasty. The control group was consisted of individuals with neither kidney diseases nor *CsA* therapy, but with a verified periodontal disease where the average loss of attachment was 1.98. The level of attachment is the distance between the base of the pocket and a fixed point on the crown, such as the cementoenamel junction. Changes in the level of attachment can be due only to gain or loss of attachment and afford a better indication of the degree of periodontal destruction [13]. The medical history of all patients excluded use of any medicament causing GO. Patients' mean age at time of renal transplantation was 36.2 ± 9.5 years. The mean duration of therapy was 42.4 ± 36.2 months. According to the average *CsA* daily dose, all patients were divided into 4 sub-groups with 21 patients each: daily *CsA* dose of 100 mg; 125 mg; 150 mg and 175 mg dose of *CsA*. The post-transplant immunosuppressive therapy of all patients was *CsA* (Neoral®; Novartis; 2-4mg/kg/day until a satisfactory C2 level (concentration in serum 2 hours after administration) of the medicament is reached, prednisolone (0.1 mg/kg/day, Merck), mycophenolate mofetil (Cellcept ® 1.5-2g/day, Roche), Diltiazem® (2 x 90 mg, Alkaloid), the last improving the absorption of *CsA*. All subjects from the control and research group underwent the scheduled clinical and para-clinical examinations.

Clinical examinations

The clinical examinations were made by applying a few indexes:

- Plaque Index (PI) according to Silness - Löe [14]: Each of the four surfaces of the teeth (buccal, lingual, mesial and distal) is given a score from 0-3. The scores from the four areas of the tooth are added and divided by four in order to give the plaque index for the tooth with the following scores and criteria: 0-No plaque; 1-A film of plaque adhering to the free gingival margin and adjacent area of the tooth. The plaque may be seen in situ only after application of disclosing solution or by using the probe on the tooth surface; 2-Moderate accumulation of soft deposits within the gingival pocket, or the tooth and gingival margin which can be seen with the naked eye; 3-Abundance of soft matter within the gingival pocket and/or on the tooth and gingival margin.
- Gingival Inflammation Index (GI) according to Löe-Silnes [15]: 0-normal; 1-mild inflammation, slight color change and edema, no bleeding; 2-moderate inflammation, redness, edema, bleeds on probing; 3-severe inflammation, marked redness and edema, ulceration, spontaneous bleeding.
- Gingival overgrowth index (GOI) according to MacGaw et al. [16]. The vertical component measures the degree of gingival enlargement in an apico-coronal direction for every gingival unit graded by means of a 4 point scale (0-no gingival hyperplasia; 1-blunting of gingival margin; 2-less than 1/2 crown length; 3-more than 1/2 crown length).

Para-clinical examinations

The level of *CsA* content in the blood of all the examinees was measured at the Institute of Pharmacology at the Medical Faculty in Skopje, as part of the periodical medicamentous tests of the renal-transplant recipients. Blood samples were drawn from the cubital vein, 2 hours after taking the morning dose of *CsA*. Blood *CsA* concentration (C2) was determined by applying a Fluorescence Polarization Immunoassay method (FPIA) on TDx analyzer (ABBOT company) along with commercial sets. Each blood sample (2ml blood + 0, 2ml EDTA) was previously subjected to pre-treatment, in order to minimize the interference of the natural fluorescence with the components which are bonded to proteins. During the pre-treatment, solubalization reagent (50µl) and precipitating reagent (300µl) was added in the blood sample in order to dissolve the cells and precipitate the protein. Following the vortexing and spinning (9 500 x 5 min)

a clear supernatant was obtained, which was used to measure the level of monoclonal cyclosporine A in the blood. According to this method, the normal concentration of *CsA* (C2) in the blood is from 600-800 ng/ml, at a few months after transplantation.

Tissue Processing and Histochemistry

In the first phase of the periodontal treatment, after informing and obtaining consent of each patient (control and the study group), bioptical material from the overgrown interdental papilla was taken under infiltrated anesthesia and was fixed in 10% neutral formalin, and a standard patohistologic processing was made at the Institute for Pathological Anatomy at the Medical Faculty in Skopje. With the control group, the sample was taken during teeth extraction from orthodontic reasons or any other indication. Later, the tissue samples were placed into the paraffin moulds, out of which tissue cross-sections with 4-6 μm thickness were obtained. These tissue cross-sections were placed on glasses in a standardized manner and stained with hematoxylin eosin (HE), while the cross-sections for the immunohistochemical stained were placed on silane glasses and colored with (ABC-Avidin Biotin Complex method, LSAB + variant).

Immunohistochemistry (ABC-Avidin Biotin Complex method)

Primary antibody, with a determined antigene determinant, is added to the tissue sample, which is thus incubated at room temperature (30 min). Later the sample is rinsed with phosphate buffer, then deluded into 10% normal serum and rinsed again with phosphate buffer. The secondary antibody, which is complexed with biotin is added. The Avidin-Biotin Complex contains HRP enzyme (Horse radish peroxidase), which bonds with the biotin molecule of the secondary antibody, and that bonds with determinants of the primary antibody. The samples are incubated at room temperature for 30 minutes and then rinsed with phosphate buffer. The next step involves adding AVS reagent and rinsing with phosphate buffer. The final result as a positive antigene antibody reaction is followed by forming of a brown precipitate from the polymerized substrate. Immunohistochemical staining were performed using, bcl-2(clone 124; 1:50 dilution) and p53(clone DO-7; 1:50 dilution) DAKO production. We used the *in situ* assay to detect apoptotic fibroblasts using ApopTag Plus Peroxidase kit (Apoptosis detection kit-Chemicon), following the manufacturer's instructions. Following the immunohistochemical staining by applying a light microscope on the tissue cross-sections, detection and counting of the apoptotic cells was done, bcl-2 and p53, expressed as average number of cells per 10 high power field (X 400). The level of expression of p53 and bcl-2 as well as the apoptotic cells of each slide was graded on a semiquantitative manner using a graduation 0-3+; (0)=no staining; (1+)=stained cells comprising up to 10% of the inflammatory infiltrate and fibroblasts; (2+)=stained cells comprising up to 30% of the inflammatory infiltrate and fibroblasts; (3+)=stained cells comprising > 30% of the inflammatory infiltrate and fibroblasts.

Statistical analysis

Differences between the *CsA*-treated group and the control group with respect to clinical parameters and histopathological findings were analyzed using the Student t-test. Attributes statistical series were analyzed by defining the coefficient of relations, proportions and rates with determination of statistical significance between the detected differences. Statistical significance was defined as $p<0.05$. Correlations between histopathological findings and clinical parameters were tested using Spearman's rank correlation coefficient.

RESULTS

The difference in the percentage of the PI between the subgroup taking 175 mg *CsA*, the other sub-groups and the control group is statistically significant as $p=0.001$. The results also showed significant difference for the (GI) index between the subgroup of 100mg and the subgroups of 150mg and 175mg ($p<0.05$). Also there was a statistical difference between the control group and the subgroup with 150mg. The difference in the percentage of apoptotic cells between the group taking 175mg, other subgroups and the control group was statistically significant as $p<0.05$ (Figure 1). There was also a positive correlation between the GI and apoptosis as $p<0.05$ (r=0.308) (Table 1). The observed difference in

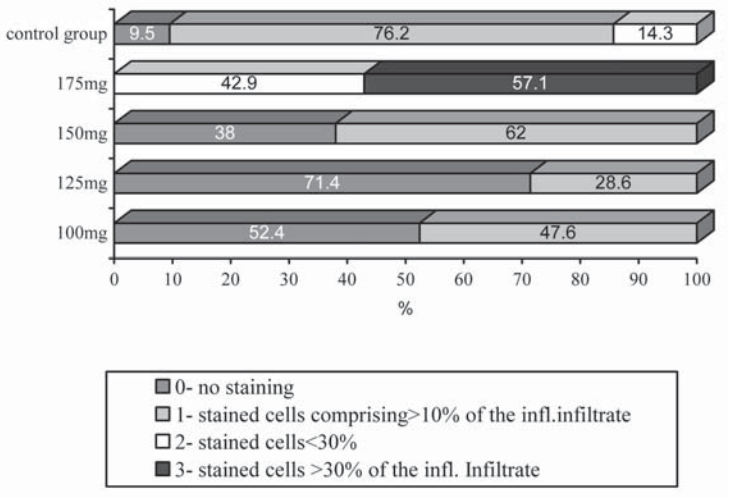

FIGURE 1. Distribution of apoptosis depending on the medicamentous dose

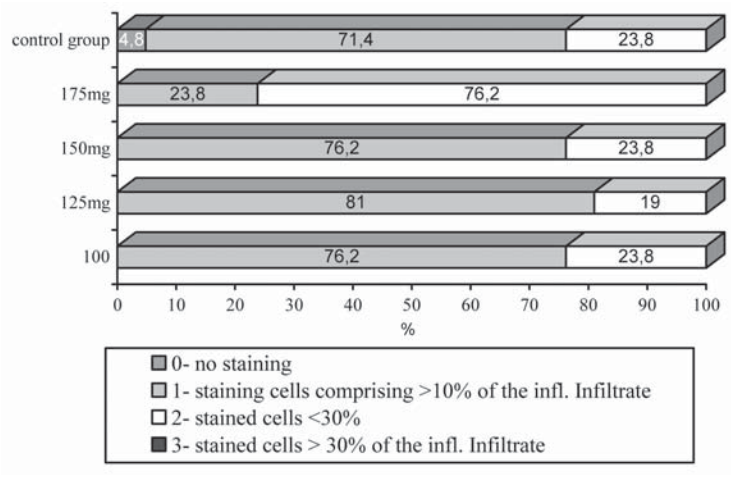

FIGURE 2. Distribution of bcl-2 depending on the medicamentous dosage

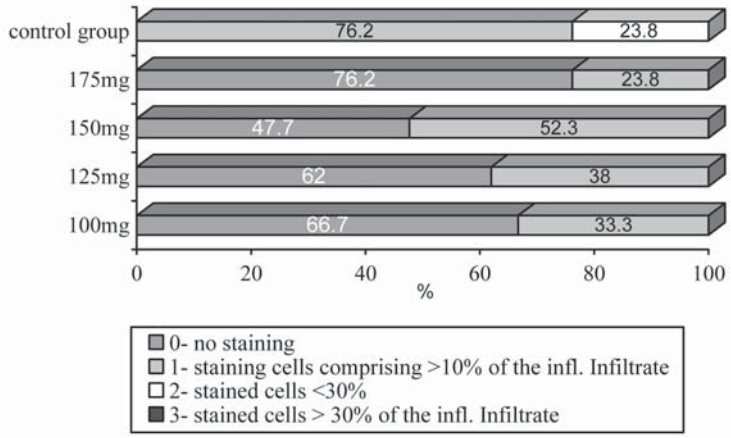

FIGURE 3. Distribution of p53 depending on the medicamentous dosage

percentage for bcl-2 (Figure 2) between the subgroups taking 175 mg compared to the other three subgroups and the control group is statistically significant (p=0.001). Finally, a significant difference (p<0.05) was observed for the percentage of p53 (Figure 3) when the control group was compared to the subgroups taking 100 mg, 125 mg and, 175 mg. However, there was no significant positive correlation between the blood concentration of *CsA* with apoptosis for p>0.05 (r=0.187). On the other hand, a significant positive correlation between the medicament dose, p53, apoptosis, and bcl-2 was observed at p<0.05 (Table 1).

TABLE 1. Correlation of Gingival Overgrowth Index, cyclosporine dosage and apoptosis, bcl-2 and p53

	Correlation Rank of GOI	Cy dose
	Spearman Rank Correlations p<0.05	Spearman Rank Correlations p<0.05
apoptosis		0.660386
bcl-2	0.389151	0.377778
p53	0.424015	0.526378

There was also a significant positive correlation of GOI, bcl-2 and p53 at p<0.05 (Table 1).

DISCUSSION

The concept which generally prevails in theories on gingival overgrowth is more individual disorder than sensitiveness to *CsA* or its metabolites. These findings may be a result of differences in the sub-populations of fibroblasts or different gingival susceptibility to invasion of microorganisms from dental plaque, or an interference of *CsA* with the T-cell immunity. Our study shows that in all patients, regardless of the applied *CsA* dose (100, 125, 150 or 175mg) there was a low level of oral hygiene, and the highest plaque values were noted in the group taking the highest dose of cyclosporine. The patients were not motivated to practise a good oral hygiene, probably because of the primary disease. Namely, gingival overgrowth leads to formation of gingival pockets which cause increased accumulation and detection of dental plaque. Although Seymour [18] believed that plaque control does not prevent the development of the GO in sensitive patients yet, the elimination of the inflammatory component leads to a reduction of the GO. They also considered that plaque control and the removal of plaque retention factors are necessary for the gingival health of renal-transplant patients, but these measures themselves do not prevent the development of GO or recurrence following surgical intervention. Despite the fact that dental plaque is a predisposing factor for development of the GO, Somacarrera [19] believed it is not a vital one, since they have registered high dental plaque and gingival inflammation indexes, as well as a high values of serum *CsA* concentration in patients with no gingival changes were observed. Our hypothesis is that the high GI in the group taking the highest *CsA* dose is caused by the suppressed local immune response, hence the control of the gingival inflammation is considered as crucial for these patients, thus preventing the recurrence of bacterial infections. Daley et al. [20] reported an association between previously present plaque and gingival inflammation which correlates with the *CsA*-caused gingival overgrowth. The inflammation leads to tissue damage through different mediators which are released by the activated inflammatory cells. There is a high level of remodeling in the inflammatory tissue and the effect of *CsA* acts synergistically with the endogen signals, resulting in an increased reparation or overgrowth [21]. The inflammatory mediators thereto act as cofactors in inducing GO. These

findings are in accordance with the findings of Echelard et al. [22] who established a significant inflammation in the attachment tissue of the medicamentously treated gingiva compared to a healthy gingiva. Oral bacteria and gingival cells and tissues in patients on *CsA* therapy, react in a specific manner manifested through a relatively high level of inflammation and cellularity compared to other forms of GO [23]. Tonetti et al. [24] was the first to promote the role of the apoptotic process in chronic inflammation, and its association with bacteria-induced gingival inflammation. Considering the fact that this mechanism participates in the GO pathogenesis, Budunelli et al. [25] claimed in their studies that the number of divisible and apoptotic cells have not shown significant difference between the examiners with gingivitis and the examineres who made up the control group, while a reduced number of apoptotic cells was detected in patients of the *CsA* group. The conducted research of our study showed a significant increase of the apoptotic processes in the gingival epithelium of the patients treated with the highest *CsA* dose, compared to the other sub-groups and the controls ($p<0.01$), confirming the role of the apoptotic processes in the ethiopathogenesis of GO. A significant positive correlation between the medicament dose, p53, apoptosis, and bcl-2 was observed (Table 1). We believe that this apoptosis is rather a consequence of the plaque, gingival inflammation and periodontal disease, than a cause of it. From the obtained results, it can be concluded that the *CsA* that causes immunosupression and inappropriate immunological response of the acquired (lymphocite) response, follows in prolonged and inefficient defence assisted by the unspecific congenital immunity (macrophage and neutrophils). Because of the prolonged chronic inflammation, there is an activation of the fibroblasts which lead to fibrosis. At the same time, the epithelium is also damaged. Fujimori et al. [26], reported that cell death has been inhibited and it occurred depending on the medicamentous concentration. Furthermore, we found a statistically insignificant positive correlation between the blood *CsA* concentration and apoptosis ($p>0.05$), (Table1). Bulut et al. [27] recorded only a significant positive correlation between the serum *CsA* level and the degree of bcl-2 expression, while no correlation was registered between the serum concentration, apoptosis and p-53 expression. There was also a significant positive correlation of GOI , bcl-2 and p53 at $p<0.05$ (Table1). The anti-apoptotic protein bcl-2 is mostly prevalent in the gingival epithelium of patients with highest dose of immunosuppressive drugs, as compared to the other subgroups and controls. It seems that the apoptosis is at a critically high level in patients taking 175mg, which "alarms" urgent activation of the apoptotic mechanisms (increased bcl-2 expression), which is actually a mechanism of a negative feedback. According to our results, it was established that the significant difference between p53 and bcl-2 is dose dependent. It was noted that the largest dose (175 mg) results in enlargement of the antiapoptotic activity and reactive enlargement of p53 in an attempt to block the cell cycle. Other authors [28], who examined the inhibition of gingival epithelium cell apoptosis in the group treated with *CsA*, failed to prove significant difference between the examined group and the controls (patients with periodontal disease) with respect to immunolocalization of p53 i bcl-2. Our results are in line with those of Saito et al. [29], who detected high bcl-2 expression in the gingival of GO. Pandilova [30] in her research also registered significant differences for the bcl-2 between a clinically healthy tissue and the groups (with parodontitis, only for attachment loss up to 3 mm. According to these results, larger bcl-2 positive epithelium cells presence in biopsies from patients treated with medicaments, compared to other biopsies which were not treated with any medicament, indicating that there is a possibility that bcl-2 participates in the GO development. We believe that the high expression of p53 with the control group and the subgroup treated with 150 mg, is a result of existing inflammation where the cell turnover is at a pathological level. The greater the antiapoptotic activity (bcl-2 i p53), the greater the gingival thickness, despite the rective enlargement of p53. The function of p53 is to inhibit the cell function in order to provide the damaged cells enough time to regenerate, and later to enter into a cell division, thus preventing any cells with possible DNA damage to divide. This is one of the mechanisms preventing malign proliferation. Saito et al. [31] detected a positive expression in the epithelium cells nucleus, while no expression was registered with the patients from the control group. It is considered that bcl-2 may lead to cell accumulation and acantosis, and therefore p-53 may be involved in the pathogenesis of the medicament-induced gingival overgrowth through DNA damages. The findings of our research showed moderate positive significant correlation ($p<0.05$) of the GI with the apoptotic index (Table 1.) These results support the idea that inflammation causes apoptosis, and that relatively overgrown gingiva is a result of the compensational epithelium proliferation. Similar results were reported in the research done by Pandilova [32], who proved the existence of a strong positive correlation between inflammation and apoptosis, which participates in the loss of attachment, but does not participate in the recession occurrence and progression. Taking into account the obtained results for the increased mononuclear cell infiltrate in tissues treated with *CsA*, compared to the

non-treated ones, Bulut et al. [33] supported the idea about the participation of inflammation and local immune stimuli in the development of GO, which nonetheless remains a multifactorial process. Cell composition and the existence of inflammatory cells reflect its chronic nature, which may result in a long-term local stimulation process leading to the GO.

CONCLUSION

Pathogenesis of *CsA*-induced GO might involve apoptosis and over-expression of bcl-2 in patients with high *CsA* doses. The most important factor for induction of apoptosis in gingival tissues is inflammation. The removal of the local irritating factors and the reduction of gingival inflammation, results in reduction of apoptosis and GO. Joint collaboration between dentists and nephrologists is necessary, who by carrying out preventive periodontal programmes, will bring improvement of oral health and hence the general health.

ACKNOWLEDGMENT

We would like to thank Prof. Dr. Milijana Tolovska and Dr.Slavica Kostadinovska-Kunovska for their assistance with immunohistochemical analyses of stained histology slides, and Katerina Hadziska-Nicheska for technical assistance. The study is financed by personal finding.

DECLARATION OF INTEREST

The authors declare that they have no competing interests.

REFERENCES

[1] Seymour RA, Heasman PA. Drugs and the periodontium. J Clin Periodontol 1988;15: 1-16.
[2] Rees TD. Drugs and oral disorders. Periodontol 2000. 1998;18:21-36.
[3] Seymour RA, Ellis JS, Thomason JM. Risk factors for drug-induced gingival overgrowth. J Clin Periodontol 2000;27:217-223.
[4] Thomason JM, Seymour RA, Ellis JS, Kelly PJ, Parry G, Dark J. et al. Determinants of gingival overgrowth severity in organ transplant patients. An examination of the role of HLA phenotype. J Clin Periodontol 1996; 23:628-634.
[5] Türkmen A, Ak G, Furuncuoglu Y, Akar U, Seyhun Y, Türk S. et al. Relationship between gingival hyperplasia and class II histocompatibility antigens in renal transplant recipients. Nephron 2000; 84(1): 29-31
[6] Schincaglia GP, Forniti F, Cavallini R, Piva R, Calura G, del Senno L. Cyclosporin-A increases type I procollagen production and mRNA level in human gingival fibroblasts in vitro. J Oral Pathol Med. 1992; 21(4):181-185.
[7] Bulut S, Ozdemir BH, Alaaddinoğlu EE, Oduncuoğlu FB, Bulut OE, Demirhan B. Effect of cyclosporin A on apoptosis and expression of p53 and bcl-2 proteins in the gingiva of renal transplant patients. J Periodontol. 2005; 76(5):691-695.
[8] Hassell TM, Hefti AF. Drug-induced gingival overgrowth: old problem, new problem. Crit Rev Oral Biol Med. 1991; 2(1):103-137.
[9] Cohen JJ. Programmed cell death in the immune system. Adv Immunol 1991; 50:55-85.
[10] Wyllie AH. Apoptosis and the regulation of cell numbers in normal and neoplastic tissues: an overview. Cancer Metastasis Rev. 1992; 11(2):95-103.
[11] Bulut S, Ozdemir BH. Apoptosis and expression of caspase-3 in cyclosporin-induced gingival overgrowth. J Periodontol. 2007; 78(12): 2364-2368.
[12] Reed JC. Bcl-2 and the regulation of programmed cell death. J Cell Biol. 1994;124(1-2):1-6.
[13] Newman MG, Takei HH, Carranza FA. Carranza's clinical periodontology, 9th ed. Philadelphia: WB Saunders, 2002: 347.
[14] Sillnes S, Löe HP. Periodontal diseases in pregnancy.II.Correlation between oral hygiene and periodontal condition. Acta Odontol Scand 1964; 22:131.
[15] Loe H, Silness J. Periodontal disease in pregnancy. I. Prevalence and severity. Acta Odontol Scand. 1963; 21:533-551.
[16] McGaw T, Lam S, Coates J. Cyclosporin-induced gingival overgrowth: correlation with dental plaque scores, gingivitis scores, and cyclosporin levels in serum and saliva. Oral Surg Oral Med Oral Pathol. 1987; 64(3):293-297.
[17] Seymour RA, Thomason JM, Ellis JS. Risk factors for gingival overgrowth in patients medicated with cyclosporine in the absence of calcium channel blockers. J Clin Periodontol 2005; 32:273-279.
[18] Seymour RA, Ellis JS, Thomason JM. Risk factors for drug-induced gingival overgrowth. J Clin Periodontol. 2000; 27(4):217-223.
[19] Somacarrera ML, Hernández G, Acero J, Moskow BS. Factors related to the incidence and severity of cyclosporin-induced gingival overgrowth in transplant patients. A longitudinal study. J Periodontol. 1994; 65(7):671-675.
[20] Daley TD, Wysocki GP, Day C. Clinical and pharmacologic correlations in cyclosporine-induced gingival hyperplasia. Oral Surg Oral Med Oral Pathol. 1986; 62(4):417-421
[21] Modéer T, Domeij H, Anduren I, Mustafa M, Brunius G.Effect of phenitoin on the production of interleukin-6 and interleukin-8 in human gingival fibroblasts. J Oral Pathol Med 2000; 29: 491-499.
[22] Echelard S, Hoyaux D, Hermans M, Daelemans P, Roth J, Philippart P, et al. S100A8 and S100A9 calcium-binding proteins: localization within normal and cyclosporin A-induced overgrowth gingiva. Connect Tissue Res 2002; 43:419-414.
[23] Trackman PC, Kantarci A. Connective tissue metabolism and gingival overgrowth. Crit Rev Oral Biol Med 2004; 15(3):165-175.
[24] Tonetti MS, Cortellini D, Lang NP. In situ detection of apoptosis at sites of chronic bacterially induced inflammation in human gingiva. Infect Immun. 1998; 66(11):5190-5195.
[25] Buduneli E, Genel F, Atilla G, Kütükcüler N. Evaluation of p53, bcl-2, and interleukin-15 levels in gingival crevicular fluid of cyclosporin A-treated patients. J Periodontol 2003; 74(4):506-11.
[26] Fujimori Y, Maeda S, Saeki M, Morisaki I, Kamisaki Y. Inhibition by niphedipine of adherence- and activated macrophage-induced death of human gingival fibroblasts. Eur J Pharmacol 2001;415:95-103.
[27] Bulut OE, Sökmensüer LK, Bulut S, Tasman F, Müftüoğlu S. Immunohistochemical study of cyclosporin-induced gingival overgrowth in renal transplant recipients. J Periodontol. 2004;75(12):1655-1662.
[28] Bulut S, Uslu H, Ozdemir BH, Bulut OE. Analysis of proliferative activity in oral gingival epithelium in immunosuppressive medication induced gingival overgrowth. Head Face Med. 2006; 2:13.
[29] Saito K, Mori S, Tanda N, Sakamoto S. Expression of p53 protein and Ki-67 antigen in gingival hyperplasia induced by nifedipine and phenytoin. J Periodontol. 1999;70(6):581-586.
[30] Pandilova M. Comparative analysis of apoptotic cell death during periodontal disease. Doctoral Dissertation, Skopje, 2003;45-46.
[31] Saito K, Mori S, Tanda N, Sakamoto S. Immunolocalizaiton of c-Myc and bcl-2 proto-oncogene products in gingival hyperplasia induced by nifedipine and phenytoin. J Periodontol. 2000; 71(1):44-49.

[32] Minovska A, Pandilova M, Janevska V. Apoptotic cell death in correlation with attachment loss during periodontal disease. Mak Stom Pregl 2004;28(1-4):17-22.

[33] Nurmenniemi PK, Pernu HE, Knuuttila ML. Mitotic activity of keratinocytes in nifedipine- and immunsuppresive medication-induced gingival overgrowth. J Periodontol 2001;72:167-173.