ResearchGate

See discussions, stats, and author profiles for this publication at: https://www.researchgate.net/publication/286519667

Ki-67 Expression in Gingival Overgrowth: An Immunohistochemical Study

Article *in* Life Science Journal · January 2011

CITATIONS
2

READS
22

2 authors:

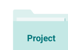
Eman Elfirt
3 PUBLICATIONS **6** CITATIONS

SEE PROFILE

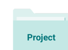
Dalia Ghalwash
The British University in Egypt
17 PUBLICATIONS **60** CITATIONS

SEE PROFILE

Some of the authors of this publication are also working on these related projects:



RD research View project



Rania Hanafi M., Ebtihal Hamdy and Eman ElFirt View project

All content following this page was uploaded by Dalia Ghalwash on 14 March 2019.

The user has requested enhancement of the downloaded file.

Ki-67 Expression in Gingival Overgrowth: An Immunohistochemical Study

Eman Y. El-Firt *[1] and Dalia M. Ghalwash [2]

[1]Departement of Oral Medicine and Periodontology, Faculty of Oral and Dental Medicine, Cairo University, Cairo, Egypt
[2]Departement of Oral Medicine and Periodontology, Faculty of Dentistry, October University for Modern Sciences and Arts, 6th October, Egypt
*elfirt@yahoo.com

Abstract: Ki-67 is a well-recognized nuclear proliferation marker. Considering that an unusual cell proliferation may have a role in the pathogenesis of gingival overgrowth with different etiologies. The study involved 4 patients with cyclosporine induced gingival overgrowth (CGO), 6 patients with phenytoin induced GO (PGO) and 5 patients with hereditary gingival fibromatosis (HGF). Healthy tissue samples without clinical signs of periodontal inflammation were also included as control samples. Immunohistochemistry against the proliferation antigen Ki-67 was performed and optical density measured and compared in both epithelium and connective tissue. Ki-67 was expressed both in the epithelium and corium of the four studied groups. The expression patterns of Ki-67 were significantly higher (p<0.00) in CGO, while no significant difference between HGF and PGO groups was detected and both showed lower values than CGO. Control group showed the significantly lowest mean of Ki-67 level and the expression was mainly in the basal layer of epithelium. In conclusion; increased cell division may have a role in the pathogenesis of gingival overgrowth induced by cyclosporine and phenytoin or inherited as HGF as reflected by increased expression of Ki-67.
[Eman Y. El-Firt and Dalia M. Ghalwash **Ki-67 Expression in Gingival Overgrowth: An Immunohistochemical Study]** Life Science Journal,. 2011; 8(4):221-226] (ISSN: 1097-8135). http://www.lifesciencesite.com.

Keywords: Cyclosporine; phenytoin; hereditary gingival fibromatosis; gingival hyperplasia/pathogenesis; Ki-67

1. Introduction

Gingival enlargement may be caused by a variety of etiologic factors. Some drugs such as cyclosporin A; the drug of choice in preventing transplant rejection; and phenytoin; the most commonly used drug for managing epileptic seizures; are commonly associated with the adverse effect of gingival overgrowth (Rateitschak et al., 1983; Bulut et al., 2004; Lin et al., 2007). Gingival overgrowth can also be inherited as an autosomal dominant disorder, or occasionally as an autosomal recessive mode of inheritance, in a condition known as hereditary gingival fibromatosis (HGF) (Singer et al., 1993; Coletta and Graner, 2006). Both types of gingival overgrowth (drug induced or familial) are characterized histologically by thickened, parakeratinized epithelium with elongated rete-pegs and increased extracellular matrix within the connective tissue (Mariani et al., 1993; Martelli et al., 2000; Vardar et al., 2005).

Squamous cell carcinomas may arise in some cases of drug induced gingival hyperplasia although it has been long thought that these conditions are not related to tumorigenesis (Varga and Tyldesley, 1991; McLoughlin et al., 1995; Saito et al., 1999). Oral cancers and increased proliferative activity of oral tissues have been analyzed for many years by monoclonal antibodies to specific antigens such as Ki-67 (Zoeller et al., 1994). Ki-67 is a proliferation associated antigen that serves as a marker for estimation of tissue growth as it is present in the nuclei of proliferating cells located in G1, S, G2, and M phases of the cell cycle and absent in quiescent cells lagging in G0 phase, suggesting a role for Ki-67 in the early steps of rRNA synthesis (Schlter et al., 1993; Buduneli et al., 2007). The mean rate of ki-67 positive cells in phenytoin-induced gingival overgrowth is proven to be more than 10% of immune-stained sections, which is comparable to that of dysplastic oral mucosae (Saito et al., 1999).

As for the HGF, although they usually represent a totally benign condition, yet one case of epithelial dysplasia of the overgrown tissue has been reported (Gunhan et al., 1995). HGF epithelial cells demonstrated higher proliferation rates than normal gingivae and increased expression of proliferation markers as proliferating cellular nuclear antigen (PCNA) and Ki-67 of HGF mesenchymal fibroblasts has been detected *in vitro* (Saygun et al., 2003; Martelli et al., 2005).

The present study aimed to evaluate the state of imbalance in homeostasis of the proliferative activity of gingival epithelium and connective tissue cells by comparing the immunohistochemical expression of a commonly used proliferation marker, Ki-67, in cyclosporine and phenytoin gingival overgrowth as well as cases of HGF.

2. Material and Methods

Study Population

Gingival biopsies were collected from 15 subjects (seven females and eight males with age ranges from 10-32 yrs) with moderate to severe gingival overgrowth (GO) during gingivectomy procedures. The study involved 4 patients with cyclosporine induced gingival overgrowth (CGO), 6 patients with phenytoin induced GO (PGO) and 5 patients with hereditary gingival fibromatosis (HGF). Diagnosis was based on patients' history and clinical examination to differentiate between different causes of gingival overgrowth shown in figure 1 (A, B and C).

Healthy tissue samples without clinical signs of periodontal inflammation were also obtained from the marginal gingiva of four unrelated HGF patients (two males and two females, aged 16 - 28 yrs) when the subjects underwent routine dental treatment (tooth extraction for orthodontic reasons or crown-lengthening procedures). All patients signed a consent form after being advised of the nature of the study.

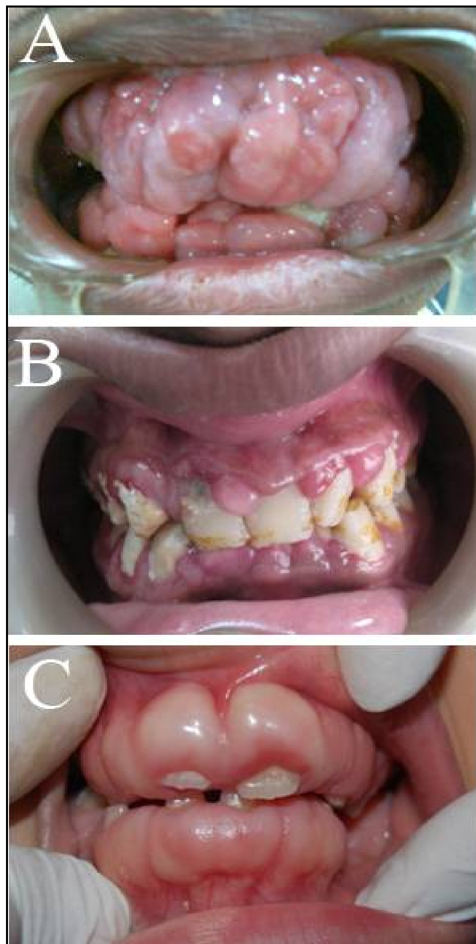


Figure (1): Clinical view of three patients with severe gingival overgrowth: A: CGO, B: PGO and C: HGF

Tissue Processing

As previously described by Buduneli *et al*., (2007), tissue samples were fixed in 4% paraformaldehyde and embedded in paraffin. Sections with 5-µm thickness were cut at the central region of each specimen to obtain maximum standardization of the cutting surface. One of the sections was stained with hematoxylin and eosin to evaluate the histopathologic presentation of gingival enlargement.

For Ki-67 staining, sections were deparaffinized by passing through xylene and alcohol, and rehydrated in 96% ethanol, then immersed in 3% hydrogen peroxide to block endogenous peroxide activity. The sections were incubated with a mouse anti-human Ki-67 antibody (Zymed, CA, USA) at 4°C overnight. Normal serum was used as a negative control. Subsequently, the standard streptavidin–biotin–peroxidase complex method was performed using SP kit (Zhongshan Goldenbridge Biotechnology, Beijing, China) for immunohistochemical detection of the proliferation marker Ki-67. Reaction products were visualized by immersing the sections for 5 min in diaminobenzidine solution. Nuclei were lightly counterstained with hematoxylin. Each step was followed by thorough washes with phosphate buffered saline (PBS).

Assessment of immunostaining

Ordinary light microscope was first used to detect the positive and negative immunostaining and localization of the positive reaction within the tissues. Image analyzer computer system (Leica Qwin 500 image analyzer computer system, Wetzlar, Germany) was used to measure the optical density (OD) of the immunostained sections. Five sections were used for each subject and three fields of a gingival section were chosen randomly for the analysis of Ki-67 staining using a magnification of (x400) so that a total of 15 microscopic fields were analyzed for each subject.

Statistical Analysis

Data were presented as mean and standard deviation (SD) values. One-way Analysis of Variance (ANOVA) was used for comparison between the four groups. Tukey's post-hoc test was used for pair-wise comparison between the groups when ANOVA test is significant. Paired t-test was used to compare between Ki-67 levels in epithelium and connective tissue. The significance level was set at $P \leq 0.05$. Statistical analysis was performed with PASW Statistics 18.0 (Predictive Analytics Software) for Windows (IBM Company, Chicago, IL, USA).

3. Results

Histopathology and Immunohistochemistry

As shown in H&E stained sections in figure 2 (A&B), the histopathological features did not differ greatly between different cases of drug induced GO (CGO and PGO). They shared a common histopathology of a significant papillary hyperplasia and parakeratinized epithelial layer with acanthosis and deep ridges penetrating into the underlying connective tissue, with wide variable levels of inflammatory cell infiltration and chorion fibrosis.

Connective tissue alterations were more marked in specimens from HGF group manifested by increased amount of collagen fiber bundles and fewer fibroblasts. Mild chronic inflammatory infiltrates were also frequently observed in the subepithelial connective tissue samples. These changes are shown in fig (3).

Nuclear immunoreactivity for Ki-67 antigen was easily identified, and nuclei with a clear brown color, regardless of the intensity of staining, were interpreted as positive, but this positive reaction was more marked and widely distributed within epithelial cells than within connective tissue cells. In healthy control tissues, Ki-67-positive cells were observed only in the basal cell layer of epithelium while the majority of gingiva samples from the CGO group showed deep and widely distributed Ki-67 positive cells throughout epithelial layers. The PGO and HGF groups showed almost similar expression of Ki-67 antigen which was mainly located in the basal and suprabasal layers of the epithelium. In the lamina propria, Ki-67 expression was observed in fibroblasts of hyperplastic gingival tissues mainly in tissue sections belonging to the CGO, while the control gingiva revealed week immunostaining of fibroblasts. These findings of the immune stained sections are shown in figure (4).

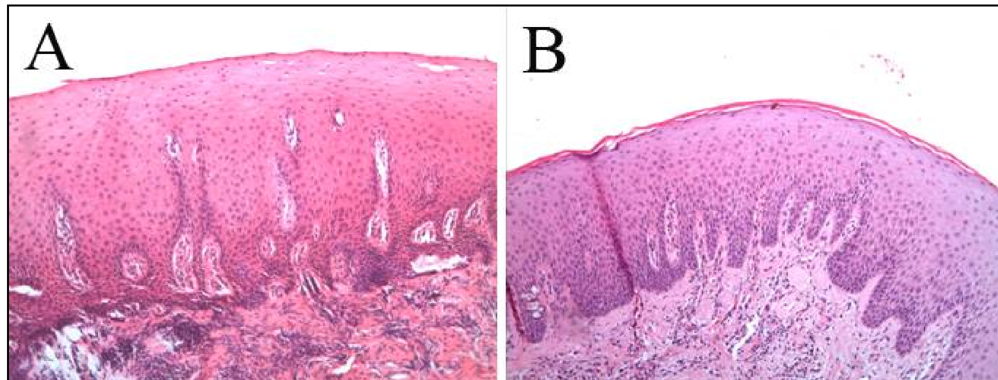


Figure (2): Histopathologic presentations of drug induced GO: A - Section from CGO showing irregular acanthosis and chorion fibrosis with marked inflammatory cellular infiltrate. B - Section from PGO group showing acanthosis with mild parakeratosis and papillomatosis with chorion fibrosis and lymphomononuclear infiltrate (H&E; original magnificationx100).

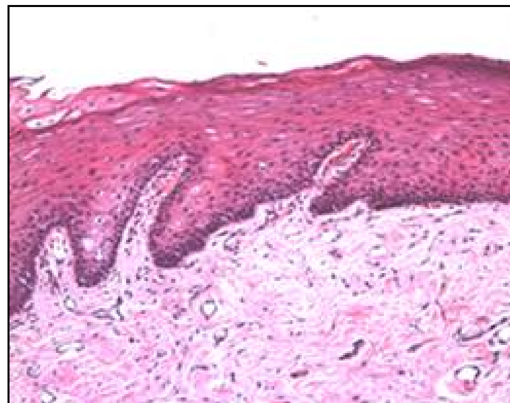


Figure (3): Histopathologic presentation of HGF showing dense connective tissue predominantly consisting of thick and irregularly arranged collagen fibers underlying a well structured epithelium (H&E; original magnification x100).

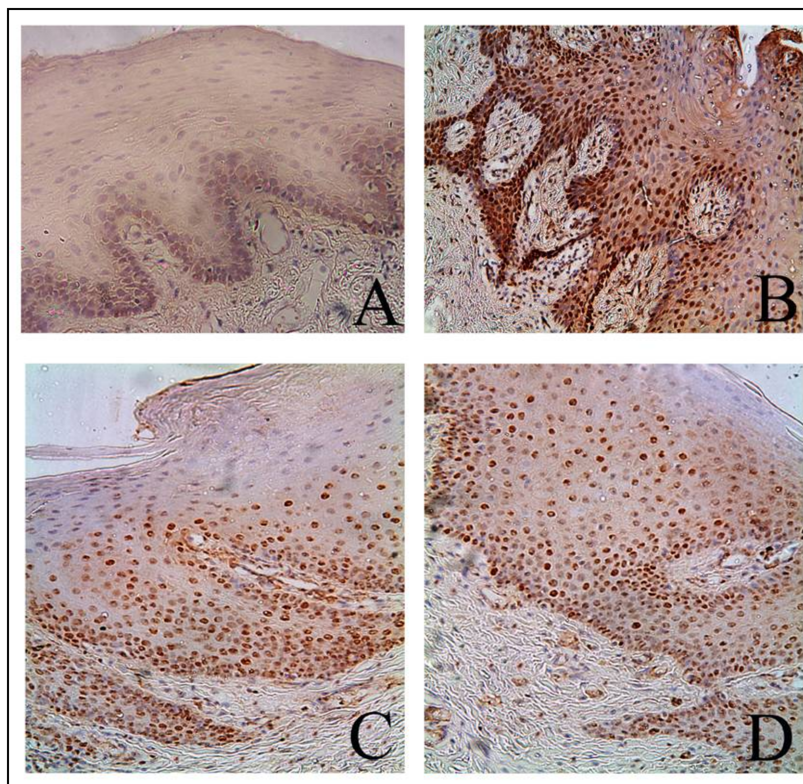


Figure (4): Ki-67 antigen expression in tissue samples of all study groups. A: Ki-67 antigen –positive nuclei observed mainly in the basal layer of control gingival epithelium. B: Deep immune staining of the nuclei of almost all layers of the hyperplastic epithelium in CGO. C: immune stained section of PGO and D: HGF showing comparable patterns of Ki-67 antigen expression in the basal and suprabasal layers of the epithelium. Note that in all sections connective tissue expression of Ki-67 antigen is less marked than epithelium (immunostaining; original magnification x200).

Optical Density

As shown in table (1), Ki-67 was expressed both in the epithelium as well as the corium of the four studied groups with the epithelium showing the significantly higher means of Ki-67 OD than connective tissue in CGO and PGO groups as well as control tissue samples at P values of 0.003, 0.006 and 0.014; respectively. As for HGF group, there was no significant difference in the mean values of Ki-67 OD between epithelium and connective tissue (P=0.747).

As shown in table (2), The OD of Ki-67 in the keratinocytes within CGO group showed the significantly highest mean of Ki-67 level (73.4±4.4) at a P value <0.001. There was no significant difference between HGF and PGO groups; both showed lower values than CGO, while control tissue samples showed the lowest mean of Ki-67 level (33.4±11.8). Similar findings were detected in the corium of the test and control groups with the significantly highest mean of Ki-67 seen in the corium of CGO group (60.4±0.4).

Table (1): The mean, standard deviation (SD) values and results of paired t-test for comparison between Ki-67 OD levels in epithelium and connective tissue within each group

	Control	HGF	PGO	CGO
Epithelium	33.4±11.8	46.7± 2.9	50.9±2.1	73.4±4.4
Connective tissue	16±7.9	45±10.6	30.9± 9	60.4±0.4
P-value	0.014*	0.747	0.006*	0.003*

*: Significant at P ≤ 0.05

Table (2): The mean, standard deviation (SD) of optical density values and results of ANOVA test for comparison between Ki-67 levels in the four groups

	Control	HGF	PGO	CGO	*P*-value
Epithelium	33.4±11.8[c]	46.7±2.9[b]	50.9±2.1[b]	73.4±4.4[a]	<0.001*
Connective tissue	16±7.9[c]	45±10.6[b]	30.9±9[b]	60.4±0.4[a]	<0.001*

*: Significant at P ≤ 0.05, Means with different letters are statistically significantly different according to Tukey's test

4. Discussion

Although it has been thought that drug-induced gingival hyperplasia is not related to tumorigenesis, recent case reports have shown that squamous cell carcinoma may arise in gingival hyperplasia induced by cyclosporine (Varga and Tyldesley, 1991) and phenytoin (McLoughlin et al., 1995) and also as unusual histologic finding with HGF (Gunhan et al., 1995).This possible implications between the pathogenesis of GO and tumorigenesis suggested the aim of the present study which was the examination of the expression of a tumor-related marker, Ki-67, in hyperplastic gingival tissues induced by cyclosporine and phenytoin as well as cases of HGF and compare them to healthy control tissues.

Currently, more than 15 drugs have been identified as possible causative agents of gingival overgrowth. However, phenytoin and cyclosporine are more commonly involved (Lin et al., 2007; Silverstein et al., 1997). One property that is common for these two different classes of drugs is that they directly affect cellular calcium metabolism. Since cellular production of collagenase is modulated by calcium influx, fibroblasts from patients treated with these drugs may produce an inactive form of collagenase, being responsible for an increase in the extracellular matrix (Brunet et al., 1996). Combined with this reduction in extracellular matrix degradation; enhanced proliferation of keratinocytes and/or resident fibroblasts were reported (Saito et al., 1999; Nurmenniemi et al., 2001). These previous findings align with the histopathologic changes and the significant increase in the optical density of Ki-67 staining reported in the current study within the epithelium and corium of both cyclosporine and phenytoin induced GO groups when compared to the control tissue samples.

Nurmenniemi *et al.,* (2001) also reported a significant increase in numbers of Ki-67–labeled cells in patients with CGO compared to healthy controls. **Saito *et al.,* (1999)** found that mean rates of Ki-67–positive cells in PGO were significantly higher as well than healthy tissues. These conflicts with a previous study reported that the acanthosis observed in cyclosporine-treated patients is not caused by enhanced keratinocytes proliferation but rather by prolonged cell life caused by an antiapoptotic effect of cyclosporine (Niimi et al., 1990). **Bulut *et al*.,(2004)** revealed that epithelial proliferation rates may be unchanged in renal transplant patients with CGO when compared to healthy controls.

As for HGF, most attention has been focused on the proliferative potential of mesenchymal fibroblasts. In the present study, both epithelium and connective tissue cells were studied and no significant difference was found in the proliferative potential of epithelium and connective tissue as reflected by Ki-67 optical density which was higher than control tissues and comparable to that of PGO group. In corroboration, a study with 12 different cell lines from patients of a Brazilian HGF family demonstrated a significantly higher proliferation rate of HGF fibroblasts compared to fibroblasts from normal gingivae (de Andrade et al., 2001). On the other hand, **Saygun *et al.,*(2003)** suggested that the underlying mechanism of HGF are not involved with increased cellular proliferation and that lack of proliferation is caused by unfavorable cellular environment lacking key nutrients caused by excessive extracellular matrix deposition.

Findings from the current study confirmed that increased cell division and proliferation may have a role in the pathogenesis of drug-induced gingival overgrowth as well as HGF, however; several factors, including age, genetic predisposition, pharmacokinetic variables and plaque-induced inflammatory changes are believed to be important in the onset and severity of gingival overgrowth. Accordingly, further studies with larger sample size will provide more conclusive data on the possible role of enhanced proliferative activity of cells in the pathogenesis of gingival overgrowth.

Acknowledgements

Histopathologic and immunohistochemical procedures and evaluation were performed at the Department of Oral Pathology, Faculty of Oral and Dental Medicine, Cairo University.

Corresponding author
Eman Y. El-Firt
Departement of Oral Medicine and Periodontology, Faculty of Oral and Dental Medicine, Cairo University, Cairo, Egypt
elfirt@yahoo.com

References

Brunet, L.; Miranda, J.; Farré, M.; Berini, L. and Mendieta, C. (1996): Gingival enlargement induced by drugs. Drug Saf.; 15:219-231.

Buduneli, N.; Buduneli, E.; Cxınar, S.; Lappin, D. and Kinane, D.F.(2007): Immunohistochemical evaluation of Ki-67 expression and apoptosis in cyclosporin A-induced gingival overgrowth. J Periodontol.; 78:282-289.

Bulut, Ö.E. ; Sökmensüer, L.K.; Bulut, S.; Tasman, F. and Müftüoǧlu S.(2004): Immunohistochemical study of cyclosporin-induced gingival overgrowth in renal transplant recipients. J Periodontol.; 75:1655-1662.

Coletta, R.D. and Graner, E.(2006): Hereditary Gingival Fibromatosis: A Systematic Review. J Periodontol.; 77:753-764.

de Andrade, C.R.; Cotrin, P.; Graner, E.; Almeida, O.P.; Sauk, J.J. and Coletta, R.D.(2001): Transforming growth factor beta-1autocrine stimulation regulates fibroblast proliferation in hereditary gingival fibromatosis. J Periodontol.; 72:1726-1733.

Gunhan, O.; Gardner, D.G.; Bostanci, H. and Gunhan, M.(1995): Familial gingival fibromatosis with unusual histologic findings. J Periodontol.; 66:1008-1011.

Lin, K.; Guilhoto, L.M.F. and Yacubian, E.M.T.(2007): Drug-induced gingival enlargement – Part II. Antiepileptic Drugs: not only phenytoin is involved. J Epilepsy Clin Neurophysiol.; 13:83-88.

Mariani, G.; Calastrini, C.; Carinci, F.; Marzola, R. and Calura, G.(1993): Ultrastructural features of cyclosporin A-induced gingival hyperplasia. J Periodontol.; 64:1092-1097.

Martelli-Junior, H.; Lemos, D.P.; Silva, C.O.; Graner, E. and Coletta, R.D.(2005): Hereditary gingival fibromatosis: Report of a five-generation family with cellular proliferation analysis. J Periodontol.; 76:2299-2305.

Martelli-Junior, H.B.G.; Graner, E.; Bozzo, L. and Coletta, R.D. (2000): Microscopic and proliferative comparison of gingival fibroblasts from patients with normal gingiva and with hereditary gingival fibromatosis. Pesqui Odontol Bras.; 14:123-129.

McLoughlin, P.; Newman, L. and Brown, A. (1995):Oral squamous cell carcinoma arising in phenytoin-induced hyperplasia. Br Dent J.; 178:183-4.

Niimi, A.; Tohnai, I.; Kaneda, T.; Takeuchi, M. and Nagura, M. (1990): Immunohistochemical analysis of effect of cyclosporine-A on gingival epithelium. J Oral Pathol Med.; 19:397-403.

Nurmenniemi, P.K.; Pernu, H.E. and Knuuttila, M.L.E.(2001): Mitotic activity of keratinocytes in nifedipine- and immunosuppressive medication-induced gingival overgrowth. J Periodontol.; 72:167-173.

Rateitschak-Plüss, E.M.; Hefti, A.; Lörtscher, R. and Theil G.(1983): Initial observation that cyclosporin-A induces gingival enlargement in man. J Clin Periodontol.; 10:237-246.

Saito, K.; Mori, S.; Tanda, N. and Sakamoto, S.(1999): Expression of p53 protein and Ki-67 antigen in gingival hyperplasia induced by nifedipine and phenytoin. J Periodontol.; 70:581-586.

Saygun, I.; Ozdemir, A.; Gunhan, O.; Aydintug, Y.S. and Karslioglu, Y.(2003): Hereditary gingival fibromatosis and expression of Ki-67 antigen: A case report. J Periodontol.; 74:873-878.

Schlüter, C.; Duchrow, M.; Wohlenberg, C.; et al. (1993): The cell proliferation-associated antigen of antibody Ki-67: a very large, ubiquitous nuclear protein with numerous repeated elements, representing a new kind of cell cycle-maintaining proteins. J. Cell Biol.; 123:513-22.

Silverstein, L.H.; Garnick, J.J.; Szikman, M. and Singh, B.(1997): Medication induced gingival enlargement: a clinical review. Gen Dent.;45:371-376.

Singer, S.L.; Goldblatt, J.; Hallam, L.A. and Winters, J.C.(1993): Hereditary gingival fibromatosis with a recessive mode of inheritance. Case reports. Aust Dent J.; 38:427-432.

Vardar, S.; Baylas, H.; Zihniog˘lu, F.; Buduneli, N.; Emingil, G. and Atilla, G.(2005): Gingival tissue proteoglycan and chondroitin sulphate levels in cyclosporin A-induced gingival overgrowth and the effects of initial periodontal treatment. J Clin Periodontol.; 32:634-639.

Varga, E. and Tyldesley, W.R.(1991): Carcinoma arising in cyclosporin-induced gingival hyperplasia. Br Dent J.; 171:26-27.

Zoeller, J.; Flentje, M.; Sinn, P. and Born, I.A.(1994): Evaluation of AgNOR and Ki-67 antigen as cell kinetic parameters in oral dysplasias and carcinomas. Anal Cell Pathol.; 7:77-88.

10/24/2011

View publication stats