Efficacy of Two Different Mouth Washes on the Incidence of Rats Gingival Overgrowth Induced By Cyclosporine-A

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Abstract

Background and objectives: Drug induced gingival overgrowth is known as an adverse effect with three types of drugs. This study was carried out to evaluate the efficacy of mouthwashes containing krameria tincture compared to those of chlorohexidine gluconate (CHX) on (CHX) the incidence of cyclosporine (Cs A) induced gingival overgrowth (GO).

Study design: Sixty adult male albino rats were selected and divided into 4 equal groups. Control group received no treatment. The rats of group II were administrated Cs A. Meanwhile, rats of groups III and IV were handled as group II and treated with chlorohexidine (CHX) and mouthwash containing krameria respectively. Routine tissue processing was carried out for staining with keratinocyte growth factor (KGF) and connective-tissue growth factor (CTGF). The results of this study were analyzed statistically.

Results: It was found that control group exhibited mild gingival overgrowth (GO) with knife edge gingival crest while group II yielded moderate gingival overgrowth GO with blunt gingival crest (20%) and sever gingival overgrowth GO with bulbous gingival crest. Rats of groups III and IV revealed mild gingival overgrowth (GO) with knife edge gingival crest. Also, Group II had the highest mean values for keratinocyte growth factor (KGF) and connective tissue growth factor (CTGF) while control group showed the lowest values. Statistically, there was an overall significant difference between the studied groups as well as between each two groups except between groups III and IV.

Conclusion: From the findings of the present study, we can conclude that chlorohexidine (CHX) and krameria tincture containing mouthwashes might play essential biological role in the reduction of drugs induced gingival overgrowth (DIGO) progression through their effects on the expression of keratinocyte growth factor (KGF) and connective tissue growth factor (CTGF).

Keywords: Drugs Induced Gingival Over Growth (DIGO); Cyclosporine (CsA); Keratinocyte Growth Factor (KGF); Connective Tissue Growth Factor (CTGF); Chlorohexidine (CHX) and Krameria

Introduction

Gingival overgrowth (GO) is an abnormal growth of the periodontal tissue. It’s characterized by the accumulation of extracellular matrix (ECM) in gingival connective tissues, particularly collagenous components, with varying degrees of inflammation [1]. This fibrotic overgrowth of gingival is caused by a variety of etiological factors [2].

Drug-induced gingival overgrowth (DIGO) remains the most widespread unwanted effect of systemic medication in the gingival. It is known as an adverse effect with three types of drugs: phonation (PNT), anti-epileptic drug cyclosporine (CsA), an immunosuppressive drug; and calcium channel blocker [3].

CsA is a potent immunosuppressive compound that has been used increasingly in conjunction with kidney, heart and other transplants, and has been reported to be better tolerated than conventional therapy such as corticosteroids and cytotoxic drugs [4]. The major adverse reactions to cyclosporine therapy are Nephrotoxicity, hepatotoxicity, tremors, hypertension, mild anemia, GO and, in rare instances, lymphoma [5].

The exact mechanism of action is not known. Experimental evidence suggests that its effectiveness is due to specific and reversible inhibition of immune competent lymphocytes in G0- or G1-phase of the cell cycle especially, T lymphocytes [6]. Induction of GO by CsA occurs via its stimulatory effects on expression of TGF-β which induces fibroblast differentiation into my fibroblast [7]. My fibroblast is characterized by expression of specific isoform α-SMA and when activated, synthesizes elevated levels of extra cellular matrix (ECM) proteins [8].

Those cells are considered the main cellular type involved in ECM deposition in fibrotic diseases [1,7]. CsA may reach connective tissue of the gingiva through the blood stream or from the oral cavity through the sulcular epithelium [9]. Tooth brushing and interdental cleaning are the mechanical aids for treatment and chemical plaque control. Among these chemical agents used as antiplaque agents is chlorohexidene (CHX) which considered as the gold standard because of its property of substantivity in preventing plaque formation. The mouth rinses of CHX gluconate (0.12%) demonstrates great reduction in supragingival plaque [10].

Chlorohexidine is considered the most effective antimicrobial agent, if used as mouthwash in dentistry, due to its broad spectrum action against Gram-positive and Gram-negative bacteria. This effect is a result of the dicationic nature of the CHX molecule, which also affords the agent the property of a persistent antimicrobial effect on the tooth surface by both bactericidal and bacteriostatic actions [11].

The dried root of krameria Triandra (Rhatany roots), containing less than 10% tannis has astringent properties similar to those of tannic acid. The tincture has been used as astringent mouthwash for the mucous membrane [12]. Rhatany is sometimes used as a mouthwash or gargle for mild mouth and throat irritation, swollen gums, cracked tongue and cancer sores. It is also applied to the skin for leg ulcers and for swelling and itching caused by cold and damp weather [13].

Keratinocyt growth factor KGF is a member of fibroblast growth factor family also known as FGF7. It is a growth factor present in the epithelialization-phase of wound healing. In this phase, keratinocytes are covering the wound, forming the epithelium. Keratinocyte growth factor are a family of at least 23 structurally related polypeptides known to play a critical role in angiogenesis and mesenchymal cell mitogenesis [14].

Among them, KGF acts not only as a potent mitogen for primary human keratinocytes, but also promoting their differentiation program, and protecting them against apoptosis induction [15]. Furthermore, KGF is involved in experimental and in vivo wound healing models, stimulating migration of keratinocytes, and inducing reorganization of actin cytoskeleton, therefore increasing epithelial cell motility [16].

The CCN family consists of six multifunctional members including CCN1 (Cyr61), CCN2 (CTGF), CCN3 (Nov), CCN4 (WISP1), CCN5 (WISP2), and CCN6 (WISP3) [18]. The functions of this family include embryogenesis, wound healing, and regulation of ECM production. CTGF is a cystein-rich peptide that plays a variety of important roles in cell development and differentiation and acts to promote fibrosis in many different tissues in cooperation with other growth factors and ECM proteins [19]. So, this study will be conducted to investigate impact of mouth washes containe krameria tincture compared to those of CHX on incidence CsA induced GO.

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Materials and methods

Animal subjects

Sixty adult male albino rats, weighing 150 to 200g were selected. These animals were housed and cared according to the guidelines of the Medical Experimental Research Center, Faculty of Medicine, Mansoura University. They were kept in a light controlled room with (12:12-h) light dark cycle and temperature (± 22°C). Relative humidity (65-70%) was kept constant. They were received commercial soft diet and water. The rats were classified randomly into 4 equal groups:

Group I (control group): The rats of this group received no treatment.

Group II: The rats of this group administered Cs A in mineral oil via gastric feeding daily for 7 weeks [19].

Group III: The rats of this group were handled as those of group II and concomitantly received CHX mouthwash twice daily, for the same period.

Group IV: The rats of this group were handled as those of group III and received karamira mouthwash instead of CHX, for the same period.

Medicine supplementation

Cs A: Soft gelatinous capsules of Cs A (100 mg, Sand immune, Novartis Pharmaceuticals Corporation, Hanover, Germany) was dissolved in 16.5 cm³ mineral oil. The dissolved drug was given by gastric syringe, each rat received 30 mg/kg daily.

Mouthwashes: CHX gluconate (Heritor; Sigma Pharmaceutical Industries, Egypt), karamira (phenol keramine) were used topically.

At the end of the experiment, all the rats will be scarified and their gingival will be excised. Routine tissue processing will be carried out for staining with:

I-Hematoxylin and eosin stain.

II-Immune histo chemical staining

The immunohistochemical staining for KGF7 and CTGF will be performed according to the manufacturers instruction using avoiding complex method.

The immunohistochemical staining will be assessed and scored.

Scoring and evaluation of GO using H&E stained slides

A modified scoring method was done according to the index described by Banthia, et al. [21]. The crown length was divided into three equal parts (cervical, middle and occlusal). The hyperplastic index measured the degree of gingival enlargement in an apico-coronal direction by means of 4-point scale:

0): no GO (gingiva still in the cervical one third,
1): mild (gingiva overgrown to the middle one third),
2): moderate (gingiva overgrown to the lower half of the occlusal one third) and
3) Sever (gingival overgrown to the upper half or above the occlusal one third).

Statistical analysis

The data were tabulated, coded, and then analyzed using SPSS version 17.0. For the analytical statistics, significant differences were tested using multivariate analyses of variance to compare between more than two groups for the numerical parametric data, followed by post hoc LSD for multiple comparisons. Moreover, the Kruskal-Wallis test was used to compare between more than two groups for categorical data, followed by the Mann-Whitney U test to compare two groups. The statistical tests were based on a type 1 error value of 5% (α = 0.05) and on a power of 0.85 sample size. P value ‘0.05 represents level of significance.

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Results

The degree of gingival overgrowth in Group II and group III rats showed GO with variable extents over the crown of teeth. Control group exhibited mild GO with knife edge gingival crest. Group II yielded moderate GO with blunt edge (20%) and severe GO with bulbous gingival crest (80%). Rats of groups III and IV revealed 100% mild (score 1) gingival hyperplasia with knife edge gingival crest. The highest mean rank of GO was for group II (52.60), while the lowest mean rank was for the control group (8.87). In addition groups III and IV mean ranks were equal (30.27). The Krystal-Wallis statistical test revealed an overall significance between the different groups (P < 0.001). The Mann-Whitney U test revealed a significant difference between every two groups (P < 0.001) except between groups III and IV (Table 1).

The Hematoxylin and eosin finding in control group, the gingival tissue of control group showed keratinized stratified squamous epithelium covering a core of fibrous tissue. Group II showed hyperkeratosis epithelium with thin and slender or with bulbous and broad elongated rute processes. The underlying connective tissue was hypo vascular with dense and coarse collagen bundles running in tangentially wavy pattern. Blood vessels were small slit like channels (Figure 1 AB). Group III and IV the epithelium in these groups revealed slight hyperplasia. Rate processes were slightly elongated as compared to those of the control group. The connective tissue showed collagen bundles and spindle shaped fibroblasts and hypo as celerity. The inflammatory Cells were less prominent (Figure 1 CD).

In immune history chemical finding Group II had the highest mean values of KGF (30.1 ± 1.2) while control group had the lowest one (9.6 ± 1.5). KGF was expressed mildly in control group and the antibody was detected only at basal cells. Group II immune reactivity for KGF was noticed throughout the epithelial thickness and the majority of basal cells was positive (Figure 2 AB). Groups III and IV exhibited moderate reactions for KGF. The reaction for KGF in group III was seen mostly at basal cells and within few scattered spinout cells. Meanwhile, most of the reaction in group IV was observed within spinout cells and mildly within some basal cells (Figure 2 CD).

Regarding CTGF, group II had the highest mean value (75.9 ± 2.5) while control group had the lowest one (7.9 ± 2.6). It was expressed with moderate level within connective tissue of control group while the epithelial cells of this group were negative. Fibrous tissue of group II revealed intense reaction for CTGF while the epithelial cells of this group showed mild CTGF immune reactivity (Figure 3 AB). Groups III and IV had intense reaction for CTGF within their fibrous tissues and mild reaction within their epithelia (Figure 3 CD).

Statistically, One-way MANOVA test for KGF and CTGF revealed an overall significant difference between the different groups (P < 0.001). While, LSD post hoc test for multiple comparisons revealed a significant difference between each two groups except between groups III and IV. (Table 2)

**Figure 1:** A. group I showing cuboidal basal cells with hyperchromatic nuclei (arrow), the basement membrane is flat (crooked arrow) with no rute processes. The connective tissue appears with normal arrangement of fibers.
B. Group II showing more elongated rete processes. The basal cells show disturbance and crowding (arrow). The spinous cell layer show extensive acanthosis and hyperchromatism (curved arrow). The connective tissue show increase collagen fibers and inflammatory cells (arrowhead).
C. Group III showing mild acanthosis and slightly elongated rete pegs (arrow).
D. Group IV showing basal cell layer with normal arrangement (arrow), the spinous cell layer shows infrequent mitosis (curved arrow) (H&E X400).

**Figure 2:**
A. Group I shows mild expression of KGF mainly at basal cells and few scattered superabasal cells.
B. Group II shows intense expression of KGF throughout the epithelial layers.
C. Group III shows moderate expression of KGF, most of the positive reaction is at basal cells.
D. Groups IV shows moderate expression of KGF mainly within cells. (IHC staining DAB chromogen X400).

**Figure 3:**
A. control group section shows moderate expression of CTGF Within connective tissue core.
B. Group II section reveals intense reaction for CTGF within connective tissue, the epithelium shows mild expression of CTGF.
C. Group III section shows intense reaction for CTGF within its connective tissue, the epithelium shows mild reaction for CTGF.
D. Group IV section shows intense reaction for CTGF within its connective tissue, the epithelium shows mild reaction for CTGF. (IHC staining DAB chromogen X100).

### Table 1: Non-parametric statistical tests used to determine the degree of gingival overgrowth for all groups.

*Kruskal–Wallis test

**Mann-Whitney U test

<table>
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<tr>
<th>Groups</th>
<th>Mean Rank</th>
<th>Groups</th>
<th>Mann-Whitney U</th>
<th>Z</th>
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<tr>
<td>I</td>
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<td>I&amp;II</td>
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<td>.001</td>
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<tr>
<td>II</td>
<td>52.60</td>
<td>I&amp;III</td>
<td>6.50</td>
<td>-4.92</td>
<td>.001</td>
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<tr>
<td>III</td>
<td>30.27</td>
<td>I&amp;IV</td>
<td>3.00</td>
<td>-4.92</td>
<td>.001</td>
</tr>
<tr>
<td>IV</td>
<td>30.27</td>
<td>II&amp;III</td>
<td>3.00</td>
<td>-4.92</td>
<td>.001</td>
</tr>
<tr>
<td>Chi-Square</td>
<td>53.045</td>
<td>II&amp;IV</td>
<td>3.00</td>
<td>-4.92</td>
<td>.001</td>
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<tr>
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<td>3</td>
<td>III&amp;IV</td>
<td>112.50</td>
<td>0.00</td>
<td>1.000</td>
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</tbody>
</table>

| P1 value* | 0.001 |

### Table 2: One way-MANOVA and LSD post hoc test for KGF and CTGF for all groups at the examination periods.

*One way-MANOVA test

**LSD post hoc test

<table>
<thead>
<tr>
<th>Groups (F ratio and P value)</th>
<th>Mean ± SD</th>
<th>Groups</th>
<th>(Mean difference and P value)</th>
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<tr>
<td>(1.857, 0.001*)</td>
<td>KGF</td>
<td>CTGF</td>
<td>I&amp;II (195, 0.001** ) (680, 0.001** )</td>
</tr>
<tr>
<td>One way- MANOVA</td>
<td>Groups</td>
<td>KGF</td>
<td>CTGF</td>
</tr>
<tr>
<td>KGF</td>
<td>(384.27, 0.001*)</td>
<td>I</td>
<td>9.6 ± 1.5</td>
</tr>
<tr>
<td>CTGF</td>
<td>(2181.37, 0.001*)</td>
<td>II</td>
<td>030.1 ± 1.2</td>
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<tr>
<td></td>
<td></td>
<td>III</td>
<td>020.0 ± 1.8</td>
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<td></td>
<td></td>
<td>IV</td>
<td>019.4 ± 1.7</td>
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### Discussion

In this study, we examined alterations in gingival tissue clinically and histologically due to drug-induced gingival overgrowth (DIGO) using cyclosporine A (CsA) and evaluated expression of connective tissue growth factor (CTGF) and keratinocyte growth factor (KGF) as markers of gingival overgrowth healing [20].

In Hematoxylin and eosin results, we found that buccolingual sections in mandibular molars showed microscopic examination of the studied groups confirmed that gingival overgrowth was obvious in CsA treated groups, while enlargement appeared less prominent in mouth washes treated groups. This is partially in accordance with [21,22] who found that gingival overgrowth buccally was sever in groups treated with CsA in comparison with healthy controls.

Regarding examination of histological findings of the studied groups confirmed that the gingiva of the control group showed normal thickness of epithelium and keratin with normal arrangement of the four epithelial layers, the basal cells, the spinous cells, the

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granular cells, and the keratin were well adhered to the underlying layers. The connective tissue showed normal arrangement of fibers and blood vessels as described by (Garant) [23].

The experimental Group II which received cyclosporine A (CsA) showed acanthotic epithelium with hyperorthokeratosis and elongated rete processes within the underlying collagenous and vascular connective tissue. The inflammatory cells were distributed throughout the connective tissue. These results were in agreement with previous studies done by [23-25]. They indicated that thickening of the epithelium and elongated rete processes with highly vascular connective tissue and focal accumulation of inflammatory cells appeared to be a characteristic feature of CsA induced gingival overgrowth (GO). Also [26], reported that the epithelial hyperplasia may be referred to proliferation and hypertrophy of the spinous cell layer.

In the present study, the group III that was treated with application of chlorhexidine (CHX) containing mouthwash aided in reducing the severity of gingival overgrowth (GO). We found the epithelium with mild hyperorthokeratosis and acanthosis. The rete pegs are small, the connective tissue shows numerous vascular channels and collagen bundles. This finding is consistent with [27,28], who recorded that chlorhexidine (CHX) can reduce the severity of GO triggered by CsA administration. Since, chlorhexidine (CHX) mouthwash is considered as gold standard with concentration of 0.2% to 0.12% shown to be effective in preventing plaque accumulation. Thus, adequate plaque control can improve gingival condition [29] while GO was exacerbated when dental plaque was retained [30].

The present study, the group IV that was treated with krameria mouthwash reduced the severity of gingival overgrowth (GO) induced by Cyclosporin A (CsA), and we found less prominent in gingival enlargement, the epithelium show mild acanthosis and mild hyperkeratosis, the underlying connective tissue shows mild inflammatory cell infiltrate and hypovascular. This is in agreement with [31], who found that krameria tincture had anti-inflammatory activity in the acute inflammation models when administered topically in gingival overgrowth induced by CsA. In group II shows increase in expression of (KGF) and (CTGF) these finding were in agreement with [32], who reported that some cytokines and growth factors were found in higher levels in gingival overgrown tissues, including interleukin-6 (IL-6), IL-1, Keratinocyte growth factor (KGF), fibroblast growth factor-2 (FGF-2), transforming growth factor-β (TGF-β) and connective tissue growth factor (CTGF).

In the present study, our results for (KGF) and (CTGF) expression the present study, it was obviously increased in group II, and decreased in groups III and IV. In accordance [19,21], Found that Cyclosporin A (CsA) treatment caused high expression of keratinocyte growth factor (KGF), whereas chlorhexidine (CHX) treatment twice a day diminished this expression.

Conclusions
In the light of the findings of the present study, we can conclude the followings:
1 – cyclosporine-A induced GO incidence might be minimized through strict oral hygiene program and plaque control.
2 – Krameria tincture and CHX mouth rinses could reduce the severity of the CsA induced GO through their anti-inflammatory effects.
3 – Krameria tincture mouth wash could be used as an alternative to CHX for individuals who have an interest in naturally based products and to reduce the side effect of CHX resulting from long term use of CHX as teeth staining and mucosal irritation.

Bibliography

17. Leask A and Abraham DJ. " Center for Rheumatology, Department of Medicine, Royal Free, University College London, Rowland Hill Street, London NW3 @PF, U.K Biochem Cell Biol". Dec; 81.6 (2003) : 355-363.


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