[Downloaded free from http://www.njcponline.com on Saturday, April 4, 2020, IP: 46.196.90.72]

Original Article

The Gingival Crevicular Fluid Levels of Growth Factors in Patients with Amlodipine-Induced Gingival Overgrowth: A Pilot Study

KN Kose, S Yilmaz[1], U Noyan[2], B Kuru[3], HS Yildirim, OB Agrali, HO Ozener, L Kuru

Department of Periodontology, Faculty of Dentistry, Marmara University, [1]Private Practice, 34437, [2]Department of Oral and Dental Healthcare, Acıbadem Hospitals, [3]Department of Periodontology, Faculty of Dentistry, Yeditepe University, Istanbul, Turkey

Received: 03-Oct-2019;
Revision: 09-Nov-2019;
Accepted: 16-Dec-2019;
Published: 04-Apr-2020

Abstract

Background: Amlodipine, calcium channel blocker (CCB), is used in the management of cardiovascular diseases which causes gingival overgrowth (GO). The growth factors may have a role in the pathogenesis of amlodipine-induced GO. **Objectives:** This pilot study aimed to investigate the growth factors including transforming growth factor-β1 (TGF-β1), platelet-derived growth factor-BB (PDGF-BB), and basic fibroblast growth factor (bFGF) in gingival crevicular fluid (GCF) of patients with amlodipine-induced GO and compare with of healthy subjects. **Methods:** GCF samples were collected from 56 sites presenting GO (GO + group) and from 38 sites not presenting GO (GO- group) of 5 patients using amlodipine for more than one year, and from 45 sites (control group) of 5 healthy subjects. The levels of TGF-β1, PDGF-BB, and bFGF were determined by using ELISA kits. **Results:** The mean concentration of TGF-β1 in GCF samples of GO + group (9.50 ± 7.30 ng/ml) was higher than both GO- group (2.07 ± 0.50 ng/ml) and control group (2.74 ± 1.01 ng/ml) ($P = 0.014$). No significant difference was found among the groups in the GCF levels of PDGF-BB ($P = 0.767$). bFGF was detected in only 33% of the sites from patients. **Conclusion:** These preliminary results suggest that TGF-β1 may play a crucial role in the pathogenesis of amlodipine-induced GO.

Keywords: *Amlodipine, Gingival overgrowth, growth factors*

Introduction

Hypertension, as a "silent killer," taking an effective role in stroke, dementia, ischemic heart disease, vision loss, and heart and kidney failures, is a common disease all over the world and its estimated prevalence in 2010 was 1.39 billion persons, representing 31% of all adults.[1] This disease is a cause of 7.5 million deaths per year, 12.8% of the total death in all over the world. While the overall prevalence of hypertension was highest in Africa, around 46% for both sexes combined, it was the lowest in America, around 35% for both sexes.[2]

Hypertension is widely managed by the help of antihypertensive drugs including calcium channel blockers (CCBs) in current medical practice.[1] These drugs block the slow calcium channels in cell membranes, regulate the Ca^{+2} concentration which is involved in the initiation of both smooth and cardiac muscle contraction and in the propagation of the cardiac impulse. It is well documented that CCBs have systemic side-effects including headache, facial flushing, dizziness, edema, nausea, and dyspepsia which are mainly attributable to their vasodilative effect as well as the local side-effect, gingival overgrowth (GO).[3-6]

GO, a common adverse side-effect of CCBs, with a varying prevalence rate from 21% to 93%,[4] presents remarkable enlarged gingiva, which covers almost half of the teeth crown and exhibits a pebbly surface. In a study by Andrew *et al.*,[7] the prevalence of GO in patients using CCBs was 31.5% in Kenyan population, which was statistically higher than the hypertension patients using

Access this article online

Quick Response Code:

Website: www.njcponline.com

DOI: 10.4103/njcp.njcp_532_19

Address for correspondence: Dr. KN Kose, Department of Periodontology, Faculty of Dentistry, Marmara University, Marmara University Basibuyuk Medical Campus, Basibuyuk, Maltepe, 34854 Turkey.
E-mail: kemkose@superonline.com.tr

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

How to cite this article: Kose KN, Yilmaz S, Noyan U, Kuru B, Yildirim HS, Agrali OB, *et al.* The gingival crevicular fluid levels of growth factors in patients with amlodipine-induced gingival overgrowth: A pilot study. Niger J Clin Pract 2020;23:561-7.

[Downloaded free from http://www.njcponline.com on Saturday, April 4, 2020, IP: 46.196.90.72]

non-CCBs (7%). It occurs within one month starting from the drug use and reaches its maximum level in 12 to 18 months. This tissue enlargement extends facially, lingually, and coronally and may interfere with oral hygiene practice, mastication, and speech. Interestingly, GO may not necessarily exhibit around all teeth in the mouth and may be exacerbated by the accumulation of microbial dental plaque.[6,7]

CCBs-induced GO was first reported by Ramon *et al*. for nifedipine in 1984.[8] Since then, numerous reports have been published regarding nifedipine-induced GO and its pathogenesis.[4,9-11] On the other hand, there is far less evidence present in the literature regarding amlodipine-induced GO, most of it has come from the case reports[10,11] and only a few from prevalence studies.[4-6,9,12-14] Amlodipine is a relatively new, third-generation CCB having less severe side-effects compared to other CCBs.[15] Amlodipine- associated GO was first reported by Ellis in 1993.[12]

While the precise mechanism of drug-induced GO is not yet fully understood, it is affected by age, genetic predisposition, pharmacokinetic variables, alterations in gingival connective tissue homeostasis, and locally acting growth factors and cytokines.[11] Over the last couple of decades, it has been shown immunohistochemically that the expression of several growth factors are elevated in overgrown gingival tissues and this may contribute to the pathogenesis of drug-induced GO.[16-18] The activation of transforming growth factor-β1 (TGF-β1) and connective tissue growth factor (CTGF) partnership was demonstrated in the fragments of gingival tissues collected from patients presenting GO after prolonged administration of nifedipine and amlodipine.[19] Conversely, microarray analyses suggested a significant downregulation in TGF-β1 levels in the CCBs-induced GO tissue samples.[20] It is clear that there is a need for further research in the era of amlodipine-induced GO and the mechanisms underneath.

Gingival crevicular fluid (GCF) represents a rich collection of cellular and biochemical mediators which can be utilized as potential diagnostic or prognostic identification tool of the periodontium in health and disease status.[16] Seymour *et al*.[14] demonstrated the sequestration of amlodipine into the GCF of patients with amlodipine-induced GO and its significance in relation to this side effect. Furthermore, increased TGF-β1 levels detected in the GCF samples of drug-induced GO patients have been reported to be a possible indicator for GO.[21] However, to our knowledge, there are no published studies investigating the growth factors levels in the GCF samples obtained from patients with amlodipine-induced GO, so far.

TGF-β is an important member of a superfamily of growth regulatory proteins which have a wide variety of biological effects including cell proliferation, control of development, and tissue recycling and repair. TGF-β may stimulate or inhibit proliferation, block or affect entry into differentiation pathway, and promote or prevent cell migration depending on the tissue context.[22] On the other hand, TGF-β has been shown to be stimulatory for extracellular matrix formation while inhibitory for proteolytic matrix degradation.[23] It is thought that the increased anabolism and decreased catabolism of gingival tissue is mainly responsible for drug-induced GO.[24] Saito *et al*.[17] demonstrated significant immunostaining for TGF-β and its receptors in overgrown gingival specimens and concluded that TGF-β could be one of the growth factors important in drug-induced GO. Recent studies have shown that both TGF-β and CTGF, a reliable marker for tissue fibrosis, are important mediators in drug-induced GO.[18,25] Platelet-derived growth factors (PDGF) are potent mitogen and chemotactic for the cells of mesenchymal origin and are considered to be one of the principle local regulatory hormones of connective tissues.[26,27] Iacopino *et al*.[28] indicated that the clinical presentation of drug-induced overgrown gingival tissues is associated with specific macrophage phenotypes expressing high levels of essential polypeptide growth factor, PDGF-BB. One of the most important angiogenic factors is the basic fibroblast growth factor (bFGF) which plays a crucial role in the formation of granulation tissue and tissue fibrosis.[29] It was shown to stimulate the proliferation of a variety of cells including fibroblasts, keratinocytes, and osteoblasts;[29] in addition, increased bFGF levels were found in the serum of patients with drug-induced GO.[29] Moreover, Saito *et al*.[17] showed an increased synthesis of TGF-β and bFGF in drug-induced GO tissues immunohistochemically. However, in the light of current evidence, the possible role of bFGF particularly in the amlodipine-induced GO has not been clearly identified yet.

In the present pilot study, we aimed to determine the levels of mesenchymal growth factors TGF-β1, PDGF-BB, and bFGF in GCF samples of patients with amlodipine-induced GO in order to be able to predict the possible relation between them and gingival enlargement.

Subjects and Methods

Subject population

Between July 2018 and December 2018, five adult patients (2 males) with mean age of 53.40 ± 7.43 years visited to our department using amlodipine for at

[Downloaded free from http://www.njcponline.com on Saturday, April 4, 2020, IP: 46.196.90.72]

least 1 year for their cardiovascular problems and exhibiting moderate to severe GO were included into the study. Five systemically and periodontally healthy individuals (2 males) with 53.0 ± 6.89 years of age were included in the study as a control group who had no sign of gingival inflammation and exhibited no GO. All subjects were informed about the nature and purpose of the study, and their consents were obtained. The Research Ethical Committee of Marmara University Faculty of Medicine approved the study protocol with the reference number 09.2018.521. Exclusion criteria were pediatric patients, pregnancy, use of any other medication, and patients with any other systemic disease.

Clinical measurements

Clinical assessments including full mouth and site-specific plaque index (PI),[30] gingival index (GI),[31] bleeding on probing (BOP), and probing depth (PD) were recorded following a session including complete oral hygiene instructions prior to any periodontal intervention. In addition, upper and lower full mouth impressions were taken from each subject to assess their hyperplasia scores (HS) using the method described by Seymour *et al*.[14] The sites with GO presenting more than 2 degrees of HS were selected for GCF sample collection for the GO+ group.

GCF collection

GCF samples were collected from 56 sites presenting GO (GO+ group), from 38 sites not presenting GO (GO- group) of the same 5 patients, and from 45 sites of healthy subjects (control group) by using paper strips (Periopaper; Proflow Inc., Amityville, NY, USA). Briefly, after isolation of selected sites with cotton rolls, teeth surfaces were cleaned from saliva and supragingival plaque by using a high-power suction tip and a periodontal probe, respectively, to prevent saliva and/or plaque contamination. Paper strips were placed at the entrance of the gingival crevice and left for 30 sec. The strips with any visible signs of saliva or blood contamination were discarded. The volumes of GCF samples were determined by Periotron 8000 (Oraflow, Inc., Smithtown, NY, USA). The samples were kept at –40°C until their use.

Elution of GCF and determination of growth factors

The GCF-blotted paper strips were allowed to thaw at room temperature for 30 min. To elude the GCF samples from the paper strips, 50 µl of phosphate-buffered saline (PBS) was added to each tube containing the strips and centrifuged at 11000 rpm for 15 min.[32] This step was repeated one more time and the total volume of 100 µl was stored at 4°C for up to 24 h prior to use. Each GCF sample was used for the analysis of only one growth factor.

The level of each growth factor including TGF-β, PDGF-BB, and bFGF was analyzed by using commercially available Sandwich ELISA kits (Quantikine DB 100, Quantikine DBB 00, Quantikine DFB 50, R and D Systems; Minneapolis, MN, USA) according to the manufacturer's directions. In the case of TGF-β1, acidification and neutralization procedures were performed prior to the experiment to activate the latent growth factor.[26,32] Briefly, an aliquot of 40 µl of each sample was transferred to a polypropylene tube and the latent TGF-β1 was activated by adding 40 µl of 2.5 M acetic acid/10 M urea. After mixing and incubation at room temperature for 10 min, the acidified sample was neutralized by adding 40 µl of 2.7M NaOH/1M HEPES buffer.

An aliquot of 200 µl of known concentrations (0, 31.2, 62.5, 125, 250, 500, 1000, and 2000 pg/ml) of the activated recombinant human TGF-β1 standard (R and D Systems) and 200 µl of GCF sample were added to each well of the ELISA plate which had been pre-coated with a recombinant human soluble receptor II which binds specifically to human TGF-β1. The plate was incubated at room temperature for 3 h. After washing 3 times, 200 µl of the detecting antibody (horseradish peroxidase-conjugated polyclonal antibody against TGF-β1) was added and the plate was incubated at room temperature for 1.5 h. After washing steps, 200 µl of the substrate solution (tetramethylbenzidine containing H_2O_2) was added into each well and incubated at room temperature for 20 min. Following the addition of 50 µl of 2 M H_2SO_4 to stop the reaction, the absorbance was measured at 450 nm (A_{450}) using a spectrophotometer (Microplate Reader Elx 800, Biokit, Barcelona, Spain). Similar procedures were repeated for PDGF-BB and bFGF according to the manufacturer's instructions except the acidification procedure.

Statistical analysis

All statistical analyses were performed by using a commercially available statistical software, SPSS version 15 (SPSS, Inc., Chicago, IL, USA). Comparisons of the data among the groups were performed by using Kruskal–Wallis test with post-hoc Bonferroni correction. In the presence of a significant difference, Mann–Whitney *U* test was used for the paired-comparison of any two groups. Statistical significance was set at $P < 0.05$ level.

Results

There were no differences between the test and control groups regarding age and sex ($P = 0.000$). Since the GO+ and GO- data were assessed in the same individuals, no significant differences are detected in

[Downloaded free from http://www.njcponline.com on Saturday, April 4, 2020, IP: 46.196.90.72]

the test groups regarding daily dosages and duration of usage of amlodipine, as expected [Table 1].

The intraoral clinical appearance of a patient exhibiting lobulated and fibrotic GO with a pebbly surface initiated at the interdental papillae which is typical for drug-induced GO is presented in Figure 1.

The mean clinical measurements of the sites which were sampled for TGF-β1 are summarized in Table 2. Clinical assessment of selected sites revealed that all parameters of the GO+ group were significantly higher than those of the control group (P = 0.000). Although the PI, GI, PD, and HS values of the GO+ sites were higher than the GO- sites, a statistically significant difference was observed only for HS (P = 0.000).

The concentration of TGF-β1 in GCF from GO+ sites (9.5 ± 7.3 ng/ml) was significantly higher than that from both GO- (2.07 ± 0.50 ng/ml) and control sites (2.74 ± 1.01 ng/ml) (P = 0.014) [Table 2]. Regarding the TGF-β1 amount, only the difference between the GO+ and control group was statistically significant (P = 0.005). Although TGF-β1 amount of the GO + group was higher than the GO- group, the difference did not reach the level of significance (P = 0.067).

The mean values of clinical measurements, the concentration, and the amount of PDGF-BB at the selected sites exhibited the highest scores in GO+ sites [Table 3]. While all clinical parameters of the GO+ sites were higher than those of the control sites (P = 0.000), only the HS of the GO+ sites was higher than the other two groups. With regard to the concentration and amount of PDGF-BB in the GCF, the differences among the 3 groups were not statistically significant (P = 0.767, P = 0.605).

On the other hand, bFGF was detected in only 33% of the sites from patients, while in 40% of the sites from healthy subjects. Therefore, there was not enough data to perform any statistical evaluation for the values related to the bFGF levels. The clinical measurements at the selected sites for the bFGF experiments were similar

Table 1: Dose and duration of amlodipine usage in the test group patients

Subjects	Dose of amlodipine (mg/day)	Duration of usage (years)
No. 1	10	3
No. 2	10	5
No. 3	20	4
No. 4	10	3
No. 5	10	1



Figure 1: A male patient using amlodipine for the last 5 years. The intraoral clinical appearance exhibits lobulated and fibrotic GO with a pebbly surface initiated at the interdental papillae, which is typical for drug-induced GO. Overgrown tissue covers almost half of the crown of some teeth. Plaque accumulation and calculus were also evident

Table 2: Comparisons of clinical measurements, concentration, and amount of TGF-β1 at the selected sites

	GO+ group n=56 sites	GO- group n=38 sites	Control n=45 sites	P¥
Plaque index	2.00±0.60***	1.66±1.32	0.16±0.40	0.000
Gingival index	2.21±0.65***	2.00±1.00**	0.13±0.29	0.000
Probing depth (mm)	5.00±1.10***	3.50±1.30**	1.33±0.44	0.000
Hyperplasia score	3.57±1.01***###	0.00±0.00	0.00±0.00	0.000
Concentration of TGF-β1 (ng/ml)	9.50±7.3*#	2.07±0.50	2.74±1.01	0.014
Total amount of TGF-β1 (pg)	3.53±2.64**	1.86±0.42	1.63±1.30	0.005
GCF (µl)	0.58±0.50	0.46±0.10	0.36±0.10	0.067

¥Kruskal-Wallis Test, P<0.05. *Mann-Whitney-U Test with post-hoc Bonferroni Correction. *P<0.05, **P<0.01, ***P<0.001 compared with control. #Mann-Whitney-U Test with post-hoc Bonferroni Correction. #P<0.05, ###P<0.001 compared with the GO– group

Table 3: Comparisons of clinical measurements, concentrations, and amount of PDGF-BB at the selected sites

	GO+ group n=56 sites	GO- group n=38 sites	Control n=45 sites	P¥
PI	1.89±0.69***	1.54±0.45	0.12±0.22	0.0000
GI	2.36±0.77***	1.92±0.66	0.13±0.29	0.0021
PD	4.63±0.95***	3.25±0.81	1.16±0.32	0.0000
Hyperplasia score	3.68±1.20***###	0.00±0.00	0.00±0.00	0.0000
PDGF-BB concentration (ng/ml)	3.48±2.60	3.54±1.93	3.88±1.19	0.767
Total amount of PDGF-BB (pg)	1.90±1.43	1.73±1.21	1.01±0.38	0.6054
GCF (µl)	0.62±0.30**	0.52±0.21	0.35±0.10	0.0299

¥Kruskal-Wallis Test, P<0.05. *Mann-Whitney-U Test with post-hoc Bonferroni Correction. **P<0.01, ***P<0.001 compared with control. #Mann-Whitney-U Test with post-hoc Bonferroni Correction. #P<0.05, ##P<0.01, ###P<0.001 compared with GO–

[Downloaded free from http://www.njcponline.com on Saturday, April 4, 2020, IP: 46.196.90.72]

to the values determined for the other two growth factors (data not shown).

Discussion

Understanding the importance of the growth factors and cytokines in both normal and pathological cellular events has enlightened the unknown mechanisms behind many diseases including cancer, neurological disorders, AIDS, and fibrotic disorders. The underlying pathogenesis of drug-induced GO, as a fibrotic disorder, is not completely clarified. Several possible mechanisms have been suggested including the role of matrix metalloproteinases, fibroblast genetics, and growth factors/cytokines.[25] In the present pilot study, we aimed to probe one of the mechanisms involving in the pathogenesis of drug-induced GO, by evaluating collectively GCF levels of growth regulatory molecules, namely, TGF-β1, PDGF-BB, and bFGF in relation to amlodipine-induced GO.

Compared with GO- and control sites, the results verified significantly higher expression of TGF-β1 in the GCF samples obtained from the GO+ sites of amlodipine using patients. Provided evidence may suggest that TGF-β1 is a crucial growth factor playing a role in the manifestation of amlodipine-induced GO. To the best of our knowledge, this is the first study to evaluate GCF levels of TGF-β1, PDGF-BB, and bFGF in order to determine the relationship between amlodipine-induced GO.

Amlodipine was detected in GCF and its significant sequestration was followed in patients exhibiting GO.[12] Linden *et al*.[33] demonstrated that TGF-β1 gene polymorphism can affect the severity of GO associated with concomitant use of cyclosporine A and a CCB. In our study, there is a wide variation among the TGF-β1 level of the GO+ samples. This could also be explained by the presence of TGF-β1 gene polymorphism. Saito *et al*.[17] showed a higher level of TGF-β1 in the tissues of patients with GO but the basic mechanism of such presence and its relation to the local gingival changes stayed uncertain. Furthermore, the presence of TGF-β1 has been demonstrated in the GO+ tissue fragments of patients using amlodipine,[19] whereas microarray analysis did not confirm this existence.[17] Our results were in line with the evidence proposing the upregulation of TGF-β1 in the case of amlodipine usage. This finding supports the assumptions about the pathogenesis of drug-induced GO of which that higher level of TGF-β stimulates extracellular matrix deposition both directly by promoting matrix synthesis[26] and indirectly by inhibiting the enzymatic degradation of matrix molecules,[24] and as a result, drug-induced GO takes place in patients using amlodipine. Chantry *et al*.[34] presented the inhibitory effect of TGF-β on the production proinflammatory cytokines such as IL-1. In addition, Turner *et al*.[35] demonstrated that TGF-β1 induced the production of IL-1 receptor antagonist. It is well known that IL-1 is a strong catabolic cytokine playing an indispensable role in tissue turnover and induction of tissue metalloproteinase. Perhaps, the presence of a high local concentration of TGF-β1 may indirectly inhibit the extracellular matrix degradation causing accumulation of collagen resulting in drug-induced GO.

Subjects with GO usually have more plaque formation than those without GO. Our subjects did not differ in their plaque scores for the GO+ and GO- groups. This might be due to the screening time because we, first, gave the oral hygiene instructions and at the second visit, measured the plaque scores. Therefore, our experimental method not only decreased the influence of local factors on clinical parameters and the growth factor levels in all study groups but also facilitated the sample collection by decreasing the possible plaque contamination and bleeding.

In the presented study, we did not see any differences in the concentrations and the amounts of PDGF-BB among all three groups. Even though the amount of PDGF-BB was higher in both GO+ and GO- groups than the control group, these differences were not statistically significant. Iacopino *et al*.[28] demonstrated that the clinical presentation of hyperplastic gingival tissues from patients using phenytoin is associated with specific macrophage phenotypes which express the essential mesenchymal growth factor PDGF-BB. Since there were no differences in the PI and GI scores of sites between GO+ and GO- groups in our study, the effect of local inflammation could be excluded from the factors effecting the amlodipine-induced GO and so the effect of macrophage-originated PDGF-BB. By giving the oral hygiene instructions before the sample collection, we were able to prevent the effect of local inflammation-induced PDGF expression. Similar PDGF levels among our three groups revealed that this growth factor may not have a direct effect on this pathology and indeed, we may assume that complex interactions between various inflammatory mediators and growth factors are most likely involved in the pathogenesis of amlodipine-induced GO agreeing with the recent literature.[19,25]

Keeping in mind, this is a pioneering work, it is necessary to say that the most important limitation of the current study is the number of patients recruited to the observation. However, gaining GCF samples

[Downloaded free from http://www.njcponline.com on Saturday, April 4, 2020, IP: 46.196.90.72]

from a number of areas of the same patients' GO+ and GO- sites eliminates the patient-related factors affecting the obtained results. The revealed a significantly different amount of various growth factors in GCF from patients with GO may be essential in terms of contribution to the current literature.

In conclusion, this pilot study indicates that TGF-β1 might be a key growth factor in the amlodipine-induced GO.

Financial support and sponsorship

This work was supported by Yeditepe University Research Fund.

Conflicts of interest

There are no conflicts of interest.

References

1. Bloch. MJ. Worldwide prevalence of hypertension exceeds 1.3 billion. J Am Soc Hypertens 2016;10:753-4.
2. Global Health Observatory Data. Available from: http://www.who.int/gho/ncd/risk_factors/blood_pressure_prevalence_text/en/.
3. Gaur S, Agnihotri R. Is dental plaque the only etiological factor in Amlodipine induced gingival overgrowth? A systematic review of evidence. J Clin Exp Dent 2018;10:e610-9.
4. Ellis JS, Seymour RA, Steele JG, Robertson P, Butler TJ, Thomason JM. Prevalence of gingival overgrowth induced by calcium channel blockers: A community-based study. J Periodontol 1999;70:63-7.
5. Umeizudike KA, Olawuyi AB, Umeizudike TI, Olusegun-Joseph AD, Bello BT. Effect of calcium channel blockers on gingival tissues in hypertensive patients in Lagos, Nigeria: A pilot study. Contemp Clin Dent 2017;8:565-70.
6. Vidal F, de Souza RC, Ferreira DC, Fischer RG, Goncalves LS. Influence of 3 calcium channel blockers on gingival overgrowth in a population of severe refractory hypertensive patients. J Periodontal Res 2018;53:721-6.
7. Andrew W, Evelyn W, Francis M, Mark J, Mark C. Pattern of gingival overgrowth among patients on antihypertensive pharmacotherapy at a Nairobi Hospital in Kenya. Open J Stomatol 2014;4:169-73.
8. Ramon Y, Behar S, Kishon Y, Engelberg IS. Gingival hyperplasia caused by nifedipine--a preliminary report. Int J Cardiol 1984;5:195-206.
9. James JA, Marley JJ, Jamal S, Campbell BA, Short CD, Johnson RW, *et al*. The calcium channel blocker used with cyclosporin has an effect on gingival overgrowth. J Clin Periodontol 2000;27:109-15.
10. Aldemir NM, Begenik H, Emre H, Erdur FM, Soyoral Y. Amlodipine-induced gingival hyperplasia in chronic renal failure: A case report. Afr Health Sci 2012;12:576-8.
11. Seymour RA, Thomason JM, Ellis JS. The pathogenesis of drug-induced gingival overgrowth. J Clin Periodontol 1996;23:165-75.
12. Ellis JS, Seymour RA, Thomason JM, Monkman SC, Idle JR. Gingival sequestration of amlodipine and amlodipine-induced gingival overgrowth. Lancet 1993;341:1102-3.
13. Jorgensen MG. Prevalence of amlodipine-related gingival hyperplasia. J Periodontol 1997;68:676-8.
14. Seymour RA, Ellis JS, Thomason JM, Monkman S, Idle JR. Amlodipine-induced gingival overgrowth. J Clin Periodontol 1994;21:281-3.
15. Hosie J, Bremner AD, Fell PJ, James GV, Saul PA, Taylor SH. Side effects of dihydropyridine therapy: Comparison of amlodipine and nifedipine retard. J Cardiovasc Pharmacol 1993;22(Suppl A):S9-12.
16. Embery G, Waddington R. Gingival crevicular fluid: Biomarkers of periodontal tissue activity. Adv Dental Res. 1994;8:329-36.
17. Saito K, Mori S, Iwakura M, Sakamoto S. Immunohistochemical localization of transforming growth factor beta, basic fibroblast growth factor and heparan sulphate glycosaminoglycan in gingival hyperplasia induced by nifedipine and phenytoin. J Periodontal Res 1996;31:545-55.
18. Uzel MI, Kantarci A, Hong HH, Uygur C, Sheff MC, Firatli E, *et al*. Connective tissue growth factor in drug-induced gingival overgrowth. J Periodontol 2001;72:921-31.
19. Pisoschi CG, Stanciulescu CE, Andrei AM, Berbecaru-Iovan A, Munteanu C, Popescu F, *et al*. Role of transforming growth factor beta-connective tissue growth factor pathway in dihydropyridine calcium channel blockers-induced gingival overgrowth. Rom J Morphol Embryol 2014;55:285-90.
20. Shimizu T, Kubota T, Nakasone N, Abe D, Morozumi T, Yoshie H. Microarray and quantitative RT-PCR analyses in calcium-channel blockers induced gingival overgrowth tissues of periodontitis patients. Arch Oral Biol 2011;56:277-84.
21. Kuru L, Yılmaz S, Kuru B, Köse KN, Noyan Ü. Expression of growth factors in the gingival crevice fluid of patients with phenytoin-induced gingival enlargement. Arch Oral Biol 2004;49:945-50.
22. Barnard JA, Lyons RM, Moses HL. The cell biology of transforming growth factor beta. Biochim Biophys Acta 1990;1032:79-87.
23. Lu H, Mackenzie IC, Levine AE. Transforming growth factor-beta response and expression in junctional and oral gingival epithelial cells. J Periodontal Res 1997;32:682-91.
24. Hallmon WW, Rossmann JA. The role of drugs in the pathogenesis of gingival overgrowth. A collective review of current concepts. Periodontology 2000 1999;21:176-96.
25. Trackman PC, Kantarci A. Molecular and clinical aspects of drug-induced gingival overgrowth. J Dent Res 2015;94:540-6.
26. Graves DT, Kang YM, Kose KN. Growth factors in periodontal regeneration. Compendium 1994:S672-7; quiz S714-677.
27. Kose KN, Xie JF, Carnes DL, Graves DT. Pro-inflammatory cytokines downregulate platelet derived growth factor-alpha receptor gene expression in human osteoblastic cells. J Cell Physiol 1996;166:188-97.
28. Iacopino AM, Doxey D, Cutler CW, Nares S, Stoever K, Fojt J, *et al*. Phenytoin and cyclosporine A specifically regulate macrophage phenotype and expression of platelet-derived growth factor and interleukin-1 *in vitro* and *in vivo*: Possible molecular mechanism of drug-induced gingival hyperplasia. J Periodontol 1997;68:73-83.
29. Sasaki T, Maita E. Increased bFGF level in the serum of patients with phenytoin-induced gingival overgrowth. J Clin Periodontol 1998;25:42-7.
30. Silness J, Loe H. Periodontal disease in pregnancy. II. Correlation between oral hygiene and periodontal condtion. Acta Odontol Scand 1964;22:121-35.
31. Loe H, Silness J. Periodontal disease in pregnancy. I. Prevalence and severity. Acta Odontol Scand 1963;21:533-51.
32. Agrali OB, Kuru BE, Yarat A, Kuru L. Evaluation of gingival crevicular fluid transforming growth factor-beta1 level after treatment of intrabony periodontal defects with enamel matrix

[Downloaded free from http://www.njcponline.com on Saturday, April 4, 2020, IP: 46.196.90.72]

derivatives and autogenous bone graft: A randomized controlled clinical trial. Niger J Clin Pract 2016;19:535-43.

33. Linden GJ, Haworth SE, Maxwell AP, Poulton KV, Dyer PA, Middleton D, *et al*. The influence of transforming growth factor-beta1 gene polymorphisms on the severity of gingival overgrowth associated with concomitant use of cyclosporin A and a calcium channel blocker. J Periodontol 2001;72:808-14.
34. Chantry D, Turner M, Abney E, Feldmann M. Modulation of cytokine production by transforming growth factor-beta. J Immunol 1989;142:4295-300.
35. Turner M, Chantry D, Katsikis P, Berger A, Brennan FM, Feldmann M. Induction of the interleukin 1 receptor antagonist protein by transforming growth factor-beta. Eur J Immunol 1991;21:1635-9.