Immunoexpression of interleukin-6 in drug-induced gingival overgrowth patients

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Abstract

Background: To analyze the role of proinflammatory cytokines in drug-induced gingival enlargement in Indian population. **Aim:** To evaluate for the presence of interleukin-6 (IL-6) in drug-induced gingival enlargement and to compare it with healthy control in the absence of enlargement. **Materials and Methods:** Thirty-five patients selected for the study and divided into control group (10) and study group (25) consisting of phenytoin (10); cyclosporin (10) and nifedipine (5) induced gingival enlargement. Gingival overgrowth index of Seymour was used to assess overgrowth and allot groups. Under LA, incisional biopsy done, tissue sample fixed in 10% formalin and immunohistochemically evaluated for the presence of IL-6 using LAB-SA method, Labeled-Streptavidin-Biotin Method (LAB-SA kit from Zymed- 2nd generation LAB-SA detection system, Zymed Laboratories, CA). The results of immunohistochemistry were statistically analyzed using Kruskaal–Wallis and Mann–Whitney test. **Results:** The data obtained from immunohistochemistry assessment shows that drug-induced gingival overgrowth (DIGO) samples express more IL-6 than control group and cyclosporin expresses more IL-6 followed by phenytoin and nifedipine. **Conclusion:** Increased IL-6 expression was noticed in all three DIGO groups in comparison with control group. Among the study group, cyclosporin expressed maximum IL-6 expression followed by phenytoin and nifedipine.

Keywords: Cytokines, drug-induced gingival overgrowth, fibrosis, immunohistochemistry, interleukin-6, tissue inhibitor of metalloproteinase

Introduction

Many systemic diseases require long-term medical management with drugs regardless of any adverse effects and manifestations. Gingival overgrowth is one of the known side effects of a few drugs administered over a prolonged period. The most common drugs causing this condition are immunosuppressants like cyclosporin, anticonvulsants like phenytoin and calcium channel blockers like nifedipine. Although the pharmacologic effects of these drugs differ widely, they all seem to affect the gingiva similarly, causing gingival overgrowth.[1]

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Several studies have reported that local, genetic, and systemic factors may contribute to the development of drug-induced gingival overgrowth (DIGO).[2,3] Studies regarding the pathogenesis of DIGO have revealed considerable information on connective tissue metabolism involved in DIGO.[4,5] Many such studies have shown that elevated levels of various cytokines like interleukin-6 (IL-6), IL-1β, transforming growth factor-β (TGF-β); fibroblast growth factor and platelet-derived growth factor (PDGF) are present in DIGO and that these cytokines play a significant role in the pathogenesis of drug-induced gingival overgrowth.[6-8]

IL-6 is a pleiotropic cytokine produced by a wide range of cell types including lymphocytes, monocytes, fibroblasts, and endothelial cells.[9] The gingival fibroblast, the predominant cell type in the gingiva, is responsible for the synthesis and turnover of collagen and glycosaminoglycans in the extracellular matrix of the gingival tissues.[10,11] IL-6 was found to stimulate gingival fibroblasts and play a major role in extracellular matrix synthesis.[7,12] Elevated levels of IL-6

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were also found in pulmonary and renal fibrosis and to be associated with scleroderma.[13-15]

Several studies by various methodologies were conducted about pathogenesis of DIGO with the motive to control and prevent it with the aim of improving esthetics and restoring function.[16,17] However, the complexities of the events that contribute to drug-induced gingival overgrowth are yet to be elucidated clearly. As just a handful of studies have investigated the role of IL-6 in DIGO and considering the paucity of studies in the Indian population on DIGO the present comparative drug effect study was undertaken.[8,18,19] Thus, the objective of this study was to evaluate the involvement of IL-6 in DIGO by highly specific LAB-SA immunohistochemistry so as to assess the role of IL-6 in the pro-inflammatory processes involved in DIGO.

Materials and Methods

After obtaining the Ethical Clearance from the Institutional Ethical Review Board, patients attending the departments of neurology, cardiology, and transplant medicine and who were undergoing treatment with phenytoin; cyclosporin and nifedipine were screened for gingival overgrowth. From them, thirty-five patients were selected for the study and divided into two groups, the control and study groups. The control group of ten patients was from systemically healthy patients reporting for esthetic gingivoplasty procedures and crown lengthening procedures prior to restorations. The control patients had not been prescribed any drugs known to cause gingival overgrowth while for the study group apart from taking phenytoin, cyclosporin or nifedipine, these patients had not been prescribed any other drugs known to cause gingival overgrowth.

The study group consisted of groups A, B, and C.

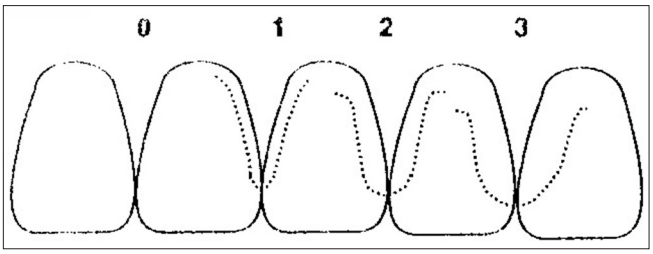
- Group A: Phenytoin-induced gingival overgrowth (ten patients)
- Group B: Cyclosporine-induced gingival overgrowth (ten patients)
- Group C: Nifedipine-induced gingival overgrowth (five patients)

The study procedure was explained to all patients, and informed consent was obtained prior to the study to satisfy institutional ethical requirements. Thorough history taking and clinical examination assessing gingival overgrowth by using gingival overgrowth index (GOI) of Seymour was done in all patients. GOI scores 2 or 3 only were taken for study groups. All patients underwent routine hematological and biochemical investigations. All patients underwent scaling and obtained fitness from their regular physician prior to biopsy.

Gingival overgrowth index by Ellis and Seymour (2004)

Criteria for assessing gingival overgrowth

- No encroachment of interdental papilla onto tooth surface



- Mild encroachment of interdental papilla
- Moderate encroachment involving lateral spread of papilla across buccal tooth surface of less than one-quarter of tooth width
- Marked encroachment of papilla, i.e., more than one-fourth width. Loss of normal papilla form

The GOI of Seymour[20] is the index most commonly used for clinically[20] quantifying the overgrowth seen in DIGO. Marked on a scale of 0–3 it provides an easy reference to quantify the severity of overgrowth and also to plan the surgical intervention necessary for management of the overgrowth.

Immunohistochemical evaluation

After anesthetizing the sample site using 2% lignocaine, gingival biopsy samples [Figure 1] from interdental papilla, gingival margin, and attached gingiva were obtained using a Bard-Paker blade no 15 [Figure 1]. The tissue samples were labeled, fixed in 10% neutral buffered formalin and embedded in paraffin.

Using a microtome, tissue sections of 4 μ were sectioned from the paraffin embedded, formalin fixed, and tissue blocks. Two slides were made from each tissue specimen, one slide for hematoxylin and eosin (H and E) staining and one slide for immunohistochemistry analysis for both control and study groups. All slides were given code numbers to mask the control and study groups so as to prevent bias.

The H and E staining was done to examine for gingival hyperplasia and fibrosis. Moreover, finally using the primary antibody IL-6 (IL-6 antibody from GeneTex, USA - rabbit polyclonal to human IL-6, dilution–1: 400) and the secondary antibody histostain plus kits for LAB-SA Detection system - From Zymed laboratories) immunohistochemical staining and evaluation with control slides stained with positive tissue control (esophageal carcinoma specimen containing maximum IL-6) and negative tissue control (specimen treated with a nonimmune serum instead of same concentration of primary antibody) were done. To prevent any examiner bias the results of immunohistochemistry were analyzed and graded for standardization by two independent oral pathologists for expression of IL-6 in the study and control samples based on severity of staining as per the IHC gradings for tissue samples.

Results

The statistical package SPSS PC+ (Statistical Package for Social Sciences, Ver. 14.01 IBM industries, Armonk, New York) was used for statistical analysis. The mean values and test of significance were obtained using the Kruskaal–Wallis (H test) and Mann–Whitney test.

The fibrosis and IL-6 positive staining was more in the study group when compared to control group. The cyclosporin group (Group B) (ten patients) were more positive for IL-6 in comparison to nifedipine group (Group C) (five patients) and Phenytoin group (ten patients) (Group A).

Table 1 shows the mean values in control and study groups using Kruskaal–Wallis test and the *P* values showed statistical significance in the study group when compared to control group. Figure 2 shows the graphical representation of data, wherein the study group (Groups A, B, C) (phenytoin, cyclosporin, nifedipine,) expresses more IL-6 expression in comparison to control group [Figures 3-5].

Table 2 [Figure 6] shows the result of Mann–Whitney test to compare the control and study Group B (cyclosporin) and the test of significance ($P < 0.05$) showing that there is statistical significance which is very high in fibroblasts followed by blood vessels.

Tables 3 and 4 [Figures 7 and 8] show the results of Mann–Whitney test to compare control with study Group A (phenytoin) and study Group C (nifedipine) respectfully.

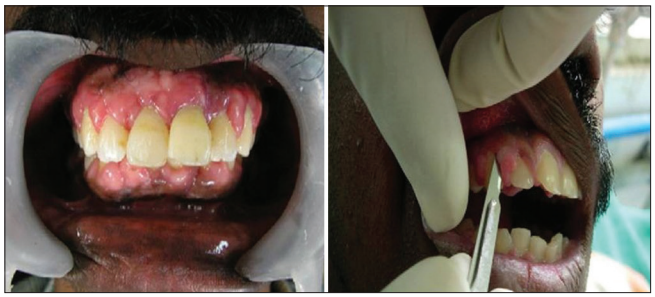


Figure 1: Photograph of biopsy procedure

On comparison, the *P* values show that there is maximum significance of IL-6 staining in cyclosporin group followed by the phenytoin and nifedipine groups.

Discussion

Drug-induced gingival overgrowth remains the most widespread side effect of systemic medications on the periodontal tissues. Three major drug categories – the anti-convulsants, the calcium channel blocking agents and the immunosuppressants have been associated with gingival overgrowth with a reported prevalence rate of 90%.[20] Incidence and prevalence studies have also reported on a wide variability in gingival response to these medications and other confounding variables and risk factors such as – age, gender, dose and duration of drug use, concomitant use of other medications, ability to maintain oral hygiene, role of local factors, and the genetically determined capacity of the host to deal metabolically with chronically administered drugs and the responsiveness of gingival fibroblasts to these drugs.[2,21]

The etiopathogenesis of drug-induced gingival overgrowth has been studied by various authors who have enlightened on the complex pathogenesis of DIGO and the various roles

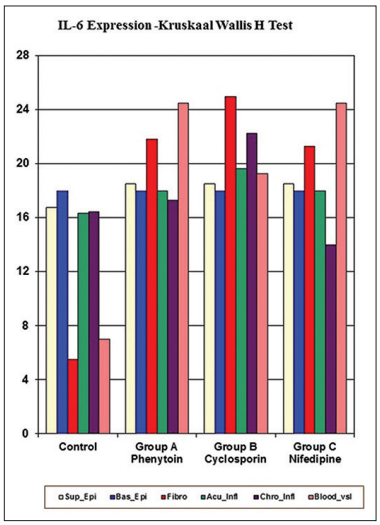


Figure 2: Kruskall-Wallis H Test

Table 1: The mean value in control and study groups and their test of significance using Kruskall–Wallis test

Group	Superficial epithelium	Basal epithelium	Fibroblasts	Acute inflammatory cells	Chronic inflammatory cells	Blood vessel
Control group	16.75	18.00	5.50	16.35	16.45	7.00
Group A (phenytoin)	18.50	18.00	21.85	18.00	17.30	24.50
Group B (cyclosporin)	18.50	18.00	25.00	19.65	22.25	19.25
Group C (nifedipine)	18.50	18.00	21.30	18.00	14.00	24.50
P	0.4753	1.0000	0.0001	0.6369	0.2197	0.0001

P≤0.05 significant

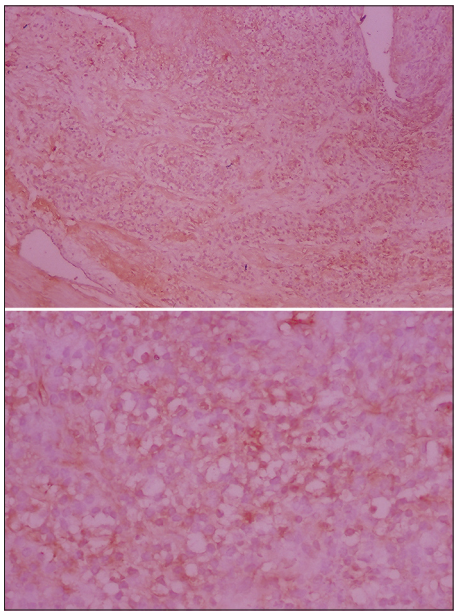


Figure 3: Photomicrograph (×10/×40) showing study Group A (phenytoin) taking up positive IHC staining for interleukin-6

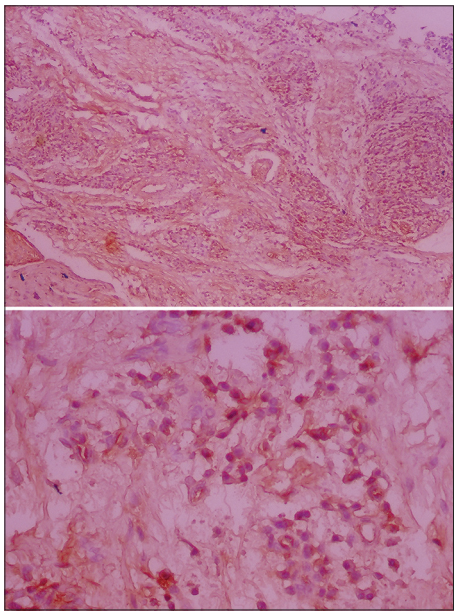


Figure 4: Photomicrograph (×10/×40) showing study Group B (cyclosporin) taking positive IHC staining for interleukin-6

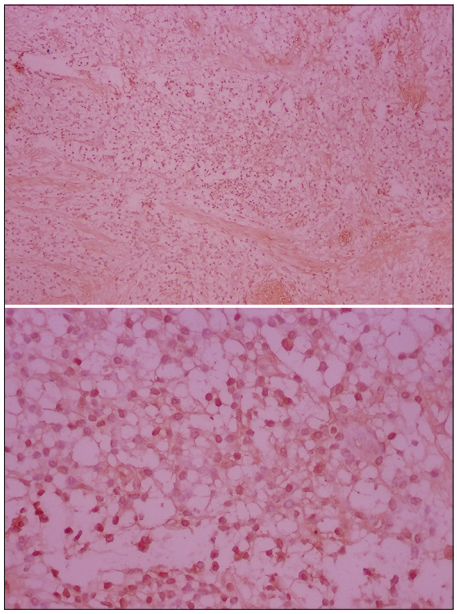


Figure 5: Photomicrograph (×10/×40) showing study Group C (nifedipine) taking positive IHC staining for interleukin-6

Table 2: The mean and *P* value of Mann–Whitney U-test of control and study Group B (cyclosporin)

	Control (mean)	Study Group B (cyclosporin) (mean)	*P*
Superficial epithelium	10.00	11.00	0.3173
Basal epithelium	10.00	11.00	0.3173
Fibroblasts	5.50	15.50	0.0001
Acute inflammatory cells	9.55	11.45	0.1681
Chronic inflammatory cells	9.00	12.00	0.1930
Blood vessel	7.00	14.00	0.0014

P≤0.05 significant

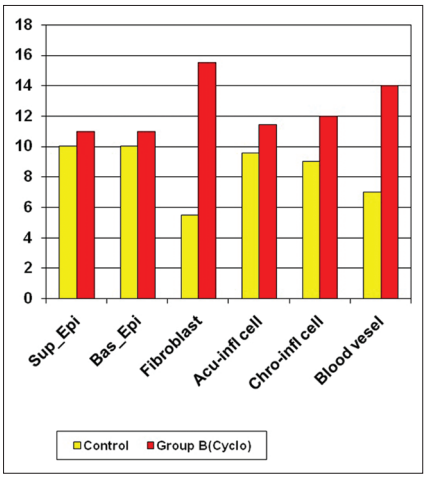


Figure 6: Mann–Whitney test–study Group B (cyclosporin)

Table 3: The mean and *P* value of Mann–Whitney U-test of control and study Group A (phenytoin)

	Control (mean)	Study Group A (phenytoin) (mean)	P
Superficial epithelium	7.60	13.40	0.0096
basal Epithelium	10.00	11.00	0.3173
Fibroblasts	5.50	15.50	0.0001
Acute inflammatory cells	10.05	10.95	0.5839
Chronic inflammatory cells	10.20	10.80	0.7651
Blood vessel	5.50	15.50	0.0001

P≤0.05 significant

played by a range of fibrogenic cytokines like IL-6, and growth factors like PDGF, TGF-β and connective tissue growth factor (CTGF) in the connective tissue metabolism in gingival overgrowth.[17,22,23]

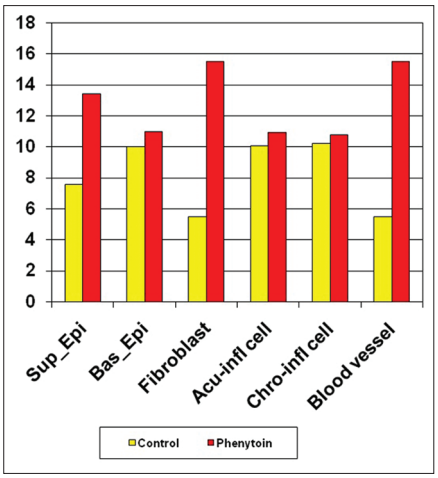


Figure 7: Mann–Whitney test–study Group A (phenytoin)

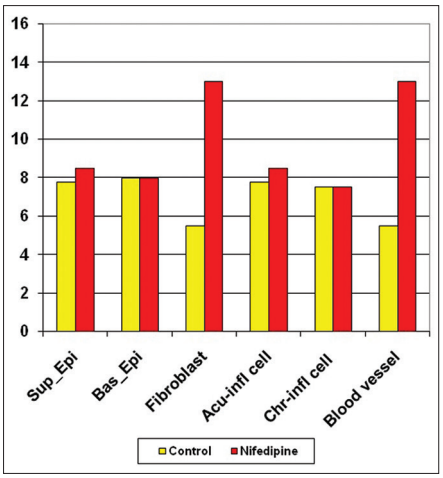


Figure 8: Mann–Whitney test–study Group C (nifedipine)

Table 4: The mean and *P* value of Mann–Whitney U-test of control and study Group C (nifedipine)

	Control (mean)	Study Group C (nifedipine) (mean)	*P*
Superficial epithelium	7.75	8.50	0.4795
Basal epithelium	8.00	8.00	1.0000
Fibroblasts	5.50	13.00	0.0002
Acute inflammatory cells	7.75	8.50	0.4795
Chronic inflammatory cells	8.25	7.50	0.6616
Blood vessel	5.50	13.00	0.0002

P≤0.05 significant

Several studies[3,7,24] have reported that a key histopathological feature of drug-induced gingival overgrowth is a dramatic elevation in the expression of specific cytokines, especially IL-6 by cells within the gingival connective tissue.[12,18,19] These reports are consistent with the findings of increased levels of IL-6 in other fibrotic diseases such as renal and pulmonary fibroses.[14,13]

Rakhi Sinha Morton and Anna Dongari Bagtzoglou reported[24] an enhanced IL-6 secretion in DIGO by Gingival Fibroblasts, thus highlighting the role of pro-fibrotic and pro-inflammatory cytokines in the pathogenesis of drug-induced gingival overgrowth.[19]

Since very few studies have been done to assess the role of IL-6 in drug-induced gingival overgrowth in the Indian population, the present study was undertaken.[19,24,25]

In this study, the IL-6 staining using IL-6 Polyclonal antibody is more in the study group than in the control group. There was statistical significance ($P < 0.05$) in the study group in concurrence with previous studies.[19,25] The IL-6 staining was maximum in the cyclosporine group followed by the phenytoin and nifedipine groups similar to the study by Rakhi Sinha Morton and Anna Dongari Bagtzoglou.[19] In their study, they state that the gingival fibroblasts respond to these drugs with an increased expression of IL-6. Williamson *et al*. reported that in DIGO there is an alteration in the cytokoine profile causing a dysregulation in the connective tissue turnover with a resultant accumulation of matrix components and fibroplasia.[25]

Of all the cytokines playing a regulatory role in the gingival and periodontal connective tissues, IL-6 appears to play an autoregulatory role for both pro-fibrotic and pro-inflammatory cytokines.[26,27] Furthermore, IL-6 targets the connective tissue cells like fibroblasts[24,28] and enhances connective tissue accumulation through increased production of tissue inhibitor of metalloproteinase[29,30] (TIMP) by fibroblasts without any effect on matrix metalloproteinase secretion[31-33] thus ultimately resulting in fibroplasia.

Since the pathogenesis of DIGO is attributed to alterations in the levels of both pro-inflammatory and pro-fibrotic cytokines like IL-6 and IL-1 in addition to growth factors like TGF-β and CTGF, further multifactorial studies with larger sample sizes are required to form a definitive conclusion on the role played by cytokines and growth factors in drug-induced gingival overgrowth.

Conclusion

This study was done to evaluate the role of inflammatory cytokines in the pathogenesis of DIGO as a way to prevent or control the overgrowth. The results of this study show clearly that the pro-inflammatory cytokine IL-6 expression was more in the DIGO groups than in the control group.

The extent of IL-6 expression was maximum in cyclosporin takers followed by those taking phenytoin and nifedipine. The gingival fibroblasts in all the study groups showed the most statistical significance along with nonspecific staining of blood vessels and epithelium which are of no statistical

significance. Future studies may focus on increasing sample size, alternative drug therapy, combination drug therapy, GCF concentration, and also the quantum of drug necessary for induction of gingival overgrowth.

Even though much depends on genetic variability, fibroblast heterogeneity, and individual host response the fact that cytokine expression is elevated in the pathogenesis of DIGO can lead to development of anti-inflammatory modalities as a necessary treatment option in the prevention and management of gingival overgrowth. This study underscores the fact that prevention of cytokine expression by early periodontal therapeutic management and oral hygiene practices may lead to resolution of inflammation and subsequent prevention of DIGO. Hence, the role of a periodontist in early screening and co-management of systemic drug takers cannot be emphasized enough.

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Conflicts of interest

There are no conflicts of interest.

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